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Qiao Niu *Editor*

Neurotoxicity of Aluminum

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Qiao Niu
Editor

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Editor
Qiao Niu
Department of Occupational Health, School of Public Health
Shanxi Medical University
Taiyuan, Shanxi, China

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Contributors

Christopher Exley The Birchall Centre, Lennard-Jones Laboratories, Keele University, Keele, Staffordshire, UK

Ruifeng Liang Department of Environmental Health, Shanxi Medical University, Taiyuan, People's Republic of China

Xiaoting Lu Occupational Health Department of Public Health School, Shanxi Medical University, Taiyuan, Shanxi, China

Jisheng Nie Department of Occupational Health, School of Public Health, Shanxi Medical University, Taiyuan, Shanxi, China

Qiao Niu Department of Occupational Health, School of Public Health, Shanxi Medical University, Taiyuan, Shanxi, China

Christopher A. Shaw Department of Ophthalmology and Visual Sciences, University of British Columbia, Vancouver, Canada

Jing Song Republic Health School, Shanxi Medical University, Taiyuan, Shanxi, China

Linping Wang Shanxi Medical University, Taiyuan, People's Republic of China

Huifang Zhang Department of Occupational Health, School of Public Health, Shanxi Medical University, Taiyuan, Shanxi, China

Qinli Zhang Shanxi Medical University, Taiyuan, China

University of Mississippi Medical Center, Jackson, MS, USA

Chapter 1
Overview of the Relationship Between Aluminum Exposure and Health of Human Being

Qiao Niu

Abstract Aluminum is a type of ubiquitously existing naturally and widely used metal in our world. It is combined with other elements and forms different compounds. In different pH and due to other conditions, it can be released into ions of different valence states. Our century is an "aluminum age"; aluminum is used in many fields of our daily life, such as vaccine adjuvant, antacids, food additives, skin care products, cosmetics, and cooking wares, and may be as elements or contaminants appeared in a lot of foods, including infant formulae, milk products, juice, wine, sea foods, and tea. It also appears in drinking water due to the water treatment process, or naturally coming from weathering rocks and soils, or released from rocks and soils caused by pollution-induced acid rain. Due to good physical and chemical property, aluminum is being tremendously utilized in many industries. In a lot of production and process procedures, aluminum particulates are seriously exposed by workers. Many factors, such as silicon, citrate, iron, calcium, fluoride, etc., can affect absorption of aluminum in human body. Human being ingests aluminum through the respiratory and digestive system and skin. Aluminum can affect our health, especially impair central nervous system. The important damage is cognitive impairment in Al-exposed peoples, Alzheimer's disease and other neurodegenerative disorders have been related with aluminum exposure, and aluminum has been proposed as etiology.

Keyword Aluminum · Dietary intake · Occupational exposure · Adverse effect · Cognitive impairment

Q. Niu (✉)
Department of Occupational Health, School of Public Health, Shanxi Medical University, Taiyuan, Shanxi, China
e-mail: niuqiao55@sxmu.edu.cn

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1.1 Aluminum in the Environment

Aluminum is a type of widely used light metal with the second position in utilization ranking of metals. It is ubiquitous, the third most common element, and the first rich metal in the earth, accounting for about 8% of the earth's crust. It is combined with oxygen, fluorine, silicon, sulfur, and other species, does not appear naturally in the elemental state [4, 11, 93], and mainly exists as bauxite rock and other aluminum salts, such as silicates and cryolite. With centuries and centuries weathering of rocks and volcanic activity as natural processes for most part of aluminum redistribution in the environment [4, 44, 93], it is released to the environment naturally as aerosols, settled in surface water and earth. From the nineteenth century, aluminum was found and extracted from rocks, and due to its excellent chemical and physical property, such as low gravity, ductility, malleability, reflectivity, high tensile strength, corrosion resistance, readily machined into shapes, and high electrical conductivity, it was quickly utilized at an incredible quantity for many purposes; we got into an "aluminum age." Bauxite is a type of aluminum salt and the most important raw material to be refined to produce alumina, from which aluminum metal is recovered by electrolytic reduction; aluminum is also recycled from scrap. Industrial activities of aluminum production and use performed by human being such as exploration, mining, smelting, manufacturing, and polishing also result in the anthropogenic release of great amount of aluminum to the environment. Only from January 2015 to September 2017, the total production of alumina over the world is 327,156 thousand metric tonnes (Fig. 1.1), and metal aluminum is 163,464 metric tonnes (Fig. 1.2). China is the greatest contributor for aluminum mining, refining, production, fabrication, manufacturing, and use (Fig. 1.3) (http://www.world-aluminium.org/).

The biggest utilizations for aluminum metal and its alloys are in production of transportation vehicles, such as cars, buses, high-speed trains, and aircrafts, materials of building and construction, packaging, and electrical equipment. Transportation vehicle uses are one of the fastest growing areas for aluminum use due to the surprisingly growing need from China and other emerging economies. Aluminum powders are used in a lot of industrial fields, such as pigments and paints, fuel additives, explosives, and propellants. Aluminum oxides are not only used as raw materials to produce metal aluminum but also as food additives and in the manufacturing of abrasives, refractories, ceramics, electrical insulators, catalysts, paper, spark plugs, light bulbs, artificial gems, alloys, glass, and heat-resistant fibers. Aluminum hydroxide is used widely in pharmaceutical and personal care products, for example, as adjuvant in vaccine, main constituent of antacids. Aluminum compounds are used in food industries as preservatives, fillers, coloring agents, anticaking agents, emulsifiers, and baking powders; soy-based infant formula can contain aluminum. Natural aluminum minerals especially bentonite and zeolite are used in water purification, sugar refining, brewing, and paper industries.

In recent decades, with the fast industrialization and burning of fossil fuel, great amount of NO, NO_2, SO_2, SO_3, and CO_2 is emitted into the air, combines with water

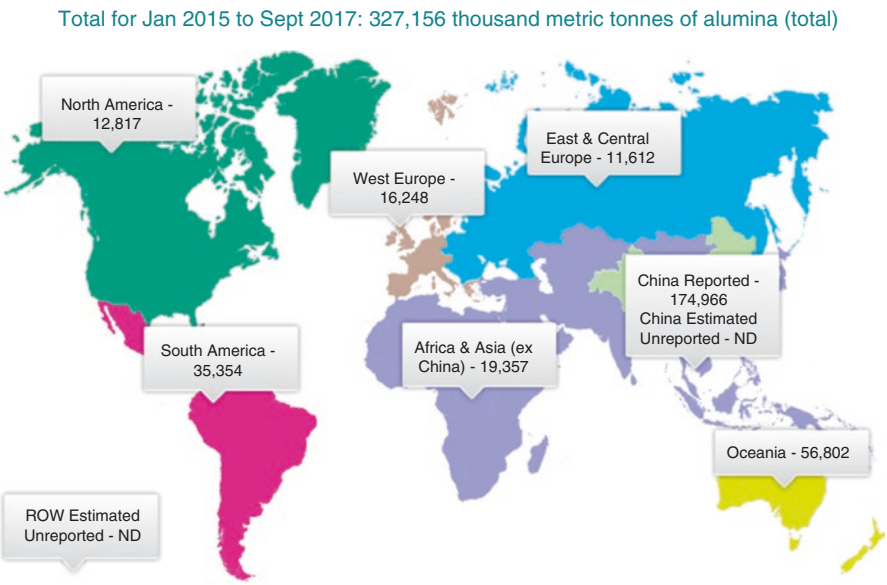

Fig. 1.1 Total quantity of alumina produced globally from January 2015 to September 2017 is 327,156 thousand metric tonnes. China is the biggest producer with the production quantity of 174,966 thousand metric tonnes, accounting for 53.48% of the global production quantity. Oceania and South America stand at the second and third positions with production quantity of 56,802 and 35,354 thousand metric tonnes, respectively, accounting for 17.36% and 10.80%, respectively. The production quantity in North America, West Europe, East and Central Europe, and Africa and Asia (ex., China) are between 10,000 and 20,000 metric tonnes

vapor, and then forms acidic rain that falls into ground earth and results in bauxite resolving and Al^{3+} releasing into soil and surface water.

In general, the forms of aluminum in metal, oxide, and hydroxide are hardly soluble in water and organic solvents, but some aluminum compounds, such as aluminum alkyls, alkyl halides, hydrides, bromide, chloride, iodide, carbide, chlorate, nitride, and phosphide, are active to react with water.

Aluminum levels in environmental media vary tremendously depending upon the location where geochemical constituents are different, the degree of industrialization, the severity of pollution, and sampling site. Generally, background levels of aluminum in the atmosphere are different, ranging from about 0.6 to 7.0 μg/m^3 [49]. Much higher levels can be routinely observed in urban and industrial areas, especially heavily industrialized cities and seriously polluted regions. Aluminum levels in surface water is usually very low (<0.1 mg/L); however, in acidic waters or water with high content of humic or fulvic acid, the concentration of soluble aluminum increases due to the increased solubility of aluminum oxide and aluminum salts [95]. Its concentration in soils and rocks varies greatly, ranging from about 7 to over 100 g/kg in different geographical and geological locations. Some natural aluminum minerals especially bentonite and zeolite are used in water purification; this process is thought

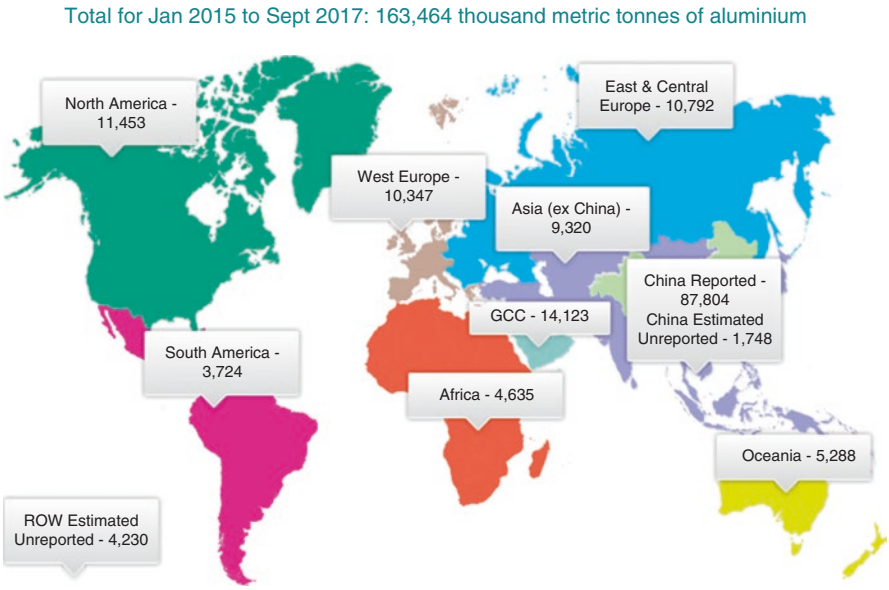

Fig. 1.2 Total quantity of metal aluminum produced globally from January 2015 to September 2017 is 163,464 thousand metric tonnes. China is still the biggest producer with the production quantity of 89,552 thousand metric tonnes, accounting for 54.78% of the global production quantity. Gulf Cooperation Council (GCC) stands at the second position with production quantity of 14,123 thousand metric tonnes, accounting for 8.64%. The production quantity in North America, West Europe, East and Central Europe, and Africa and Asia (ex., China) all are around 10,000 thousand metric tonnes, respectively. Oceania, Africa, and South America are small producers of metal aluminum; their production quantity together is 13,647 thousand metric tonnes, accounting for only 8.35% of global quantity

to leave behind Al ions in the treated water that we drink everyday. In the environment, only one oxidation state of aluminum exists, Al^{+3}, and it does not undergo oxidation reduction reactions. Al^{+3} is a type of reactive ion and can react with other matters in the environment to form various complexes. Environmental factors, such as pH, salinity, and the presence of various species with which aluminum may form complexes, can largely control the fate and transport of aluminum. In general, when the soil is rich in organic matters which are capable of forming aluminum-organic complexes and when the pH is low, such as in areas prone to acid rain or in acidic mine tailings, the solubility and mobility of aluminum in soil are greatest.

1.2 Exposure of Aluminum by Human Being

The general population is primarily exposed to aluminum through the consumption of food items, taking in antacids, ingestion of aluminum in drinking water, and inhalation of ambient air, though the latter two exposure ways are believed as only minor parts. There are other ways in which the people are exposed to aluminum.

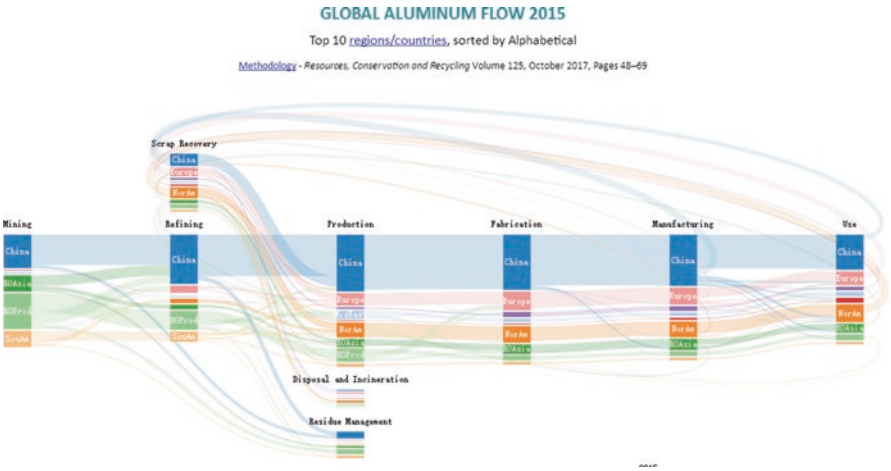

Fig. 1.3 From the global aluminum flow 2015, it is clearly showed that China is the biggest aluminum producer and user through whole production-utilization chain: mining, refining, scrap recovery, production, fabrication, manufacturing, and use

1.2.1 Dietary Aluminum Exposure

Aluminum oxides are used as food additives, such as preservatives, fillers, coloring agents, anticaking agents, emulsifiers, and baking powders. The concentration of aluminum in foods and beverages varies widely, depending upon the type of food product, the technology or type of processing performed, and the geographical areas in which food crops are grown. Based on the FDA's 1993 Total Diet Study dietary exposure model and the 1987–1988 US Department of Agriculture (USDA) Nationwide Food Consumption Survey, the investigators estimated daily aluminum intakes of 0.10 mg Al/kg/day for 6–11-month-old infants, 0.30–0.35 mg Al/kg/day for 2–6-year-old children, 0.11 mg Al/kg/day for 10-year-old children, 0.15–0.18 mg Al/kg/day for 14–16-year-old males and females, and 0.10–0.12 mg Al/kg/day for adult (25–30- and 70+-year-old) males and females. In Wuhan, Central China, 59 samples of youtiao were taken and analyzed; the aluminum contents were from 514.6 to 1578.6 mg/kg, much higher than China National Standard (GB) 2760–2014 [51]. Both the mean and median aluminum contents of youtiao, a typical, traditional, and widely consumed fried dough food in China, exceeded 100 mg/kg, which is the limit value for aluminum regulated by China National Standard (GB) 2760–2014 [87], though the median and 97.5th percentile of weekly dietary intake of aluminum from youtiao did not exceed the provisional tolerable weekly intake (PTWI) set by the joint FAO/WHO Expert Committee on Food Additives. If an adult eats 327.10 g of youtiao per week, which is very possible in China, the weekly dietary intake of aluminum would exceed the PTWI [50]. Like most substances ingested into the digestive tract, aluminum is absorbed from the upper intestine more than from the stomach. The stomach is lined by a thick, mucus-covered membrane which has a much smaller surface area than the intestine membrane has.

Aluminum absorption in the digestive tract seems to be a two-step process, a mucosal cell uptake of Al as initial and then a much slower release into the blood following. The mechanisms mediating aluminum absorption in the digestive tract have been suggested to include both passive (diffusion) and active (carrier- and vesicular-mediated) transport across intestinal cells, as well as paracellular diffusion between these cells.

1.2.1.1 Aluminum in Tea and Cookwares

Depending upon regional tea consumption habit, especially in China, Japan, eastern Asia countries, and the United Kingdom, tea may be a major source of Al ingestion. The Al concentration in fermented tea (794 ± 140 mg/kg) was higher in some degree than that in raw tea (594 ± 129 mg/kg). According to the tea consumption investigation in residents of two main cities of Yunnan, China, a main tea plantation and consumption area, mean daily Al doses taking from tea were 99–60 μg/kg bw/day [12]. Consumption of tea infusions can account for up to 50% of one's daily Al exposure [106]. Aluminum minerals bentonite and zeolite are also used in sugar refining and brewing and left in sugar which often be used in food. In developing countries, the aluminum cookwares made from scrap metal are widely used in many families; an investigation [96] reported that the mean exposure estimate for aluminum was 125 mg per serving (250 ml) with aluminum cookware, more than six times the World Health Organization's provisional tolerable weekly intake of 20 mg/day for a 70 kg adult, and 40 of 42 cookwares tested exceeded this level. Besides, the artisanal aluminum cookwares tested also released great amount of lead, cadmium, and arsenic. Apart from aluminum cookwares, some other metallic, glass, stainless steel, and ceramic utensils can leach considerable quantities of aluminum.

1.2.1.2 Aluminum Exposure from Infant Milk and Formula

Infant formula can contain aluminum; a survey in EU market [63] revealed that all 30 infant formulas sampled, both ready-to-drink milks and milk powders, were contaminated with aluminum, especially the concentration of aluminum in 2 soya-based milk products was as high as 656 and 756 μg/L. The data from other countries vary greatly, such as 440 and 730 mg/L in ready-to-use milk and soy-based formulas and 3442 μg/L in a milk-based iron-fortified ready-to-use formula, in Canada [14]; aluminum concentrations might be as low as 6 μg/L and as high as 1152 μg/L (particularly for soy-based, lactose-free, and hypoallergenic formula) in Britain, the European continent countries, Nigeria, Saudi Arabia, and the United States. The Al concentrations in milk vary greatly depending upon source, location, and local practice. The concentrations in milk are increased in a rank of complexity of processing: 0.004 ± 0.001 mg/L in raw cow's milk, 0.081 ± 0.010 mg/L in "small market" milk, 0.732 ± 0.270 mg/L in powdered milk, and 0.027–5.7 mg/kg in processed cheese which is thought to be due to the addition of anticaking additives including sodium

aluminosilicate while making cheese [1]. So the major part of aluminum in milk products should be contaminants while being processed depending to the complexity of processing technology.

1.2.1.3 Aluminum in Other Foods

High aluminum concentrations were found in a lot of foods in Europa and Japan. 21.09 mg/kg in molluscs and crustaceans, 25.5 mg/kg in shrimp and 42.9 mg/kg in mussel, 116 mg/kg in shellfish, and 88.4 mg/kg in sea urchin [3]. Millour et al. found Al up to 116 mg/kg in shellfish [60], and the edible portions of 159 species of saltwater organisms collected from 4 French coastal areas were reported a mean Al content at 1.35 mg Al/kg [38]. Aluminum were also found in market-sold fruit juices, wines, alcohol, coffee, beer, bottled water, meat, sweats, oils and fats, rice, cereals and potatoes, fruit, green and yellow vegetables, pasta, pastries, and cakes. In Brazilian market-sold juices, Al concentrations in grape juice were reported ranging from less than 0.1 to 0.19 mg/L, in peach juice ranging from 0.15 to 0.31 mg/L, in mango juice varying from less than 0.1 to 0.25 mg/L, in passion fruit ranging from less than 0.11 to 0.37 mg/L, and in guava juice ranging from less than 0.19 to 0.3 mg/L [10]. Tariba summarized the Al concentrations in wines from different countries, 0.017–0.018 mg/L from Argentina, 0.132–1.67 mg/L from the Czech Republic, 0.244–0.81 mg/L from Croatia, 0.01–1.5 mg/L from Hungary, and 0.36–9.5 mg/L from Greece [85]. Two types of German sweets the Westerner like most cocoa powders contained the highest mean Al (165 mg/kg) and chocolate contained a mean 48 mg Al/kg [83]. It can be concluded that aluminum can be found in almost all the foods no matter what they come from or what type they are and what technology they are made or processed.

1.2.1.4 Aluminum in Drinking Water

The mean Al concentrations in finished municipal tap water were reported as 20–174 μg/L in Canada [41]. Polish scientists carefully measured Al in potable water from the area of the city of Poznań using three frequently used analytical techniques (GFAAS, ICP-MS, and ICP-AES) and found that the water source pH, the temperature, the concentrations of organic carbon, and the nature of the suspended particulates together decided the chemical forms of aluminum in water [30]. The data from adults living in six Japanese cities indicated that an adult consuming 2 L of water each day could receive 80 ± 7 μg Al/day, accounting 2.2% of their total mean daily Al dietary intake (3600 ± 1370 μg/day) [68]. A median Al concentration in finished municipal drinking water (0.112 mg/L) was reported in the United States, which corresponded to a daily ingested dose of 160 μg Al/kg for a 70-kg adult (assuming water consumption of 1.4 l/day) or about 1% of the ingested Al amount by food [49]. In Taiyuan city, China, the mean Al concentration was reported as 0.014 mg/l, lower than the China National Standard and WHO recommended standard [115].

1.2.2 Aluminum Exposure by Medication and Personal Care Products

Aluminum hydroxide is used widely in pharmaceutical and personal care products. Aluminum is rich in over-the-counter medicines, such as antacids and buffered aspirin, and in a number of topically applied consumer products such as antiperspirants, first aid antibiotic and antiseptics, diaper rash and prickly heat, insect sting and bite, sunscreen and suntan, and dry skin products. Aluminum-containing adjuvants have been used in vaccines to enhance the immune response against killed, inactivated, and subunit antigens for more than nine decades, and almost whole population in the world, except for peoples living in very poor and remote areas, get many times vaccinations not only in childhood but also in adulthood. The healthy people with normal renal function can ingest much larger amounts of aluminum taking from aluminum-containing medications than from the diet and drinking water, possibly as high as 12–71 mg Al/kg bw/day from antacid/anti-ulcer products and 2–10 mg Al/kg bw/day from buffered analgesics when taken at recommended dosages, equals 3500–5200 mg/day [49], but the absorption rate is low (0.07–0.2%) [109]. Absorption of aluminum in human gastrointestinal tract is generally low, about 0.1–0.4%, although absorption of particularly bioavailable forms such as aluminum citrate and aluminum maltolate may be higher at about 0.5–5%. Although for the patients who are under antacid therapy, big doses of as much as half a gram of aluminum in the form of aluminum hydroxide can be ingested throughout the day, absorption of aluminum hydroxide is usually less 0.01% of the intake dose. Bioavailability of aluminum in human gastrointestinal tract varies greatly based mainly on the chemical form of the ingested compound (i.e., type of anion) and the concurrent exposure to some dietary compounds which can chelate aluminum, such as citric acid, ascorbic acid, or lactic acid.

1.2.3 Aluminum Exposure by Occupation

Bauxite is the most important raw material used in the production of metal aluminum, and is refined to produce alumina (aluminum oxide), which is put into electrolytic reduction process, and aluminum metal is recovered. Aluminum metal can be also recycled from aluminum-containing scraps. Aluminum hydroxide is produced from bauxite too. Along with the fast industrialization in China, Russia, India, and other developing countries, due to the need for products lightweight, the demand for aluminum in construction, shipbuilding, aircraft, automobile, high-speed train production, packaging, and electrical equipment increases in an incredible quantity over the world. China, Russia, Canada, and the United States are the main producers and users of primary aluminum. Only in 2016, China produced 57,960,000 tonnes of aluminum, more than half of production quantity of the total world. A huge occupational population is exposed to aluminum in China; though we have not got the

exact number, it is estimated in four to six million workers employed in aluminum production, processing, and manufacturing and aluminum-related industries. Aluminum powders are used in pigments and paints, fuel additives, explosives, and propellants. Besides as the material for producing aluminum metal, aluminum oxides are also used in the production of abrasives, refractories, ceramics, electrical insulators, catalysts, paper, spark plugs, light bulbs, artificial gems, alloys, glass, and heat-resistant fibers. Natural aluminum minerals especially bentonite and zeolite are also used in paper industries.

Occupational exposure to aluminum happens in industries in the form of McIntyre powder, aluminum oxide, aluminum sulfate, aluminum dust and fumes in potrooms, and aluminum fumes during welding aluminum plate while manufacturing automobiles, aircraft, trains, and ships. Aluminum hydroxide and aluminum fluoride are the main exposure source in the aluminum fluoride plant, aluminum oxide and a small amount of aluminum fluoride are exposed in the smelter potroom, and aluminum oxide and a small amount of oxidized aluminum metal fume are exposed by workers in the foundry. The air inside aluminum potrooms, smelters, foundries, welding places, and remelting plants can contain appreciable concentrations of Al oxides and Na3AlF6 [98]. According to the investigations, the aluminum dust or fume concentrations in the air of workplaces vary from 0 to several tens mg/m^3, the diameters of aluminum particles for aluminum dust can be at nanometer to micrometer scales, but those for aluminum fume are mostly at nanometer scales. During routine operations, total and respirable Al dust concentrations measured in workers' breathing zones were 0.08–2.1 mg/m^3 and 0.03 mg/m^3, respectively. The Al oxides generally constitute approximately 25–44% of the total Al in these dusts [97]. The occupational exposure of aluminum is mainly by inhalation, while the inhaled aluminum particulate can enter CNS via several ways. First, the inhaled aluminum particulates deposit in alveoli and pass through "respiratory membrane" and be delivered into the bloodstream and are transferred to organs and tissues with the systemic circulation; second, are transferred from the nasal cavity via the olfactory neuron into brain tissue [16]; and, third, are absorbed into systemic circulation by the vasculature of the nasal cavity.

1.3 Aluminum Bioavailability and Influencing Factors

Aluminum bioavailability is critically important for its absorption, transportation, metabolism, and toxic effect in creatures including human being. Oral aluminum bioavailability is increased by citrate, acidic pH, and uremia and may be decreased by silicon-containing compounds. Oral aluminum bioavailability is also inversely related to iron status. Water perhaps contribute significantly for the aluminum body burden, oral aluminum bioavailability from water has been reported to be 0.1% to 0.4% and much more than that in food, but some researchers believed the similar oral aluminum availability between water and food. Surprisingly, aluminum bioavailability from occupational inhalation exposure is ~2% [76], significantly higher

than those from water and foods. Though food provides the primary source (>90%) of aluminum for the general human, a few data on oral aluminum bioavailability from foods or beverages can be obtained. Oral aluminum bioavailability from food has been assumed to be less than that from water because the aluminum may be incorporated in high-molecular-weight, relatively insoluble complexes in foods. Oral aluminum bioavailability from milk was estimated to be <1% in rabbits [107]. Though tea leaves contain considerable quantity of aluminum and some people described tea leaves as accumulator of aluminum, the aluminum in tea leaves having low oral bioavailability has been suggested. In tea leaves, 91–100% of aluminum is combined with organic complexes which may interfere with the bioavailability of aluminum [32]. Oral aluminum bioavailability from medication is dependent on aluminum species from the drugs; the bioavailability of aluminum from ingested aluminum hydroxide seems being less than those from aluminum chloride, nitrate, citrate, and lactate from which the aluminum ions are released more than aluminum hydroxide; and sucralfate, another type of aluminum compound, just like aluminum hydroxide, almost can't solve in water but can solve in acid and base and shows oral bioavailability similar with that of aluminum hydroxide and lower than that of soluble aluminum compound such as aluminum chloride, nitrate, citrate, and lactate [108]. In conclusion, the oral absorption or availability of aluminum is mainly affected by the solubility of aluminum compound, pH, and carboxylic acids.

1.3.1 Some Important Influencing Factors on Oral Aluminum Absorption

Citrate Citrate may be one of the most important factors that affect oral aluminum absorption; it may form coordination complexes with aluminum and enhance oral aluminum absorption. Amount of studies revealed that aluminum citrate is more bioavailable than other aluminum chemical species, while oral ingested, it can increase oral absorption of aluminum and also increase aluminum distribution into and out of tissues and discharge from the creature body through renal elimination [54].

Silicon-Containing Compounds Some studies, both epidemiological investigation and animal experiments in drinking water, suggested that increased dietary intake of silicon (Si)-containing compounds could reduce aluminum absorption and facilitate aluminum excretion [7].

Fluoride Fluoride seems to increase aluminum absorption; the mechanism may be it forming numerous complexes with aluminum. In a study with speciation calculations, the authors suggested that fluoride could solubilize more than 60% of aluminum in the stomach [36]. But contradictorily, some authors believed that fluoride might decrease aluminum absorption or enhance its clearance [69].

Iron Iron (Fe) is a very active metal and have different status, which may have impacts on the absorption of aluminum and its accumulation in the brain due to its competitiveness with aluminum; thus aluminum absorption was generally increased in the deficiency of iron. Rats fed with an iron-deficient diet showed greater (0.0065%) aluminum bioavailability, and rats fed with an iron-supplemented diet manifested lower (0.0028%) oral aluminum bioavailability than controls (0.0040%) [66]. In general iron shows two valent status, divalent iron Fe(II) and trivalent iron Fe(III). Fe(II), not Fe(III), decreased the absorption of aluminum hydroxide from the intestinal tract and hence decreased the content of aluminum in portal and systemic blood, in which the authors proposed the mechanism may be Fe(II)-enhancing transferrin (Tf)-mediated aluminum uptake first and then ferritin binding the aluminum in mucosal cells [91].

Calcium and Sodium Calcium is a type of very important ion in creature. Just like iron, calcium (Ca) status influences aluminum absorption and accumulation. In a study on aluminum-treated rat, deficient dietary calcium content increased the aluminum absorption rate and extent, aluminum accumulation in tissue, and aluminum-induced neuropathology [86]. There may be a negative effect of sodium (Na) on aluminum absorption, and an increment of aluminum uptake induced by reduction of sodium has been reported [90].

Ethanol In an in vivo study, rats were combinedly treated with ethanol and aluminum chloride, the results revealed that ethanol elevates the effects of aluminum, but the mechanism on how ethanol affects aluminum toxicokinetics was not provided [77]. In our study on occupationally Al-exposed workers, it seems that the workers with alcohol drinking habit displayed more serious cognitive impairment than workers without alcohol drinking habit (unpublished data). We still cannot distinguish that is it the effect of aluminum or ethanol, because longtime and high-quantity alcohol consumption impairs cognition too.

Age There were reports that aluminum concentration was increased with the aging, not only in Alzheimer's disease patients but also in normal people [56]. Age-related aluminum content increases in the blood, bone, brain, and other soft tissues were reported in human being. Aluminum contents were ~160 mg/kg (in ash) in the lung of 0–3-month-old, ~625 in 1–12-year-old, and >2000 in 19–89-year-old adults, ~100 in the liver in 0–3-month-old, ~150 in 1–12-year-old, and ~550 in 19–89-year-old adults, and ~150 in the kidney in 0–3-month-old, ~300 in 1–12-year-old, and ~350 in 19–89-year-old adults [84]. Brain and bone aluminum increases too with age, up to ~40 years old, then showing a plateau or slight decrease to age 70 and then an increase later in life. Shimizu reported mean hippocampal and frontal cortex aluminum concentrations as 0.014 and 0.020 mg/kg (wet tissue) in 32–46-year-old and 0.402 mg/kg and 0.373 mg/kg in 75–101-year-old [81]. Serum aluminum levels in healthy 20–80-year-old people increased with age too [114]. The increment of aluminum in human body with aging may be the accumulation of aluminum getting from environment including air, water, food, medication, vaccine, and skin care products (Fig. 1.4).

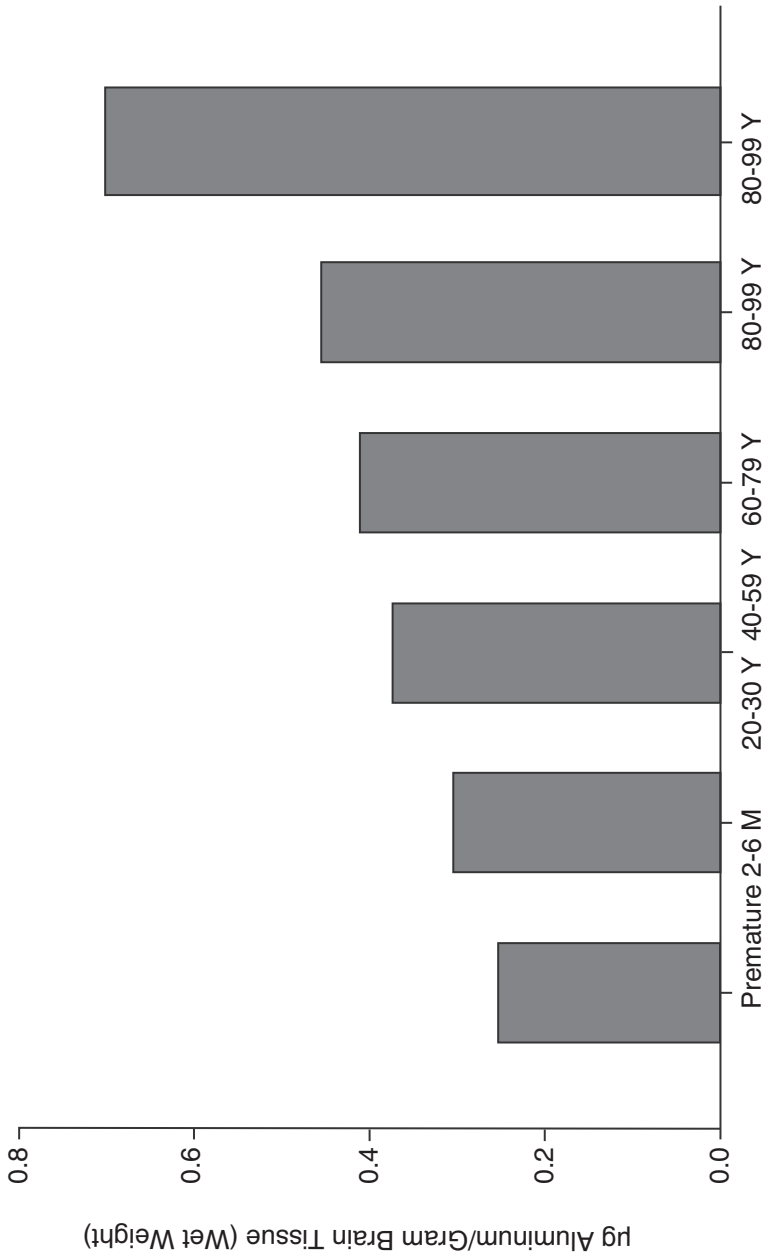

Fig. 1.4 Aluminum concentrations in brain tissues of different ages. (Quoted from Ref. [56])

Foods and Dietary Components Organic ligands in food may associate with aluminum, so presence of food in the stomach may inhibit aluminum absorption. In a study to assess influence of beverages and foods on oral absorption of aluminum, Australian scientists separately co-administered orange juice, or coffee, or wine, or meat, or carbohydrate/cereal products with aluminum sulfate solution and found that orange juice, coffee, and wine increased aluminum absorption by increasing peak serum aluminum concentrations and urinary aluminum excretion and, in contradictory, meat and carbohydrate/cereal products decreased aluminum absorption [94]. Some dietary components, such as phytate and polyphenols, can chemically associate with aluminum and affect aluminum absorption [73, 74].

1.4 Adverse Effect of Aluminum on Human Being

In biological systems including human body, Al^{3+}, like all the metal cations, looks for carboxylate and phosphate groups connected to macromolecules (i.e., proteins, RNA, and DNA) or linked into low-molecular-mass ligands, such as amino acids, nucleotides, citrates, phytates, lactates, carbonates, phosphates, and sulfates as constituents [39], and the phenolic group of the amino acid tyrosine in proteins. Most of the Al^{3+} in human serum is bound to the protein Tf, a recognized carrier of trivalent metal ions, and, in its citrate complex form, can bind to a deprotonated alcohol group and then exerts its biological effects. As regards the aluminum particles, their reactivity and related adverse effect are dependent on their size, shape, surface area, bulk density, and aluminum content. Nanoparticles of aluminum powders displayed maximal oxidation (combustion) rates than microparticles [43] and showed severer oxidative impairment on neural cells [75]. Tiny aluminum flakes are also more reactive due to their thinness and corresponding high surface area. The size of aluminum aerosols in environment air is critical for what part of respiratory system they are deposited in and where they exert their adverse effects. In occupational locations, three aerosol fractions are now thought to be health-related; they are inhalable, thoracic, and respirable fractions [65]. The inhalable fraction refers to the total amount of airborne particulates to enter the nose and/or mouth during breathing (aerodynamic diameters (dae) $\leq$100 μm); the thoracic fraction passes through the tracheoalveolar region of the lung (dae <28 μm), while the respirable fraction (dae <10 μm) goes into and deposits in the alveolar region of the lung (includes the respiratory bronchioles, the alveolar ducts, and the sacs). In recent years, exposure to tiny particles (dae <2.5 μm) has been put under spotlight for their possible relation with cardiovascular and respiratory diseases [17, 18]. The workers employed in aluminum powder processing and producing are probable to be exposed to some or all of the aerosol fractions, based on the different production process used. The aluminum refinery workers that use alumina powders and are exposed to aluminum fumes may encounter the same situation [42, 88]. The Al^{3+} ions on the aluminum particle surface possess strong Lewis acids property and react strongly with water, while hydroxyl groups on the particle surface are Lewis bases which interact with metal

ions [19]. Hence, the reactivity of Al_2O_3 particles is based on its specific crystal structure and hydrophilic/hydrophobic surface properties and the surface hydration degree. Adsorption capacity of Al_2O_3, $Al(OH)_3$, and aluminum phosphate is important for its adverse health effect; the inhalation of Al_2O_3 particles and related oxyhydroxide particles in aluminum smelting process can serve as a delivery vehicle for hydrogen fluoride adsorbed on the particle surface; in addition, the release of volatile by-products including polycyclic aromatic hydrocarbons (PAH), generated by carbon electrodes in the electrolytic reduction process, may exert additional adverse effect [4, 42, 52], and Jinzhu et al. have in vitro demonstrated the cooperative effects of aluminum and benzopyrene, a major constituent of PAH [45].

1.4.1 Neurotoxic Effects Induced by Occupational Aluminum Exposure

Workers in aluminum industries can be occupationally exposed to airborne aluminum particulate at concentrations exceeding to which the general population was exposed by approximately 350 times. Occupational aluminum intake was estimated to be 21 mg/day (6×10^{-3} mg/kg bw/day), much higher than 0.06 mg/day (1.7×10^{-5} mg/kg bw/day) of the general population's intake. Many studies have shown that these types of occupational exposures could produce elevated urine aluminum concentration, elevated serum aluminum concentration, and elevated bone aluminum content. The serum and urine of workers employed in the electrolytic aluminum production for average 3.8 years, the production of aluminum powder for 10.2 years, and the production of aluminum sulfate for 7.4 years, and in aluminum welding for 10.7 years, were compared with a control group [82]. All of the aluminum-exposed workers displayed higher (significantly or nonsignificantly) serum aluminum concentrations than that of the controls. And all of them showed significantly higher urine aluminum concentrations than that of the controls, and a significant correlation was found between weekly mean air aluminum concentration and weekly average urine aluminum content. A positive correlation was found between serum and urine aluminum concentrations. The plasma and urine aluminum concentrations of the aluminum-exposed workers measured end-of-shift were higher than those measured beginning-of-shift and higher on last workday than on first workday in a week [62]. Both plasma and urine aluminum concentrations were higher after exposure to aluminum fume than exposure to aluminum dust in similar concentrations. The reason might be that the dust particles were larger than fume particles. Post-of-shift urinary aluminum concentrations were correlated significantly with workshop air aluminum concentration. Inhaling nanoparticles of alumina is an increasing problem due to the large usage of nano-aluminum. In the in vivo and in vitro studies, Zhang et al. indicated that nano-alumina impaired neurobehavioral functions in rats and induced cell necrosis and apoptosis, likely mediated by the reduction in MMP and ROS and the induction of the caspase-3 gene. The ability of the nano-alumina

particles caused cell death, ultrastructural lesions, mitochondrial damage, and mitochondrial membrane integrity in vitro. Nanoparticles of alumina were much more toxic compared to micro-alumina particles, indicating a particle size-induced toxicity of nano-alumina; one key mechanism may be the ability of alumina to damage the mitochondria [75, 117]. In recent report, Zhang et al. also found the genotoxicity of nano-alumina, inducing DNA damage [116].

In industrial environment, workers mainly inhale aluminum fumes, dusts, and flakes via respiratory tract, though they are exposed to aluminum particulate by skin too. Several studies have reported adverse effects in respiratory tract of aluminum-exposed workers, such as asthma-like symptoms, widely known as potroom asthma, wheezing, dyspnea, and lung function impairment. But the cause of potroom asthma has been suggested to be the exposure to fluorides in the workplace air [48]. Some studies debated if there was an association between allergic status and the development of potroom asthma symptoms in aluminum-exposed workers. Furthermore, occupational exposure to aluminum dust was directly associated with the development of aluminum pneumoconiosis [13], a type of aluminum dust-induced pulmonary fibrosis in aluminum industry workers. Contact dermatitis and irritant dermatitis were symptoms reported in workers exposed to aluminum alloys and aluminum dust too. Epidemiological investigations have revealed a higher risk of developing lung cancer [55] or bladder cancer in aluminum-exposed workers compared with controls, but, the risk was ascribed to the inhalation or dermal exposure to the PAHs which are generated during aluminum production, other than exposure to aluminum compound particles.

Extensive occupational health and occupational epidemiological investigations have reported adverse neurological symptoms or signs as results of occupational aluminum exposure, even related to Alzheimer's disease [92]. An important even critical issue in these investigations is aluminum exposure assessment, otherwise the exposure-response relationship could not be achieved. The researchers utilized a number of different methods to assess the aluminum exposure, including exposure scaling for different job types, estimation for aluminum body burden, or simply years working in the aluminum industry, and even having worked or having not worked in the aluminum industry. In the aluminum-exposed workers, a variety of neuropyschiatric or neurological symptoms, including angry, depression, confusion, loss of coordination, loss of memory, and balancing problems were significantly correlated with occupational aluminum exposure, both exposure duration and exposure level or estimated exposure dose.

In a cross-sectional study [40], 33 occupationally aluminum-exposed Al electrolytic workers, who were 35.16 ± 2.95 (mean ± S.D) years old and exposed to aluminum for 14.91 ± 6.31 (mean ± S.D) years, were investigated. Air aluminum concentration in workplaces and their urinary aluminum concentration were measured by means of graphite furnace atomic absorption spectrophotometer. Matched normal reference group were selected from a flour plant. Neurobehavioral core test battery (NCTB) recommended by WHO was performed. Autonomic nervous function test battery recommended by Ewing DJ was conducted. FAC SCAN was used

to measure the lymphocyte subsets of peripheral blood. The mean air aluminum concentration in the workshop was 6.36 mg/m^3 (2.90–11.38 mg/m^3). Urinary aluminum concentration of the Al electrolytic workers (40.08 ± 9.36 μg/mg.cre) was significantly higher than that of the controls (26.84 ± 8.93 m/mg.cre). Neurobehavioral test results revealed that the scores of DSY, PAC, and PA in Al electrolytic workers were significantly lower than those of the controls and the score of POMSC, POMSF, and SRT among Al-exposed workers were significantly raised compared to those of the controls. Autonomic nervous function test results displayed that R-R interval variability of maximum ratio of immediately standing up in Al electrolytic workers were decreased compared with the control group, while the BP-IS, HR-V, HR-DB, and R30:15 did not show significant change.

In a cross-sectional case-control study conducted in Northern Italy, 64 former aluminum dust-exposed workers were compared with 32 unexposed controls from other companies matched for age, professional training, economic status, and educational and clinical features. Cognitive functions were assessed by the Mini Mental State Examination (MMSE), the Clock Drawing Test (CDT), and the auditory evoked Event-Related Potential (ERP-P300), and the time required to solve the MMSE (MMSE-time) and CDT (CDT-time) was also measured to detect early signs of mild cognitive impairment (MCI). Significantly higher internal doses of serum Al and blood Fe were found in the ex-aluminum dust-exposed workers compared to the controls. The results of neuropsychological tests displayed a significant difference in the latency of P300, MMSE score, MMSE-time, CDT score, and CDT-time between the former Al-exposed workers and the controls. P300 latency was correlated positively with Al-s and MMSE-time. Al-s concentration showed significant effects on all the tests: a negative relationship was observed between internal Al concentrations, MMSE score and CDT score; a positive relationship was found between internal Al concentrations, MMSE-time and CDT-time. All the potential confounders such as age, height, weight, blood pressure, schooling years, alcohol, coffee consumption, and smoking habit were taken into account, and their affections were ruled out by statistical analysis. Based on the findings, the authors suggest a possible role played by the inhalation of aluminum dust in preclinical mild cognitive disorder which might prelude Alzheimer's disease (AD) or AD-like neurological deterioration [67].

A total of 66 retired Al potroom workers and 70 unexposed controls were investigated by Xiaoting Lu and colleagues [53]. The cognitive functions were assessed with the Mini Mental State Examination. Since tau protein hyperphosphorylation and expression are pathological markers of Alzheimer's disease, and due to unacceptability of brain tissue of workers, the tau protein expression in peripheral blood lymphocyte of workers was analyzed with Western blot. The cognitive functions of the Al-exposed workers were significantly decreased compared to the controls. Twelve mild cognitive impairment cases in the exposed group and 14 mild cognitive impairment cases in the control group were diagnosed, and the difference is significant. Significantly higher p-tau181 and p-tau231 levels, which are somewhat similar with AD patients, were detected in the Al-exposed workers than in the control group. The study suggests that long-term exposure to Al may cause cognitive disor-

ders and that p-tau181 and p-tau231 might be useful indicators for monitoring cognitive decline in Al-exposed workers.

Some other groups of scientists performed investigations which specifically examined the relationship between occupational aluminum exposure and occurrence of AD, but significant correlation was not found. However, negative conclusion can't be drawn. The results of these investigations are limited due to the complicated exposure situations in workplaces. Hardly can a worker be only exposed to aluminum particulate without exposure to other hazards, and the workers' exposure estimation is often not clear, adequate, and accurate due to the long-term, often changing, and complicated exposure situations. There coexist many toxic substances in the air of workplaces due to the production process and material needs; other toxic substances other than aluminum as the cause of the observed effect can't be ruled out. Additionally, frequently appeared defects in epidemiological studies, such as small sample sizes, relatively young age of exposed workers, misclassification bias, inappropriate selection of exposed group and comparison group, and unable strictly controlling confounding factors, are usual weaknesses under criticism in these occupational epidemiological investigations [79].

1.4.2 Aluminum Exposure in Drinking Water and Neurological Disorders

The neurotoxic features of aluminum are well displayed in mounting of investigations in non-occupational populations, and associations between aluminum exposure and neurological disorders even Alzheimer's disease have been reported; however, the strong evidence demonstrating causality of aluminum on human neurological disorders is still not clear.

Since bioavailability of aluminum in water is higher than in other form in normal living conditions, though it was thought to be lower than that of inhaled aluminum particles that occurs in occupational settings [33], the relationship of aluminum exposure level in drinking water and prevalence of Alzheimer's disease has been extensively investigated. Though the data collected for this relationship is difficult to reach a sounded conclusion because of the big variation of study designs, the difficulty to maintain big and long-term cohort, and the unbalanced study quality in these investigations, the majority, though not all, of the epidemiological investigations identified, reported, and at least implied a positive relationship between aluminum levels in drinking water and risk of cognitive impairment, dementia, or AD [21]. Silica in drinking water has been identified as a protective agent against the development of dementia, and fluoride has also been suggested to have a potential protective effect against AD. Due to methodological issues, the results drawn from many of the epidemiological investigations studying the association between aluminum in drinking water and the risk of developing AD are limited in some degree. These methodological issues mainly include: almost all the detailed individual Al

exposure information from longtime drinking water and from other exposure ways are lacked; disease diagnosis and ascertainment are poor due to the incomplete disease records, inconsistent "diagnosis scale," and poor recall of family members; unable to adjust important confounding factors; and in general the investigated sample sizes are not large enough. A study performed in France [59] is better than other studies performed to date in methodology. The strong evidence drawn from a significant positive relationship between aluminum levels in drinking water and the development of AD in this large-scaled prospective study, plus the weak evidences drawn from positive relationships in numerous studies that have some methodological deficiency, may propose the positive and probable causality relationship between aluminum and AD and certainly can be used to encourage further investigations with well designing and better methodology.

Ferreira et al. have systematically selected and reviewed 34 existing study papers exploring evidence on relationship between Al exposure (mainly through drinking water) and the risk of developing AD and showed in their review article that 68% of them established a relationship between Al and AD, 23.5% did not get conclusion, and 8.5% did not establish a relationship between Al and AD [20]. From Ferreira PC's review article, it is clear that the majority of the investigators got the positive relationship between Al exposure and AD.

Two groups of Norwegian scientists led by Flaten [21, 22] performed ecological investigations using basically the same sources of data to measure exposure and outcome and got almost the same results. The municipalities included in the investigations were grouped according to Al contents in drinking water, and the mortality with dementia was outcome measure, which were coded from death certificates as the underlying or a contributory cause of death. After analysis, they found a dose-response relationship. The Al contents in drinking water were <0.05 (control), 0.05–0.20, and >0.20 mg/l, and age-adjusted mortality rates showed relative risks for dementia of 1.00, 1.15, and 1.32 in men and 1.00, 1.19, and 1.42 in women.

Wood et al. [100] analyzed mental test scores of 386 patients with hip fracture, while they were admitted into hospital and tested mental state between 1982 and 1985. Almost all the patients with reduced mental test scores were identified between one health district with high Al concentration (0.18–0.25 mg/l) in drinking water and two districts with low Al concentration (<0.05 mg/l) in drinking water. In general, Al treatment is a standard process for "purifying water" in water supply plants and elevates Al concentrations in the drinking water; the water supply in the high-Al district in this study had only "been treated with Al since 1982," that is, for only 0–3 years before the mental tests were performed; so considering the short term of high level Al exposure from drinking water, this study does not provide much evidence both pro and against the Al–AD hypothesis.

Martyn et al. [58] selected 88 county districts in England and Wales, integrated them as 7 computerized tomography scanning units, and utilized the records of these computerized tomography scanning units to estimate incidence rates of AD. They found that the relative risk of AD was 1.5 times higher in districts with mean Al concentration >0.11 mg/l than districts with mean Al concentration <0.01 mg/l. No obvious dose-response relationship was observed, but when the

analysis was restricted to subjects under 65 years of age, a tendency for dose response appeared.

In Ontario of Canada, Neri and Hewitt [64] performed a large-scaled case-control study. They matched 2232 patients who had been diagnosed as AD or presenile dementia and discharged from hospital, with an equal number of age and sex comparable patients discharged with a nonpsychiatric diagnosis, analyzed the data, and calculated the relative risk of AD. A dose-response relationship appeared, with the increasing of Al concentrations in drinking water (>0.01 mg/l (control), 0.01–0.10 mg/l, 0.10–0.20 mg/l, and >0.20 mg/l). The relative risks of AD increased too (1.00, 1.13, 1.26, and 1.46).

Frecker [31] examined the birthplaces of 40 individuals in 7 communities around Bonavista Bay in Newfoundland, who had died with a diagnosis of dementia recorded on their death certificates. The relative risks for dementia in these communities seemed to increase with increasing Al concentrations in drinking water. But, due to the small number of patients and defect of the ecological design, to draw the conclusions from this study was limited.

Wettstein et al. [99] selected two groups of 80–85-year-old residents who had a long-term (>15 years) residing in Zürich, Switzerland, according to the mean Al concentration in drinking water of their residence area, one group with a mean approximately 0.10 mg/l Al concentration in drinking water and the another group with <0.01 mg/l, and measured their cognitive impairment and compared the mnestic and naming skills. No difference in cognitive impairment between the two groups was found. But, we should note that a concentration of 0.10 mg Al/l is not very high. Also, the limitation in this study in contrast to most other epidemiological studies is that the data came from only two sources of drinking water and the bioavailability of Al is probable to vary with water qualities due to different Al speciation and the only high-Al source in this study might contain a low fraction of bioavailable Al.

A series of papers have been published based on the Ontario Longitudinal Study of Aging that may be the most long-term observation till now on the relationship between Al content in drinking water and AD, in which about 2000 men have been followed for about 30 years and Forbes et al. studied the relationship between cognitive function and Al, fluoride, and other constituents in drinking water [23–28]. In the initial report of the study, the OR for impaired cognitive function was 1.14 (not significant) for median Al concentration (>0.085 mg/l) in drinking water compared to lower Al concentrations. In later analyses, they took in consideration other water constituents and adjusted data, using two different logistic regression models, found significantly elevated odds ratios (OR = 1.97, 95% confidence interval [CI] 1.21–3.22, $p < 0.05$ and OR = 2.27, 95% CI 1.27–4.02 for Al $p < 0.01$) [28]. Also, they compared individuals with high Al (>0.085 mg/l) and low fluoride (<0.88 mg/l) concentrations in their drinking water with those with low Al and high fluoride in drinking water, and the OR was as high as 2.72 ($p < 0.01$).

The Paquid cohort [2, 5, 6, 15, 80] in southwestern France, which was composed of 3777 elderly men and women in the parishes of Gironde and Dordogne, left puzzle for us. The investigators used mental impairment as the outcome variable in the first three papers published from this cohort and used AD in the last paper. In the

preliminary report of the study, when an increase of 0.1 mg/l of Al in drinking water was calculated, an unbelievable high relative risk of 4.5 (95% CI 3.4–6.1) was shown. But, then the archival data from the individual waterworks were rechecked, and it was found that the Al measurements, based which the study data were analyzed and conclusion was drawn, were erroneously high. Because of these big errors, the investigators resampled all of the water sources and analyzed samples with up-to-date methods and thorough quality control in the same laboratory, after analysis, the new values of Al content in drinking water were surprisingly many times lower than the old ones. It is likely that the unbelievable high relative risk for mental impairment in preliminary report was in a great part due to using erroneous exposure data. The results got from epidemiological study using the new drinking water data were ambiguous: There was a weak positive relationship between Al content in drinking water and cognitive impairment in the elderly who drank the water with pH less than 7.3; but when the pH of drinking water was above 7.3, the relationship was negative. In the most recent analysis of the Paquid cohort, the authors used AD, diagnosed using the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria, in spite of cognitive impairment, as outcome variable. The results seemed reasonable; the relative risk of AD adjusted for age, sex, education level, residence place, and wine consumption was 2.14 (95% CI 1.21–3.80) for individuals whose drinking water Al content was >0.10 mg/l, while the relative risk of dementia was 1.99 (95% CI 1.20–3.28). Furthermore, in a subcohort whose information on bottled mineral water consumption was available, the relative risk of dementia adjusted for age, sex, education level, residence place, wine consumption, silica in drinking water, and mineral water consumption significantly increased to 3.36 (95% CI 1.74–6.49).

Forster et al. performed a case-control study composed of 109 cases of clinically diagnosed presenile AD patients (<65 years of age) in Northern England, relative risks of presenile AD for different Al concentrations varied from 0.8 to 1.3, and no significant relationship between the disease and Al contents in drinking water was found [29]. However, the Al concentrations in this study were relatively low compared to other studies, the highest concentration was only 0.125 mg/l, and few concentrations were above 0.050 mg/l. This may be the reason of low relative risk of presenile AD in this study. Another reason may be that gastrointestinal absorption of Al increases with age. Perhaps the cases in this study were not old enough. And the effect of Al on AD may be smaller in presenile stage than in senile stage.

AD was listed as the underlying cause of death in Ontario, Canada; Forbes et al. examined death certificates there [27] and reported an AD death rate ratio of 2.42 (95% CI 1.42–4.11) for Al concentration >0.336 mg/l relative to <0.067 mg/l in drinking water. Then they restricted the analysis to individuals over 75 years of age, and the rate ratio increased to 3.15 (95% CI 1.85–5.36). Furthermore, while they repeated the analysis on individuals >85 years only, the rate ratio was raised to 4.76, and they adjusted the data for drinking water source (groundwater vs. surface water) and the water contents of silicon, iron, pH, fluoride, and turbidity, and the rate ratio

surprisingly increased to 9.95. The Al concentrations in this study were higher than in other published studies, and the effect of Al in drinking water was higher too.

A case-control study on autopsy-verified material from a brain bank in Ontario was conducted by McLachlan et al. on the basis of strict neuropathologic criteria [59], with 385 AD cases (296 pure AD and 89 with other coexisting pathology) and 295 controls (125 with no brain histopathology and 170 with neurodegenerative diseases but has never been implicated with Al). The authors compared all AD cases with all non-AD controls, took the Al concentration in drinking water at last residence before death as the exposure level, and got the OR 1.7 (95% CI 1.2–2.5) associated with Al concentration >0.10 mg/l. Then they used 10-year weighted residential histories to improve the data for Al exposure, and the estimates of ORs increased to 2.5 or greater. Furthermore, when they calculated ORs using increasing Al cutoff points, ORs increased gradually: the OR was 3.6 (95% CI 1.4–9.9) at 0.125 mg/l, 4.4 (95% CI 0.98–20) at 0.150 mg/l, and 7.6 (95% CI 0.98–61) at 0.175 mg/l. The diagnostic quality of the data in this study might be ideal, but potential bias might exist due to using brain tissue from a brain bank. The brain tissues stored in a brain bank are possibly not representative of the general population whose brains generally may not sampled and stored in brain bank, but this shortcoming seems not having substantially distorted the results.

As a part of a large, multidisciplinary study of AD, Gauthier et al. [35] performed a case-control study (68 cases) in Québec, Canada, and diagnosed AD using the NINCDS-ADRDA criteria. Exposure level was calculated from water Al contents sampled at four different seasons, combined with the individual's residential locations from 1945 to onset of AD. Furthermore, they adjusted the ORs for educational level, family AD history, and presence of at least one apolipoprotein E e4 allele. Notably, the specificity of this study is that it focused on speciation of Al, the exposure data including total Al, total dissolved Al, monomeric organic Al, monomeric inorganic Al, polymeric Al, Al_{31}, and complexes of Al with hydroxide, fluoride, silicon, and sulfate. The ORs were elevated to 2.10 for onset exposure and 1.52 for long-term exposure in total Al concentration in drinking water (>0.077 mg/l), but not significantly. The monomeric organic Al measured at disease onset is the only fraction of Al that was associated with AD (OR = 2.67, 95% CI 1.04–6.90). The threshold concentration used in this study was 0.012 mg/l (measured as elemental Al). Though this study has high-quality disease data; very detailed and specific water chemistry data, especially the Al speciation; and adjustment for till now-known risk factors, the small number of subjects (only 68 cases) seriously restricts the conclusions to be drawn.

Martyn et al. performed a case-control study composed of 106 clinically diagnosed male AD cases below 75 years old in 8 regions of England and Wales and did not found evidence of an association between AD and higher Al concentrations in drinking water; also no association was found when the analyses were restricted to water supplies with low concentrations of silicon [57]. The authors used three comparison groups (other dementia, brain cancer, and other diagnoses) to match the AD cases and performed analyses using three different methods for computing Al exposure (Al concentrations averaged from age 25 years to diagnosis, from age 25 years

to 10 years before diagnosis, and over 10 years before diagnosis). Most of the 54 ORs for increased Al concentrations were below unity, 8 of them significantly so. This is a study providing the strongest evidence against the Al–AD hypothesis in all the studies published so far.

1.4.3 Antacids Ingestion and Development of AD

A typical heavily aluminum-exposed population is that who regularly ingest antacids for stomach problems. In one study [37], researchers found a significantly elevated odds ratio for AD between regular antacid ingesters and irregular ingesters; but, when only aluminum-containing acids were taken into consideration and put into analysis, the association became not significant. There was no other study that reported a significant positive association between antacid ingest and AD till now. Reports on the relationship between aluminum content in food and the risk of developing AD are limited and controversial. This situation may be due to difficulty to measure aluminum content in foods and to get accurate exposure information in dietary studies. A positive relationship between the consumption of foods with high aluminum content and the risk of developing AD was reported in a small-scaled case-control study, but the results need to be confirmed in larger-scaled cohort investigations.

1.4.4 Aluminum-Related ALS and PDT in Specific Regions

In 1945 until 1960, two syndromes featuring amyotrophic lateral sclerosis (ALS) and a parkinsonism-dementia (PD) developed in some natives in some regions of the world, including indigenous people in several western Pacific foci; the Chamorro on Guam; Japanese on the Kii peninsula of Honshu Island, Japan; and the Auyu and Jakai of southern West New Guinea. The incidence of this special ALS was 50–150 times higher than elsewhere in the world [47]. High aluminum and low calcium and magnesium concentration in the environment has been proposed to contribute to these syndromes [101]. Scientists observed carefully specific localization of manganese, aluminum, and calcium in the spinal cord of ALS patients [111]. High aluminum content in soil was found, but was not found in food [59]. Using neutron activation analysis, Yoshimasu et al. found higher concentrations of aluminum and calcium in the brain of victims of ALS than in controls whereas not elevated magnesium concentration [111, 112]. The average aluminum concentrations in brains were 33.1 mg/kg in 6 ALS cases and 36.8 mg/kg in 4 PD cases compared to 17.7 mg/kg in controls, determined by neutron activation analysis. Aluminum concentration in the ALS and PD patients was statistically higher than in the controls, and calcium concentration was elevated too in the ALS and PD patients. Similar aluminum, calcium, and manganese distribution in spinal cord of ALS patients was found with

X-ray microanalysis [110]. Increased brain aluminum contents in two Guamian ALS cases (1.7 and 8.9 mg/kg) and in two Guamian PD cases (2.0 and 3.9 mg/kg) compared to an average of 1.38 mg/kg in 4 normal subjects were measured with EAAS and reported by Traub et al. [89]. Compared with normal subjects and PD patients, aluminum, silicon, calcium, vanadium, iron, and zinc contents were increased in the frontal cortex of ALS patients [61]. Measured in 26 brain regions, aluminum contents were markedly elevated in 2 of 6 ALS patients compared to 5 patients who did not showed neurological abnormalities. Mean aluminum concentrations in brain were 88 and 136 mg Al/kg dry weight in the two ALS cases, while 26 and 23 mg Al/kg in the other four cases and controls [103, 104]. High aluminum concentrations were found, using SEM with energy-dispersive spectrometry, in NFT-bearing neurons from ALS-PD and nonafflicted patients [70], and aluminum and calcium were co-localized in the NFT-bearing neurons. Utilizing wavelength-dispersive spectrometry coupled with electron beam X-ray microprobe analysis, Garruto found co-localized aluminum and calcium in the NFTs of two Guamian PD patients but not in the non-NFT-containing regions in brain of either PD patients or two normal lifelong Guamian residents [34]. The highest calcium and aluminum concentrations were semiquantitatively estimated as 7200 and 500 mg/kg dry weight, respectively. The average brain aluminum concentration (179 mg/kg dry weight) in six Guam PD cases was higher than seven Chamorro controls (57 mg/kg dry weight) [113]. Using laser microprobe mass spectroscopy, aluminum and calcium were found in the cytoplasm of hippocampal neurons bearing NFTs [71]. Also, using secondary ion mass spectrometry, aluminum and calcium were found to be associated with NFT-bearing hippocampal neurons of PD patients [34]. Using histochemical staining, Piccardo visualized aluminum in the hippocampus, spinal cord, and frontal cortex in most of three Guamian ALS patients and five PD patients who had NFTs in brains, but not in the five neurologically and neuropathologically normal Guamian or Caucasian patients [72]. Staining for aluminum was observed in the cytoplasm, nucleoli, neuropil, white matter, and some endothelial cells and walls of cerebral vessels, and X-ray microanalysis confirmed the existence of aluminum in aforementioned tissue and cell organelles. Neutron activation analysis results showed that aluminum contents in brains were higher in three Guamian demented cases than in four non-demented controls. High calcium contents in gray and white matter and low zinc contents in gray matter were also observed in the Guamian demented cases [105]. Using PIXE, extremely high aluminum contents were detected in the lumbar spinal cord and hippocampus of ALS patients from Guam and the Kii peninsula of Japan, compared with those in sporadic ALS cases and controls [46]. Aluminum content positively correlated with iron and copper contents, negatively correlated with zinc content in the neural tissue, and negatively correlated with calcium and magnesium concentrations in the birthplace area's rivers [105]. Toenail aluminum contents, often used as an indicator of metal exposure, did not show difference between 22 ALS patients and 40 controls; the median values were 34.5 mg Al/kg in the former and 37.5 in the latter, respectively [8]. Aluminum contents of the hippocampal gyrus, caudate nucleus, globus pallidus, and substantia nigra, as well as in the liver, kidney, and spleen, in four Parkinson's

disease cases were significantly higher than those in the five patients without neurological abnormalities [102].

1.4.5 Aluminum and Neurodevelopmental Toxicity

Aluminum, due to its ubiquitous existence everywhere, may contaminate everything, including commercial intravenous-feeding solutions for premature infants, and induce potential neurotoxicity. Bishop et al. randomly assigned 227 premature infants whose gestational ages were less than 34 weeks and birth weights were less than 1850 g into 2 groups and received either standard or specially constituted, aluminum-depleted intravenous-feeding solutions before they could begin enteral feeding. The authors assessed neurologic development of the 182 surviving infants who could be tested using the Bayley Scales of Infant Development at 18 months of age. The 90 infants intravenously fed with standard feeding solution showed a mean (±SD) Bayley Mental Development Index of 95 ± 22, while 92 infants intravenously fed with aluminum-depleted solution showed 98 ± 20 ($P = 0.39$). In a planned subgroup analysis on infants, whose intravenous-feeding duration exceeded the median and who did not show neuromotor impairment, the mean value of the Bayley Mental Development Index for the 39 infants intravenously fed with the standard solutions were 92 ± 20, and that of the 41 infants intravenously fed with the aluminum-depleted solutions were 102 ± 17 ($P = 0.02$). The infants intravenously fed with the standard solutions were significantly more likely than the infants intravenously fed with the aluminum-depleted solutions to show a Mental Development Index of less than 85 (39%, vs. 17%; $P = 0.03$), increasing their risk of subsequent educational problems. In all 157 infants without neuromotor impairment, increasing aluminum exposure was associated with reducing Mental Development Index ($P = 0.03$), with an adjusted loss of one point per day for infants intravenously fed with the standard solutions. The authors concluded that, in preterm infants, prolonged intravenous feeding with solutions containing aluminum is associated with impaired neurologic development [9].

María de Jesús Ramírez-Altamirano et al. conducted a study on newborns including 8 infants with neural tube defect and 15 infants without this defect. The parents of infants with neural defects confirmed their exposure to aluminum and other 18 inorganic elements. The aluminum content in hair samples was measured with inductively coupled plasma spectroscopy (ICP-MAS). In the hair of infants with neural tube defects, the aluminum content was 152.77 ± 51.06 μg/g, doubled the value of normal infants (76.24 ± 27.89 μg/g). Association between hair metal contents (aluminum plus silver, aluminum plus potassium, silver plus potassium, and potassium plus sodium) and neural tube defects was found at 75th percentile. The authors thought the metals including aluminum may be risk factors in inducing neural tube defects [76].

1.5 Conclusions

Aluminum is a certain neurotoxic agent which exists widely in the environment, including air, water, processed foods, vaccines, medications, and skin protection products, causes neuropsychological and neurological impairment which displays cognitive impairment and even dementia, and is thought to be related to occurrence of Alzheimer's disease (AD), Parkinson's disease (PD), and even other neurodegenerative diseases such as ALS. It may affect neurodevelopment too. The aluminum exposure in living environment is associated with pollution conditions and industrialization levels and influenced by a lot of factors, while the occupational aluminum exposure depends on the air aluminum concentration in workplace. AD prevalence and incidence are increased in populations exposed to high aluminum concentrations compared to those exposed to low aluminum levels. Though the exact etiology–disease relation between aluminum and AD is still not assured, large and rigorously controlled or less rigorously controlled prospective and retrospective human studies have examined the aluminum levels in drinking water supplied to different geographic regions. Those studies have shown significantly increased risk for AD in human populations that routinely consume water containing $\geq$0.1 mg/l aluminum compared to those that routinely consume water in regions with aluminum levels below 0.1 mg/l. Though only one case of AD in occupationally Al-exposed workers was reported both clinically and pathologically, cognitive impairment in aluminum-exposed workers was widely reported in a lot of countries by many authors. In conclusion, aluminum can impair central nervous system and induce cognitive impairment and even Alzheimer's disease.

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Chapter 2
The Chemistry of Human Exposure to Aluminium

Christopher Exley

Abstract Before it is possible to begin to understand the chemistry of human exposure to aluminium, it is necessary to appreciate a few basic rules. Rule number one tells us that the form of aluminium which is bound by functional groups on biomolecules is its free trivalent aqueous cation, $Al^{3+}_{(aq)}$. Rule number two tells us that the binding of $Al^{3+}_{(aq)}$ is determined by both thermodynamic and kinetic constraints. Rule number three tells us how essential it is to understand the critical importance of the exposure regime. The application of these simple rules of aluminium chemistry allows us to understand why, for example, not all aluminium salts are equal and not all routes of aluminium exposure are equivalent.

Keywords Human exposure to aluminium · Thermodynamic and kinetic constraints · Exposure regime · Aluminium binding by biomolecules · Aluminium adjuvants

What do we need to know about the chemistry of aluminium in order to understand its biological availability in humans? There are some simple rules to follow:

Rule Number One

The form of aluminium which is bound by functional groups on biomolecules is its free trivalent aqueous cation, $Al^{3+}_{(aq)}$ [1]**.**

What could be simpler? Do not, for example, be confused by the pH-dependent hydrolytic chemistry of aluminium (Eq. 2.1).

$$Al^{3+}_{(aq)} \leftrightarrow AlOH^{2+}_{(aq)} \leftrightarrow Al(OH)_2{}^{+}_{(aq)} \leftrightarrow Al(OH)_{3(aq)} \leftrightarrow Al(OH)_4{}^{-}_{(aq)} \quad (2.1)$$

While the distribution of these monomeric forms of aluminium is dependent upon the pH of the environment (or physiological milieu), for example, the aluminate

C. Exley (✉)
The Birchall Centre, Lennard-Jones Laboratories, Keele University, Keele, Staffordshire, UK
e-mail: c.exley@keele.ac.uk

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anion ($Al(OH)_4^-{}_{(aq)}$) and the divalent cation ($AlOH^{2+}{}_{(aq)}$) being the predominant forms of soluble aluminium at pH above 7.0 and around pH 5.0, respectively [2], it is the $Al^{3+}{}_{(aq)}$ cation which will determine any subsequent chemistry and be bound by, for example, a functional group on a protein. It is the $Al^{3+}{}_{(aq)}$ which forms the strongest bonds with biomolecules. The equilibria governing the distribution of the aqueous monomers of aluminium (Eq. 2.1) are practically instantaneous. This means that as soon as $Al^{3+}{}_{(aq)}$ is bound by any functional group, the equilibrium shifts to replace it, and this will continue until a new equilibrium position is reached between the new aluminium complex and $Al^{3+}{}_{(aq)}$ and the sum of its hydrolytic forms (Eq. 2.2).

$$\text{Al-Complex} \leftrightarrow Al^{3+}{}_{(aq)} \leftrightarrow AlOH^{2+}{}_{(aq)} \leftrightarrow Al(OH)_2^+{}_{(aq)} \leftrightarrow Al(OH)_{3(aq)} \leftrightarrow Al(OH)_4^-{}_{(aq)} \tag{2.2}$$

So, when the iron transport protein transferrin binds aluminium in the blood at pH 7.4 (where $Al(OH)_4^-{}_{(aq)}$ is the predominant hydrolytic form of aluminium) or a carboxylate ligand on a fish gill epithelium binds aluminium in water at pH 5.0 (where $AlOH^{2+}{}_{(aq)}$ is the predominant hydrolytic form of aluminium) in both cases, it is $Al^{3+}{}_{(aq)}$ which is bound [3].

Rule Number Two

The binding of $Al^{3+}{}_{(aq)}$ is determined by both thermodynamic and kinetic constraints.

While $Al^{3+}{}_{(aq)}$ is biologically reactive (and is avidly bound by biochemically important functional groups) for $Al^{3+}{}_{(aq)}$ to be defined as biologically available, its binding should bring about some recognisable response in participating biochemistry and/or underlying physiology [4]. The thermodynamic properties of any aluminium complex will dictate strength of binding (sometimes referred to as a stability constant) and can be thought of as competition between rate of formation and dissolution of the complex. Since $Al^{3+}{}_{(aq)}$ is a relatively small and highly electropositive cation, it will be strongly bound by many biological ligands and especially oxygen-based functional groups [5]. Generally this means that the rate of formation will be preferred over the rate of dissolution, and, therefore, $Al^{3+}{}_{(aq)}$ will remain bound. However, to both remain bound and to bring about a biochemical response, the delivery of $Al^{3+}{}_{(aq)}$ to target groups must also be optimal. Significant numbers of $Al^{3+}{}_{(aq)}$ cations must remain bound over a particular timeframe to produce a biochemical response. The delivery of $Al^{3+}{}_{(aq)}$ to its binding sites in significant amounts over a specific timeframe is governed by kinetic constraints. To further understand these kinetic parameters, let's consider a small but significant change to Eq. 2.1.

$$Al^{3+}{}_{(aq)} \leftrightarrow AlOH^{2+}{}_{(aq)} \leftrightarrow Al(OH)_2^+{}_{(aq)} \leftrightarrow \mathbf{Al(OH)_{3(s)}} \leftrightarrow Al(OH)_4^-{}_{(aq)} \tag{2.3}$$

In any system where the solubility of aluminium hydroxide is exceeded, aluminium will be precipitated as amorphous $Al(OH)_{3(s)}$ (Eq. 2.3), and the formation and dissolution of this solid phase will determine the availability of the soluble

monomeric hydrolytic aluminium ions including $Al^{3+}_{(aq)}$. Under any condition which favours the formation of $Al(OH)_{3(s)}$, it will be the dissolution of this phase which determines the rate of delivery of $Al^{3+}_{(aq)}$ to possible target sites for binding. The rate of dissolution of $Al(OH)_{3(s)}$ will depend upon the avidity with which $Al^{3+}_{(aq)}$ is bound by competitive ligands and, importantly, the stability of $Al(OH)_{3(s)}$ with newly formed amorphous precipitates of this sparingly soluble phase dissolving more rapidly than aged, semicrystalline forms (Eq. 2.4).

$$Al(OH)_{3(\text{amorphous})} \leftrightarrow Al(OH)_{3(\text{gibbsite})} \tag{2.4}$$

In those environments where the precipitation of aluminium is favoured, thermodynamic constraints upon the biological reactivity of $Al^{3+}_{(aq)}$ may give way to kinetic constraints in much the same way as a grain of sand (silica:SiO_2) does not immediately dissolve to give silicic acid ($Si(OH)_4$) upon being dropped into a glass of pure water. Thermodynamically the grain of sand should immediately dissolve to give $Si(OH)_4$, while kinetically it remains as SiO_2. The occurrence of a solid phase as an intermediate in a delivery chain for $Al^{3+}_{(aq)}$ will be rate-limiting and might also be the difference between a biological burden of aluminium (Al_B) being biologically reactive (Al_{BR}) and also biologically available (Al_{BA}) [3].

$$Al_B \leftrightarrow Al_{BR} \leftrightarrow Al_{BA} \tag{2.5}$$

This concept is more often than not ignored in the scientific literature regardless of periodic published warnings [3, 6]. For example, stock solutions of aluminium salts are regularly prepared by researchers by simply dissolving the salt in a solvent such as water. These stocks are invariably super-saturated with respect to aluminium hydroxide or aluminium hydroxyphosphate (where phosphate-buffered saline is the solvent), and no thought is given to the evolution or ageing of their aluminium content over time. During these ageing processes, condensation reactions and aggregation phenomena affect the equilibria depicted in Eq. 2.5 and almost always ensure that the biological availability of aluminium will be different in freshly prepared as opposed to aged stock preparations [6]. One simple example of this can be found when aluminium salts are dissolved in an experimental animal's drinking water which the animal then proceeds to imbibe over hours, days, or even weeks in some instances. The biological availability of the aluminium being ingested from water which is 1 week old will be significantly different to the same water when it was only hours old. The exposure regime has changed and concomitant differences in the biological response should be expected.

Rule Number Three

Understand the critical importance of the exposure regime.

The fundamentals of the chemistry of human exposure to aluminium are relatively straightforward in that they define the delivery of $Al^{3+}_{(aq)}$ to target groups. However, human exposure to aluminium must ultimately be defined by putting

Eq. 2.5 into the context of aluminium's exposome [3], from the exposure origin to the exposure outcome, the biological response.

Where this lack of understanding is paramount is in the use of guidelines relating to human exposure to aluminium such as tolerable weekly intakes (TWI) and similar indices [7]. While there are no regulations governing human exposure to aluminium, there are many examples of these meaningless indices, the provenance of which seems to be to reassure populations of the safety of aluminium products in their myriad applications. Perhaps one of the best examples of this convenient ignorance is where comparisons are made between exposure to aluminium through the gastrointestinal tract and exposure to aluminium through its use as an adjuvant in vaccination and immunotherapy [8]. How many times have we been informed that:

> …the aluminium content of any vaccination schedule (over any particular timeframe) is insignificant in comparison to the aluminium content of an everyday diet….

the inference being that it is only the amount of aluminium which is important in understanding its potential toxicity in humans. Neither the form of the aluminium nor the route of exposure is considered to be of any particular importance. This is a convenient and often cited argument supporting the safety of aluminium adjuvants in vaccines. However, whether by right or through ignorance, this argument is entirely spurious. The aluminium content of a single vaccine is usually in the range 0.25–1.25 mg/mL [9]. This is a very high total concentration of aluminium, for example, 1.0 mg/mL is equivalent to 37 mM. A typical injection volume is 1.0 mL, so the concentration of now systemic (inside the body) aluminium at the injection site immediately following vaccination will be approximately 37 mM since the volume of the diluent (e.g. the muscle interstitial fluid) at the injection site will be negligible compared to the injection volume. Aluminium under injection site conditions is extremely cytotoxic [10]. While the majority of the total aluminium might be defined as aluminium burden, Al_B (see Eq. 2.5), the biologically reactive fraction, Al_{BR}, is high enough and is sustained for long enough to be biologically available, Al_{BA}, and brings about the cell death that is observed as inflammation (red mark) at the injection site. It seems to be often forgotten, again perhaps conveniently so by some, that part of the success of aluminium adjuvants in stimulating immunity is in their toxicity at the injection site. However, the toxicity of aluminium adjuvants is not necessarily limited to the delivery of $Al^{3+}_{(aq)}$ at the injection site. The toxicity in the immediate vicinity of the injection site attracts many forms of invading cells, and these set about 'cleaning up' the cytotoxic debris including internalisation of much if not all of the remaining particulate aluminium (Al_B) [11]. We now know that these immunoreactive cells can load up their cytoplasm with aluminium adjuvant without immediate detriment to their viability [10]. They become couriers and transport their aluminium load throughout the body. At some point their aluminium load, which initially is contained in lysosome-like vesicles, is released causing death of the courier cell. This cytotoxicity is occurring at locations which are distant from the injection site and could include lymph nodes or even brain tissue [12]. Their fate will be bad news for the tissue of their destination as not only will their cell death be detrimental but the release of significant amounts of biologically

reactive aluminium will conceivably add to any toxicity. The amount of aluminium in any particular vaccine preparation might be considered as insignificant relative to the amount of aluminium which enters the gastrointestinal tract, but the potential for this aluminium to produce toxicity is inevitable at the injection site of the vaccine and possible at target organs distant from the injection site and target organs such as the brain. Rule number 3, understanding the exposure regime, helps us to understand why aluminium adjuvants are responsible for vaccine-related adverse events in predisposed individuals.

In understanding the chemistry of human exposure to aluminium, it is important to both recognise and account for the simple facts that not all aluminium salts are biologically equal and that not all routes of exposure to aluminium are equivalent in bringing about a biological response or toxicity.

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Chapter 3
Entry and Deposit of Aluminum in the Brain

Linping Wang

Abstract Aluminum, as a known neurotoxicant, contributes to cognitive dysfunction and may contribute to Alzheimer's disease. The important reason is that aluminum can enter and be deposited in the brain. There have been three routes by which aluminum could enter the brain from systemic circulation or the site of absorption. Aluminum fluxes into brain across the blood-brain barrier (BBB), the choroid plexuses and the nasal cavity. Some factors, such as the increasing of the blood-brain barrier permeability, citric acid and parathyroid hormone (PTH), and vitamin D, can promote aluminum to enter the brain. But the redistribution of aluminum out of the brain is slow, so aluminum can be deposited in the brain for a long time.

Keywords Aluminum · Entry · Deposit · Brain

Aluminum, as a known neurotoxicant, enters and is deposited in the brain, where it contributes to cognitive dysfunction and may contribute to Alzheimer's disease. High concentration of aluminum has been found in senile plaques and neurofibrillary tangles, which occur in the brains of subjects with Alzheimer's disease. There are certain evidences from clinical and experimental studies that oral, subcutaneous, abdominal cavity and respiratory aluminum exposure can increase brain aluminum. Human brain aluminum increased with exposure age, and animal brain aluminum increased with duration of aluminum exposure.

L. Wang (✉)
Shanxi Medical University, Taiyuan, People's Republic of China
e-mail: sxwlp5945@sxmu.edu.cn

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3.1 Aluminum Enters into the Brain

3.1.1 Aluminum Enters into the Brain Across BBB

Aluminum entering into the brain across the blood-brain barrier has been defined to be the primary route of brain aluminum uptake. The anatomical basis of the BBB is primarily attributed to the tight junctions between the cerebral microvascular endothelial cells that line the microvessels that perfuse the brain. Additional impediments to permeation through the BBB come from the absence of fenestrations and the low transcytotic activity of the endothelial cells, the pericytes that surround 30% of their surface, and the astrocyte foot processes that cover 99% of the surface of the endothelial and pericyte cells. Substances must either diffuse through the membranes of these cells or be transported by cell membrane carriers to penetrate the BBB. The surface area of the 400 miles of brain capillaries that are the site of the BBB is roughly 12 m^2. There is much greater opportunity for rapid exchange between the blood and brain through the BBB [1]. The potential mechanisms of distribution of substances across the BBB are the same as those across any cell membrane: diffusion, carrier-mediated and receptor-mediated transport by facilitated diffusion, and active transport. And aluminum could enter into the brain through all these ways.

3.1.1.1 Aluminum Influxes into the Brain Across BBB Directly as a Small Molecular Weight Pieces

It is generally believed that the BBB is restrictive for small molecules at capillary endothelial cells and for large molecules at the interendothelial tight junctions. It has been defined that some substances can influx into the brain penetratingly through BBB. The brain capillary permeability coefficient (P) is affected by molecular weight and the octanol/water partition coefficients. The relationship of permeability to octanol/water partition coefficient and molecular weight was found to be predictable for drugs with molecular weights less than 400. The roles of lipophilicity (hydrogen-bonding potential, polar surface area) and molecular weight as predictors for diffusion of small molecules across the BBB have been well described, providing the opportunity to estimate the permeability rates of aluminum and its complexes across the BBB from their octanol/buffer partitioning coefficient and molecular weight [2].

3.1.1.2 Aluminum Entry into the Brain Is Mediated by Transferrin (Tf)-Transferrin Receptor (TfR), Which Is an Important Iron Carrier Receptor

The prominent rate and extent mechanism by which aluminum transports across the blood-brain barrier are mediated by carrier receptor. The system of Tf-TfR is one of the important carrier receptors by which aluminum transports across BBB.

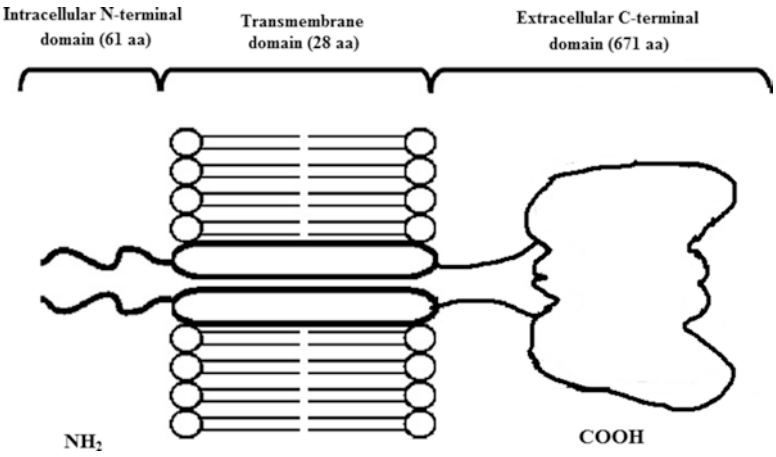

Fig. 3.1 TfR1

It is a possible route of entry for aluminum into the cells of the central nervous system via the same high-affinity receptor-ligand system that has been postulated for iron delivery to neurons and glial cells. It is defined that aluminum can enter into the brain under normal physiological conditions.

Tf is part of a family of proteins that includes serum Tf, ovotransferrin, and lactoferrin, which binds circulating Fe^{3+} and prevents it from traveling throughout the body in its toxic form. The Tf consists of a polypeptide chain of 679 amino acids in humans, and its monomer (80 kDa) is a glycoprotein that consists of two subunits (40 kDa each) known as the N-lobe and the C-lobe separated by a short spacer sequence. There is an iron binding site in between each N- and C-terminal sequences' globular lobe. Two tyrosines, one histidine, and one aspartic acid bind the iron ion to the transferrin in both lobes. Therefore each Tf molecule (apo-Tf) can transport one (monoferric Tf) or two (diferric Tf) iron atoms. The association constant for dimeric Tf and the TfR is 30-fold higher than that of monoferric Tf and 500-fold higher than apo-Tf. Diferric Tf represents approximately 10–30% of circulating Tf, leaving Tf to bind with other metal ions. Synthesis of Tf occurs primarily in hepatocytes, but small amounts are also synthesized in Sertoli, ependymal, and oligodendroglial cells. Tf transports and delivers iron into cells via interactions with its receptor [3].

The TfR (CD71) is a type II transmembrane glycoprotein that is found primarily as a homodimer (180 kDa) consisting of identical monomers joined by two disulfide bonds. Each monomer has 760 amino acids; its molecular weight is 90–95 kDa. It comprises a large extracellular C-terminal domain (671 amino acids) with an O-linked glycosylation site at threonine 104 and 3 N-linked glycosylation sites on arginine residues 251, 317, and 727 known as the ectodomain that includes the site of Tf-binding, a single-pass transmembrane domain with 28 amino acids and a short intracellular N-terminal domain with 61 amino acids (Fig. 3.1). The ectodomain includes a O-linked glycosylation site and three N-linked glycosylation sites. Glycosylation at these sites is required for adequate function of the receptor. It has

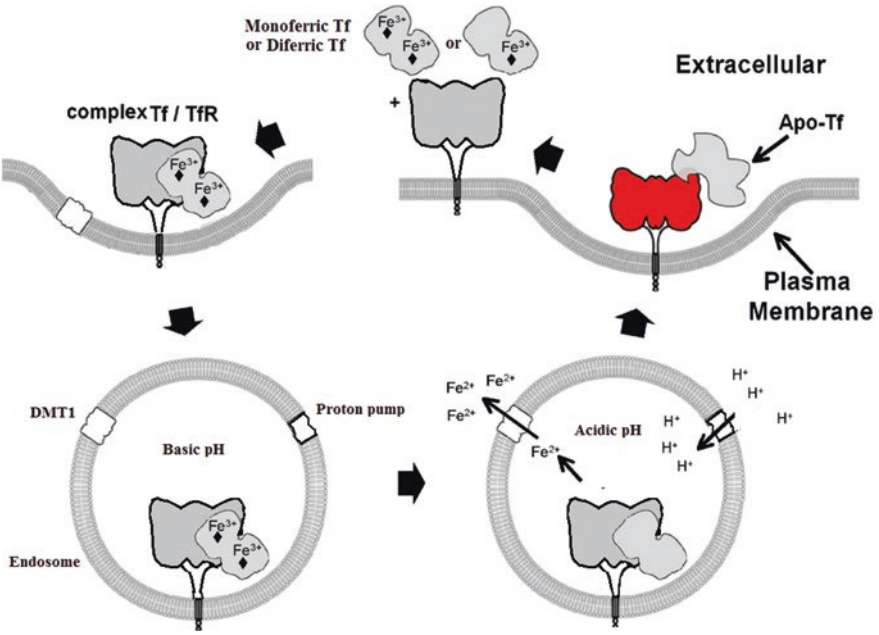

Fig. 3.2 Cellular uptake of iron through the Tf-TfR system

been previously demonstrated that the TfR phosphorylation on serine 24 in the intracellular domain is not required for internalization or recycling of the receptor. It is only an essential protein involved in iron uptake and the regulation of cell growth. Delivery and uptake of iron from Tf into cells occurred by the internalization of iron-loaded Tf are mediated by the TfR [3].

TfR is widespread in the central nervous system. The expression of TfR has also been observed on nonproliferating cells, including those of the vascular endothelium of brain capillaries. It exists in the hippocampus, pontine nucleus, reticular formation, arcuate nucleus, red nucleus, substantia nigra, several cranial nuclei, deep cerebellar nuclei, and cerebellar cortex, as well as in the cerebral cortex and brainstem neurons, choroid plexus cells, and brain capillary endothelial cells.

Cellular uptake of iron takes place through the Tf system via receptor-mediated endocytosis. There are two steps in the course of Fe^{2+} transporting into the nervous system: the first step is that a complex forms TfR on the endothelial cells combining to the Tf-Fe^{3+} and the second step is the release of iron. Endocytosis of the diferric or monoferric Tf/TfR complex occurs via clathrin-coated pits, and the complex is delivered into endosomes. Protons are pumped into the endosome resulting in a decrease in pH that stimulates a conformational change in Tf and its subsequent release of iron. The iron is then transported out of the endosome into the cytosol by the divalent metal transporter 1 (DMT1). Apo-transferrin remains bound to the TfR while in the endosome and is only released once the complex reaches the cell surface [3] (Fig. 3.2).

An ion like aluminum is easily bound to many substances and structures in the organisms. The ligands are often nonspecific and can bind other metal ions. Once aluminum enters the circulation, it is associated with several endogenous ligands. The major aluminum binding fraction of plasma has been shown to be transferrin (Tf), the chief iron transport protein in vertebrates. Tf specifically binds Al^{3+} with a high affinity, approaching its affinity for iron (Fe^{3+}); Tf-Fe^{3+} normally enters tissues throughout the body by receptor-mediated endocytosis of the TfR-$(Tf)_2$ complex. About 81% of aluminum in circulation was coffiplexed with transferrin.

It is identified that the system of Tf-TfR might mediate aluminum citrate transport across the BBB, a possible route of entry for aluminum to neurons and glial cells of the central nervous system via the same high-affinity receptor-ligand system that has been postulated for iron delivery. And aluminum is able to gain access to the central nervous system in normal physiological conditions [4].

Al^{3+} has been demonstrated to complex to specific binding sites on human Tf at physiological pH, and this association is ligand concentration dependent and reversible Al^{3+}; it's capable of gaining access to the cells in the central nervous system via this Tf-TfR interaction under normal physiological conditions. Some Tf complex could disrupt normal iron regulatory processes by binding in a relatively nondisplaceable manner with the TfR; the first part was the same high affinity of Tf-Fe^{3+} and Tf-Al^{3+} in the brain for the Tf receptor; the second part was to determine that the ligands were, indeed, interacting with the same receptor. Tf-Al^{3+} and Tf-Fe^{3+} demonstrate that they are acting interchangeably with the same receptor; the interactions of both Tf-Al^{3+} and Tf-Fe^{3+} with the receptor are completely reversible over the time periods indicated [4].

Tf-Al^{3+} and Tf-Fe^{3+} have the same high affinity for the Tf receptor. Cells in the brain possess a specific high-affinity receptor for Tf that is independent of the metal being transported. The Tf-TfR system is postulated to be the prominent route whereby the brain can access iron from the general circulation to satisfy its high metabolic requirements. It is defined that about only 30% of the ion binding sites that plasma Tf has available in the circulation are saturated with iron at any time [5], leaving the remaining 70% available to other ions. Some analysis of batches of available "iron-saturated" Tf by this laboratory and others has also demonstrated that up to 30% of these binding sites are actually occupied by Al^{3+} [6]. Study has demonstrated that a metal ion other than iron is capable not only of binding to Tf but also of utilizing this interaction to gain access to cells in the brain via the Tf-TfR system. And Al^{3+} can enter into the brain though Tf-TfR system in a normal physiological path as the same cellular routes of Fe^{3+}.

Furthermore, Al^{3+} may be capable of interfering with normal cellular iron homeostasis and could disrupt iron-dependent cellular processes (e.g., oxidative phosphorylation) in the central nervous system. In this regard, studies have defined that ferritin isolated from the brains of Alzheimer's disease patients, which is the chief iron storage protein, has a sixfold higher Al^{3+}content than normal age-matched controls. Studies found that the binding activity of Tf increased significantly in Alzheimer's patients, and it also suggested that increasing of Tf-iron binding activity may also play a role in Al^{3+} entry into the brains of these patients [7].

3.1.1.3 Aluminum Citrate Enters into the Brain Mediated by Monocarboxylic Acid Transporters (MCTs), Which Is Also a Family of Transporters That Moves Monocarboxylic Acids Across Membranes

The presence of proton-coupled MCTs was first recognized by lactate and pyruvate transport into human red blood cells with transport being significantly inhibited by α-cyano-4-hydroxycinnamate. Currently, the family of transporters is defined that it contains 14 members, and 4 members (MCT1, MCT2, MCT3, MCT4) have been demonstrated to mediate the proton-dependent transport of monocarboxylates such as lactate, pyruvate, and ketone bodies. MCT8, earlier known as XPCT (X-linked PEST containing transporter) with 12 putative transmembrane domains, with both N- and C-terminal ends located on the inside of the plasma membrane, contains a PEST domain in its N-terminal and is a thyroid hormone transporter. Studies demonstrated that MCT8 transports both the thyroid hormones (T3 and T4) with high affinity with Km values of 2–5 μM. MCT8 is distributed extensively in many tissues including the heart, brain, pituitary, liver, kidney, skeletal muscle, and thyroid. MCT10 is an aromatic amino acid transporter and also is a T-type amino acid transporter1 (TAT1). The functional characterization of other members of this family has not been done and they are known as orphan transporters. MCTs have 12 transmembrane domains with C- and N-termini within the cytoplasm and an intracellular loop between TMDs 6 and 7. The conservation of sequence between different isoforms of the mammalian MCTs is the greatest for MCT1-4, whereas sequence is least conserved between other members of the family. The TMDs are highly conserved between the family members with high variations in the C- and N- termini including the intracellular loop. The variations in the sequences of different isoforms may lead to differences in substrate specificity and regulation of MCTs. The regulation of MCTs has been demonstrated to occur both by transcriptional and posttranscriptional mechanisms [8].

The MCTs have been found in the membranes of erythrocytes, the brain capillary endothelial cells that comprise the blood-brain barrier, and various other cells [9]. They can transport lactate and other monocarboxylates across mammalian plasma membranes. Its primary endogenous substrate is L-lactate; pyruvate, acetate, propionate, and butyrate are also substrates [10]. Valproate and salicylate are substrates for the blood-brain barrier MCT [11]. MCTs provide electroneutral cotransport of monocarboxylates along with protons in a stoichiometric ratio of 1:1, and the direction of transport is determined by the relative intra- and extracellular concentrations of monocarboxylates and hydrogen ions. As a transporter, the function of MCT1 is dependent on a proton gradient, and it acts as a proton-dependent cotransporter/exchanger. Transport followed an ordered, sequential mechanism. The first step is that a proton binds to the transporter and then binds to lactate. The second part is that the proton and lactate are further translocated across the membrane with their sequential release on the other side. The return of the free trans-

porter binding site across the membrane determines the net flux of lactate and thus forms the rate-limiting step of transport. Transport can be stimulated by a pH gradient (low to high). This may indicate either influx or efflux of substrate depending of the intracellular and extracellular substrate concentrations and the existing pH gradient across the plasma membrane.

It has been defined that aluminum citrate, which possesses a free monocarboxylic acid moiety, can be transported across the blood-brain barrier mediated by the MCT located [12]. Once aluminum enters the circulation, it is associated with several endogenous ligands. Two to four percent was bound to the small molecular weight ligand citrate except for 81% of aluminum in circulation which was coffiplexed with transferrin [13]. Other researchers demonstrated that 11% of the aluminum in serum is bound to citrate [14]. Aluminum citrates were the predominant aluminum species under the conditions employed. And the aluminum citrate complex is the predominant small molecular weight species found in serum. Aluminum is complexed to citrate by two of its three carboxylates and its alkoxy group, leaving a free carboxylate. Aluminum citrate transport across the blood-brain barrier involves either an uncharacterized monocarboxylate transporter MCT isoform expressed in the brain such as MCT7 or MCT8 or one of the many members of the organic anion transporting protein family, some of which are known to be expressed at the blood-brain barrier.

Many substrates and inhibitors of MCT1 and organic anion transporters, e.g., BSP and fluorescein, reduced aluminum citrate uptake into b. End5 cells which showed expression of MCT1, but not MCT2 or MCT4.

The process that aluminum cirate uptakes into the brain by MCTs depends on ATP [12]. The uptake of aluminum citrate can be reduced by inhibitors of mitochondrial respiration and oxidative phosphorylation. It suggested that it is an ATP-dependent mechanism. But it is not inhibited by ouabain, suggesting no role for Na/K-ATPase [12].

The aluminum citrate uptake through MCTs is sodium and pH independent [12]. Many members of the MCT appear to be sodium independent. And the uptake of aluminum citrate was pH independent. Citrate uptake increased at pH 6.9 compared to 7.4, whereas citrate uptake did not. At physiological pH, aluminum citrate is a better substrate if the uptake of aluminum citrate and citrate is mediated by the same carrier. The uptake of citrate enhancing at pH 6.9 may be due to reduce ionization of citrate. The lower pH increases the concentration of aluminum, which may serve as a better substrate for a monoanion carrier. The different response to pH reduction may show different transporters for citrate and aluminum citrate. Inhibition of aluminum citrate uptake by some compounds, e.g., furosemide which is a nonselective cation channel blocker, anion-exchange inhibitor and possible substrate for the MCT, and organic anion transporters, may reflect a requirement for another ion in the transport process but more likely reflect nonspecific effects [12].

3.1.1.4 Aluminum Citrate Enters into the Brain via System Xc–, Which Is Known to Be a Na^+-Independent Glutamate Transporter, at the BBB

System xc− or the cystine/glutamate antiporter is composed of transporter protein and a heavy chain subunit. The former is a light chain-specific subunit, xCT, which is encoded by the slc7a11 gene. The latter is a cell surface antigen protein 4F2hc that is encoded by the slc3a2 gene [15]. It exchanges glutamate for cystine in a 1:1 ratio and according to the respective concentration gradients [16]. Under physiological conditions, cystine is imported and intracellularly reduced to cysteine, a building block of the antioxidant GSH. In vitro, cystine supply via system xc− is very crucial for survival of certain cell types as they can only survive in the absence of system xc− when the medium is supplemented with reducing agents [17]. In vivo, however, it has been defined that genetic deletion of system xc− does not necessarily lead to any gross abnormality in the CNS, and there are no any signs of increased oxidative stress since GSH from other sources can be supplied by different cell types to sensitive cells [18]. While cystine is imported, glutamate is obligatorily exported, and system xc− has been identified as the major source of extracellular glutamate in several rodent brain regions [19]. Glutamate released via system xc− physiologically modulates synaptic transmission via activation of pre- and postsynaptic metabotropic glutamate receptors located in the vicinity of the synaptic cleft [16]. Moreover, studies have recently shown that the released of glutamate via system xc− regulates glutamatergic synapse strength through reducing the number of postsynaptic alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate receptors (AMPA receptors). Additionally, this glutamate could also activate extrasynaptic N-methyl-D-aspartic acid receptors (NMDA receptors) and as such in high concentrations may induce excitotoxicity [16].

System xc− is a Na^+-independent glutamate transporter and an l-glutamate/l-cystine exchanger. It has been found that it widespread distributes in the brain [20]. System xc− is expressed at high levels in the brain parenchyma and the meninges and the ependyma. The central nervous system cell types contribute to system xc− activity in acute brain slices by cystine uptake or in living animals by microdialysis. It is showed that system xc−-dependent cystine uptake in microglia is higher than in astrocytes and neurons, microglia>astrocytes>neurons. Study has shown that system xc− activity in cortical astrocytes is higher compared to neurons and microglia. The activity of system xc− increased in microglia with sAPP, leading to compromised synaptic density in hippocampal neurons in co-culture.

System xc− transports an anionic form of l-cystine in exchange for l-glutamate. Studies have shown that aluminum citrate is transported by the same transporter, system xc−, as well as l-glutamate and l-cystine, at the BBB, in RBEC1 (a BBB model cell line). The Na^+-dependent uptake of aluminum citrate was shown in RBEC1. It suggested that Na^+-dependent transport systems such as the EAATs are

involved in the aluminum citrate uptake in RBEC1 [21]. System xc− activity is very important for maintenance of the intracellular glutathione level and the redox balance between cystine and cysteine in the extracellular milieu [22]. Under several inflammatory factors including LPS, oxidative stress, and TNFα can also activate or increase the expression of macrophage/microglial system xc− [23]. Possible physiological roles for the induction of system xc− are acting as a detoxifying system in the brain and BCEC1 by supplying l-cystine/l-cysteine from the circulating blood for the synthesis of glutathione. Therefore, chronic inhibition of system xc− at the BBB by its blockers and/or substrate inhibitors such as aluminum citrate is speculated to cause decreases in the l-cystine levels in BCEC1 and the brain, and then vulnerability of the BCEC1 and neurons to oxidative stress, resulting in BBB dysfunction and neurodegenerative diseases [24]. Such a process is consistent with the direct demonstration that depletion of glutathione in primary murine cortical cells enhances the extent of NMDA-mediated excitotoxicity.

Aluminum citrate can be taken up into RBEC1 via system xc−, so this system might play an important role in aluminum citrate transport at the BBB. The uptake of aluminum citrate showed temperature and concentration dependency, and it did not require an inwardly directed Na^+-gradient as a driving force, ruling out the involvement of Na^+-dependent glutamate transporters in its transport [21].

3.1.2 Aluminum-Containing Compounds Could Enter into the Brain Through the Olfactory Mucosa/Olfactory Bulb Barriers

The three rabbits, which remained free of neurological deficits, are exposed to aluminum lactate through the nasal cavity. There are granulomas in the left olfactory bulb and cerebral cortex. The cortical involvement was bilateral but more severe on the left. Two animals had granulomas in the pyriform cortex and one had a lesion in the hippocampus. The granulomas consisted of accumulations of macrophages, lymphocytes, and occasional plasma cells. A granuloma was identified within the fiber layer of the left olfactory bulb of one of the animals receiving Al chloride, but granulomas were not seen in the cerebral hemispheres of these animals. The animals exposed to sodium lactate were free of comparable lesions; the result indicated that aluminum lactate could enter into the brain through olfactory mucosa/olfactory bulb barriers [25].

Aluminosilicates comprise the bulk of inhaled aerosol contaminants in the air. Aluminum-containing compounds could enter into the brain through olfactory mucosa/olfactory bulb barriers under physiological conditions and a defect in the normally very effective olfactory mucosa/olfactory bulb barriers leading to excessive influx into the brain of aluminum-containing compounds.

3.1.3 Little Aluminum Enters the Brain Through Choroid Plexus

Although metals could enter the brain across the choroid plexus, it is not a prominent way for aluminum entering the brain. There is a choroid plexus in each of the four cerebral ventricles of the brain. They synthesize most of the cerebrospinal fluid (CSF) that fills the brain ventricles and the subarachnoid space that surrounds the brain and spinal cord. The total surface area of the choroid plexuses is approximately 10cm^2. About 1/1000 of the surface area of brain capillaries are the site of the BBB. Brain atlases of the rat and human show brain regions as far as 1 mm away from the nearest component of the CSF. There is little opportunity for the choroid plexuses and CSF compartment than through rapid exchange between blood, and the brain through the BBB aluminum citrate was administered via the femoral vein. Peak aluminum concentrations were seen within the first 10 min at all three sites, the frontal cortical brain, blood, and lateral ventricle. Aluminum frontal cortical brain/blood ratios (oBBRs) were significantly higher than those for the lateral ventricle. The result suggested that the primary site of aluminum permeation across the BBB is at cerebral capillaries [26]. So, aluminum primarily enters the brain from blood through the BBB rather than through the choroid plexuses, and little aluminum enters the brain through choroid plexus.

3.2 Aluminum Effluxes from the Brain

Study has showed that aluminum can be remove from brain ECF, either into brain cells or blood through a carrier, and it is effective and energy-dependent [26]. But more researchers defined brain entry of aluminum, presumably from blood, and some degree of aluminum persistence in the brain. The redistribution of aluminum out of the brain is slow. The concentration of aluminum in human brain increases with age. The half-life of brain aluminum could not be accurately calculated but was estimated to be about 150 days [2].

Researchers observed that rat brain aluminum concentrations decreased only slightly from 1 to 35 days after systemic aluminum injection, in the absence or presence of the aluminum chelator desferrioxamine. It suggested that aluminum could be retained in the brain for a long time. Part of aluminum flows out the brain across the BBB shortly after the aluminum enters the brain. But once aluminum enters the cells, it may be retained for a long time [27].

3.3 The Influence Factors of Aluminum Entry and Deposition in the Brain

3.3.1 Parathyroid Hormone (PTH) and Vitamin D

Some studies defined that in individuals with normal renal function, PTH and vit D can promote the absorption of aluminum in the liver, brain, and parathyroid [28].

3.3.2 Permeability of the BBB

The primary lesion in Alzheimer's disease and dialysis dementia has been postulated to be an impaired BBB permeability that allows neurotoxins like aluminum to reach the central nervous system. Actually aluminum itself affects the permeability of the BBB of rats to small peptides. Intraperitoneal injection of aluminum chloride increased the permeability of the BBB to iodinated N-Tyr-delta-sleep-inducing peptide and beta-endorphin by 60–70% [29]. The results of immunohistochemistry and Western blot analysis showed that aluminum induced a decrease in the expression of F-actin and occludin. All these results suggested that aluminum toxicity might be related to the change of the permeability and the integrity of BBB [30].

Short time and low dose of aluminum might not change the ability of learning and memory in juvenile rats; however, the permeability and ultrastructures of the BBB might be significantly changed [31].

Aluminum chloride and aluminum lactate can increase the permeability of the blood-brain barrier, while aluminum hydroxide can gradually increase the concentration of human or animal blood aluminum after prolonged and repeated consumption [32]. It increases the rate of transmembrane diffusion and selectively changes saturable transport systems [33].

So aluminum can increase permeability of the BBB, and then more aluminum enters the brain.

3.3.3 Citric Acid

Study has defined concentrations of aluminum in the cerebral cortex, hippocampus, and cerebellum of rats which were treated with 100 mg aluminum/kg body weight in the form of aluminum citrate. And aluminum concentrations in the cerebral

cortex in the animals fed citric acid increased because of possible absorption of the citrate chelate presumably formed with the aluminum present in the diet. But there was no significant increase in tissue aluminum concentrations in all brain regions in Sprague-Dawley or Wistar rats after treatment with aluminum hydroxide. It suggested that citric acid can promote influx of aluminum into the brain [34].

Aluminum, as a known neurotoxicant, can enter the brain through BBB, the choroid plexuses, and sensory nerve from the nasal cavity, but the efflux of aluminum from brain is very slow. So aluminum can be deposited in brain for a long time, then it is a main reason which induces neurotoxicity. Some compounds of aluminum can injure BBB, through which the raising of the blood-brain barrier permeability can promote aluminum to enter into the brain.

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Chapter 4
Aluminum as a CNS and Immune System Toxin Across the Life Span

Christopher A. Shaw

Abstract In the following, I will consider the impact of aluminum on two major systems, the central nervous system (CNS) and the immune system, across the life span. The article will discuss the presence of aluminum in the biosphere, its history, and the sources of the element. These include food, water cosmetics, some vaccines, and a range of other sources. I will also consider aluminum's unique chemistry. Finally, in humans and animals, I will consider how aluminum may impact the CNS at various levels of organization and how it may be involved in various neurological disease states across the life span. These disorders include those of infancy and childhood, such as autism spectrum disorder (ASD), as well as those in adulthood, such as in Alzheimer's disease. The bidirectional nature of CNS–immune system interactions will be considered and put into the context of neurological disorders that have an autoimmune component. I will argue that the exposure to humans and animals to this element needs to be reduced if we are to diminish some CNS and immune system disorders.

Keywords Aluminum bioavailability · Central nervous system · Immune system · Autoimmunity · Autism spectrum disorder

4.1 Introduction: Neurological Diseases and Causality Factors

An ongoing debate in any of the subfields of neurological disease research concerns the relative contributions of the putative factors to the origin and progression of any such disease. This debate occurs regardless of whether the disease in question is Alzheimer's disease (AD), Parkinson's disease (PD), and Lou Gehrig's disease (amyotrophic lateral sclerosis (ALS)) in older individuals or autism spectrum disorder (ASD) in children. In each case, the debated etiologies tend to include the

C. A. Shaw (✉)
Department of Ophthalmology and Visual Sciences, University of British Columbia, Vancouver, Canada

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following: genetic mutations/deletions or polymorphisms, environmental toxins, and some combination of both of these factors.

Included in environmental considerations are the emerging concepts about the role of a broad range of environmental impacts across the life span, i.e., the "exposome" [86]. This last may include potential toxic contributions from the microbiome of those so affected [23].

Until recently, most conceptualizations of neurological disease etiologies have focused rather narrowly on abnormal genetic factors. In relation to neurological diseases associated with aging, the concentration of effort in seeking genetic causes is arguably not warranted by the numbers of the so-called "familial" forms of AD, PD, or ALS compared to those forms considered to be "sporadic", or of unknown cause. The latter are usually considered to arise from environmental exposures to some toxin(s).

For these disorders, the percentages derived from autosomal mutations never exceed 10% of the total, regardless of how many new contributing mutations are found for those in the familial category [126]. One clear exception is Huntington's disease which has a clearly linked mutation.

As with the above diseases, developmental neurological disorders, for example, ASD and juvenile schizophrenia, do not show a uniformly dominant genetic etiology (as discussed in [122]). The studies to date on ASD, for example, do show some interesting genetic variations in those with the disorder, but these do not tend to be uniform across the ASD patient population. Some researchers have described each case as being like "snowflakes," meaning that each, while showing genetic deviations from the normal population, is nonetheless unique.

At the same time, studies of any of the sporadic neurological diseases cited above have failed to find a single environmental factor (see details and references in Shaw [122]), although such have been clearly indicated in several non-related neurological disease clusters, for example, those involving methyl mercury poisoning or lathyrism (see Shaw [122]).

An emerging view, albeit not necessarily a consensus one, is that most age-dependent neurological diseases at any stage of the life span likely arise due to some complex interplay of genetic susceptibility factors (and there can be more than one) and toxic exposures in any individual exposome (many more than one such factor) that may be relatively unique to any affected individual (for additional references, see Shaw [122]).

These considerations apply in particular to current views about ASD, an early onset neurological disorder characterized, in part, by abnormal social interactions and language development. This is partially because of evidence suggesting a rising incidence to those on this spectrum. It should be noted that not all of those in the field agree that incidence has changed, instead attributing the measured changes in rate to a combination of expanded diagnostic criteria and greater social and medical awareness of the condition. While these latter conjectures have become popular in some circles, rigorous evidence that either or both contribute to persistent incidence changes has not yet been produced. Indeed, some of those holding such views seem particularly concerned to move the discussion away from environmental factors,

and, in particular, from vaccines and the various components of vaccines, including aluminum (Al) adjuvants.

4.1.1 Aluminum Toxicity: General Considerations for the Impact on Human Neurological Diseases Across the Life Span

With the above as a general background, it will be worthwhile to consider aluminum in its various forms and routes of administration as a potential neurotoxin generally. More specifically, it is important to consider the role that aluminum exposure through various sources, including through routine pediatric vaccinations, may play in disorders of the developing central nervous system (CNS).

Finally, it will be important to discuss the intimate interrelationship, particularly in CNS development, with the immune system and how aluminum as a neurotoxin can impact both systems.

4.2 Aluminum in the Biosphere and Forms of Human Exposure

One element to which humans are currently heavily exposed is aluminum, whose ubiquity in the human biosphere has been steadily increasing for well over 100 years. Not only does aluminum toxicity has clear impacts on the CNS of animals and humans; it also negatively impacts other organ systems in both. It can also be toxic to plants (for reviews, see Tomljenovic [125] and Shaw et al. [139]).

The scientific literature is replete with examples of such toxicity, some of them going back almost to the earliest exposures to bioavailable aluminum derived from human activity. As noted by William Gies [52] over a century ago:

> These studies have convinced me that the use in food of aluminum or any other aluminum compound is a dangerous practice. That the aluminum ion is very toxic is well known. That aluminized food yields soluble aluminum compounds to gastric juice (and stomach contents) has been demonstrated. That such soluble aluminum is in part absorbed and carried to all parts of the body by the blood can no longer be doubted. That the organism can "tolerate" such treatment without suffering harmful consequences has not been shown. It is believed that the facts in this paper will give emphasis to my conviction that aluminum should be excluded from food. (p. 816)

Gies was also referring to even earlier studies, some from the early nineteenth century, starting with observations on the parenteral administration of aluminum salts [102] and with animal studies (Siem, as cited in Dollken [35]). Dollken [35], for example, showed instances of degeneration in the rabbit CNS following aluminum exposure.

Much of the literature on aluminum neurotoxicity will be discussed in Sect. 4.3 below, but it is important to note at the outset that it is still widely held, by some in both the medical and lay communities, and that aluminum is both inert and harmless. This view is then elaborated to propose that any potential for aluminum CNS toxicity to occur has been "debunked," to use a lay/journalistic term (see, e.g., Lidsky [84]), when in fact quite the opposite is the case.

Apart from careless scholarship, there are other reasons leading to the view that aluminum is not involved in neurological diseases. In part, some of the objections have arisen from what was perceived to be a lack of evidence for earlier claims that aluminum from various environmental sources posed a health risk, as suggested by McLachlan et al. [93]. Critics of McLachlan's work contended that human exposure to ionic aluminum exposure was, and remains, fairly minimal under most circumstances and thus could not play a significant role in neurological diseases. The McLachlan review may, however, have been prescient, in that while metallic aluminum is relatively inert, as are the various aluminum–silicate complexes, aluminum ions (Al^{3+}) can be released in acidic environments and are anything but benign.

4.2.1 Aluminum Chemistry and the Intersection of Aluminum with the Biosphere

Aluminum is the third most common element after oxygen and silicon on earth and the most abundant metal in the earth's crust. The abundance of aluminum on earth and the recent historical and current ubiquity in the biosphere have fostered attitudes such as that already mentioned, namely, that if it is so common, it cannot be harmful. However, as shown by Exley and colleagues [19–21] and others [139], this element was not widely bioavailable until recent historical times.

In regard to this last point, it may be notable that due in part to its historical lack of bioavailability, aluminum seems to have been "selected out" of involvement in terrestrial biochemical evolution [19, 43]. This situation changed with the industrial extraction of aluminum, primarily from bauxite, from the 1820s onward, and its myriad current materials applications have brought human beings into ever-increasing contact with various forms of the element.

Chemically, aluminum avidly binds to oxygen, carbon, phosphorous, and sulfur, all key elements in biochemical reactions in biological systems, and thus provides the potential to significantly impact such systems. In spite of claims often made in the context of aluminum's various industrial and medical applications, the element is therefore certainly not inert – nor, as will be shown below, is it harmless. It is also manifestly not an "essential" element, as erroneously previously claimed by some medical websites (e.g., the Children's Hospital of Philadelphia [22]).

4.2.2 Sources of Aluminum in the Biosphere

As already noted, aluminum has been linked to various disorders in plants, animals, and humans [42, 112, 120, 139–141], not least of which are those involving the CNS in animals and humans.

Aluminum in the biosphere, particularly that which may affect humans, arises from various sources [118]. It has a significant presence in processed foods, both through deliberate addition for its chemical properties and due to contamination during the manufacturing process. Salts of aluminum show up in a great variety of medicinal products, including antacids, various coatings for pills, and some vaccines. In regard to the latter, aluminum salts serve as adjuvants to improve the immunogenicity of antigens [141]. Aluminum salts are also used as mordants in cosmetics and in antiperspirants.

The release of ionic aluminum can occur in acidic conditions such as in acid soil or in various food preparations where acidic solutions are in contact with metallic aluminum. The former includes soils of volcanic origin and, increasingly, in soils exposed to acid rain. The latter has become an emerging feature of the human biosphere with the consequence that aluminum has become even more bioavailable than in previous decades (Exley, pers. comm.).

Food remains the most common source of human exposure to aluminum [158]. The second most common source appears to be from aluminum vaccine adjuvants [141]. In both cases, aluminum can readily enter the body by way of its soluble salts.

In the case of food, aluminum is absorbed through the gastrointestinal (GI) system with an average daily human range of 3–10 mg [158]. Intestinal absorption is influenced by compounds that increase absorption (e.g., citrate and fluoride) or is decreased by substances such as milk [137].

In addition, the acidity of some foods cooked in aluminum pans may serve to release ionic aluminum. Additionally, as most “tin” cans are actually made of aluminum and have been for a number of years, any acidic solution that breaches the protective epoxy coating, a bisphenol-A epoxy resin, will release potentially large amounts of ionic aluminum (not to mention bisphenol-A). This concern applies to cans containing fruit juices and various “soft” drinks. The structural integrity of the coating on aluminum cans can also be compromised by mechanical stress and/or heat [54].

Far beyond the possible release of aluminum ions from older cookware and current aluminum cans, aluminum finds its way into a variety of products for human use, as presented in the following subsection. In each case, the relative absence of obvious acute effects has led to a view similar to that which greeted the McLachlan study, namely, that human exposure to aluminum from most sources is unlikely to have a significant impact on human health. That this perception is largely incorrect is abundantly demonstrated every 2 years at the Keele University conference on aluminum (e.g., Keele University [75]).

Table 4.1 Sources of bioavailable aluminum in humans

Major sources of Al exposure in humans	Daily Al intake (mg/day)	Weekly Al intake (mg/day)	÷ PTWI[a] (1 mg/kg/bw; for an average 70 kg human PTWI = 70 mg)	Amount delivered daily into systemic circulation (at 0.25% absorption rate)
Natural food	1–10	7–70	0.1–1	2.5–25 μg
Food with Al additives	1–20 (individual intake can exceed 100)	7–140 (700)	0.1–2 (10)	2.5–50 μg (250 μg)
Water	0.08–0.224	0.56–1.56	0.008–0.02	0.2–0.56 μg
Pharmaceuticals (antacids, buffered analgesics, anti-ulceratives, antidiarrheal drugs)	126–5000	882–35,000	12.6–500	315–12,500 μg
Vaccines (HepB, Hib, Td, DTP)	0.51–4.56	NA	NA	510–4560 μg[b]
Cosmetics, skin care products and antiperspirants[c]	70	490	NA	8.4 μg (at 0.012% absorption rate)
Cooking utensils and food packaging	0–2	0–14	0–0.2	0–5 μg

[a]PTWI (provisional tolerable weekly intake) is based on orally ingested Al; generally only 0.1–0.4% of Al is absorbed from the GI tract; however, Al may form complexes with citrate, fluoride, carbohydrates, phosphates, and dietary acids (malic, oxalic, tartaric, succinic, aspartic, and glutamic), which may increase its GI absorption (0.5–5%). Co-exposure with acidic beverages (lemon juice, tomato juice, coffee) also increases Al absorption as well as conditions of Ca^{2+}, Mg^{2+}, Cu^{2+}, and Zn^{2+} deficiency

[b]A single dose of vaccine delivers the equivalent of 204–1284 mg orally ingested Al (0.51–4.56 mg), all of which is absorbed into systemic circulation. Al hydroxide, a common vaccine adjuvant, has been linked to a host of neurodegenerative diseases; it also induces hyperphosporylation of MAP tau in vivo

[c]The risk of antiperspirants is both from dermal exposure and inhalation of aerosols. Inhaled Al is absorbed from the nasal epithelia into olfactory nerves and distributed directly into the brain (From Shaw [122])

Table 4.1 highlights some of the key sources of bioavailable aluminum to which humans are exposed (excerpted from Tomljenovic [139]. See original article for relevant references).

Aluminum can appear in drinking water following the use of aluminum sulfate as a flocculant, but its overall impact seems to be low (0.3%) [158], except in unusual circumstances such as the large aluminum sulfate spill into the water supply in Camelford (United Kingdom) in 1988. High concentrations can also arise naturally in well water near volcanic or acidified soils.

The addition of fluoride to drinking water as part of a campaign against dental caries raises concern from two neurological perspectives, notwithstanding the

presumed – and, apparently, incorrect – value of water fluoridation for the prevention of tooth decay. First, fluoride promotes GI disorders [146]. Second, the joint presence of aluminum and fluoride can form aluminofluoride compounds which can act as phosphate analogues [137].

Aluminum can also enter the body by inhalation, with an estimated daily uptake of 4.4 μg in industrialized areas [101]. Aluminum metal workers may show higher levels in blood, urine, and bone [40, 53, 85]. Health outcomes of inhaled aluminum can include respiratory tract infections with asthma-like symptoms [78] and cognitive disorders [116], the latter implicating uptake into the CNS. A recently characterized apparent "cluster" of neurological diseases has been described in former miners who were deliberately exposed to aluminum powder by inhalation in an unsuccessful attempt to prevent silicosis [92].

Given patent kidney function, most dietary/waterborne aluminum ions will be excreted through the kidneys relatively rapidly. An additional major route of aluminum excretion is through sweat [95]. Notably, the same is not true for aluminum bound up in fluoride complexes or for aluminum that has a different route of administration, such as by injection into muscle or skin. Nor is it clear how much aluminum from inhalation is removed.

In regard to CNS levels, the amount of aluminum in the normal adult human brain is less than 2 μg/g [3], with the distribution reflecting higher concentrations in gray compared to white matter [18]. Along with bone, the brain has the highest potential to accumulate aluminum [42, 43]. Postmortem brain samples of individuals exposed during the Camelford incident showed an aluminum concentration of from 0.75 μg/g in frontal white matter to 49 μg/g in the choroid plexus [45]. The association of aluminum with the hallmark abnormal protein entities in Alzheimer's disease, amyloid beta (Aβ) plaques, and neurofibrillary tangles (NFTs) has been well documented [15].

There is disagreement about how much aluminum entering the brain is later removed [156] versus [76], although the differences in outcome may reflect the route of administration. However, retained aluminum seems to be stored in five main compartments: the blood–brain barrier, the brain interstitial fluid, neurons, glia, and, in pathological neurological diseases, in inclusions such as Lewy bodies, NFTs, and Aβ plaques [4, 79, 117].

4.2.3 Aluminum in Vaccines

As mentioned, one main source of aluminum, particularly in the very young, is its widespread use as a vaccine adjuvant, or "helper," acting to stimulate an immune response. There are a variety of aluminum adjuvant preparations, but the two most common are aluminum hydroxide and aluminum phosphate [16].

Although a single vaccine may contain only a relatively small amount (usually less than 0.5 mg of the adjuvant compound, not elemental aluminum), aluminum adjuvants may cumulatively constitute an important source of the overall aluminum

body burden. For example, the administration of 20 or more vaccines containing 0.5 mg aluminum compound as adjuvants would add up to an extra 10 mg aluminum compound to the body burden, equivalent to a normal dietary intake of aluminum of over 4000 mg/day [101].

Two considerations apply here. First, the circumstances in which aluminum from vaccines may be given in such amounts include the typical pediatric vaccine schedule of many Western countries and from various sources in war-time conditions. In the latter case, it is notable that Gulf War syndrome was associated, at least in part, with multiple vaccines given to potentially deploying soldiers. Many of these vaccines were aluminum-adjuvanted [66]. The second consideration is that aluminum adjuvants are not subject to the same pharmacokinetics as that of dietary/water aluminum exposure and do not seem to be efficiently excreted.

In regard to aluminum excretion, there are two key caveats to consider. The first is that the form in which aluminum is found is a major factor in its potential toxicity. Thus, not all aluminum adjuvants are likely to be identical in their potential impact. Nor have detailed studies compared the various forms [125]. Second, companies making such adjuvants usually employ proprietary forms of these compounds, which may have quite different properties to those that are more commercially available. The neurological pathologies associated with aluminum-adjuvanted vaccines administered to commercial sheep, to be described below, do not, however, support the notion that such proprietary forms necessarily have lesser neurological impacts than commercial forms of the same molecules [88].

4.3 Human and Animal Studies of Aluminum Neurotoxicity

In addition to the early evidence for aluminum's toxic actions on the CNS, more recent studies have clearly implicated this element in various human neurological disorders. A now-famous example termed "dialysis-associated encephalopathy" (DAE) occurred when kidney dialysis patients were accidentally given dialysis fluids containing high levels of aluminum [117]. The outcomes were typically of relatively rapid onset and severity. The resulting neurological signs included cognitive dysfunctions resembling Alzheimer's disease and epileptic seizures. Postmortem histology showed some of the hallmark pathological features of Alzheimer's disease, including NFTs and Aβ plaques. It is likely that the mechanism by which aluminum ions were transported into the brain involved one or more of the various carrier proteins, including ferritin and transferrin [119, 157].

Aluminum has been further linked to other neurological disorders across the life span, from Alzheimer's disease (see review by Tomljenovic [139]) in old age and to ASD in children [125, 140].

A variety of other CNS disorders of an autoimmune nature have also been associated with aluminum injections. These include macrophagic myofasciitis (MMF) [50, 51]which is a deteriorating neuromuscular disorder that follows intramuscular injections of adjuvant aluminum hydroxide. A sequela to MMF is often a form of

mild cognitive impairment (MCI) [115], sometimes viewed in other circumstances as a precursor to Alzheimer's disease. MMF also features a variety of disturbances in interhemispheric functions. Variations on the "autoimmune syndrome/inflammatory syndrome induced by adjuvants" (ASIA) disorders [69, 130], including MMF, may also occur.

Animal models of neurological disease using aluminum are available for ALS [109, 124], Alzheimer's disease [149–153], and, as cited in Tomljenovic [139], ASD [123, 126, 140]. In the first instance, subcutaneous injections of aluminum hydroxide in young male mice induce apoptotic neuronal death in motor neurons in the spinal cord and motor cortex, accompanied by degraded motor function.

Similarly negative CNS outcomes in more extensive experiments have been reported [29]. In addition, subcutaneous aluminum hydroxide injections in newborn mice induce significant weight increases in some cases and a range of behavioral changes associated with increased anxiety [123]. Aluminum-treated mice also show deficits in social interactions [127].

Adding yet another species, Lujan et al. [88] reported a neurological disorder in commercial sheep after a mass vaccination campaign against "blue tongue." The adjuvant in the vaccine was aluminum hydroxide. Chronic adverse effects were observed in 50–70% of flocks and up to 100% of animals within an affected flock. The behavioral disturbances and neurological signs included restlessness, compulsive wool biting, generalized weakness, muscle tremors, loss of response to external stimuli, ataxia, tetraplegia, and stupor.

As with human DAE, coma and death in the treated sheep could follow. On histological examination, inflammatory lesions in the brain and spinal cord were found associated with the presence of aluminum. These lesions included multifocal meningoencephalitis, demyelination, multifocal neuronal necrosis, and neuron loss in the spinal cord.

The disorder was made worse by cold weather conditions, perhaps suggesting some synergy with other environmental factors. These initial observations were successfully reproduced under experimental conditions following the experimental administration of aluminum-containing vaccines.

The veterinary studies by Lujan et al. [88] seem largely to confirm the general nature of the negative CNS outcomes previously seen in mice following aluminum adjuvant administration. In both mice and sheep, motor and cognitive function changes were noted. Degeneration of neurons in the CNS followed in both cases, particularly among motor neurons.

A key question is how aluminum might be transported from the site of injection into the CNS. The answer has been provided by the work of the Gherardi group which showed that aluminum hydroxide administered intramuscularly in mice does not stay localized in the muscle, but rather migrates to different organs. The path by which it does so is now clear from various tracking experiments with fluorescent markers, notably rhodamine- and nano-diamond-labeled aluminum hydroxide. These studies demonstrated that a significant proportion of the nanoparticles escape the injected muscle within immune cells (macrophages), travel to regional draining lymph nodes, and then exit the lymphatic system to reach the bloodstream, eventu-

ally gaining access to distant organs, including the brain. Such a "Trojan horse" transport mechanism, in which aluminum-containing macrophages enter the brain, predictably results in the gradual accumulation of aluminum due to lack of recirculation [29, 76]. These studies clearly refute previous notions that injected aluminum adjuvant nanoparticles remain localized at the injection site and only act on the immune system through some "depot effect."

The examples in mice and sheep have obvious relevance for human exposure to aluminum adjuvants, and the noted CNS pathologies are worth considering in the context of the development of age-related neurological disorders of all kinds.

In particular, the work on MMF and the in vivo models of the same [29, 30, 76] show that the bioaccumulation of aluminum in the CNS can occur at a very slow rate under many different conditions, especially by periodic vaccination with aluminum-adjuvanted vaccines.

Aluminum accumulation may be expected to be equally slow when the source is drinking water or food, and its deleterious eventual outcomes will be the result of cumulative body/brain burden and age. In the latter regard, there is evidence from older literature that aluminum in the brains of the elderly, of Alzheimer's disease patients, and of those with various forms of dementia is often associated with NFTs [61, 106–108, 136, 147]. The source of the CNS aluminum in these cases is not known and could be any of those mentioned in this section.

What will be obvious from a consideration of these data is that while aluminum has the potential to be both acutely toxic, as in DAE, and chronically toxic, as perhaps in Alzheimer's disease, the range of impacts on the CNS can be extremely varied, both in CNS area affected and the time course of any resulting pathology. In this regard, aluminum neurotoxicity is disseminated both spatially and temporally in a manner that may more closely resemble the typical phenotype of multiple sclerosis. What is equally clear is that aluminum has the capacity to impact the CNS at multiple levels of organization, from DNA all the way though to higher systems interactions [125].

From the preceding, one way to view aluminum neurotoxicity may be to consider it as a generally neurotoxic element with spatial variations in CNS subsystem impacts that are extremely diverse. In this view, the precise outcome may depend on a variety of intrinsic and extrinsic factors. Concerning the former, age, sex, and individual genetic polymorphisms and biochemistries (including the microbiome; see Scheperjans et al. [121]) are likely to be key players. Extrinsic factors include the type of aluminum compound, the amount of exposure, and the route of exposure (e.g., by food, water, intramuscular versus other types of injection, inhalation, etc.).

For all of these reasons, the toxicity of aluminum in the CNS appears to depend on a number of variables, which include both direct and indirect cellular mechanisms. In either case, factors include the form of aluminum complex, the size of the particles, the route of administration, and the dose. Dose itself may not be the major consideration [30]. In animal models, species and even strain may influence aluminum outcomes [29]. Finally, it should now be apparent that interactions with the immune system, to be discussed below, will determine, at least in part, the influence of the other factors.

The full range of CNS impacts of aluminum is shown in Table 4.2.

4.3.1 Aluminum-Triggered Genetic Alterations and Protein Expression Levels

An emerging area of study is that of "epigenetics" or the changes in gene expression regardless of the mutational state of the gene. Epigenetic modifications can occur in several ways. The first is when a stable but reversible alteration of gene function is mediated by histone modification, cytosine methylation, the binding of nuclear proteins to chromatin, or interactions among any of these elements. Such modification does not require, or generally involve, any changes in the DNA sequence itself.

A second way epigenetic modification can occur is through "epimutation" or a heritable change in gene expression that does not affect the DNA sequence. Instead, epimutation involves the silencing of a gene that is not normally silenced or, conversely, the activation of a gene that is not normally active. It is useful in this regard to consider the probability demonstrated in the literature that some factor, perhaps a toxin, can be the trigger of such epigenetic changes, whose net consequence is to cause some gene to under- or over-express downstream protein production. A recent example ties back to the discussion of aluminum toxicity and is based on older observations that aluminum can bind to and alter DNA [74].

The key point here is to illustrate what may serve as a new way of looking at the interaction between genes and toxins in that genes do not have to be modifiable in their DNA structure in order to be modified in their expression. The fact that some toxins, such as aluminum, can do so in the CNS may prove to be a factor in the impact of such toxins on neurological disease.

4.3.2 miRNA Alterations in Gene Expression

A less direct means of altering gene expression occurs in the impact that various other genes or molecules may have on the transcriptional machinery of the cell, notably transfer and messenger RNA. RNA transcriptional errors have been implicated in ALS and other neurological diseases [100, 135]. Gene expression is also affected by microRNA (miRNA), which can act to silence various gene expression patterns. Again, aluminum may be one of the contributors to this outcome. Changes in miRNA have been implicated in Alzheimer's disease, as one example [89]. In context to the aluminum-induced gene expression changes, the impact of aluminum on miRNA cannot be discounted.

Table 4.2 Aluminum's CNS impacts in animals and humans

Al's neurotoxic effects related to AD
Effects on memory, cognition, and psychomotor control
Significantly decreases cognitive and psychomotor performance in humans and animals
Impairs visuomotor coordination and long-term memory and increases sensitivity to flicker in humans and rats
Impairs memory and hippocampal long-term potentiation (LTP) in rats and rabbits in vivo (electrophysiological model of synaptic plasticity and learning)
Effects on neurotransmission and synaptic activity
Depresses the levels and activity of key neurotransmitters known to decline in AD in vivo: acetylcholine, serotonin, norepinephrine, dopamine, and glutamate
Reproduces hallmark cholinergic deficits observed in AD patients by impairing the activity of cholinergic synthetic and transport enzymes:
Impairs acetylcholinesterase activity
Reduces neural choline acetyltransferase
Inhibits choline transport in rat brain and in synaptosomes from the cortex and hippocampus
Attenuates acetylcholine levels in rabbit hippocampus and concomitantly induces a learning deficit
May cause acetylcholine deficit by acting upon muscarinic receptors and potentiating the negative feedback controlling acetylcholine release into the synaptic cleft
Inhibits neuronal glutamate-nitric oxide (NO)-cyclic GMP (cGMP) pathway necessary for LTP
Damages dendrites and synapses
Impairs the activity of key synaptosomal enzymes dependent on Na-K, Mg^{2+}, and Ca^{2+}
Inhibits glutamate, GABA, and serotonin uptake into synaptosomes
Impairs neurotransmission by disrupting post-receptor signal transduction mediated by the two principal G-protein regulated pathways: PLC and AC (see effects on G-proteins and Ca^{2+} homeostasis)
Inhibits dihydropteridine reductase, essential for the maintenance of tetrahydrobiopterin (BH4), a cofactor important in the synthesis and regulation of neurotransmitters
Impairs ATP-mediated regulation of ionotropic and metabotropic receptors – cholinergic, glutamatergic, and GABAergic
Interferes with receptor desensitization by increasing the stability of the metal-ATP receptor complex and causes prolonged receptor activity (by replacing Mg^{2+} from the metal site)
Effects on G-proteins and Ca^{2+} homeostasis
Alters IP and cAMP signaling cascades by interfering with G-proteins (as AlF), second messengers and second messenger/Ca^{2+} targets:
Potentiates agonist-stimulated cAMP production following chronic oral exposure in rats, by inhibiting the GTPase activity of the stimulatory G-protein (Gs), leading to prolonged activation of Gs after receptor stimulation and increased cyclic AMP production by AC
Increases cAMP levels by 30–70% in brains of adult and weanling rats
Inhibits muscarinic, adrenergic, and metabotropic receptor-stimulated IP3 accumulation by inhibiting Gq-dependent hydrolysis of PIP2 by PLC
Decreases IP3 in the hippocampus in rats following chronic oral administration
Inhibits PKC
Blocks the fast phase of voltage-dependent Ca^{2+} influx into synaptosomes

(continued)

Table 4.2 (continued)

Al's neurotoxic effects related to AD
Binds to CaM and interferes with numerous CaM-dependent phosphorylation/dephosphorylation reactions
Impairs Ca^{2+}/CaM-dependent LTP
May cause a prolonged elevation in intracellular Ca^{2+} levels by:
Interfering with desensitization of the N-methyl D-aspartate (NMDA) receptor channel
Delaying the closure of voltage-dependent Ca^{2+} channels
Blocking CaM-dependent Ca^{2+}/Mg^{2+}-ATPase responsible for extrusion of excess intracellular Ca^{2+}
Elicits a Ca^{2+}-dependent excitotoxic cascade by frequent stimulation of the NMDA receptor which may result in:
Persistent further activation of NMDA receptor by endogenous glutamate and exacerbation of glutamate excitotoxicity
Mitochondrial and ER Ca^{2+} store overload
Compromised neuronal energy levels
Erosion of synaptic plasticity
Increased susceptibility to apoptosis and accelerated neuronal loss
Perturbs neuronal Ca^{2+} homeostasis and inhibits mitochondrial respiration in a complex with amyloidogenic A peptide in a triple transgenic mouse model of AD
Metabolic and inflammatory effects
Inhibits utilization of glucose in the brain
Inhibits hexokinase and G6PD
Reduces glucose uptake by cortical synaptosomes
Alters Fe^{2+}/Fe^{3+} homeostasis and potentiates oxidative damage via Fenton chemistry
Alters membrane properties by:
Decreasing the content of acidic phospholipid classes: phosphatidylserine (PS), phosphatidylinositol (PI), and phosphatidic acid (PA) in rat brain myelin by 70%
Inducing the clustering of negatively charged phospholipids, thereby promoting phase separation and membrane rigidification and facilitating brain-specific LPO
Increases the permeability of the BBB by:
Increasing the rate of transmembrane diffusion
Selectively changing saturable transport systems
Facilitates glutamate transport across the BBB and potentiates glutamate excitotoxicity
Decreases antioxidant activity of SOD and catalase in the brain
Increases cerebellar levels of nitric oxide synthase (NOS)
Augments specific neuro-inflammatory and pro-apoptotic cascades by inducing transcription from a subset of HIF-1- and NF-B-dependent promoters (APP, IL-1 precursor, cPLA2, COX-2, and DAXX)
Activates microglia, exacerbates inflammation, and promotes degeneration of motor neurons
Nuclear effects
Binds to phosphonucleotides and increases the stability of DNA
Binds to linker histones, increases chromatin compaction, depresses transcription
Inhibits RNA polymerase activity
Reduces the expression of the key cytoskeletal proteins tubulin and actin

(continued)

Table 4.2 (continued)

Al's neurotoxic effects related to AD
Downregulates the expression of the light chain of the neuron-specific neurofilament (NFL) gene in 86% of surviving neurons in the superior temporal gyrus of AD patients
Upregulates well-known AD-related genes: amyloid precursor-like protein (APLP)-1 and APLP-2, tau, and APP, in human neuroblastoma cells when complexed with A, to a larger extent than other A-metal complexes (A-Zn, A-Cu, and A-Fe)
Upregulates HIF-1- and NF-B-dependent gene expression (see metabolic and inflammatory effects)
Effects on MTs, cytoskeleton, and NFT formation
Induces neurofibrillary degeneration in basal forebrain cholinergic neurons and cortical and hippocampal neurons and accumulates in NFT-bearing neurons
Causes neurite damage and synapse loss in hippocampal and cortical pyramidal neurons by disabling their capacity for MT assembly
Directly alters MT assembly by interfering with magnesium and GTP-dependent MT-polymerization mechanisms. Actively displaces magnesium from magnesium-binding sites on tubulin and promotes tubulin polymerization
Decreases the sensitivity of MTs to calcium-induced depolymerization and effectively disables the regulatory circuits that are set to maintain the sensitive dynamics between polymerization and depolymerization cycles of tubulin and, ultimately, impairs MT assembly
Inhibits axonal and dendritic transport mechanisms by depleting MTs
Induces cAMP-dependent protein kinase phosphorylation of MAPs and NFs in rats following chronic oral exposure and enhances the formation of insoluble NF aggregates. Al-induced hyperphosphorylated NFs are resistant to dephosphorylation and degradation by calcium-dependent proteases (calpain)
Promotes highly specific nonenzymatic phosphorylation of tau protein in vitro by catalyzing a covalent transfer of the entire triphosphate group from ATP to tau via O-linkage (cAMP-dependent protein kinase phosphorylation sites), at concentrations similar to those reported in AD brains
Induces tau phosphorylation and motor neuron degeneration in vivo (as a vaccine adjuvant)
Facilitates cross-linking of hyperphosphorylated tau in PHFs, stabilizes PHFs, and increases their resistance to proteolysis
Inhibits dephosphorylation of tau in synaptosomal cytosol fractions
Decreases levels of specific MAP isoforms
Effects on amyloidosis
Elevates APP expression and induces senile plaque deposition in 30% of patients subjected to chronic dialysis treatment
Elevates APP expression and promotes A deposition and amyloidosis in hippocampal and cortical pyramidal neurons in rats and mice following chronic oral exposure
Binds the amyloidogenic A peptide and perturbs its structure from a soluble-helical form to the insoluble random turn-sheet conformation at physiologically relevant concentrations. The neurotoxic A-sheet conformation may be reversed by the addition of a natural Al binder-silicic acid, a promising therapeutic agent for AD
Promotes the formation of amyloid fibrils in complex with ATP and induces their aggregation
Induces conformational changes in A and enhances its aggregation in vitro in cultured mouse cortical neurons, following chronic (50 M,>3 weeks) but not acute exposure (10–100 M, 1 week)

(continued)

Table 4.2 (continued)

Al's neurotoxic effects related to AD
Appears to be the most efficient cation in promoting A1-42 aggregation and potentiating A1-42 cellular toxicity in human neuroblastoma cells:
Induces a specific oligomeric state of A1–42 and by stabilizing this assembly markedly reduces cell viability and alters membrane structure, an effect not seen with other metal complexes (A1-42-Zn, A1-42-Cu, and A1-42-Fe) or A1-42 alone
Strongly enhances the spontaneous increase of A1-42 surface hydrophobicity (compared to A1-42 alone, A1-42-Zn, A1-42-Cu, and A1-42-Fe), converting the peptide into partially folded conformations
May promote amyloidosis by interfering with the muscarinic acetylcholine receptor-stimulated IP3/PLC-regulated production of the neuroprotective nonamyloidogenic s-APP:
As fluoroaluminate, blocks DAG/PKC-dependent budding of secretory vesicles containing APP from the trans-Golgi network (TGN), thus inhibiting APP redistribution toward the plasma membrane where it would undergo processing by secretase to produce s-APP
May inhibit IP3/Ca^{2+} – dependent production of s-APP
May inhibit PKC-dependent APP cleavage by secretase
Inhibits proteolytic degradation of A by cathepsin D

Adapted from Tomljenovic [139] which contains the literature citations for each Al-induced change.

4.4 Overview of Innate Versus Adaptive Immune Systems and Their Roles in CNS Development and Neurological Disease

There is now a growing body of evidence suggesting that the immune and nervous systems are uniquely interrelated, both in development and in mature function. Nowhere is this linkage clearer than in considerations of ASD. (Note, much of the following sections has been excerpted from [106].

The innate ("natural") immune system is a non-specific first line of defense against infectious diseases. It is composed of various cells and molecules that can recognize invading pathogens and consists of eosinophils, monocytes, macrophages, natural killer cells, dendritic cells, Toll-like receptors, and complement system mediators (for a general overview, see Janeway et al. [70]). In this system, the first response to a given pathogen is relatively slow, but it becomes more rapid with a secondary exposure to the same entity. In contrast, the adaptive immune system is specifically directed against invading pathogens. It contains highly specialized cells such as T (thymus-derived) and B lymphocyte (bone marrow-derived) cells, generating, respectively, cellular and humoral types of immune response. T cells, also termed T-helper, T4, or CD4 cells, are white blood cells that are essential for the adaptive immune response. "CD4" refers to a glycoprotein (cluster of differentiation 4) found on the surface of T cells and other cell types (e.g., monocytes, macrophages, and dendritic cells). T-helper cells do not themselves destroy invading pathogens as they have no phagocytic or cytotoxic capabilities, but they enable other cells such as CD8 killer cells to do so. Two types of T-helper cell are recognized,

Th1 and Th2, each designed to eliminate different types of pathogen. Th1 cells produce interferon γ and act to activate the bactericidal actions of macrophages and induce B cells to make complement-fixing antibodies. These responses are the basis of cell-mediated immunity.

The Th2 response involves the release of interleukin 5 (IL-5), acting to induce eosinophils to clear parasites. Th2 also produces IL-4, which facilitates B-cell isotype switching. In general, Th1 responses are usually directed against intracellular pathogens (viruses and bacteria), while Th2 responses act against extracellular bacteria, other pathogenic parasites, and toxins.

A second crucial aspect of the adaptive immune system, particularly in response to future pathogen responses, is the production of antibodies. Antibodies are immunoglobulins (Igs): large Y-shaped proteins produced by plasma cells of which there are a variety of isotypes each with particular actions and localizations of such action.

When describing features of the innate immune system, it is necessary to consider the role of the "inflammasome." The inflammasome is an intracellular, multiprotein complex that controls the activation of proinflammatory caspases, primarily caspase-1. The complex generally has three main components: a cytosolic pattern-recognition receptor known as the nucleotide-binding oligomerization domain (NOD)-like receptor (NLR), the enzyme caspase-1 (part of the apoptosis pathway), and an adaptor protein known as apoptosis-associated speck-like protein (ASC), which facilitates the interaction between the NLR and caspase-1 (see review by Walsh et al. [148]). The NLR subfamilies include NLRP3, the best studied of this group.

The NLRP3 inflammasome is activated by various stimuli, including pathogenic signals (e.g., bacterial, fungal, viral) [36, 68], endogenous danger signals (adenosine triphosphate (ATP), Aβ, uric acid crystals) [58, 90, 91], and environmental microparticles (e.g., silica crystals, aluminum salts) [65]. The latter are of obvious importance in consideration of the impact of aluminum salts used as adjuvants that may also gain ingress into the CNS.

NLRP3 activation is a two-step process. A first signal, such as the presence of microbial Toll-like receptor ligands, primes cells by producing pro-IL-1β expression. A second signal, such as ATP, activates caspase-1 and leads it to process pro-IL-1β and pro-IL-18 [58, 90, 142]. The activation of NLRP3 is not completely understood, but three upstream mechanisms of activation have been proposed. These are ion fluxes (K+ and other ions) [110], mitochondrial-derived reactive oxygen species [160], and phagosome destabilization and the release of lysosomal enzymes (cathepsins) that digest proteins after cell death [24, 64].

The effects of NLRP3 inflammasome activation within the CNS remain unknown in many cases, but recent evidence suggests it has a role in neurological diseases and, as already mentioned, in the context of adjuvant aluminum salts.

4.4.1 HPA–Immune System Interactions in Development and Disease

IL-1β, the key proinflammatory cytokine, is released following NLPR3 inflammasome activation by aluminum adjuvants and exhibits multifactorial effects on the immune system [37, 80, 81]. IL-1β is also known to activate neurons in the central nucleus of the amygdala [17]. This nucleus plays a major role in the HPA axis response to systemic immune stimulation [155]. Abnormalities in the amygdala [62, 97] and alterations in cortisol levels indicative of a dysfunctional HPA axis are common in ASD children and may, in part, serve to explain the limited abilities of these children to react adequately to their social environment, as well as their tendency toward enhanced anxiety behaviors [59, 113].

The HPA axis is not only crucial for regulating a broad array of psychological stress responses [31, 56, 57, 71] but also regulates neuro-immune stress arising from exposure to bacterial and/or viral stimuli.

From the preceding, it is clear that the HPA axis is one of the major pathways by which the CNS regulates the immune system [38, 39, 41, 98, 154]. Alterations in HPA axis regulation can lead either to excessive immune activation, and hence inflammatory and autoimmune disorders, or to excessive immune suppression and thus increased susceptibility to infectious diseases.

In this context, it is notable that many autoimmune/inflammatory conditions have been consistently linked to adjuvant administration and/or repetitive immunizations with antigenic components, including aluminum adjuvants [88, 113, 125, 141, 143].

Cortisol, the main glucocorticoid hormone product of HPA activity, appears to have a crucial role in priming microglia toward a hyperactive state and thus a role in neurodegeneration by inducing the M1 phenotype [8]. In adult rats, prior sensitization of the microglia by cortisol potentiates the proinflammatory response to a peripheral immune challenge by lipopolysaccharide (LPS) and significantly augments the production of the inflammatory cytokines IL-1β, IL-6, and TNF-α in the brain [47].

Glutamate is the major excitatory neurotransmitter in the mammalian brain and is therefore crucial for normal brain development and function [55, 72]. However, excessive glutamate release is deleterious to neuronal viability and is thought to play a role in the pathophysiology of neurological diseases and neuropsychiatric disorders, including ASD [13, 55]. In regard to ASD, children and adults with the disorder typically display higher serum levels of glutamate [128, 129], as well as a specifically higher concentration of glutamate/glutamine in the amygdala and hippocampus [114].

The higher levels of glutamate in ASD children find a direct correlate with the levels of glutamatergic receptors: at 2 years of age, the developing human brain contains more synaptic glutamate receptors than at birth, but the number of these receptors progressively declines over the next decade. The immature brain is thus likely to be more susceptible to excitotoxic insults than that of a young adult [72].

At the other end of the age spectrum, receptor subunit composition for various glutamate receptor subtypes also changes during life [111], which may make the aged brain more susceptible to excitotoxic insults than that of younger adults. Thus, the dynamics of glutamate levels and glutamate receptor characteristics across the life span makes neuronal vulnerability more pronounced in early and later life, albeit in different ways. In turn, this complex response pattern reflects the underlying complexity contributed by neural–immune–HPA interactions.

4.4.2 Autoimmunity

Briefly stated, autoimmune disorders arise when an individual's own immune system generates antibodies that attack healthy tissues rather than the invading pathogens.

Autoimmune reactions can also cause the abnormal growth of tissues and a variety of dysfunctional states. Examples of organ systems that can be affected include blood cells and vessels, connective tissues and joints, skin and muscles, the endocrine system, and, of particular interest in what follows, the CNS.

Close to 100 autoimmune disorders are now recognized, with more added to the list every year. Well-known autoimmune disorders include systemic lupus erythematosus (SLE), celiac disease, rheumatoid arthritis, type 1 diabetes, and a host of other lesser-known disorders. In the CNS, autoimmune disorders include multiple sclerosis, Guillain–Barré syndrome, and myasthenia gravis.

Autoimmune disorders can have multisystem impacts, and individuals can have more than one such disorder at the same time. Likely examples of multisystem syndromes include fibromyalgia, chronic fatigue syndrome, and the emerging syndrome termed "autoimmune syndrome/inflammatory syndrome induced by adjuvants" ASIA. Gulf War syndrome, which in many cases includes clearly negative impacts on the CNS [32], likely also reflects a multisystem autoimmune disorder of the ASIA type. As cited above, MMF is triggered by aluminum adjuvants and leads to clear changes in cortical function in the form of MCI. The observation that aluminum salts can themselves be antigenic lends support to the notion that MMF may have autoimmune as well as inflammatory features [51] and places it firmly within the ASIA spectrum of disorders.

The link between abnormal immune system function and ASD is illustrated in Table 4.3.

It should be mentioned that the potential link between the various autoimmune/inflammatory CNS disorders and adjuvants, particularly aluminum adjuvants in vaccines, has led some investigators to question whether these disorders actually exist [60]. Such views sometimes appear to reflect more the perceived need to provide continued public assurance about vaccine safety, rather than any actual reservations about whether such disorders are aluminum-induced and/or autoimmune in nature.

Table 4.3 Immune system role in ASD

Types of abnormalities	Type of immune stimuli/time of stimulation	Species	Outcome	Autism
Neurobehavioral	Poly I:C/early postnatal	Mouse, rat	Deficits in social interaction, increased anxiety [67, 77]	Impaired social skills, increased anxiety and stereotypic behavior [138]
	LPS/early postnatal	Rat	Altered responses to novel situations (i.e., reluctance to explore a novel object) [133]	Anxiety to novel situations, preference for routine [71]
	Poly I:C/early postnatal	Mouse	Cognitive dysfunction (i.e., memory deficits [67]	Cognitive dysfunction and mental retardation [46, 99]
Neuroanatomical	Poly I:C/prenatal	Mouse	Compromised neurogenesis and abnormal formation of the cerebral cortex [132]	Abnormal neuronal morphology and cytoarchitecture of cerebral cortex [62]
	Complete US pediatric vaccine schedule/ postnatal, according to schedule	Monkey	Failure to undergo normal maturational changes in amygdala volume [63]	Impaired amygdala development [62, 97]
Neurochemical	Poly I:C/early postnatal	Mouse	Increased extracellular glutamate in the hippocampus [67]	Increased glutamate in the amygdala-hippocampal region [114]
	LPS/early postnatal	Rat	Increased seizure susceptibility [48]	Increased seizures and epilepsy [7, 144]
Immune	LPS/early postnatal	Rat	Abnormal cytokine profiles [134]	Abnormal cytokine profiles [5, 6, 94, 104, 145]
	Al-adjuvant/ early adulthood	Mouse	Increased astrocyte and microglia reactivity [11, 12]	Increased astrocyte and microglia reactivity [5, 6, 94, 104, 145]
	LPS/early postnatal	Rat	Exacerbation of inflammatory conditions [134]	Immune hypersensitivity [33]

Shared aspects between autism and abnormal neurobehavioral, neuroanatomical, neurochemical, and immune system outcomes resulting from repeated peripheral immune stimulation (From Ref. [122])

Poly I:C polyriboinosinic–polyribocytidilic acid, a synthetic analogue of double-stranded RNA (viral antigen), *LPS E. coli* lipopolysaccharide

The precise mechanisms of action of aluminum adjuvants are still being resolved almost 90 years after their introduction [44]. Whatever else one wishes to say about the role of aluminum adjuvants in autoimmune disorders, aluminum is distinctly not inert, nor are the cumulative amounts received necessarily trivial for CNS health, as detailed above. Indeed, as discussed in the present section, the interactions between the immune and nervous systems virtually ensure that adjuvant aluminum will impact both, even if the intent is only to modify the former.

Autoimmune disorders often display differences based on sex. For example, multiple sclerosis [103], MMF [115], and ASIA in general [159] appear much more often in women than in men (3.2:1; 7:3; 7:3, respectively), a point of some relevance to ASD which mostly occurs in males. Many autoimmune disorders also show an age window, that is, a particular range of ages during which they are most likely to arise. The underlying reasons for both of these observations are unclear.

4.4.3 Aluminum and Failed Biosemiosis

Because the nervous system utterly depends on signaling from the gene (or any part of the DNA that induces protein production) up to neuronal and neural systems outputs, anything that degrades biological signaling, termed "biosemiosis," may be highly deleterious.

In this regard, aluminum, with its demonstrated potential to impact the various levels of organization, may be one of the more destructive toxins to the CNS from the perspective of multilevel biosemiosis. Insofar as aluminum can alter DNA and RNA, such actions will lead to altered proteins which, in turn, will impact cellular function. Moving upward in levels in the CNS, dysfunctional cells cannot help but alter neural circuit function, neural systems function, and, ultimately, behavior. In this manner, aluminum alone may induce a "multiple-hit" outcome in the CNS on its own, making it perhaps uniquely toxic among the various substances known to negatively impact the CNS [125].

4.4.4 Aluminum's Role in Immune System Signaling Errors with a Focus on ASD

Which factors might serve as triggers to abnormal immune function as it relates to the CNS in development or in adulthood? The above material details much of the information on aluminum toxicity in the context of neurological disease, including developmental disorders such as ASD. The maternal immune activation (MIA) studies cited below further bolster this notion given aluminum's clearly demonstrated adjuvant, and thus immune-stimulating, actions.

As already noted, however, the subject of aluminum involvement in ASD remains highly controversial. In part, some of this controversy may arise from addressing developmental disorders in relation to a possible causal impact of this element. As also noted above, a certain part of the medical community has discounted any deleterious role for aluminum for human health in general. More specifically, hesitation to accept a role for aluminum in ASD may arise because it is virtually impossible to avoid considering one major source of aluminum exposure: aluminum-adjuvanted pediatric vaccines.

So explosive is the potential impact of such a linkage that it often forces investigators to assert that aluminum could not be involved in ASD at all, in spite of the rather large literature on aluminum neurotoxicity, in part cited above. This position appears primarily to be an attempt to avoid any discussion of vaccine safety. From a strictly scientific perspective, albeit not necessarily from that of those concerned with reassuring the public about vaccine safety per se, this position would appear to be problematic.

In spite of such reservations, the available literature clearly shows that the neurotoxicity of aluminum in the CNS manifests itself in symptoms such as deficits in learning, memory, concentration, speech, and psychomotor control, as well as increased seizure activity and altered behavior (i.e., confusion, anxiety, repetitive behaviors, and sleep disturbances) [139]. All of these are features of the overall spectrum of disorders included in ASD.

In regard to aluminum adjuvants, the prolonged hyperactivation of the immune system and chronic inflammation triggered by repeated exposure, combined with the unexpectedly long persistence of such adjuvants in the human body, are thought to be principal factors underlying the toxicity of these compounds. In regard to the latter point, one reason aluminum salts such as the hydroxide are so effective as adjuvants is the relative inability of the body to excrete or degrade them in comparison to aluminum derived through dietary exposure. This clearly demonstrated point from the literature is often overlooked or ignored when assessing vaccine safety, sometimes leading to spurious comparisons between the amounts of aluminum found in a standard vaccine and those in various food products or in the diet overall.

Over the last decade, in vivo studies in animal models and humans have indicated that aluminum adjuvants have an intrinsic ability to induce adverse neurological and immune-inflammatory outcomes [28, 82, 105, 109]. Some of these studies have led to the description of the ASIA syndrome, which is known to comprise a wide spectrum of adjuvant-induced conditions characterized by a mis-regulated immune response [94, 130].

The ability of aluminum adjuvants to cross the blood–brain and blood–cerebrospinal fluid barriers [76, 88, 124] may in part explain the adverse manifestations following some vaccines which tend to be neurological in nature, with an underlying immunoinflammatory component [26, 131, 159]. Thus, as cited above, aluminum's impact on the CNS is likely a component of the bidirectional aspects of CNS–immune system interactions.

The data for MMF cited here may also suggest that some forms of neurological or immune system dysfunction could also arise in children, particularly considering

the potential body burden of aluminum that the children can accumulate. While an adult MMF patient may have received up to 17 vaccines in the 10 years prior to diagnosis, the average child in the United States following the Centers for Disease Control and Prevention (CDC)'s vaccination schedule will receive the same number of aluminum-adjuvanted vaccines in their first 18 months of life [140, 141]. Given that early postnatal life in humans is a period of intense neurological development, anything that has the potential to interfere with such development is going to place the system as risk.

It should be stressed in this context that toxins other than aluminum have also been proposed to be involved in ASD [33]. This possibility does not diminish the potential impact of aluminum itself, however. The ability of aluminum to adversely affect both the immune and the nervous system in an interactive manner makes it a strong candidate risk factor for triggering developmental disorders such as ASD in which the two principal features are precisely those of neurological and immune system signaling dysfunctions.

It should be clear from the above that the etiology of ASD is not a simple process involving only genetic factors, but rather involves a multiple-hit type of etiology in which both immune and nervous system interactions driven by a combination of genetic susceptibilities and environmental agents play important roles. This notion is not particularly surprising given the existing literature on neurodegenerative diseases associated with aging (e.g., AD, PD, and ALS), which often comes to many of the same conclusions.

4.4.5 Pathogen and Aluminum Activation of the Immune System in Relation to the CNS

Repeated administration of bacterial and viral antigenic protein fragments, many of which are adsorbed to adjuvant aluminum salts, is clearly analogous both in nature and timing to peripheral immune stimulation with microbial mimetics in experimental animals during early periods of developmental vulnerability. If administered during these periods (including early postnatal life), such potent immune stimuli can not only produce adverse neurodevelopmental outcomes in these animals but can also permanently impair immune responses to subsequent immune challenges later in life [14, 49]. These MIA outcomes can have profound effects, some of which are linked to ASD.

Many cytokines induced as part of an immune response, including those arising from adjuvants, can act as "endogenous pyrogens"; that is, they can induce a rapid-onset fever by acting directly on the hypothalamus, without the need for the formation of another cytokine (i.e., IL-1β, IL-6, TNF-α) [9, 10, 27, 34]. While transient fever is an essential component of the early immune response to infection, a prolonged febrile response is a hallmark of many inflammatory and autoimmune diseases [34].

Fever-promoting cytokines produced in peripheral tissues by immune stimulation can enter the brain by way of the circumventricular organs (CVOs) [34]. CVOs

are structures in the brain with an extensive vasculature and are among the few sites devoid of protection by the blood–brain barrier. They provide one link between the CNS and peripheral blood flow and thus are an integral part of neuroendocrine function.

The absence of a blood–brain barrier to CVO molecule release allows the CVOs to provide an alternative means for the release of hormones and various peptides from the CNS into peripheral circulation. As well, the structural connections now demonstrated between the lymphatic system and the CNS only add to the potential for immune–CNS bidirectional ingress [87]. In this context, persistent inflammation of the CNS appears to play a prominent role in neurodevelopmental and neurodegenerative disorders [2, 89, 104, 145].

4.5 Summary and Final Considerations

The data cited in the above sections clearly shows that aluminum, far from being either inert or safe, is actually "insidiously unsafe" [76] in any of its manifestations or routes of ingress into the bodies of humans or animals [125]. In adult humans or animals, the impacts include those of various organ systems, particularly the CNS and immune system, and can lead to a variety of multisystem disorders. In children, especially early in CNS development, exposure to aluminum from various sources, possibly significantly from vaccines containing aluminum adjuvants, may have profound deleterious consequences. One of these consequences may be ASD.

We live in a period described by some authors as the "age of aluminum." Aluminum, once relatively inaccessible in the biosphere, has become ubiquitous. Given the dangers that elemental aluminum poses to the various organ systems, it would behoove us to limit our exposures to this toxic element in food, water, cosmetics, and various medicinal products, including in vaccines.

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Chapter 5
Occupational Exposure to Aluminum and Cognitive Impairment

Xiaoting Lu

Abstract Aluminum industry has been producing more than a century of history. Aluminum is a light metal which is widely used in industrial applications. Occupational aluminum workers are an important source for high exposure Al population. With the world's demand for aluminum and the increasing production, between 2007 and 2016, primary Al production in China increased by 251% which shows that a growing number of workers are being exposed to Al. Occupational aluminum exposure to the health of the population has become increasingly prominent. Al is a well-established neurotoxicant and is suspected to be linked with various neurodegenerative diseases including Alzheimer's disease (AD) and MCI. Studies on workers exposed to aluminum have revealed disturbances in cognitive function. This chapter reviews the relationship between occupational exposure to aluminum and cognitive impairment.

Keywords Aluminum · Occupational exposure · Biological monitoring · Cognitive function

5.1 Introduction

Aluminum (Al) is a ubiquitous element in the Earth. Therefore, exposure is unavoidable even in the general population. Exposure that greatly exceeds that of the general population is experienced owing to occupation and drinking water. Occupationally, Al can be found in various industries, e.g., the Al powder industry and the metal industry, and in Al foundries. Aluminum resistance to chemicals is due to a protective layer of Al oxide that spontaneously forms on the surface. Aluminum and its alloys with other elements (copper, magnesium, manganese, silicon, and zinc) are used in vehicles, electric devices and wiring, building materials, packaging, and for corrosion protection of structural steel. Aluminum powders are

X. Lu (✉)
Occupational Health Department of Public Health School, Shanxi Medical University, Taiyuan, Shanxi, China
e-mail: luxiaoting@sxmu.edu.cn

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used in pigments, automotive paints, rocket propellants, explosives, and fireworks. Bauxite is the main raw material for aluminum production. It is refined to Al hydroxide and further to Al oxide, which is electrolytically reduced to aluminum (primary aluminum production). Large volumes of aluminum are produced by recycling scrap aluminum in the smelter (secondary aluminum production) [22]. Neurotoxicity is the critical effect of exposure to aluminum. Al is a well-established neurotoxicant and is suspected to be linked with various neurodegenerative diseases including Alzheimer's disease (AD) and MCI [14, 17, 20]. In recent years, studies suggested an association between cognitive impairment and other neurological effects and occupational exposure to aluminum dust or fume. This chapter reviews the relationship between workers' exposure to aluminum and cognitive impairment.

5.2 Exposure Assessment

Occupational exposures during the primary smelting of aluminum, foundry work, production of aluminum flake powder, or the welding of aluminum with the metal inert gas (MIG) method can be associated with an increased uptake of aluminum [23]. The existing form of aluminum in the workplace is mainly fume and dust. So that it is primarily absorbed from the air via the lungs at the workplace. Ingestion is possible, but according to current scientific knowledge, the inhalation route dominates.

Data on the Al half-times of occupationally exposed persons varies widely in the literature, from days to months, depending on the duration of the exposure. Certainly, the bioavailability of different types of Al has to be considered. The magnitude of Al exposure and work conditions varied widely among the different job types. At electrolysis worksites, the main pollutants were aluminum oxide and fluorides, and workers were exposed to relatively high levels of aluminum fume and dust. However, the smelting and welding workers were much less exposed to these pollutants, and many welding workers worked outdoors. The workers studied have been aluminum production workers employed in either the foundry or potroom [3, 11, 16, 24] and aluminum welders [9, 26] with normal, slightly, or moderately increased measures of body burden of aluminum. Among industrially exposed workers, welders who use the metal inert gas (MIG) technique have the highest concentrations of aluminum in urine and serum [25].

Biological monitoring of aluminum is based on the analysis of aluminum concentration in blood/serum or in urine. Al is conventionally determined in serum, plasma, whole blood, or urine after it enters the human body. Hosovski et al. found in an aluminum foundry that the mean U-Al was 1.7 μmol/L among 87 workers with a job seniority of about 10 years [11]. In a group of 17 welders (job seniority about 4 years), the mean S-Al and U-Al concentrations were 0.21 and 2.8 μmol/L, respectively [9]. In another study, the high-exposure group of 30 welders mainly working on aluminum tanks and ships for about 14 years revealed higher levels: the mean serum Al and urine Al concentrations were 0.46 and 7.1 μmol/L, respectively [23].

Bast-Pettersen et al. reported a median urinary Al concentration of 40.5 μg/l in 20 Al welders aged 21–52 years with an average exposure time of 8.1 years [4]. In a foundry, among 119 workers with a mean exposure history of 15 years, low concentrations of aluminum were measured in blood (B-Al) (median 0.037 μmol/L) and urine (median 0.15 μmol/L) [12]. Similar observations were made among welders of train bodies and truck trailers with a job seniority of over 10 years three times for 4 years: in the first examination, the mean P-Al and U-Al concentrations of 44 welders were 12.5 μg/l and 110.7 μg/g Cr, respectively; in the second examination, the mean P-Al and U-Al concentrations of 33 welders were 13.8 μg/l and 120.0 μg/g Cr, respectively; in the third examination, the mean P-Al and U-Al concentrations of 20 welders were 13.9 μg/l and 81.5 μg/g Cr, respectively [13]. Sjorgen et al. reported 38 welders (age = 39 years, work years = 17 years) whose median urinary Al concentration was 24 μg/g Cr and blood Al concentration was 3.0 μg/l [26]. Altogether, the exposure time and Al levels in serum and urine were within the range of studies on occupational exposed Al workers. The obvious conclusion to be drawn is that occupational exposed aluminum can with time lead to significantly increased levels in aluminum biomarkers.

5.3 Cognitive Impairment

There is no doubt that aluminum accumulation and its subtle effects occur among occupationally exposed individuals. Studies on workers exposed to aluminum have revealed disturbances of cognitive processes. Occupational exposure to Al seems to have different effects on workers in different industries. The measurable effects on the central nervous system might only develop after a protracted exposure, and the intellectual domain, mainly affected, varies. Many of the studies concerned exposures in primary aluminum production, foundries, or smelting of scrap aluminum.

The first studies on neurotoxic effects of Al exposure in humans were carried out on patients who had died of dialysis-related encephalopathy. Alfrey et al. found, for example, elevated levels of Al in the brains of patients that had died of dialysis-related encephalopathy [2]. A case study by McLaughlin et al. first suggested that occupational aluminum exposure can cause both lung and brain effects. The patient was a 49-year-old man who had worked for 13 years as a ball-mill operator in a flake powder factory. He was diagnosed with lung fibrosis and rapidly progressing deterioration of CNS functions: forgetfulness, speech disorder, myoclonic jerks, hemiparesis, convulsive attacks, and dementia. Postmortem analysis of aluminum in the brain yielded 5 mg/kg (wet weight) which was a 10–20 times higher concentration than the levels found in brain samples from nonexposed individuals, which were analyzed in parallel [18]. Longstreth reported three patients with a progressive neurologic disorder. The exposure years of three patients were 12, 15, and 16, respectively. Neurologic findings showed that all had incoordination and an intention tremor. Neuropsychological test scores found that two patients had cognitive

deficits, and the most severely affected patient also had spastic paraparesis. The author believed that these patients' diseases probably are related to an occupational exposure in the potroom [16]. After that a few epidemiological studies were conducted on workers occupationally exposed to Al. In Canadian miners who used McIntyre powder by inhalation as prophylaxis against silicosis, Rifat et al. [21] conducted a morbidity prevalence study on Al-produced neurotoxic effects in these mines between 1988 and 1989. The exposed group was consisted of 261 miners and the control was 346 unexposed miners. Cognitive test scores and proportions impaired indicated a disadvantage for exposed miners. With adjustment confounding, the estimated relative risk of impairment of cognitive function among exposed miners was 2.6 [21]. Hosovski investigated 87 workers (age = 41 years, job seniority = 19 years) in an aluminum foundry and 60 nonexposed workers (age = 42 years, job seniority = 18 years). Al values in blood in exposed group were 136.85 μg/l and that in the control was 58.09 μg/l. Al values in urine in exposed group were 45.38 μg/l and that in the control was 7.25 μg/l. The author measured the psychic abilities of subjects with psychomotor ability tests, Wechsler's test of intelligence, and Bender's test for estimation of cerebral damage. Slower psychomotor reaction and dissociation of oculomotor coordination were found in the exposed workers. Exposed workers had reduced memory ability and their mental and emotional balance was disturbed. The observed changes in psychomotor and intellectual abilities could be a consequence of the long-lasting toxic effects of aluminum [11]. White reported the investigation of 25 symptomatic workers from an aluminum smelting plant. The mean age was 47 years of 25 workers and the average duration of employment was 19 years. Neuropsychological test results showed preservation in certain spheres of functioning, such as verbal IQ, with substantial impairment in others, particularly memory functioning. On memory tests, 70–75% showed mild or greater impairment. This study supported the existence of a syndrome characterized by incoordination, poor memory, impairment in abstract reasoning, and depression. Aluminum exposure in the potroom was considered the most likely cause [27]. A cross-sectional study was conducted at a Norwegian primary aluminum plant. Thirty-eight retired workers aged 61–66 years comprising 14 potroom workers, 8 foundry workers, and 16 controls volunteered to participate. They were tested with a neuropsychological test battery. Workers in potrooms with Søderberg electrolytic cells were found to show signs of impairment of the nervous system. A test for tremor discriminated significantly between the potroom group and the controls. There was a suggestion of increased risk of impaired visuospatial organization and a tendency to a decline in psychomotor tempo in the potroom workers. Bast et al. suggest that the above findings may be related to long-term occupational exposure in the potroom and further to chronic low-dose exposure to aluminum [3]. Sjogren studied the effect on the nervous system among welders exposed to aluminum and manganese. This study chose 38 welders (age = 39 years, work years = 17 years) as exposed group and 39 nonexposed Al welders (age = 40 years, work years = 14 years) as control group. The blood Al concentration in exposed group was 3.0 μg/l and that in the control was 1.0 μg/l. The urinary Al concentration in exposed group was 24 μg/gCr and that in the control was 4.7 μg/gCr. Nervous symptom showed that the

welders exposed to Al reported more symptoms from the central nervous system at the time of the test and the most prominent symptom was fatigue. Psychological examination showed that the welders exposed to Al achieved a significantly lower score in non-dominant hand tapping speed, Luria-Nebraska motor scale task item3 and item4, and dominant hand pegboard than did the control group. This study suggested the neurotoxic effects in the welders exposed to aluminum and manganese are probably caused by the aluminum and manganese exposure, respectively [26]. The investigation in 1999 was a cross-sectional study of asymptomatic aluminum welders and a reference group of mild steel welders. Based on urinary aluminum concentrations, welders were classified into a reference (n = 28, average age 38 years), low (n = 27, average age 37 years)-, and high (n = 24, average age 41 years)-exposure group. The mean urinary aluminum concentrations were 0.46, 2.25, and 9.98 μmol/l, respectively. A comprehensive neuropsychological examination was undertaken to assess psychomotor function, simple visual reaction time, attention-related tasks, verbal and visual or visuospatial abilities, as well as verbal and visual learning and memory. The result showed that the low exposed group performed poorer on the memory for designs and on more difficult block design items demanding preliminary visuospatial analysis. The time-limited synonym task, embedded figures, digit symbol speed, and the backward counting component of the divided attention task showed exposure-response relations. In general terms, therefore, the present results suggest that aluminum is associated with detrimental effects on certain cognitive functions. What seems common to the tasks showing impairments is the involvement of time-limited processing in visuospatial tasks where working memory demands are great [1]. Workers from one of the largest aluminum production plants in China founded in 1958 were studied; 167 male workers aged 25–60 years (mean age 37.6 years) were selected to use the WHO-recommended neurobehavioral core test battery (NCTB). An Al-exposed group included 104 workers who had been exposed to aluminum, while working in electrolysis, smelting, or welding, for at least 5 years. Al urine concentration in exposure group was 41.79 μg/g Cr, and that in non-exposure workers was 17.73 μg/g Cr. In this study, all results were adjusted for education and duration of employment to reduce the effects of these factors. After adjustment for work duration and educational level, notable changes in mood as well as neurobehavioral performance still existed in the Al-exposed groups, and age-dependent characteristics were obvious. Younger Al-exposed workers had short memory and elderly workers an impairment of motor activity and accuracy to a certain extent. It should be noted that in the present study, we only found that the young Al-exposed workers had considerably impaired cognitive functions and the elderly notably retarded motor ability, instead of cognitive impairment. These results indicate that occupational aluminum exposure, at the measured level, might interfere with normal behavioral functions. These effects seem to be age-dependent, which might be attributable to age-related changes in susceptibility to environmental chemicals as well as the duration of aluminum exposure [8]. Pollizi conducted a cross-sectional case-control study in northern Italy. The group of 64 exposed workers was to be retired from work for at least 10 years and composed of former aluminum dust-exposed workers with long-term exposure

to the metal from an aluminum remelting plant, and the control group of 32 blue collar workers was composed of demographically similar subjects. The median exposure level of aluminum, in the respirable fraction, was 14.70 μg/m^3 with a range of 7.46–39.26 μg/m^3. Mean serum Al in exposed group (14.1 μg/L) is significantly higher than that of the control group (8.2 μg/L). The neuropsychological tests resulted there is a significant difference in the latency of P300, MMSE score, MMSE-time, CDT score, and CDT-time between the exposed and the control population. P300 latency correlates positively with Al-s and MMSE-time. Al-s has significant effects on all tests: a negative relationship was observed between internal Al concentrations, MMSE score, and CDT score; a positive relationship was found between internal Al concentrations, MMSE-time, and CDT-time. The authors suggest aluminum may be an essential hazard for the central nervous system and raise the question whether preclinical detection of aluminum neurotoxicity and consequent early treatment might help to prevent or retard the onset of AD or AD-like pathologies [20]. Buchta et al. surveyed the longitudinal study included 98 Al welders (mean age 37 years) in the car-body construction industry, with a median of 6 years of occupational exposure to Al welding fumes, and an education-matched, gender-matched, age-matched control group of 50 car-production workers (mean age 36 years) at the same plant. Two cross-sectional studies were done in 1999 and 2001. In the second cross-sectional study, 97 welders and 50 controls could be examined. Al concentration in plasma and urine was measured. All subjects were tested the neurobehavioral, which was included a symptom questionnaire, modified Q16, and computerized and non-computerized tests: psychomotor performance (steadiness, line tracing, aiming, tapping), verbal intelligence (WST), simple reaction time, digit span, block design (HAWIE), symbol-digit substitution, switching attention (European neurobehavioral evaluation system, EURO-NES), and standard progressive matrices. The median Al urine concentration in exposure group was 57.6 μg/gcr (1999) and 52.4 μg/gcr (2001), and median plasma Al level in exposure group was 10.3 μg/L (1999) and 4.3 μg/L (2001). Median respirable air dust was 0.47 mg/m^3 (1999) and 0.67 mg/m^3 (2001). Significant difference in reaction time was seen between welders and non-welders. Regression analyses reveal a significant relationship between reaction time and Al excretion in urine that was confounded by other factors. The results suggest that reaction time could be a first indicator for possible neurological changes in Al welders, as it is significantly related to exposure and age [5]. Buchta conducted the longitudinal study comprised of two cohorts, Al welders and controls in 1999 and 2000. A group of 33 aluminum welders (age = 43 years, Al welding = 11 years) and a control group of 26 production workers (age = 40 years) participated in two examinations in this longitudinal study. In the first examination, Al-preshift and Al-postshift in plasma of exposed workers were 9.6 μg/l and 11.6 μg/l. Al-preshift and Al-postshift in urine of exposed workers were 92.1 μg/gCr and 97.0 μg/gCr. In the second examination, Al-preshift and Al-postshift in plasma of exposed workers were 10.6 μg/l and 14.3 μg/l. Al-preshift and Al-postshift in urine of exposed workers were 90.1 μg/gCr and 143.9 μg/gCr. Cognitive performance showed that welders conducted significantly poorer performance in symbol-digit substitution, block design, and to some extent

in switching attention [6]. He et al. conducted the study on the alteration of neurobehavioral parameters, autonomic nervous function, and lymphocyte subsets in aluminum electrolytic workers of long-term aluminum exposure. Thirty-two exposed workers came from an aluminum plant and 32 control workers were selected. The age of exposed group was 35 years and the length of service was 15 years. Urinary Al in exposed group was 40.08 μg/gCr and that in the control was 26.84 μg/gCr. All subjects were tested nervous function by NCTB (neurobehavioral core test battery) and autonomic nervous function test battery. NCTB result showed that there are significant differences in POMSC, POMST, SRT, SRTF, DSY, PAC, and PA between two groups. This study suggests that Al exposure exerts adverse effects on neurobehavioral performances, especially movement coordination and negative mood [10]. Monika conducted and summarized a meta-analysis of data on the effect of occupational Al exposure on cognitive and motor performance. The final sample consisted of nine studies examining 449 exposed and 315 control subjects. The study found that urinary Al concentration below 135 μg/l has an impact on cognitive performance and cognitive performance was negatively related to U-Al [19]. N.H. Zawilla undertook this research to test the cognitive status of workers ($n = 54$) exposed to Al dust and matched control workers ($n = 51$) by using the ACE-R. The serum Al in the exposed workers was 20.27 μg/l and that in the control was 4.43 μg/l. The ACE-R is a brief cognitive test battery that includes five cognitive functions, namely, attention, memory, verbal fluency, language, and visuospatial abilities. There are significant difference in total ACE-R test score between exposed group and control group [29]. Concetto et al. investigated exposure sample of 86 male Al welders and control group of 90 clerical staff came from the same company. The median age of exposure workers is 38 years and length of service is 22 years. The serum Al in the exposed workers was 24.19 μg/l and that in the control was 6.93 μg/l. The results showed exposed workers decreased cognitive with response with regard to attention and memory performance using WMS and the Stroop test. This study confirmed that occupational exposure to Al causes alteration in cognitive responses that are more evident in complex functions [7]. Lu et al. analyzed the relation between cognitive functions and tau-protein expression in peripheral blood lymphocytes of retired aluminum (Al)-exposed workers. Sixty-six retired Al potroom workers (age = 62 years, length of service = 30 years) and 70 unexposed controls (age = 61 years) were investigated. The serum Al in the exposed workers was 25.18 μg/l and that in the control was 9.97 μg/l. There is significant difference in total MMSE scores, orientation in time and place, short-time memory, and calculation ability. This study suggests that long-term exposure to Al may cause cognitive disorders [17]. Yang investigated 366 Al-exposed workers in aluminum potroom and assessed their cognitive function with Mini-Mental State Examination (MMSE). Serum Al in Al-exposed workers was 48.99 μg/l. This study suggested the total scores of MMSE decreased with the increase of serum Al level and long-term exposure to Al may cause MCI. MCI induced by Al was significantly associated with global DNA methylation in blood [28]. The above study of occupational population in various industries has found that occupational exposed Al may cause different degree of cognitive impairment in different aspects (see Table 5.1).

Table 5.1 Occupational exposure aluminum and cognitive impairment

Author	Publication year	Work type	Exposure years(years)	Sample size(population age)	Blood aluminum	Urine aluminum	Cognitive function impairment
Rifat	1990	Miners		Control 346			MMSE
				Exposed 261			CPM (colored progressive matrices test)
							SDMT(symbol digit modalities test)
Hosovski	1990	Foudary	19	Control 60 (42 years)	Control 58.09 μg/l	Control 7.25 μg/l	Complex reaction time
				Exposed 87(41 years)	Exposed 136.85 μg/l	Exposed 45.38 μg/l	Oculomotor coordination
							Sum of manipulative test
							Memory
							Coding
							Picture completion
							Object assembling
Sjogren	1996	Welder	17	Control 39 (40 years)	Control 1.0μg/l	Control 4.7μg/gCr	Tapping speed(non-dominant hand)
				Exposed 38 (39 years)	Exposed 3.0μg/l	Exposed 24.0 μg/gCr	Luria –Nebraska motor scale item3, item4
							Pegboard (Dominant hand)
Ritva Akila	1999	Welder	23	Reference 28 (38 years)	None	Reference 0.46 μmol/l	The memory for designs
				Low exposure 27 (37 years)		Low exposure 2.25 μmol/l	Difficult block design items
				High exposure 24 (41 years)		Highexposure 9.98 μmol/l	

Guiwen Guo	1999	Electrolysis, smelting, welding	17	Control 64 (40 years)	None	Control 17.73 μg/g Cr	Tension, depression, anger and fatigue
				Exposure 103 (37 years)		Exposure 41.79 μg/g Cr	Digit span forward
							Digit symbol
							Total pursuit aiming
Salvatore Polizzi	2002	Foundry	25(exposure years)	Control 32 (67 years)	Control 8.2 μg/l	None	The latency of P300, MMSE score, MMSE-time, CDT score and CDT-time
			11 (retirement years)	Exposure 64(68 years)	Exposure 14.1 μg/		
M. Buchta	2003	Welder	6	1999	1999	1999	Reaction time
				Reference 50	13.47μg/l	57.77μg/g Cr	
				Exposure 98	2001	2001	
				2001	6.4μg/l	52.4μg/g Cr	
				Reference 50 (36 years)			
				Exposure 97(37 years)			
He	2003	Electrolytic	15	Control 32		Control 26.84 μg/gCr	POMSC
				Exposed 34(35 years)		Exposed 40.08 μg/gCr	POMST
							SRT
							SRTF
							DSY
							PAC
							PA

(continued)

Table 5.1 (continued)

Author	Publication year	Work type	Exposure years(years)	Sample size(population age)	Blood aluminum	Urine aluminum	Cognitive function impairment
Buchta	2005	Welder	11	1999	Exposed	Exposed	Symbol-digit substitution
				Reference26	1999	1999	Block design
				Exposure 33	Preshift 9.6 μg/l	Preshift 92.1 μg/g Cr	Switching attention
				2001	Postshift 11.6 μg/l	Postshift 97.0 μg/g	
				Reference 26 (40 years)	2001	Cr	
				Exposure 33(43 years)	Preshift 10.6 μg/l	2001	
					Postshift14.3 μg/l	Preshift 90.1 μg/g Cr	
						Postshift 143.9 μg/g	
						Cr	
N. H. Zawilla	2014	Smelter	22	Control 51(46 years)	Control 4.43 μg/l		ACE-R
		Other production line					
				Exposed 54(46 years)	Exposed 20.27 μg/l		
Concetto	2014	Welder	16	Control 90(38 years)	Control 6.93 μg/l		Wechsler Memory Scale
				Exposed 86(46 years)	Exposed 24.19 μg/l		The Stroop Test
Lu xiaoting	2014	Potroom	30	Control 70 (61 years)	Control 9.97 μg/l		MMSE
				Exposed 66 (62 years)	Exposed 25.18 μg/l		Orientation in time and place
							Short-time memory
							Calculation ability
Yang xiaojuan	2015	Potroom	21	45	48.99		MMSE
							Orientation in time and place
							Short-time memory
							Attention and language skills

However, some studies are inconsistent with the above content. The role of Al in neurology is controversial. Letzel et al. showed no measurable cognitive decline in 32 dust-exposed workers in a German Al powder plant. He conducted two cross-sectional studies at an Al powder plant to evaluate possible nervous system effects from occupational Al exposure. The first study selected 32 Al dust-exposed workers and 30 unexposed control workers to test biological monitoring, neuropsychological testing, and evaluation of P300 potentials. Five years later, in the second investigation, all available workers from both groups (15 still exposed workers, 6 formerly exposed workers, and 15 unexposed workers) were reassessed using the same methods except for the P300 potentials. In the first study, Al concentration in plasma of exposed workers was 8.7 μg/l and that of the control was 4.3 μg/l. The urinary Al concentration in exposed group was 87.6 μg/g Cr and that in the control was 9.0 μg/g Cr. In the second study, Al concentration in plasma of exposed workers was 6.7 μg/l and that of the control was 4.3 μg/l. The urinary Al concentration in exposed group was 19.8 μg/g Cr and that in the control was 4.5 μg/g Cr. In the two cross-sectional studies, no significant exposure-related differences between the two study groups were found for the psychometric test and the P300 parameters [15]. Iregren et al. studied effects on the nervous system in a group of potroom and foundry workers, Al welders, and a small group of flake powder production workers. There were 119 smelters (age = 46 years, length of service = 15 years), 16 flake powder production workers (age = 35 years, length of service = 8 years), and 38 welders (age = 38 years, length of service = 15 years) as groups exposed to aluminum and 39 mild steel welders (age = 39 years) as control group. The serum Al in the smelters, flake powder production workers, and welders was 1.0 μg/l, 9.0 μg/l, and3.0 μg/l, respectively. That in the control was 1.0 μg/l. Al urine concentration in the smelters, flake powder production workers, and welders was 4.2 μg/g Cr, 59 μg/g Cr, and 24 μg/g Cr, respectively. That in non-exposure workers was 4.7 μg/g Cr. In the potroom and foundry workers, no effects on the nervous system related to Al exposure were detected, whereas the welders, who had been exposed to high levels of Al, showed a reduced performance, though not significant, in four tests of motor function and one pegboard test. However, in the highly exposed flake powder production workers, no effect on the central nervous system was seen [12]. Bast investigated 20 Al welders (age = 33 years, exposed to Al years = 8 years) and 20 construction workers as control group. The urinary Al concentration in welders was 0.18 μmol/lCr. Neuropsychiatric symptoms showed that welders reported more symptoms than referents did. Results of the static steadiness test showed that the welders performed statistically significantly better than the reference. The author explained the result may be a positive selection of workers with high manual skills into welding working and a possible job-related training effect on hand steadiness [4].

Variation in findings may be due to differences in the methods of assessment and the magnitude of exposure to aluminum. Certain methodological weaknesses have made it difficult to identify the role of aluminum in some of the conclusions drawn. For example, workers have been exposed to several potential toxicants other than aluminum, no measures of aluminum uptake or body burden were reported, no reference groups were used, or findings based on very small samples have been reported.

5.4 Conclusion

Occupational exposure to Al seems to have different effects on workers in different industries. The measurable effects on the central nervous system might only develop after a protracted exposure, and the intellectual domain, mainly affected, varies. The current focus in most neurotoxicological research is on low-level exposure, and consequently the impairments reported are often subtle because they reflect marginal or subclinical changes. The present study suggests that to detect, and more importantly understand, the earliest signs of central nervous system dysfunction it is necessary to apply a theoretically based cognitive approach to the analysis of performance especially for empirically sensitive tasks. The selection of test methods allowing component analysis to be undertaken offers the most likely prospect of showing the elementary cognitive processes underlying impaired performance. For the future, a cohort study of large sample occupational population exposed aluminum will be established, and neurobehavioral test will be standardized, so that the relation of occupational exposure aluminum and cognitive function is more convincing.

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Chapter 6
Exposure to Aluminum in Daily Life and Alzheimer's Disease

Jisheng Nie

Abstract Aluminum is the third most abundant element on the earth's crust and has been considered a constituent of rather inert minerals. Therefore, it has often been regarded as not having a significant health hazard. Consequently, aluminum-containing agents have been used in processing, packaging, and storage of food products and also in the treatment of drinking water as flocculants. Recently, acid rain due to environmental pollution has transported more aluminum-containing minerals into residential drinking water resources. It is therefore not surprising that aluminum burden in the human body has increased. Research data showed that aluminum is not as safe as was previously thought and that aluminum may contribute to the initial advancement of Alzheimer's disease. Aluminum-mediated neurodegeneration resulting in cognitive dysfunction has been associated with amyloidβ (Aβ) deposition, formation of intraneuronal neurofibrillary tangles (NFTs), and apoptotic neuronal death characterized histopathologically in AD. The origin of Alzheimer's disease is generally not known; its development is likely triggered by unknown environmental factors. Although it is inconsistent with the link between human exposure to aluminum in everyday life and its contribution to Alzheimer's disease, a growing body of evidence points to aluminum as being one such significant influence.

Keywords Aluminum · Daily life exposure · Alzheimer's disease

6.1 Introduction

Aluminum (Al) is very abundant on the earth. Al-containing materials have long been extensively used in food additives, water purification, medications, Al-adjuvanted vaccines, and many other products [3]. The reduced pH of bodies of water due to acid rain has transported more aluminum-containing minerals into various environmental media. Thus, human body is readily exposed to a significant

J. Nie (✉)
Department of Occupational Health, School of Public Health, Shanxi Medical University, Taiyuan, Shanxi, China
e-mail: niejisheng@sxmu.edu.cn

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amount of Al in our daily life. New evidence showed that brain Al concentration can reach 0.35 mg/kg, about 100 times over plasma concentration [1]. This selective accumulation has been raising concern over the aluminum's potential adverse effects in neurotoxicity and neurodegeneration. Alzheimer's disease (AD) is a progressive neurodegenerative cerebral disorder. Al-mediated neurodegeneration resulting in cognitive dysfunction has been associated with amyloid**β** (A**β**) deposition, formation of intraneuronal neurofibrillary tangles (NFTs), and apoptotic neuronal death characterized histopathologically in AD [19]. Although the associations between Al exposure in daily life and AD are not consistent, many studies support Al exposure is a risk factor for the pathogenesis of AD.

6.2 Natural Sources of Aluminum Exposure

Aluminum (Al) is the third most abundant element on the earth's crust with 7.5% where it is frequently found as aluminosilicates, hydroxides, phosphates, sulfates, and cryolite. Soils and weathered rocks constitute the major sources of aluminum in environmental media. The transform and transport of aluminum is marked effected by environmental factors such as pH, salinity, and the presence of various species with which it may form complexes. In general, the solubility and mobility of aluminum in soil is not only dependent on soil content of organic matter capable of forming aluminum-organic complexes but also low pH such as in areas prone to acid rain or in acidic mine tailings. Natural processes account for most of the redistribution of aluminum in the environment. Aluminum is also released due to anthropogenic activities such as mining and industrial uses, in the production of aluminum metal and other aluminum compounds [6].

Aluminum levels in environmental media vary widely depending upon the location and sampling site. In general, the concentration in soils varies widely, ranging from about 7 to over 100 g/kg. Background levels of aluminum in the atmosphere are low, typically ranging from about 0.005 to 0.18 μg/m^3 [13]. However, reports showed higher Al levels were seen in urban and industrial locations. Nowadays Al concentration in surface water is generally lower than 0.1 mg/L; however, when PH in water is decreased, the level of aluminum increases due to the increased solubility of aluminum compounds [13].

6.3 Anthropogenic Sources of Aluminum Exposure

In 1825 aluminum was isolated in its elemental form by the Danish physicist Hans Oersted. Due to its excellent material properties such as castability in any shape, plasticity, heat conduction, low density, low melting point, oxidative passivation, and suppleness with concurrent toughness, aluminum metal and its alloys have many modern applications, especially in transportation, building and construction,

packaging, and electrical equipment. Aluminum powders are used in pigments and paints, fuel additives, explosives, and propellants. Aluminum oxides are used as food additives and in the manufacture. Aluminum hydroxide is used widely in pharmaceutical and personal care products. Food-related uses of aluminum compounds include preservatives, fillers, coloring agents, anticaking agents, emulsifiers, and baking powders; soy-based infant formula can contain aluminum. Natural aluminum minerals especially bentonite and zeolite are used in water purification, sugar refining, brewing, and paper industries. Currently, the use of aluminum makes us live in the aluminum age ensuring an accelerated exposure to aluminum in our daily lives and a burgeoning body burden of aluminum for each and every one of us [3].

6.4 Aluminum Exposures of General Population in Daily Life

The general population is primarily exposed to aluminum through the consumption of food items [26]. Aluminum in drinking water represents another, minor, source of exposure. Additional exposures may arise from the use of aluminum compounds in pharmaceuticals and consumer products. Uptake through inhalation is negligible for the general public, although workers who are exposed to higher Al dust in their workplace have an increased tendency to contract pulmonary aluminosis (restrictive lung disease).

Under normal conditions, it is clear that aluminum concentration is relatively low in most unprocessed foods. Mean fresh weight concentrations (in μg/g) in common food types are shown below: beverages (1.5); fruit (2.7); fish (fresh or tinned, 3.2); milk and dairy products (4.5); meat, sausage, and offal (5.4); vegetables (5.7); sugar and sugar-rich products (6.7); bread, cake, and pastries (7.4); and edible seeds (beans, peas, etc., 9.3) [13]; it is well known that there are big differences in the aluminum content of the individual food types between and within various countries. Many reports have estimated Al dietary exposure in different countries and regions, such as the United States, Greece, Belgium, South China, and the European Union. These data show large variations.

In general, human exposure to aluminum from food contact materials is negligible. However, the use of aluminum household utensils for acidic or salted foods, such as apple puree, rhubarb, tomato puree, or salted herring, could result in an additional aluminum exposure due to the increased solubility of aluminum. Also, the use of aluminum bottles for acidic beverages such as apple juice with mineral water or tea might moderately increase the aluminum exposure. High aluminum levels in food were also seen in convenience stores and fast-food restaurants especially those that contain tomato, different types of pickles, and vinegar when using aluminum vessels and trays [30].

Total dietary aluminum exposure from all sources has been estimated in a number of European countries (Netherlands, Hungary, Germany, Sweden, and Italy). Cereals and cereal products, vegetables, and beverages accounted for 10% more of

the dietary aluminum exposure in the general population. Mean dietary exposure from water and food in nonoccupational exposed adults ranged from 1.6 to 13 mg aluminum per day. This amount corresponds to an exposure of 0.2–1.5 mg/kg body weight (bw) per week for a 60 kg adult [13]. It must be noted that there are large variations in the average contamination between the different countries and, within a country, between different surveys. And large aluminum variations in individual exposure can occur for differences in living environment, soil contamination, dietary habits, or the consumption of foods with aluminum additives [26].

Due to higher food intake than adults, children may be the highest potential exposure group when expressed as aluminum per kg body weight. The estimated aluminum exposure at the 97.5 percentile in children aged 3–15 years was 0.7 mg/kg bw/week in France and that for toddlers (1.5–4.5 years) was 2.3 mg/kg bw/week and that for those aged 4–18 years was 1.7 mg/kg bw/week in the United Kingdom. And the potential estimated exposure for infants aged 0–3, 4–6, 7–9, and 10–12 months were 0.10, 0.20, 0.43, and 0.78 mg/kg bw/week, respectively. The mean potential exposure to aluminum in 3-month-old infants from a variety of infant formulae was up to 0.6 mg/kg bw/week for milk-based formulae and was 0.75 mg/kg bw/week for soya-based formulae.

6.5 Absorption, Distribution, and Excretion of Aluminum

Although consumption of food items comprises the primary source of aluminum for the general population, studies in humans and experimental animals show that the oral bioavailability of aluminum from drinking water is about 0.3%, whereas the bioavailability of aluminum from food and beverages is about 0.1%. Aluminum chemical forms and ligands in dietary constituents contribute to the bioavailability of aluminum. At least tenfold variation was found in the oral absorption of aluminum from food depending on the chemical forms present in the intestinal tract. Ligands in food can either enhance the uptake by forming water-soluble complexes (e.g., with carboxylic acids such as citric and lactic acids) or reduce it by forming insoluble compounds (e.g., with phosphate, dissolved silicate, phytate, or polyphenols) [13].

After absorption, aluminum distribution is unequal in all tissues in humans, and there is accumulation in some tissues. The total aluminum is in the range of 30–50 mg/kg bw in healthy human subjects. Normal serum aluminum concentrations are about 1–3 μg/L. In the human body, approximately one-half of the aluminum accumulates in the skeleton, and approximately one-fourth accumulates in the lungs (from accumulation of inhaled insoluble aluminum compounds). Aluminum level in human skeleton is in the range of 5–10 mg/kg. Aluminum also exits in the human skin, lower gastrointestinal tract, lymph nodes, adrenals, parathyroid glands, and most soft tissue organs. In rats higher aluminum levels were found in the spleen, liver, bone, and kidneys than in the brain, muscle, heart, or lung. Moreover, aluminum can cross the placenta and distribute into the developing fetus and even

distribute to the milk of lactating mothers. Available studies indicate that aluminum levels increase with aging in a number of tissues and organs (bone, muscle, lung, liver, and kidney) of experimental animals.

Aluminum excretion via the kidneys constitutes a primary route. And aluminum is excreted minorly by the bile. Unabsorbed aluminum is eliminated through alimentary tract in the feces. It takes a very long time for various organs and tissues of experimental animals and humans to eliminate aluminum. There are big differences in the elimination half-life of aluminum, ranging from hours, days, and months to years, suggesting that there is more than one compartment of aluminum storage from which aluminum is eliminated. Although aluminum persists longer time in humans than in rodents, there is little information on allometric scaling of aluminum elimination rates that can be used to extrapolate these results from rodent to the human.

Al in the environment was originally considered harmless, because aluminum exists in only one oxidation state (+3) and does not undergo oxidation reduction reactions, and in solution, Al^{3+} salts form monomeric hydroxy compounds which start to form polymeric and colloidal particles as the solution ages. Because of the formation of these insoluble aluminum species, it was assumed that absorption would be limited and thus the metal would be innocuous [4]. However, Al^{3+} can enter the nervous system by transport across the blood-brain barrier using receptor-mediated endocytosis of transferrin. Approximately 0.005% of the aluminum-protein complexes enter the brain by this means. New evidence showed that brain Al concentration can reach 100 times over plasma concentration. This selective accumulation may result from major bioconcentration by the cerebral vasculature [1]. The ensuing content of Al in the brain is within molarity range of 4–15 mM. This is over ten times the concentration of Al that is toxic to isolated human neuronal and glial cells. For this reason, there has been a rising concern over the aluminum's potential adverse health effects. In 2007, the provisional tolerable weekly intake (PTWI) of aluminum was reduced from7.0 mg per kg body weight to 1.0 mg per kg body weight because of the adverse effects of aluminum on the reproductive and nervous system in experimental animals. However, in 2011, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) revised the PTWI to 2.0 mg per kg body weight as a result of new bioavailability and toxicological data.

6.6 Aluminum-Induced Neurotoxicity and Aluminum Hypothesis in Etiology of AD

Aluminum has no any definite biological function, suggesting that the element possesses properties which are neutral or incompatible with fundamental life processes. Aluminum as a neurotoxic metal was initially established in the early 1970s after years of uncertainty. Studies in vitro and in vivo have clearly established the potential of aluminum to cause significant neurotoxicity and neurodegeneration. However,

in humans, the proposed connection between aluminum exposure and neurotoxicity was established as a result of studies of dementia in patients undergoing long-term dialysis chronically exposed parenterally to high concentrations of aluminum, first linking Alzheimer's disease (AD) to aluminum exposure. AD is a progressive mental deterioration manifested by memory loss, inability to calculate, visual spatial disturbances, confusion, and disorientation. The neuropathological characteristics include formation of intraneuronal neurofibrillary tangles (NFTs), deposition of amyloid beta peptide (Aβ) in neuritic plaques or senile plaques (SPs), loss of neurons, and synapses in the cerebral cortex and certain subcortical regions. The causes for AD are still mostly unknown, although unproven etiological factors have included genetics, head trauma, oxidative stress, infectious agents, and environmental factors including aluminum toxicity. AD has decades-long prodromal phase, suggesting that there may exist slow but progressive accumulation of a toxic or infective agent over time. Such potential environmental agents are slowly increased in susceptible neural cell types of AD-vulnerable brain regions to adverse levels till old age, giving rise to AD neuropathology without rapid neuronal lysis. Chronic aluminum neurotoxicity best matches this profile [2, 12].

The Aluminum Hypothesis, the idea that aluminum exposure is a causal factor in promoting Alzheimer's disease, dates back to 1965 when administration of aluminum salts into the brains of rabbits induced cognitive deficits in association with the formation of neurofibrillary changes that, after silver staining, seemed similar to the neurofibrillary tangles which exists in the brains of AD cases. Crapper et al. soon replicated these results in cats. Then the following important evidence found that there is a high level of aluminum in the brain of AD patients with long-term dialysis, which was the first report for its linkage with AD. Since then numerous reports have prompted the suggestion that aluminum is a possible cause of AD [12]. Subsequent study using a low dose of aluminum salts to rabbits by intracerebral injection found NFTs similar to AD patients in rabbits' brain, and it is the first chronic neurotoxicity model of aluminum. Then the Aluminum Hypothesis had been the focus of intensive research efforts for decades long [12]. However the hypothesis has some unsatisfy parts: First, high aluminum levels in the brain are not found in all AD patients, and as a common characteristic in AD, the SPs are not seen in experimental Al toxicity. Second, aluminum-induced NFTs are not the same as NFTs in AD when components' details of NFTs were analyzed. Third, with increased aluminum levels, only transit dialysis dementia in renal patients is elevated, and no rising incidence of cognitive impairment and AD symptoms were seen. Yet, the Aluminum Hypothesis continues to attract the attention of a group of scientists, and aluminum continues to be viewed with concern by some of the public. More recent studies showed the neurodegenerative effects of extended exposure of experimental animals to levels of aluminum that have relevance for the human population. Moreover, aluminum-mediated neurodegeneration resulting in cognitive dysfunction has been associated with elevated amyloid precursor protein (APP) expression, amyloidβ (Aβ) deposition, hyperphosphorylation of tau, formation of intraneuronal neurofibrillary tangles (NFTs), and apoptotic neuronal death resembling those that are found with AD brain, which can highlight the relevance of Al in AD [5].

6.6.1 Aluminum and Aβ

Aβ is a 39 to 43 amino acid-long peptide derived from a larger transmembrane protein; the amyloid precursor protein (APP) has an intrinsic tendency to form insoluble aggregates. Mountains of studies have focused on the structure, aggregational properties and neurotoxicity of Aβ, and their roles in AD. Aluminum influences the aggregation and toxicity of Aβ [33]. In physiological buffers, Al, Fe, and Zn at 10 mM concentration strongly promoted Aβ aggregation (a rate enhancement of 100- to 1000-fold). Al appears to be the most efficient cation in promoting Aβ aggregation in vitro increasing Aβ neurotoxicity dramatically. And aluminum also inhibits proteolytic degradation of Aβ peptide by cathepsin D in vitro. It is widely believed that amyloid-Al complexes are more toxic than Al or amyloid on their own and consequently play a key role in the etiology of AD [37].

Systemic aluminum can induce AD-like behavioral deficits in treated rats. Chronic exposure to dietary Al not only results behavioral deficits, but leads to elevated levels of amyloid precursor protein, and these elevated levels have been correlated to α- and β-secretase subtypes, which together appeared to have led to increased levels of Aβ1-42. In addition, with increasing accumulation of aluminum in the brain, an elevated burden of amyloid plaques was observed in patients with renal failure. Yumoto et al. [36] examined the presence of Al at autopsy of five AD patients using energy-dispersive X-ray spectroscopy combined with transmission electron microscopy (TEM-EDX). The results demonstrated colocalization of Al and Aβ peptides in amyloid fibers in the cores of senile plaques. There is also evidence that Al may alter the dynamics of Aβ. The core center of amyloid plaques is known to contain an overabundance of Aβ42, which is less soluble than the more abundant Aβ40. There is now clear evidence that when Al complexes with Aβ42, it reduces solubility, increases precipitation of β-sheets, and facilitates Aβ flux across the BBB. Aluminum is also known to enhance the processing of APP. It has been shown that Al accumulated in AD brain accelerates the generation of Aβ due to the faulty proteolysis of normal APP. It has been shown that APP has a domain homologous to inhibitor of bovine pancreatic trypsin, and Al inhibited the activity of serine protease inhibitors. Thus Al is indirectly involved in activating serine proteases such as a-chymotrypsin, enhancing processing of APP and leading to accumulation of Aβ and plaque formation.

6.6.2 Aluminum and NFTs

As a marker for neurodegenerative diseases such as Alzheimer's disease (AD) and ALS, NFTs are the aggregates of phosphorylated tau protein. The phosphorylated tau protein has its ability to self-assemble into filamentous structures that are the pathological hallmark of tauopathies. Many reports show that Al promotes phosphorylation of the tau protein and causes the formation of NFTs. Al exerts

hyperphosphorylation of tau depending not only on protein phosphatase-2A (PP2A) activity inhibition but caspase activation which truncates hyperphosphorylated tau. Al then binds to the truncated hyperphosphorylated tau and aggregates it into granules. High local concentrations of Al/truncated hyperphosphorylated tau may trigger polymerization to form NFTs. Some of the NFTs are getting larger enough to kill the neurons [31]. However, there are a few differences in reports about tau aggregates and its toxicity. The reports show that tau aggregates by Al are in amorphous form, not in common fibrillary form. Al can produce toxicity to the fibroblasts that expressed tau. However, tau did not aggregate in these cells, but neurofilaments do aggregate in aluminum-treated cells [14]. Also the Al-induced increased in tau immunoreactivity was observed in human neuroblastoma cells, without an effect on cell viability [21].

Some studies reported that Al causes neurofilament monomers of tau in soluble form which results in the formation of aggregates into nonfibrillar material. Al also induces to form fibrillary bundles of neurofilaments and to form NFTs [14, 28].

6.6.3 Aluminum and Cell Death

Apoptosis is one of the mechanisms contributing to neuronal loss in AD. Neurons in the cortex and hippocampus of the AD brain show evidence of DNA damage, nuclear apoptotic bodies, and chromatin condensation. Multiple studies have shown that Al induces cell death stimulus similar to that of AD [10, 24, 25]. Al induces cytochrome c release from mitochondria, a decrease in Bcl-2 in both mitochondria and endoplasmic reticulum, Bax translocation into mitochondria, activation of caspase-3, and DNA fragmentation [11]. The released cytochrome c from mitochondria binds to Apaf-1 and initiates Al-induced apoptosis cascade [24]. The formed complex activates caspase-9, which in turn activates the effector caspase that is caspase-3. The released cytochrome c is involved in three distinct pathways like opening of the mitochondrial transition pore (MTP), translocation of mitochondria of the proapoptogenic Bax which can form the channel by itself, and interaction of Bax with the voltage-dependent anion channel (VDAC) to form a larger channel which is permeable to cytochrome c. The primary event in the apoptosis is considered as the mitochondrial changes following cytotoxic stimuli [29]. Furthermore, the studies show that the activation of SAPK/JNK (stress-activated protein kinase or c-Jun N-terminal kinase) signal transduction pathway is also caused by the induction of Al and results in apoptosis [9]. Apoptosis is believed to be the general mechanism of Al toxicity to the cells. Treatment with Al shows some characteristic features of apoptosis like shrinkage of cell bodies, hypercondensed and irregularly shaped chromatin, and extensive fragmentation of chromatin and DNA [11, 16]. Al induces apoptosis in the astrocytes further leading to the neuronal death by the loss of the neurotrophic support [27].

6.7 Epidemiological Evidence of a Relation Between Aluminum Intake in Daily Life and Alzheimer's Disease

Early reports on neurodegenerative effects of Al such as those with dialysis dementia involved relatively brief exposure to high levels of Al. More recently and more controversially, adverse effects in daily exposures to lower levels of Al have been described. Because of the difficulties in accurately assessing chronic dietary Al exposure, there have only been few epidemiological studies on the effects of aluminum in food in the general population. A pilot study including 23 case-control pairs reported potential positive results. Although the crude odds ratio for AD in subjects who consumed foods containing high levels of aluminum was 2.0 compared to those who preferred a fresh food diet, ORs were unstable and not statistically significant in this study [15]. Moreover, some important confounders such as renal function and vitamin deficiencies were not considered. Some foods containing high levels of Al like tea may contribute up to 50% of the total daily Al intake in some countries [35]. Yet, several studies found that there is no significant link between tea consumption and risk of AD. So, it is controversial to the possibility of a link between aluminum in the diet and AD.

Although drinking water is a minor contributor to the whole Al exposure in humans, numerous population studies link Al content of drinking water to risk of AD. In 1989, Martyn et al. performed a study of the incidence of AD in relation to aluminum levels in drinking water over the previous 10 years. The study found that the incidence of probable AD was 1.5 times higher in areas where the mean aluminum concentration exceeded 0.11 mg/L than in areas with concentrations of <0.01 mg/L, and there is no relationship between other types of dementia or epilepsy and aluminum levels in water [17]. It should be noted that not all AD patients had an equal probability of being included in the analysis; the population was not representative.

The association was found between aluminum in drinking water, and death rates from the neurodegenerative disease in Norway that showed relative risks for dementia in males are 1.00, 1.15, and 1.32 for low, medium, and high levels of aluminum in drinking water, respectively; the corresponding values for women were 1.00, 1.19, and 1.42. Frecker [8] confirmed geographic distributions of dementia mortality in Newfoundland related to aluminum levels in drinking water. However, the authors cautioned that these associations were ecological, serving to generate hypotheses for further study.

A case-control study conducted by Neri and Hewitt using hospital discharge data found a relative risk of 1.46 for aluminum concentrations of ≥0.200 mg/L compared to <0.01 mg/L [22]. The study was really ecological in that no additional adjustments were made for confounding factors except for age and sex. Based on the Ontario Longitudinal Study of Aging where 2000 men have been followed for about 30 years, Forbes et al. explored the relationship between Al, fluoride, and other constituents in drinking water and cognitive function. The research data showed that

the RR was 2.72 for men in areas with high Al and low fluoride concentrations in drinking water, compared to those with low Al and high fluoride levels. McLachlan et al. conducted a case-control study to investigate the relationship between AD and exposure to aluminum in drinking water. AD was diagnosed by autoptical histopathological analysis. Al concentration in drinking water at last residence before death was used as the measure of exposure [20]. Research data showed an OR was 1.7 for subjects in areas where levels of aluminum are ≥100 μg/L in drinking water. The authors later obtained even larger estimates (OR of 2.5 or greater) on the weighted residential history in the analysis [20].This is the only study based on neuropathologically confirmed cases of AD, which is a strength.

In France, Rondeau et al. [23] utilized the data from the Paquid cohort study to examine the link between aluminum and silica in drinking water and the risk of dementia and AD [23]. The analysis included 2698 subjects, aged 65 years and over. Al concentrations in drinking water ranged from 0.001 to 0.459 mg/L, and, for silica, 4.2–22.4 mg/L in drinking water. Over an 8-year follow-up, all new cases of dementia and AD were recorded. The analysis of data adjusted for age, gender, educational level, place of residence, and wine consumption revealed that the RR of dementia was 1.99 (95% CI: 1.20–3.26) for individuals who lived in areas with aluminum concentration >0.1 mg/L. For AD the adjusted RR was 2.14 (95% CI: 1.21–3.80). The concentration of silica in drinking water appeared to exert a protective effect in the development of AD (RR = 0.73, 95% CI 0.55–0.99, $P = 0.04$). Although no dose-response effect was found, the conclusions were made that Al concentration >0.1 mg/L in drinking water may be a risk factor for dementia and AD.

Several studies showed lack of significant association between AD in human populations and aluminum levels in their drinking water. Wettstein et al. [34] compared the cognitive skills between two groups in districts with high (98 μg/L) or low (4 μg/L) aluminum concentrations in their drinking water. No substantial differences were found in cognitive impairment between the high- and low-exposure groups. Urinary aluminum and serum aluminum levels showed no significant difference between ten AD patients and ten controls in both areas. However, the significance of these negative results might be limited by the fact that the highest concentration of aluminum in drinking water was below 100 μg/L [34]. Likewise, Martyn et al. [18] found no association between the risk of AD and higher Al concentrations in drinking water in a case-control study. One hundred and six men with early-onset AD were identified as cases in the study. And 99 men with other dementing illnesses, 226 men with brain cancer, and 441 men with other diseases of the nervous system were included as controls that may be deceased research sensitivity. And it should be noted that cases of early-onset AD are more affected by their genetic background than patients with sporadic AD [18]. Forster DP et al. got similar negative results whose study was also based on early-onset AD patients. Early-onset AD patients are more likely to contain mutations in their AbetaPP and/or presenilin genes, being more affected by their genetic constitution rather than by environmental influences [7].

Despite a voluminous literature, there are completely opposite assertions on the relation between AD and aluminum. Some researchers thought that chronic aluminum intake can cause Alzheimer's disease; the opposition concluded that lifetime exposure to Al is not likely to be an important risk factor for AD. The contradictory results are in part due to the great difficulty in unambiguous interpretation of epidemiological findings. Above studies are limited by methodological issues in investigation on the relationship between aluminum in drinking water supplies and the risk of developing AD. The issues include the absence of individual exposure data, poor outcome ascertainment, failure to adjust for important confounders, and small sample sizes. Thus, findings from well-defined laboratory conditions and those from population studies are not yet sufficiently and conclusively correlated so as to result in a unanimous recognition of the hazards of environmental aluminum. Moreover it is controversial whether research data concerning aluminum's role in AD satisfy Hill's criteria for causality or not [32]. These conditions illustrate the need for more study rather than more debates.

6.8 Conclusions

Based on the above data and arguments, the neurotoxic effects of Al are beyond any doubt, and Al as a factor in AD cannot be discarded. This is mainly because AD is a multifactorial disease, and to date the specific etiologies of AD are unknown. Thus, the Al hypothesis, along with other hypotheses, continues to survive. Since the accumulation of Al may occur in prodromal stages of AD, we propose that Al in daily life may initiate and promote the AD disease process; even at the very least it exacerbates the neurodegenerative process.

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Chapter 7
Animal Model of Aluminum-Induced Alzheimer's Disease

Jing Song

Abstract Lack of a satisfactory animal model for Alzheimer's disease (AD) has limited the reach progress of the pathogenesis of the disease and of therapeutic agents aiming to important pathophysiological points. In this chapter, we analyzed the research status of animal model of aluminum-induced Alzheimer's disease. Compared with other animal models, Al-maltolate-treated aged rabbits is a more reliable and efficient system in sharing a common mechanism with the development of neurodegeneration in Alzheimer's disease.

Keywords Aluminum · Alzheimer's disease · Animal model · Rabbits

Alzheimer's disease (AD) is a progressive neurodegenerative disorder in the geratology that presently affects more than tens of millions of individuals in the world. The complex neuropathological and biochemical abnormalities seen in the brain of patients with Alzheimer's disease include cortical and subcortical atrophy, formation of intraneuronal neurofibrillary tangles (NFT), and deposition of amyloid-β (Aβ) peptide in neuritic plaques, neuropil threads, synaptic loss, oxidative stress, and eventually neuronal loss, probably via a mechanism involving apoptosis.

During the past many decades, drug development for AD aimed at various potential targets has experienced tremendous, global setbacks, which have been persistent enough to make the efforts to find anti-AD drugs appear to be an ineffective strategy; for example, the failure rate of anti-Aβ drugs in clinical trials is approximately 100% [1]. Discovering new disease mechanisms and investigating the disease network of AD will aid in the identification of the pathogenesis of AD and potential treatments for this disease.

Animal models have significantly contributed to the study of AD, and suitable animal models are essential way to understand the pathophysiology of AD and the development of new therapeutics. Despite the intensive investigations on multiple animal models (rats, rabbits, zebra fish, transgenic mice, as showed in Table 7.1 [2]

J. Song (✉)
Republic Health School, Shanxi Medical University, Taiyuan, Shanxi, China

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Table 7.1 Various animal models employed for studies on neuropathology and therapeutic strategies

Neuropathology	New Zealand rabbits		Aβ deposition, NFT formation, oxidative stress, apoptosis
	Transgenic animal models	APP/PS1 transgenic mice	Plaque formation
		Zebra fish	Disruption of synaptic function (h Tau), caspase-3 activation, expression of human protein tau-P301L, hyperphosphorylation, and conformational changes of tau
Therapeutic events	htau mice	Decrease in number of NFTs and Aβ deposition	
	Rat model	Amelioration of Aβ-induced cognitive decline	
	AD 11 mice	Amelioration of cholinergic and behavioral deficit	
	Transgenic zebra fish	Improvement of hyperphosphorylation of tau through GSK3β inhibitors	
	Transgenic mice	Amelioration of memory and synaptic transmission	
	APP/PS1 mice	Amelioration of cognitive function via calpain inhibition	
	Vaccination	Transgenic mice	Microglial clearance of Aβ
		Tg2576	Clearance of Aβ
		DNA epitope vaccine	Amelioration of Aβ pathology, reduction of gliosis, improvement of behavioral deficits

for a few decades, neither the etiology of the disease has been completely unraveled nor has a substantial therapeutic strategy so far.

Introduction of aluminum salts into aged New Zealand rabbit brain could demonstrate neurofibrillary tangle (NFT) formation in 1965. This outstanding contribution substantiated the role of aluminum (Al) in AD in turn becoming the basis further molecular studies in rabbits [2]. This chapter will focus on animal model of aluminum-induced AD.

7.1 The Involvement of Aluminum in AD

In 1897, Dollken began the pioneering studies on neurotoxicity of Al in experimental animals. The potential toxicity of Al in experimental animal models and in humans under different clinical conditions was made known by many scientific studies [3]. But the usage of Al in experimental animal came to light following the extraordinary discovery of Klatzo et al. and Terry and Peña in 1965 and 1986 who showed that injections of Al salts into rabbit brain led to the formation of NFTs which appeared similar to the NFTs of AD [4]. Later, these results were replicated in cats by Crapper et al. in 1973 [5]. Because of the complex chemistry of Al and no readily available radioisotope for experimental purposes, the connection between

this element and the etiology of AD had not obviously shown up until the studies by Priest [6] on humans and Yumoto [7] on animal. Using the Al radioisotope, they confirmed that by way of systemic administration, this element can indeed enter the central nervous system. In addition, there is documented evidence that Al is neurotoxic, both in human disease and in experimental animals [8]. In 1984 Uemura [9] testifies that intranuclear Al accumulation led to neurofibrillary changes in chronic animals. In 1985 Wen and Wisniewski [10] localized Al in rabbit CNS in histochemistry. Thereby the formation of neurofibrillary aggregates (NFAs) in animals induced by Al salts provided an important basis to the controversy that Al is one of the contributing factors to several neurodegenerative disorders, mainly related to AD. However, this issue remains argumentative.

Recently, Savory et al. [11] reported that Al-maltolate treatment mimicked AD-like neuropathology in aged rabbits which emerged β-amyloid deposition, neurofibrillary tangles, apoptosis, and oxidative stress in hippocampus, forebrain, and midbrain regions. In addition, important circumstantial evidences were provided by Rao [12] that Al-maltolate-treated rabbits are similar in the case of AD on the neuropathological features. Therefore, this animal model might be a promising model of AD with more recognition for further researches.

7.2 The Aluminum Compounds for Animal Model

7.2.1 The Complex Chemistry of Aluminum

The role of Al in Alzheimer's disease still remains a mystery even after many decades of research, probably due to conflicting data in the literature, reflecting the complex chemistry and ubiquitous nature of Al. Understanding the role of chemical speciation in biological systems is the intrinsic difficulty. Hence, to understand the mechanism of Al-induced neuropathology, the selection of an appropriate Al compound is important.

The choice of an appropriate Al compound is important for obtaining consistent results since at neutral pH many of these compounds form insoluble precipitates. Thus, it is necessary to consider the hydrolysis equilibria of Al.

Al speciation chemistry is a very complex phenomenon. In aqueous solution at pH <5.0, Al exists as an octahedral hexahydrate, $Al(H_2O)_6^{3+}$, usually abbreviated as Al^{3+}. As the solution becomes less acidic, $Al(H_2O)_6^{3+}$ undergoes successive deprotonations to yield $Al(OH)^{2+}$ and $Al(OH)_2^{+}$. Neutral solutions give an $Al(OH)_3$ precipitate that redissolves, because of the formation of tetrahedral aluminate, $Al(OH)_4^{-}$, the primary soluble Al(III) species at pH >6.2. Only two species dominate over the entire pH range, the octahedral hexahydrate $Al(H_2O)_6^{3+}$ at pH <5.5 and the tetrahedral $Al(OH)_4^{-}$ at pH >6.2, while there is a mixture of hydrolyzed species and coordination numbers between 5.5 <pH <6.2 [13]. Hence, if not taking hydrolysis reactions into account, the soluble Al concentration of the solution cannot be calculated simply despite a known quantity adding of an Al compound to water. For

example, when Al inorganic salts such as perchlorite, sulfate, hydroxide, or chloride are dissolved in water at a calculated concentration of 10 mM, however, after pH adjustment and filtration, the exact Al concentration is only about 50 μM [14]. Thus, the chemistry of Al is indeed complex and should be fully understood before using Al as a toxic element to science research.

7.2.2 The Aluminum Salts and Complexes

From the beginning of aluminum toxicological studies, Al salts and complexes have been applied wildly, in which the counterion was either the conjugated base of a strong acid (Cl^-, $S0_4^{2-}$, NO_3^-) or a weak a-hydroxy carboxylic acid (lactate, citrate, tartrate). The former undergoes extensive hydrolysis in water giving rise to acidic solutions, which after neutralization unavoidably produce $Al(OH)_3$. The same occurs for the latter when analytical concentrations are in the sub-millimolar range.

Administration of different Al compounds to a certain extent induces AD neuropathology, but compared to other compounds, Al maltolate seems to be more effective. The studies on aged rabbits with many Al salts, such as $AICl_3$, Al lactate, $AlSiO_4$, and AlF, showed that there are no neurofibrillary aggregates (NFAs) appearing in the nervous system in a large scale; only the large neurons of the nucleus of the motoris lateralis are minimally involved [15]. These results indicate that Al-inorganic complexes do not mimic AD neuropathology in its distribution of pathology. In addition, different animal groups like dogs, ferrets, and cats treated with Al-organic and Al-inorganic complexes did not mimic the AD neuropathology either. But, there is a turnaround displayed in Al-maltolate-treated aged rabbits which showed NFTs in the axons imaged in hippocampal neurons (Fig. 7.1), which

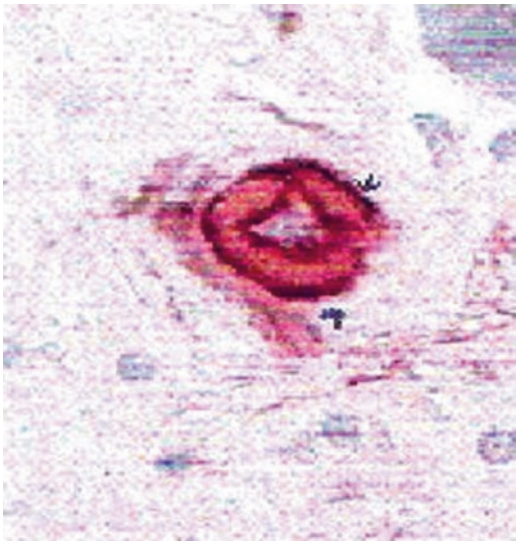

Fig. 7.1 Represents the localization of Aβ (vascular region) in the Al-maltolate-treated aged rabbits which is also observed in the vascular region of demented people

follows the distribution of these lesions in AD [16–19]. A research about mRNA fraction displayed that compared to Al lactate exposed and the control young rabbits, the brain polysomal RNA in Al maltolate exposed is more active [20]. This research also supported the phenomenon that compared with the other Al complexes, Al maltolate is more efficient because Al maltolate enhances the bioavailability of Al in the brain. Thus, both in AD and in experimental NFAs rabbits, the positively charged Al maybe promote the formation and stabilization of the NFAs.

But why? Because Al maltolate is water soluble at neutral pH and can deliver a significant amount of free aqueous Al without the complications of $Al(OH)_3$ precipitation. Most other Al salts, such as $Al_2(S0_4)_3$ and $AlCl_3$, produce insoluble complexes at pH 7.0. However, from pH 3.0 to 10.0, Al maltolate is soluble and stable and especially keeps hydrolytic stability at pH 7.0, and it has no speciation chemistry problems. For example, compared to other Al salts like Al aspartate or Al lactate (soluble Al concentration is 55–330 μM), Al maltolate increases the soluble Al concentration to 4–6 mM.

Most studies carried out in the writer's laboratory have employed the Al maltolate originally synthesized [21, 22]. Al maltolate is easily synthesized as described in the original paper, and the purified crystalline material can be stored at room temperature for extended time periods. For intracerebral or intraperitoneal injection, sterile solutions in physiological saline are prepared immediately prior to administration in experimental animals.

In summary, Al maltolate is suitable over other Al compounds because of its following properties: (a) very high metal solubility at pH 7.0 and (b) prominent kinetic restrictions to ligand exchange reactions in neutral, hence suitable for toxicological studies and also to understand the neuropathology.

7.3 The Aged Rabbits Are More Effective as Animal Model

For several decades of study, different animal groups like rats, cats, ferrets, and dogs have been administrated with some Al complexes to make the animal model of the AD neuropathology, but no satisfied model was found. When the aged rabbits were treated with Al maltolate, AD-like neuropathology was observed in the axons imaged in hippocampal neurons. Rabbits are particularly sensitive to aluminum neurotoxicity, and they develop severe neurological changes that are dependent on dose, age, and route of administration. The most prominent feature induced by aluminum in rabbit brain is a neurofibrillary degeneration that shares some similarity with the neurofibrillary tangles found in Alzheimer's disease patients.

But, why choose the aged rabbits? What about the young?

7.3.1 The Susceptibility of Aged Rabbits in Inducing AD Neuropathology Compared to Young Ones

Many studies by Savory et al. showed that the aged rabbits were more susceptible to Al neurotoxicity than the young rabbits [23]. The NFTs, oxidative stress damage, and apoptosis were observed in the hippocampus of aged rabbits treated with Al maltolate. But these pathological changes are not found in the hippocampus of Al-maltolate-treated young rabbits. The following extensive study by Savory et al. and Rao et al. [19] about Al-induced oxidative damage, redox-active iron (Fe) accumulation, and their relationship to apoptosis indicated that the anti-apoptotic Bcl-2 and the pro-apoptotic Bax proteins respond in Al-maltolate-treated aged rabbits which could constitute a key defect in aged neuron, leading to increased susceptibility to oxidative damage and apoptosis as observed in AD, suggesting that Al-maltolate-induced aged rabbits mimic AD pathology. But, an increased Bcl-2 response and minimal Bax immunopositivity appeared in young rabbits. In addition, some studies by Markesberry [24] and Lovell [25] show the evidence from clinical and animal model studies displayed that Al content in the brain increases with age, maybe related to the increased exposure to Al and/or the decreased ability to remove Al from the brain with age. Hence, the aged rabbits are considered to be more susceptible in reproducing AD neuropathology compared to young ones.

7.3.2 The Al-Induced Neurodegeneration

The study by Katsetos et al. showed that widespread argyrophilic NFAs were found in a number of brain regions in Al-treated aged and young rabbits; quantitatively the aged animals are affected to a much greater extent [26]. Studies from Savory's group have reported that intracisternal Al administration induces NFD most strikingly in the medulla and upper spinal cord, as similar to regions affected in AD. The brain regions are less affected in the case of Al-maltolate-treated young rabbits compared to the aged [27–29].

7.4 The Similar Features in Al-Maltolate-Treated Rabbits with AD Patients

7.4.1 Neurofibrillary Degeneration

After intraventricular administration of Al maltolate to rabbits, widespread neurofibrillary degeneration was found in pyramidal neurons of the isocortex and allocortex, nerve cells of the brain stem and spinal cord, and projection neurons of the diencephalon, especially the perikarya and proximal neurites [26]. When the

intraventricular administration of Al maltolate is longer than 12 weeks, NFAs appeared in the pyramidal neurons and the oculomotor complex of the occipital cortex. Compared with motor neuron disease and the senile dementia of the Alzheimer type, widespread argyrophilic NFAs are seen in a number of brain regions in Al-treated aged and young rabbits. Compared with the less water-soluble Al compounds, intraventricular Al maltolate produces similar but more widespread degeneration of projection-type neurons. In addition, compared with the young rabbits, the aged animals are damaged to a much wide range by the Al. This may be related to an active mechanism which is involved in suppressing Al-maltolate toxicity and is decreased in the aging rabbit brain.

NFAs are observed mostly in the superior and the inferior hippocampus, lateral and inferior cerebral cortices, the superior cortex, and the stratum pyramidale subiculum [23, 30]. NFD is observed in cerebral cortical neurons and in the inferior segment of the hippocampus of aged Al-treated rabbits. NFD induced by intracisternal Al administration appeared mostly in the medulla and upper spinal cord. Compared with the aged rabbits treated with Al maltolate, the young rabbits are less affected in brain regions. NFTs were observed by confocal imaging of axons in hippocampal neurons (Fig. 7.2) from Al-treated aged rabbits [19].

Using a method of computer-controlled electron beam X-ray microanalysis and wavelength dispersive spectrometry, Gamito et al. successfully got the imaging of Al in the hippocampus NFT of Guamanian patients [31]. The elemental images showed that Al is distributed in cell bodies and axonal neurons which display NFT either. The result that Al deposits occur within the same NFT-bearing neurons and the result that compared with control case, no obvious increase of Al concentrations was imaged in non-NFT-bearing neurons in the pyramidal cell layer indicated that Al maybe involved in NFT formation.

In addition, Savory and his co-workers made many researches on the quantitation of Al in the brain and neurofilament protein expression and phosphorylation effected by Al [32]. The accumulation of Al in different brain regions of aged rabbits treated with Al maltolate was detected, yielding about 10 μg/g dry tissue in the

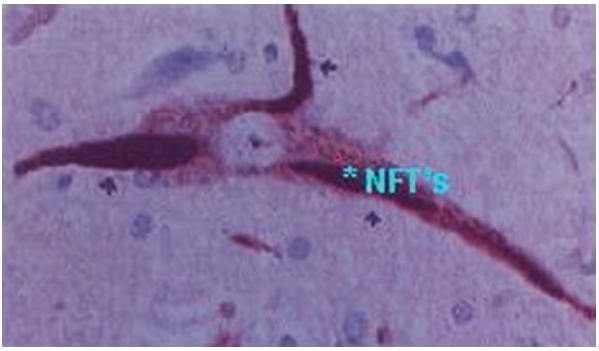

Fig. 7.2 NFT in axons imaged in a single neuron from hippocampal region of Al-treated aged rabbits

brain and spinal cord but only 2.1 μg/g dry tissue in the lumbar cord. The result also showed that in perikarya and proximal neurites of neurons of the lumbar and sacral cord areas, argyrophilic tangles were detected either. But, immunoblot studies showed negative changes in three neurofilament protein isoforms, the total phosphate content of these proteins, and the genes encoding for the 200 and 68 KDa neurofilament proteins. These results provided new evidence for the involvement of Al in AD.

The neurofilament protein phosphorylation in aged rabbits treated by Al maltolate was different from the AD patient. In Al-maltolate-treated aged rabbits, neurofilament proteins like tau, a-1-antichymotrypsin, and ubiquitin are unphosphorylated, while in AD neurofilament protein is hyperphosphorylated [27, 29]. Using different kinds of monoclonal antibodies which can recognize nonphosphorylated and phosphorylated tau, quantity of the abnormally phosphorylated tau present in these NFAs are detected. The results showed that both nonphosphorylated and phosphorylated tau are displayed.

From the thermodynamic view, cytoskeletal protein hyperphosphorylation and the associated negative charges may result in the destabilization of these aggregates. So, a hypothesis is made that phosphorylation of cytoskeletal proteins promotes the formation of the NFAs particularly in AD [28]. Thus, it is also reasonable to propose that both in AD and in experimental Al-maltolate-induced NFAs, some positively charged substance such as metal ions promotes the formation and stability of the NFAs. In the experimental Al-maltolate-induced NFAs, Al may play the role of one promoter [33].

Although the biochemical and morphologic features between NFTs induced by Al in rabbits and the neurofibrillary tangles of AD are not fully same at both gross and ultrastructural levels, there are many similarities displayed. First of all, the distribution of both tangles is different, while both types of tangles are shown in the cortex and hippocampus [11, 34]. Tangles induced by Al are found in the perikaryon and proximal parts of the dendrites and axon in the rabbits [35, 36], while tangles of AD are found throughout the axons including the terminals and throughout the neuron including the entire length of the dendrites. Secondly, the protofilament building blocks of tangles are also different in the diameter. Tangles induced by Al are 2.0 nm, while those of AD are 3.2 nm. Finally, the biochemical composition of tangles is not the same at all. The peptide composition of Al-induced tangles is chiefly neurofilament protein. In contrast, the paired helical filaments of AD tangles are much more complicated, composed of three proteins at least such as hyperphosphorylated tau, a microtubule-associated protein, and ubiquitin. Similar results about Al-induced tangles in rabbits and those of AD were reported by Klatzo et al. [4]. In addition, if the tissue is treated with silver staining, Al-induced tangles and AD pathology appeared similar [35, 37, 38].

The similarities and differences between Al-maltolate-induced tangles in New Zealand aged white rabbits and the neurofibrillary lesions of AD are summarized by Bharathi et al. [39] in Table 7.2.

Table 7.2 Characteristics of tangles associated with Al-maltolate-treated aged New Zealand white rabbits and AD

Tangle characteristics	Aluminum-induced AD in aged rabbits	Alzheimer's disease
Protein composition	Neurofilament protein, Tau (unphosphorylated)	Hyperphosphorylated Tau, a microtubule-associated protein, and ubiquitin
Configuration	Single straight filaments	Paired helical filaments
Regional		
Localization	Forebrain, spinal cord	Forebrain
Intraneuronal localization	Cell body	Proximal portion of the dendrites and axons, entire neuron
Diameter	10 nM	20–24 nM

7.4.2 Oxidative Stress

Oxidative stress occurs in both rabbits treated with Al and in AD, and the time and extent of oxidative damages also overlap to a certain degree. That aids to understanding of the pathogenesis of neurodegeneration occurring in AD.

Savory et al. [23] reported that oxidative stress products are released in the nucleus lateralis dorsalis thalami region and stratum pyramidale hippocampi, and the oxidative stress product accumulation in hippocampal neurons occurs very rapidly, within a period of 3 h, and increased in intensity at 72 h. The oxidative stress products released in the neurons are as follows: carbonyls, malondialdehyde, nitrotyrosines, peroxynitrites, and enzymes like heme oxygenase and SOD. It was proposed that Al-maltolate injection may cause microtubule transport and synaptic vesicles to decrease. Using a specific immunocytochemical technique which was an antibody system against DNP linked in situ 2,4-dinitrophenylhydrazine, Smith et al. [40, 41] detect the carbonyl reactivity both in Al-treated rabbits and AD. Smith et al. [42] found that carbonyls emerged in NFTs both of aged rabbits and of AD; meanwhile it also appeared in the glia and non-NFT-bearing neurons. And Al levels are also estimated in glia, microglia, and astrocytes and enhance the production of carbonyls [43].

As a non-redox-active metal, Al is believed to result in a lot of damages by increasing the redox-active iron concentration in the brain which is mainly through a Fenton reaction. Al is simultaneously an activator of SOD and an inhibitor of catalase; therefore superoxide radicals are readily converted to H_2O_2 and breakdown to H_2O and O_2 by catalase is slowed down, leading to the production of hydroxyl radicals [44, 45]. Thus, Al significantly plays a role in neurodegeneration through oxidative stress. In spite of the controversy of Al about the involvement in AD, these oxidative stress studies played an effective compensatory role for Al in AD. Hence, instead of being the direct cause of AD, oxidative stress may play a significant role in regulatory process of AD.

Since this animal model mimics AD pathology to some extent, it is reasonable to believe that Al-maltolate-treated aged rabbits may be the human brain's attempt to

compensate in part for designing animal models for AD. Thus, in fact, it is difficult to understand the complete neuropathological mechanism in AD by the way of this animal model. For that reason, in order to reproduce the neuropathology of AD, a reliable model which can meet most conditions still needs to be developed in the future.

7.4.3 Apoptosis

Apoptosis or programmed cell death is the tightly controlled pattern of cell death necessary for typical growth and development in multicellular organisms. Defective apoptosis can result in abnormal development and pathogenesis. Apoptosis is also an important pattern of brain cells dying in normal physiological conditions and neurotoxic situations. Loss of neurons is a hallmark of neurodegenerative disorders, and there is increasing evidence suggesting that apoptosis is a key mechanism by which neurons die in these diseases. It was reported that some neuron deaths associated with AD are related to the increased levels of Bax, decreased levels of Bcl-2, and high concentrations of peroxynitrite products which are the key biochemical markers in apoptosis [46, 47]. An amount of researches show that apoptosis is one way to mediate neuron death induced by Al and apoptosis is believed to be the general mechanism of Al toxicity to the neurons. The characteristic features of the apoptosis induced by Al in neurons were reported as follows: shrinkage of cell body, over-concentrated and irregularly shaped chromatin, and extensive fragmentation of chromatin and DNA.

The apoptosis mechanisms induced by Al maltolate in aged rabbits were summarized in the following parts which can help to understand apoptotic mechanism in AD.

7.4.3.1 Mitochondrial Permeability Transition Pore Is Involved in Al-Induced Apoptosis

Following cytotoxic stimulation, the alterations in mitochondrial morphology and function represent a primary event in cell apoptosis. It was reported that Al can accumulate in neurons following cell depolarization and then inhibited Na^+/Ca^{2+} exchange leading to an excessive accumulation of mitochondrial Ca^{2+} [48]. The increase of intramitochondrial Ca^{2+} levels is a trigger of the following events: cytochrome *c* release, an opening of the mitochondrial permeability transition pore (MTP), and subsequent apoptosis resulting from activation of the caspase family of proteases. Cytochrome *c* released from the mitochondria into the cytoplasm has been believed to be related to three different pathways: (1) opening of the mitochondrial transition pore (MTP), (2) translocation of mitochondria of the pro-apoptogenic Bax which can form the channel by itself, and (3) interaction of Bax with the voltage-dependent anion channel (VDAC) to form a larger channel which is

permeable to cytochrome *c*. On the other side, Bcl-2, as an anti-apoptotic regulator, has the ability to block the release of cytochrome *c* from mitochondria into the cytoplasm, and the mechanisms such as a direct blockade of the MTP opening or functioning as a docking protein may be involved [49]. In addition, cyclosporin A is a specific inhibitor of the MTP opening, and some researches showed that this inhibitor can remarkably reduce the Al-induced cytochrome *c* release. In other words, the translocation of cytochrome *c* induced by Al from mitochondria to cytoplasm is by the way of opening of the MTP. The use of pharmacological agents that prevent or reverse the apoptotic effects of Al can provide valuable mechanistic information on the effects of Al on cellular protein targets. Incubation of human teratocarcinoma (NT2) precursor cells with Al maltolate resulted in strong evidence of apoptosis, presumably as a result of mitochondrial injury, since cytochrome *c* is released from mitochondria into the cytoplasm [50]. A study in Al-treated rabbits displayed that the glial cell-line-derived neurotrophic factor (GDNF) protects rabbit hippocampus from the neurotoxic damages of Al to certain degrees, but the release of cytochrome c which is the trigger of Al-induced apoptosis is not prevented. This interesting result may attribute to an anti-apoptotic protein, Bcl-XL, which when overexpressed has the power to control Apaf-1 resulting to inhibition of Apaf-1-dependent caspase-9 activation. In above study, GDNF treatment increases the level of the Bcl-XL. A summary from Savory et al. [29] shows that chronic treatment of rabbits with lithium in the drinking water results in increased levels of the anti-apoptotic proteins Bcl-XL and Bcl-2, inhibition of the Al-induced cytochrome c release, decreased levels of the pro-apoptotic protein Bax, and inhibition of caspase-3 activation [51–53] and DNA fragmentation [54, 55] as observed in AD.

7.4.3.2 The Endoplasmic Reticulum Plays a Role in Al-Induced Apoptosis

Although opening of the MTP may be a precondition for the neuronal cell apoptosis induced by Al, studies from Savory et al. [11] provided evidence supporting the opinion that the endoplasmic reticulum also is an important cell organelle in monitoring neuron death. The endoplasmic reticulum is the major storage location of calcium and the container of members of the *Bcl-2* family of proteins, such as Bcl-2, Bcl-XL, and Bax. The results showed that Al maltolate induces a redistribution of the apoptosis regulatory proteins, with Bax being present at higher levels in the endoplasmic reticulum than in the cytosol and decreased amounts of Bcl-2 in the endoplasmic reticulum [53]. Another study showed that the endoplasmic reticulum stress induced by Al was by the way of the activation of *gadd 153* which is specifically activated by agents that perturb endoplasmic reticulum function [52]. In vivo, Al may disturb Ca^{2+} homeostasis or protein processing in the endoplasmic reticulum, leading to apoptotic cell death. As the apoptosis regulatory proteins redistributed by Al, Bax presents at higher levels in the ER than in the cytosol, and Bcl-2 displays at decreased amounts in the ER.

Although the effect of Al on ER function has made some progress, the signaling mechanisms remain unclear. Which signal molecules are aimed by Al maltolate

leading to perturbation of ER homeostasis? The Ca^{2+} might be one of these initiators for apoptotic cell death induced by Al [11].

7.5 Summary

The understanding of AD neurochemistry and neuropathology is a big challenge due to unavailability of a suitable animal model, which mimics AD pathology. In this chapter, we reviewed the reasons why Al-maltolate-treated rabbits is a promising animal model of AD, including the difference of using various Al compounds treated to various animal groups and the similar features in Al-maltolate-treated rabbits with AD patients. We also focused on the similarities and dissimilarities between Al-induced neurofibrillary degeneration and paired helical filaments from AD.

Al-maltolate-treated aged rabbits might act as a reliable and efficient system in sharing a common mechanism with the development of neurodegeneration in Alzheimer's disease, and this model should continue to be of value as more mechanistic schemes are uncovered. Additionally, the model could also be of considerable value in the identification of early diagnostic markers and the development of preventative and therapeutic strategies for Alzheimer's disease.

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Chapter 8
Aluminum-Induced Neural Cell Death

Qinli Zhang

Abstract Aluminum (Al), an abundant element in the earth's crust, is well-known for its neurotoxicity. Nonetheless, its causal role in neurodegenerative diseases, particularly in Alzheimer's disease (AD), is still in debate. Ample studies have shown that neural cell death and cognitive deficits induced by Al are similar to those in AD. In the present chapter, we demonstrate separately the Al-induced cell death in neuron, neuroglia cells, and co-cultured neural cells from newborn rats to illustrate the neurotoxic effects. Moreover, we not only examine the classic cell death pathways of apoptosis and necrosis but also compare with autophagy and a newly discovered cell death pathway known as necroptosis, which demonstrates its crucial roles in Al-induced neural cell death. Finally, we verify the cell death pathways attributed to the neural cell death in Al-induced AD-like mice model. The series research could provide an underlined mechanism and potential therapeutic agents to Al-induced neurodegenerative diseases.

Keywords Aluminum · Neural cells · Cell death · Apoptosis · Autophagy · Necroptosis

8.1 Introduction

Aluminum (Al) is the third most common element, comprising about 8% of the Earth's crust, exceeded only by oxygen and silicon. The widespread use of products made from or containing Al is ensuring its presence in our body, which has gained considerable attention due to its neurotoxic effects [1, 2], and has been linked etiologically and epidemiologically to several neurological disorders, including Alzheimer's disease (AD) [3–5], Parkinson's disease (PD) [6], Guamanian–Parkinsonian complex, and amyotrophic lateral sclerosis (ALS) [7]. Neural cell loss is the major

Q. Zhang (✉)
Shanxi Medical University, Taiyuan, China

University of Mississippi Medical Center, Jackson, MS, USA

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characteristic of neurodegenerative diseases [8], which is also an effect of Al neurotoxicity; they are both linked to neural cell death. Therefore, defining the mechanisms governing neural cell death might lead to the discovery of therapeutic agents and targets and provide a richer understanding of neurodegenerative diseases.

In the present chapter, we aim to investigate the Al-induced neural cell death. Firstly, we demonstrate Al-induced cell death in neuron, neuroglia cells, and co-cultured neural cells separately to illustrate the neurotoxic effects. Apart from toxic effects of Al on neurons, those on neuroglia and co-cultured neural cells from newborn rats are also considered due to their critical roles in several aspects of signal transmission as well as synaptic plasticity. Deficits in various neural cells and their dysfunctions may lead to neurodegeneration.

Secondly, we test the modes of Al-induced cell death by using signal pathway inhibitors. We not only examine the classic cell death pathways of apoptosis and necrosis but also compare the cell death effects with that of autophagy and explore a newly discovered cell death pathway in Al-induced neural cell death, known as necroptosis.

Finally, we verify the cell death pathways in Al-treated AD-like mice models. In vivo experiments are significant for Al neurotoxicity research; it could provide an underlined mechanism in Al-induced neural cell death and furthermore give potential therapeutic strategies to neurodegenerative diseases.

8.2 Aluminum-Induced Neural Cell Death

Currently, the definite mechanism of Al-induced neural cell death is not known, though it is suggested as one of the major pathological characteristics of neurodegenerative diseases. How does Al induce neurotoxic effects and which type of neural cells is damaged are still highly controversial.

Rodella L found that Al did not cause neuron loss or apoptosis in the cerebral cortex [9], while Fu HJ insisted that Al induced the apoptosis of cultured cortical neurons qualitatively and quantitatively in a dose-dependent manner [10]. Also there was a report that Al-induced neurotoxicity had an indirect effect mediated by astrocytes rather than a direct effect on neurons [11]. But Brenner S [12] and Ghribi O [13] argued that Al did induce neuron apoptosis, and there were several apoptotic events such as oxidative stress, release of calcium and cytochrome C, and activation of caspases. Moreover, the effective dosages were various in different studies. Liang and Guo's study showed that exposure to Al at low levels (100 μM or 200 μM) for up to 6 days did not result in the apoptosis of astrocytes, but only at the concentration of 400 μM could Al cause significant cell death [14]. Aremu DA reported a lower dose of Al (0.0125 mM) might induce astrocytes apoptosis [15]. While Lankoff A revealed 1–25 mg/ml Al could induce DNA damage in a dose-dependent manner, but at the dose of 25 mg/ml, the level of damage declined [16].

Though neurons are the main functions of brain units, the toxic effects of Al on neuroglia cells cannot be ignored because they constitute more than 50% of the

total cell count and outnumber neurons tenfold [14, 15]. Instead of being considered to provide only mere passive support to neurons, neuroglia cells assist neurons in the overall function of the nervous system. It is now known that various neural cells play critical roles in signal transmission as well as synaptic plasticity; defects in these functions may lead to neurodegeneration. Therefore, the present study addresses Al-induced neural cell death not only in primarily cultured neurons alone but also in cultured neuroglia cells alone and co-cultured neural cells from newborn rats.

8.2.1 Methods

To investigate the neural cell death induced by Al, we had cultured primary cortical neurons, neuroglia cells, and co-cultured neural cells obtained from newborn rats, respectively; then treated them with Al at final concentrations of 0, 0.5, 1.0, and 2.0 mM; and cultured with Al for additional 72 h. Golden standards of transmission electron microscopy (TEM) and scanning electron microscopy (SEM) for evaluating neural cell death were used to observe the characteristic morphology alternations. Acridine orange-ethidium bromide (AO-EB) staining was performed to examine morphological changes of nuclei. Flow cytometry of annexin V-PI was performed to quantify the apoptotic and necrotic neural cells; in situ cell death detection kit (TUNEL staining) was used to determine the apoptotic neural cell death in rat cortical cells cultured with Al.

8.2.2 Results

Morphology, as a publicly recognized evaluating method for apoptosis, was examined to distinguish apoptosis and necrosis in the present study. Cellular structure, nuclei appearance, and ultrastructure of cortical neural cells were observed under light microscope, fluorescent microscope, and electronic microscope.

8.2.2.1 Cellular Morphology in Al-Treated Neural Cells

Cellular morphology and survival status in cultured cortical cells were assessed by observation under inverted phase microscope (Fig. 8.1). After Al treatment, cell processes retracted and intercellular junction reduced, which were associated with a significant decrease in the number of surviving cells. There was an observable effect on neurons after Al treatment, the round or oval shape of nuclei (aI-aII) were fragmented (aIII), and apoptotic bodies formed (aIV) with the increment of Al concentrations under light phase microscope. However, in Al-treated glial cells, there were no typical morphological changes in the nuclei (bI-bIV) and there was no

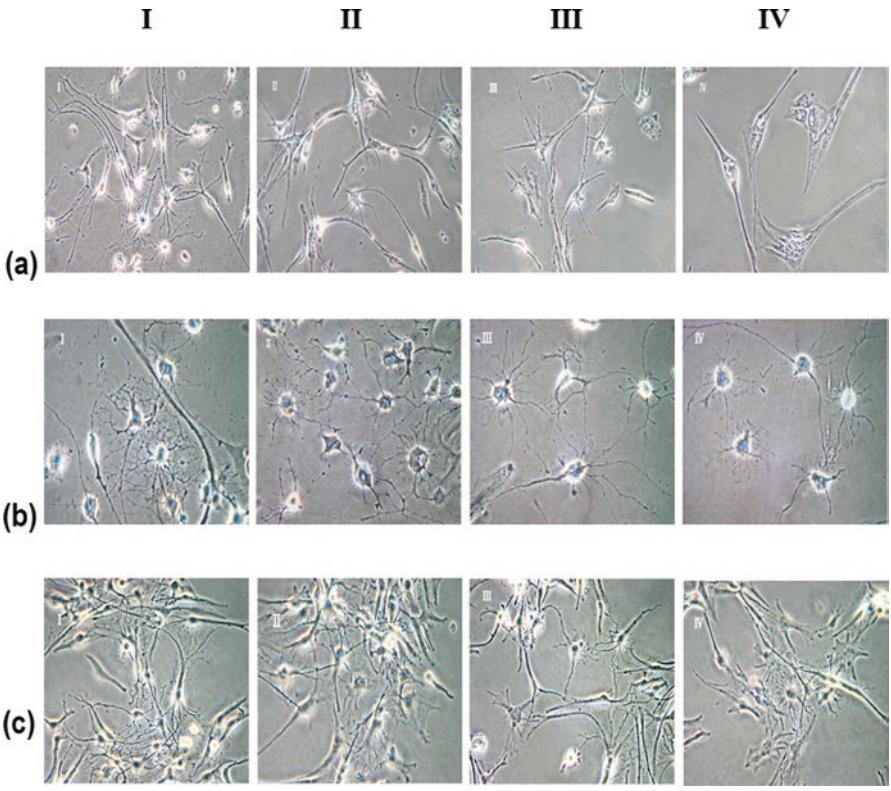

Fig. 8.1 Morphology of primarily cultured cortical cells treated with Al (×400). (**a**) Neurons, (**b**) neuroglial cells, (**c**) co-cultured neural cells (*I*, control; *II*, 0.5 mM Al3+; *III*, 1.0 mM Al3+; *IV*, 2.0 mM Al3+). Confluent neural cells were exposed to Al as described previously for 72 h and observed under light phase microscope. In Al-treated neurons, the round or oval shape of nuclei (aI-aII) was fragmented (aIII), and apoptotic bodies formed (aIV). However, in Al-treated glial cells, there were no typical morphological changes in the nuclei (bI-bIV). In Al-treated co-cultured neural cells, a few hyper-condensed chromatins and shrunken nuclei were observed (cI-cIV)

hyper-condensed or irregularly shaped or extensive chromatin fragmentation found in most nuclei of Al-treated astrocytes. In co-cultured neural cells, a few hyper-condensed chromatins and shrunken nuclei were observed (cI-cIV).

8.2.2.2 Nuclei Morphology in Al-Treated Neural Cells

To examine morphological changes of nuclei, AO-EB staining was performed (Fig. 8.2). In the control group, normal nuclei showed a bright green color with regular contours which were round and large in size (aI). The nuclei of Al-treated neurons appeared shrunken and irregular with aggregation and fragmentation of chromatin (aII-aIII). Finally, apoptotic bodies formed and released in the 2.0 mM Al

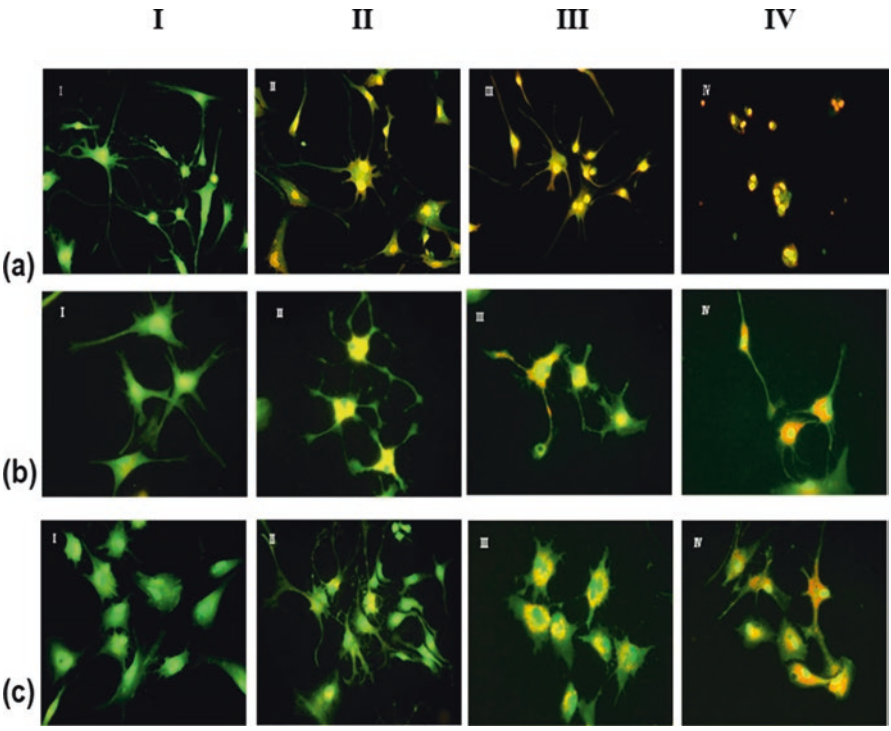

Fig. 8.2 AO-EB staining of primarily cultured cortical cells treated with Al (×400). (**a**) Neurons, (**b**) neuroglial cells, (**c**) co-cultured neural cells (*I*, control; *II*, 0.5 mM Al3+; *III*, 1.0 mM Al3+; *IV*, 2.0 mM Al3+). Being stained with AO-EB and observed under fluorescent microscope, the Al-treated neurons showed characteristic shrinkage and degeneration of nuclei (aII-aIV) but not in the controls (aI). However, no chromatin condensation or fragmentation was found in the neuroglial cells (bII-bIV) nor did the nuclei of co-cultured neural cells (cI-cIV)

group (aIV). However, the nuclei of glial cells showed no condensation and fragmentation (bI-bIII). Finally, the cells died and were dyed red (bIV). Although a little condensation (cIII) and fragmentation (cIV) occurred in co-cultured neural cells, no cell disruption or apoptotic bodies appeared.

8.2.2.3 Apoptosis Detection by TUNEL

Neurons treated with Al exhibited DNA damage, a key marker of apoptosis, as indicated by TUNEL-positive nuclei (Fig. 8.3). Neurons were observed following 72 h incubation in control medium; it showed nearly no green spots that represent TUNEL-positive cells (I). A few TUNEL-positive neurons were viewed at 0.5 mM Al3+ group (II). However, at 1.0 mM Al3+ group, more positive cells appeared (III). The number of TUNEL-positive neurons increased greatly at 2.0 mM Al3+ group (IV). Histogram demonstrated a significant increment of apoptotic rate in neurons treated with Al at the various concentrations compared with the controls.

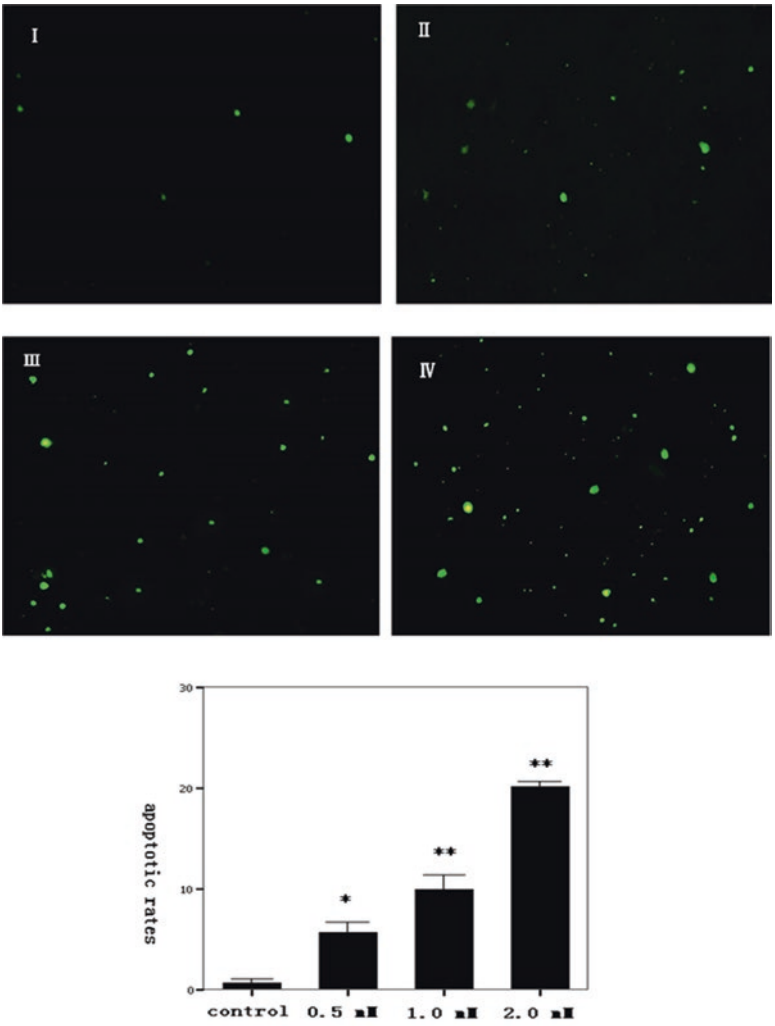

Fig. 8.3 TUNEL detection in primarily cultured neurons treated by Al (×400). *I*, control; *II*, 0.5 mM Al3+; *III*, 1.0 mM Al3+; *IV*, 2.0 mM Al3+. Histogram showed apoptotic rates detected by TUNEL assay (* and ** indicate statistical differences from control at $P < 0.05$ and $P < 0.01$)

8.2.2.4 Ultrastructure of Al-Treated Neurons

Chromatin condensation and nuclear fragmentation were further confirmed by TEM (Fig. 8.4). Al-treated neurons contained nuclei with hyper-condensed chromatin and cap-like chromatin margination (I). In addition, swelling of mitochondria and endoplasmic reticulum was demonstrated in the cells (II), and there was a mixture of nuclear fragments and ruptured organelles in the cytoplasm of apoptotic cells (III).

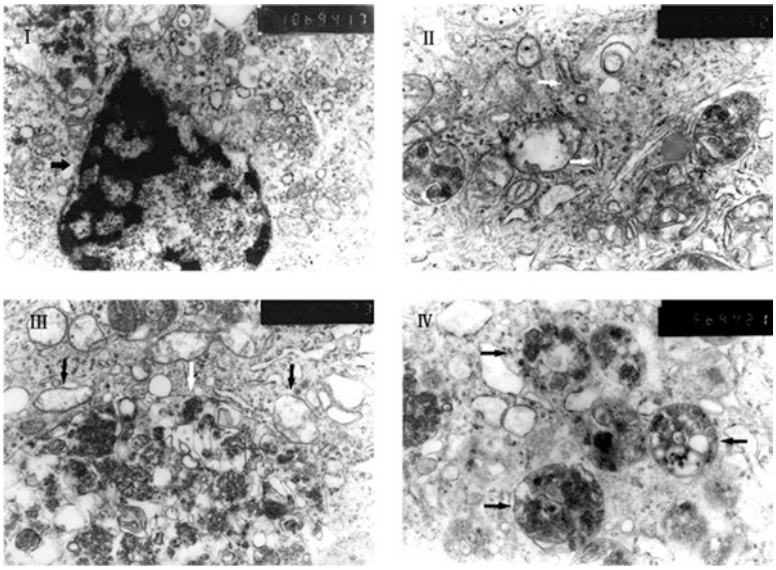

Fig. 8.4 Transmission electron micrographs of primarily cultured neurons treated by Al (*I*, ×8000; *II*, ×15,000; *III*, ×15,000; *IV*, ×15,000). Electron microscopy confirmed apoptotic characteristics such as chromatin condensation, swelling of MI and ER, and organelle disruption and showed apoptotic bodies in Al-treated neurons. (cited from: Zhang et al. [17])

Many of the separated nuclear fragments were surrounded by integrated membrane forming apoptotic bodies (IV). SEM, different from TEM, described the cell surface and gave information about shape modifications and membrane specializations with a relatively low detailed resolution. As shown in Fig. 8.5, nuclei of neurons in the control group appeared healthy and round, fully enclosed within the nuclear membrane (I). In Al-treated groups, the cells were shrunken and there were buds on the surface of the cells (II), followed by breakdown in cell membrane integrity (III) with the subsequent release of apoptotic bodies (IV).

8.2.2.5 Cell Death Rates Detected by Flow Cytometry

To measure the apoptotic rate of cultured cortical cells quantitatively, flow cytometry was used as described above. Plots of intensity of annexin V and cell counts showed a correlation between increased intensity of annexin V fluorescence in neurons and increment of Al concentration, accompanied by a decrease in the number of surviving cells. The figures show the percentage of apoptosis in neurons and astrocytes (Fig. 8.6). As shown in Fig. 8.6a, there was a significant increment of apoptotic rates in Al-treated neurons compared with that in the controls ($F = 4.775$, $P < 0.05$). Statistical analysis showed that there was a strongly positive correlation between Al concentration and early apoptotic rates ($r = 0.878$, $P < 0.01$) and total

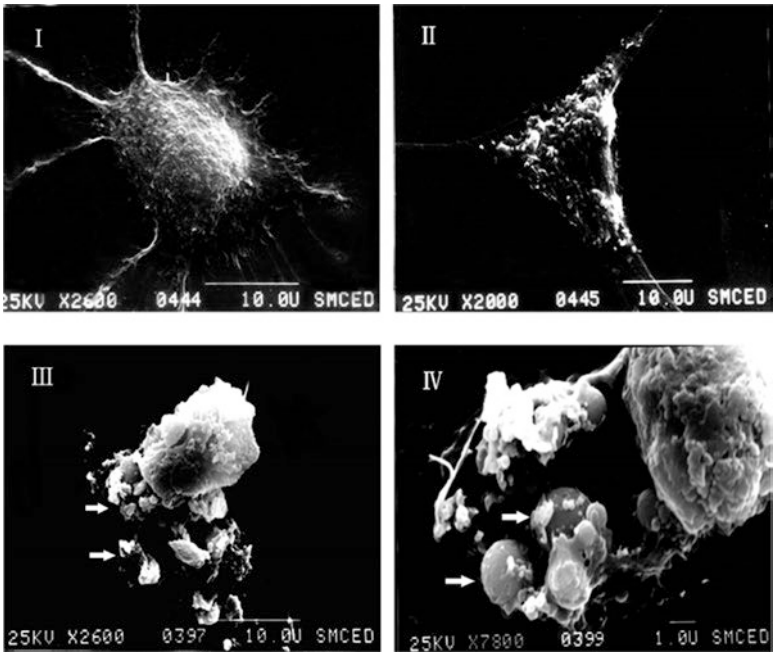

Fig. 8.5 Scanning electron micrographs of cultured neurons treated by Al (I, ×2600; II, ×2000; *III*, ×2600; *IV*, ×7800). Scanning electron microscopy confirmed apoptotic characteristics such as budding in cell surface, apoptotic bodies formed and released in Al-treated neurons. (cited from: Zhang et al. [17])

cell death rates ($r = 0.976$, $P < 0.01$). Although there was a slight elevation of early apoptotic rate of the astrocytes treated with Al, no significant difference could be found compared with that of the control cells by ANOVA test ($F = 1.793$, $P > 0.05$). However, there was a significant difference in necrotic rates and total cell death rates between those in the astrocytes treated with 2.0 mM Al and the controls ($t = 3.813$, $P < 0.05$ and $t = 4.026$, $P < 0.01$, respectively, Fig. 8.6b). In Al-treated neuron/astrocyte co-cultures, there were significant differences in early apoptotic rate, necrotic rate, and total cell death rate at 2.0 mM group ($F = 3.942$, $P < 0.05$; $F = 3.731$, $P < 0.05$; $F = 4.536$, $P < 0.01$, Fig. 8.6c).

8.2.3 Discussion

Al as an environmentally abundant non-redox trivalent cation has long been implicated in the pathogenesis of AD [18, 19]. However, the definite mechanism of Al toxicity in these diseases is not known. Ample studies have shown that cell death induced by Al is similar to that of AD [13, 20]. Research had also reported similar glial and neuronal cell damage in the selective brain regions of associated cortex and hippocampus in Al-treated rats and in patients with AD [13]. Based on

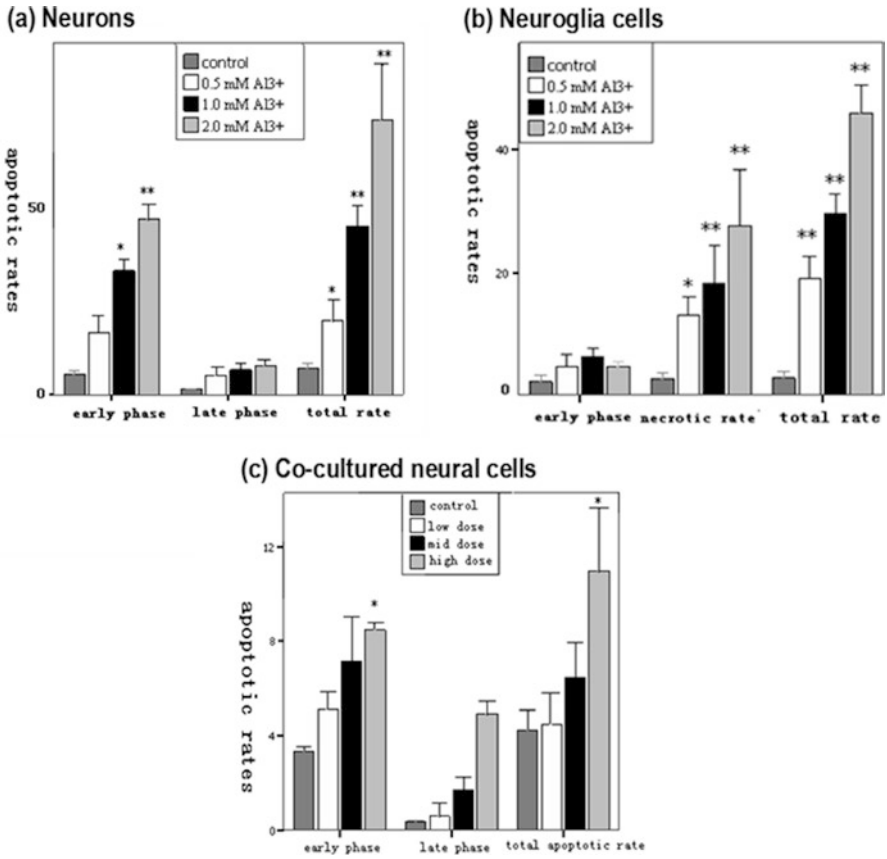

Fig. 8.6 Rates of apoptosis and necrosis examined by annexin V using flow cytometry in cultured cortical cells treated by Al. Figures show the apoptotic rates of cultured cortical cells treated with 0, 0.5, 1.0, and 2.0 mM AlCl3 for 72 h, respectively. The data showed that apoptotic rates in neurons upgrade significantly with increasing Al concentration (**a**). There were significant increases in necrotic rates and total cell death rates of Al-treated astrocytes (**b**). In Al-treated neuron/astrocyte co-cultures, the extent of the increase of apoptotic rates was not as high as that of neurons (**c**). Results are representative of four similar experiments. (cited from: Zhang et al. [17])

extensive literatures, the neurotoxic effects of Al are indisputable, and Al as a factor in AD development cannot be discarded [21].

The present study focused on the mode of Al-treated cell death not only in neurons cultured alone but also in neuroglia cells cultured alone and co-cultured neural cells. Cell shrinkage, membrane buds, and formation of membrane-bound apoptotic bodies in Al-treated neurons were observed under an inverted phase microscope, while no typical morphological characteristics in Al-treated glial cells and less typical apoptotic changes were viewed in neuroglia and co-cultured neural cells (Fig. 8.1). Under a fluorescent microscope, the apoptotic characteristics in the Al-treated neurons, such as typical cell shrinkage, cell membrane integrity loss,

chromatin condensation and fragmentation, and apoptotic bodies formed and released were shown (Fig. 8.2). TUNEL detection (Fig. 8.3) and electron micrographs (Figs. 8.4 and 8.5) further confirmed the apoptosis occurred in primarily cultured neurons treated by Al. However, neuroglia cells treated with Al only showed structure normal nuclei and cell swelling under a light and a fluorescent microscope. In the co-cultures, only a few cortical cells displayed chromatin condensation and fragmentation. To some extent, it was milder compared with that of pure neurons under the same Al environment. Apoptotic rates based on AO-EB positive percentage and apoptotic rates detected by flow cytometry had confirmed the morphological features.

Standard flow cytometry (FCM), as a well-established assay for apoptosis, offers the advantage over microscopy to acquire large numbers of events, thereby providing with a strong foundation for statistical analysis. The present results showed that early and total apoptotic rates of the Al-treated neurons were significantly higher than those of the controls, which confirmed the morphological evidences for Al-induced neuron apoptosis (Fig. 8.6). As to the apoptotic rates of the neuroglia cells, though the early apoptotic rates were slightly higher as Al-treated concentration increased, there was no significant difference between the Al-treated groups and the controls. However, there were significant differences in necrotic rates and total cell death rates between the 2.0 mM Al-treated neuroglia cells and the controls. When co-cultured neural cells were treated with Al, the apoptotic reaction of the neural cells became much milder. That is to say, Al could induce neuron apoptosis, and there was a dose-dependent relationship between the Al concentrations and the apoptotic rates, while necrosis is the major pathway of neuroglia cell death when treated with Al.

Though there are conflicting reports, the present study demonstrates that Al is capable of committing neurons to death via apoptosis in a dose-dependent manner, which consists of researches by Brenner S, Ghribi O, and Fu HJ [10, 12, 13]. Furthermore, since Al is a nonessential element in human survival, and Al content in the human brain is lower, the present study, therefore, focuses on the lower exposure of Al on cultured cortical cells. More and more researches focus on the effects of lower Al exposure [16, 22], which play more important roles in the development of Al-related diseases.

In the present work, we tried to explain some new aspects of glia–neuron interaction and discuss the implications of Al-impaired neuroglial functions on neurodegeneration. It is now known that, rather than being a mere supporter of neurons, neuroglia cells are actively involved in their modulation, and the considerable attention should be given to neuroglia cells in view of the likely implications of environmental toxicants such as Al. As shown in the paper, neurons are condemned to death by apoptosis and neuroglia cells by necrosis, but to the co-cultured cortical neurons, even there is apoptosis, the extent is milder. Therefore, loss of neuroglial regulatory and supportive roles in central nervous system (CNS) may be responsible for Al-induced neurodegeneration apart from deficits in neurons. Moreover, it is probable that neuroglia cell death precedes that of neurons in neurodegeneration, but this may be obscured by the ability of neuroglia cells to proliferate and hence replace the lost ones.

8.3 Necroptosis in Aluminum-Induced Neural Cell Death

Al is an environmentally abundant, but not essential, element in the human body. Its accumulation in brain tissue is claimed to play a role in several neurodegenerative diseases, such as dialysis encephalopathy, amyotrophic lateral sclerosis, Parkinsonism dementia, and Alzheimer's disease (AD) [20, 23, 24]. Although this association remains in debate, there is no doubt that Al induces neural cell death in vivo as well as in vitro [25]. With various pathways in cell death becoming well known, whether they are involved or may interact with one another in neural cell death cascade is just beginning to be understood. Though a dose-dependent relationship between Al content and cell death rate has been observed, the mechanism of cell death induced by Al is not clarified [20].

Involvement of apoptosis and alternative pathways of cell death induced by Al in AD and other neurodegenerative disorders has been discussed for a long time [26, 27]. Apoptotic DNA fragmentation in human brain as a sign of neuronal injury was thought to contribute to continuous neuron loss in these slowly progressive processes [28]. However, it is difficult to assess how much the neural cell apoptosis has contributed to neural cell loss, because of the chronic nature of the disease process in which only a limited number of apoptotic neurons can be detected at any time point. Some neurons exhibit morphological features of apoptosis, but many degenerative neurons do not show evidence of apoptosis, suggesting that apoptosis might not be the only mechanism of degeneration in AD [28].

Indeed, there is abundant evidence that necrotic cell death plays a prominent role in a wide range of human pathological conditions, such as myocardial infarctions and acute and chronic neurodegeneration [28, 29]. Recently, necroptosis, an alternative regulated necrotic cell death, was reported to be involved in developing neurological disorders [30]. Furthermore, screened from 15,000 compounds, one compound, necrostatin-1 (Nec-1, [2,3-dihydroxypropyl-5-bromo-N- (2-methyl-3-trifluoromethyl phenyl) anthranilate]), was identified to inhibit necroptosis specifically [31]. The data showed that necroptosis is characterized by morphologically necrotic cell death and activation of autophagy [31]. Several assays of necrotic cell death were performed, including tests of ATP levels, mitochondrial permeability, plasma membrane permeability, cell proliferation, and morphological analyses. Nec-1 also inhibited autophagy that occurred following necroptosis, but did not show effect on autophagy that takes place independently without necroptosis, suggesting that Nec-1 acts only on necroptosis and fairly early in the sequence of "necroptotic" events [31].

In order to investigate whether necroptosis is involved in Al-induced neural cell death, SH-SY5Y cell line, a type of human neuroblastoma cells, was cultured. Nec-1, a specific inhibitor for necroptosis, was then added into the culture media at different concentrations. The assays related to cell death were performed to detect the effect of the inhibitor on Al-induced cell death and in turn to elucidate whether necroptosis is involved in Al-induced neural cell death.

8.3.1 Methods

Neuroblastoma (SH-SY5Y) cells were cultured, and Al treatment was prepared by dissolving AlCl3·6H2O in triple-distilled water at desired concentrations. The influence of Nec-1 in the observed toxicity was examined using cell cultures treated with Nec-1 diluted in dimethyl sulfoxide (DMSO) at final concentrations of 0–90 μM and in turn to extrapolate the existence of necroptosis. All solutions were sterilized using 0.22 nm syringe filters immediately after preparation. Hoechst–PI staining was used to examine the status of apoptosis and/or necrosis with a fluorescent microscope following the cells' staining with Hoechst 33258 (H33258) and propidium iodide (PI). Cytometry assay was performed to quantify the cell death rate using annexin V-FITC apoptosis detection kit. Microtubule-associated protein 1 light chain 3 (LC3) is used to determine the expression of autophagy marker protein, and TEM was used to observe autophagosomes under an electronic microscope.

8.3.2 Results

8.3.2.1 Morphology and Cell Viability of SH-SY5Y Cells Treated with Al and Nec-1

SH-SY5Y cell cultures (negative control, Fig. 8.7a) appeared healthy and round with rich synapse and integrity membrane. While in the cells treated with 2 mM and 4 mM Al (b, c), there were many swelling cells combined with nuclei disruption and even disappearance (indicating necrosis) and shrunken cells with condensed nuclei (indicating apoptosis), with the administration of Nec-1 (d), the cell swelling and shrinkage reduced greatly and the cell number increased and cell synapse junction regenerated as well, which demonstrated Nec-1-dependent amelioration in cellular survival status and proliferative levels. Cell viability was consistent with the morphology of the cells treated with 0–8 mM Al and Nec-1 (60 μM) and is presented in Table 8.1. Data in SH-SY5Y cells treated with 0–8 mM Al indicated that the cell viability declined with the increment of Al concentration ($P < 0.05$), while Nec-1 (60 μM) could enhance the cell viability significantly in Al-treated SH-SY5Y cells ($P < 0.05$, $P < 0.01$). To confirm the role of Nec-1 in Al-treated cells, 4 mM Al-treated cells were administered with Nec-1 at the concentration of 0–90 μM (Table 8.2). The data showed the enhancement of cell viability with the increment of Nec-1 concentration ($p < 0.05$, $p < 0.01$).

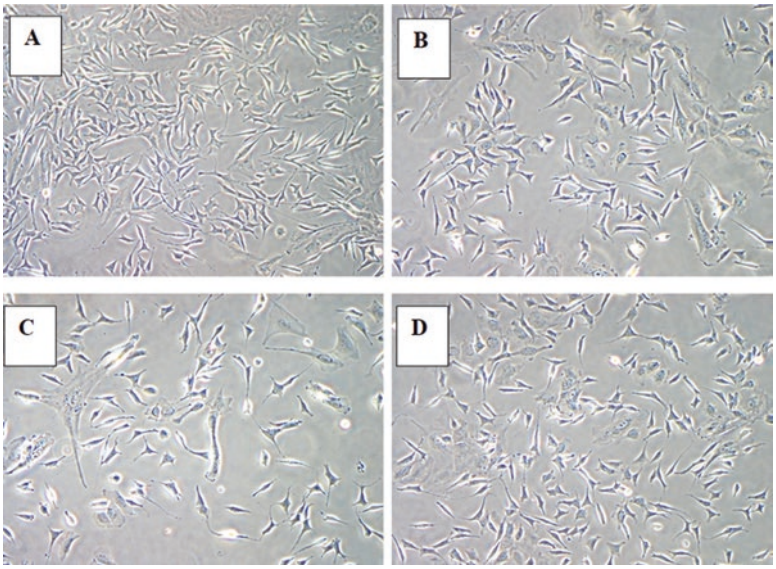

Fig. 8.7 Morphology of SH-SY5Y cells treated with Al and Nec-1 (×200). Morphology of the SH-SY5Y cells treated by Al and Nec-1 shows the Nec-1-dependent amelioration in cellular survival status and proliferative levels. (**a**) Morphology of normal control cells, (**b**) morphology of cells treated with 2 mM Al, (**c**) morphology of cells treated with 4 mM Al, (**d**) morphology of cells treated with 4 mM Al and 60 μM Nec-1. (cited from: Zhang et al. [32])

Table 8.1 Cell viabilities of SH-SY5Y cells treated with Al alone and Al plus Nec-1

	Control	2 mM	4 mM	8 mM
DMSO	0.59 ± 0.02	0.39 ± 0.08*	0.27 ± 0.04*	0.20 ± 0.01*
Nec-1	0.48 ± 0.04	0.95 ± 0.05**	0.78 ± 0.06**	0.61 ± 0.05*

Values are expressed as mean ± SD
Statistical significance by ANOVA F test = *: $P < 0.05$; **: $P < 0.01$ (compared to controls)

Table 8.2 Cell viabilities of SH-SY5Y cells treated with 4 mM Al and Nec-1

	Nec-1 concentration			
	DMSO control	30 μM	60 μM	90 μM
Cell viability	0.28 ± 0.05	0.58 ± 0.03*	0.68 ± 0.04*	1.03 ± 0.17**

Values are expressed as mean ± SD
Statistical significance by ANOVA F test = *: $P < 0.05$; **: $P < 0.01$ (compared to DMSO control)

8.3.2.2 Apoptotic Rate and Necrotic Rate Assay

Apoptosis and necrosis were determined using annexin V-PI staining and flow cytometry. The morphological changes can be visualized with H33258-PI fluorescent double staining. Under fluorescent microscope, remarkable condensation of cellular chromatin shown as condensed blue dots, which indicates typical apoptosis,

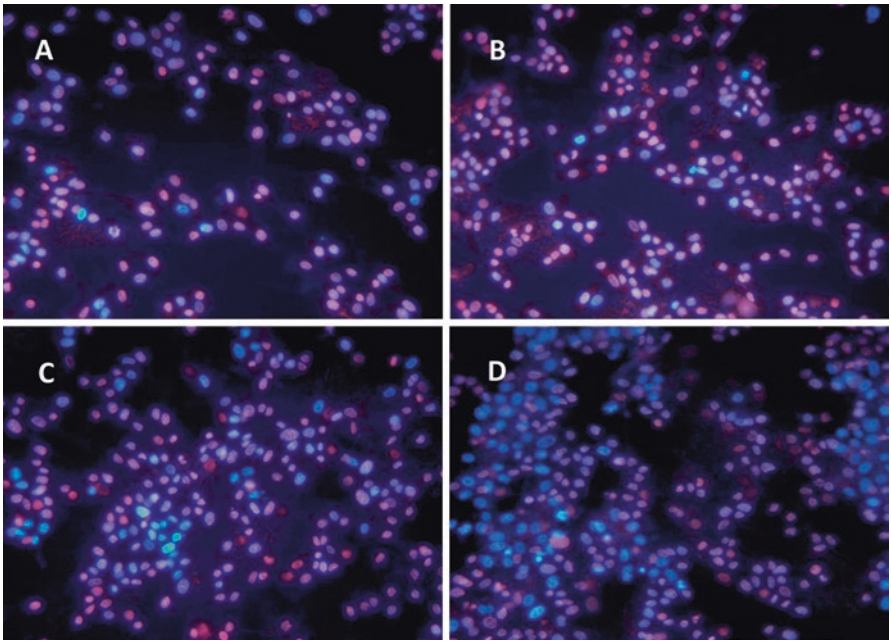

Fig. 8.8 Morphology of SH-SY5Y cells treated with 4 mM Al and Nec-1 (H33258-PI double staining, ×200). H33258-PI double staining confirmed that Nec-1 could extraordinarily decrease the number of PI-labeled necrotic cells in the cell death induced by 4 mM Al. (A) SH-SY5Y cells treated with 4 mM Al. (**b**) SH-SY5Y cells treated with 4 mM Al and 30 μM Nec-1. (**c**) SH-SY5Y cells treated with 4 mM Al and 60 μM Nec-1. (**d**) SH-SY5Y cells treated with 4 mM Al and 90 μM Nec-1. (cited from: Zhang et al. [32])

Table 8.3 Apoptotic rates and necrotic rates of SH-SY5Y cells treated with Al

	Control	2 mM	4 mM	8 mM
Apoptotic rate (%)	1.42 ± 0.12	1.33 ± 0.16	6.28 ± 0.46*	13.94 ± 0.98**
Necrotic rate (%)	6.04 ± 0.36	12.72 ± 0.95*	17.96 ± 1.02**	29.19 ± 1.32**

* and ** indicate statistical differences between cell death rates of Al-treated cells and those of control cells at $P < 0.05$ and $P < 0.01$

was present in 4 mM Al-treated cells. In the meantime, a few PI-stained red or pink cells, which indicate cell death or necrosis, were also shown (Fig. 8.8a). With the enhancement of Nec-1 concentration, the number of cells stained red or pink with PI staining reduced, while the normal cells stained dark blue with Hoechst staining increased (b, c, d). Apoptotic rates and necrotic rates (Table 8.3), quantified by flow cytometer, were all significantly enhanced in SH-SY5Y cells treated with increment of Al concentration (0–8 mM) ($P < 0.05$, $P < 0.01$). To test the role of Nec-1 on cell death rates, the cells were treated with 4 mM Al and Nec-1 at 0–90 μM (Table 8.4), the necrotic rates were significantly decreased ($p < 0.05$, $p < 0.01$), while the apoptotic rates did not change significantly at the Nec-1 concentration of 0–60 μM ($p > 0.05$).

Table 8.4 Apoptotic rates and necrotic rates of cells treated with 4 mM Al and Nec-1

	Nec-1 concentration			
	DMSO control	30 μM	60 μM	90 μM
Apoptotic rate (%)	8.68 ± 0.36	7.66 ± 0.53	5.68 ± 0.41	4.13 ± 0.41
Necrotic rate (%)	16.46 ± 0.54	10.40 ± 0.64*	5.43 ± 0.68**	6.28 ± 0.35**

Values are expressed as mean ± SD
Statistical significance by ANOVA F test = *: $P < 0.05$; **: $P < 0.01$ (compared to DMSO control)

Table 8.5 MMPs and ROS of SH-SY5Y cells treated by Al

	Control	2 mM	4 mM	8 mM
MMPs (%)	99.98 ± 5.32	88.37 ± 6.36	76.00 ± 4.12*	30.86 ± 5.96**
ROS (%)	2.60 ± 0.36	25.99 ± 2.96*	56.66 ± 3.86**	64.54 ± 3.01**

Values are expressed as mean ± SD
Statistical significance by ANOVA F test = *: $P < 0.05$; **: $P < 0.01$ (compared to controls)

Table 8.6 MMPs and ROS of SH-SY5Y cells treated by 4 mM Al and Nec-1

	Nec-1 concentration			
	DMSO control	30 μM	60 μM	90 μM
MMPs (%)	67.54 ± 6.36	49.42 ± 5.96	84.79 ± 6.86*	95.51 ± 7.01*
ROS (%)	54.07 ± 3.32	52.79 ± 2.36	54.68 ± 1.91	59.23 ± 2.96

Values are expressed as mean ± SD
Statistical significance by ANOVA F test =*: $P < 0.05$ (compared to DMSO control)

For MMPs and ROS in Al- and Nec-1-treated SH-SY5Y cells as shown in Table 8.5, MMPs in SH-SY5Y cells treated with Al significantly decreased at the concentration of 4 mM and 8 mM ($p < 0.05$, $p < 0.01$), while ROS increased significantly at the concentration of 2 mM, 4 mM, and 8 mM ($p < 0.05$, $p < 0.01$). To test the role of Nec-1 on MMP and ROS, the 4 mM Al-treated cells were administered with Nec-1 (Table 8.6). With administration of Nec1, MMPs increased significantly ($p < 0.05$), while ROS remained similar at the concentration of 0–90 μM ($p > 0.05$).

8.3.2.3 Caspase Activity of Al- and Nec-1-Treated SH-SY5Y Cells

Caspase-3 activity increased significantly in 0–8 mM Al-treated cells; however, when the Al-treated cells were co-treated with 60 μM Nec-1, it increased significantly at 2 mM Al-treated cells and then decreased significantly at 4 mM and 8 mM Al-treated cells ($p < 0.05$), as shown in Table 8.7. The data show that caspase-8 activity increased significantly in 0–8 mM Al-treated cells, but it seemed unchanged in that of Al plus Nec-1-treated cells; there was a significant difference between the caspase-8 activity of Al plus Nec-1-treated cells and that of cells treated with 4 mM Al only ($p < 0.05$, $p < 0.01$). Besides, caspase-9 activity increased significantly in

Table 8.7 Caspase activity of Al- and Nec-1-treated SH-SY5Y cells

	Control		2 mM Al		4 mM Al		8 mM Al	
	DMSO	Nec-1	DMSO	Nec-1	DMSO	Nec-1	DMSO	Nec-1
Caspase-3	1.00	1.00	1.89 ± 0.3	7.59 ± 0.3*	3.03 ± 0.4	4.61 ± 0.1*	9.29 ± 0.3	5.05 ± 0.1**
Caspase-8	1.00	1.00	1.19 ± 0.1	0.52 ± 0.1*	2.95 ± 0.2	0.89 ± 0.1**	2.24 ± 0.1	0.47 ± 0.0**
Caspase-9	1.00	1.00	1.79 ± 0.3	3.66 ± 0.2	2.24 ± 0.4	1.78 ± 0.2	0.73 ± 0.1	0.48 ± 0.1

* and ** indicate statistical differences at $P < 0.05$ and $P < 0.01$ between caspase activities in Al-treated cells and that in Al plus Nec-1(60 μM) treated cells. The activities of caspase-3 and caspase-8 were significantly decreased, indicating that caspase-3 and caspase-8 were involved in the mechanism of Nec-1 treatment

2 mM Al and 60 μM Nec-1-treated cells ($p < 0.05$) and then decreased in 4 mM and 8 mM Al plus 60 μM Nec-1-treated cells. However, there was no significant difference between the activity of 4 mM Al plus 60 μM Nec-1, 8 mM Al plus 60 μM Nec-1-treated cells, and that of cells treated with 4 mM Al only ($p > 0.05$).

8.3.2.4 LC3 Expression of Al- and Nec-1-Treated SH-SY5Y Cells

Western blot bands of LC3 protein were presented in Fig. 8.9a. LC3 expression of the cells treated with 0–8 mM Al increased significantly ($p < 0.05$) as shown in Fig. 8.9b. To test the role of Nec-1 on LC3 protein, the 4 mM Al-treated cells were incubated with various concentrations of Nec-1. Data show that the LC3 expression of the cells treated with 4 mM Al decreased significantly with the Nec-1 concentration (0–90 μM) increasing as shown in Fig. 8.9c ($p < 0.05$, $p < 0.01$). The ultrastructure of 4 mM Al-treated cells is shown in Fig. 8.9d; there were a lot of autophagosomes as indicated by black arrows. However, the autophagosomes reduced sharply when the cells were treated with 4 mM Al plus 60 μM Nec-1 (Fig. 8.9e).

8.3.3 Discussion

Despite the concept that necrosis is an uncontrolled or default form of cell death, accumulating studies have suggested that this may not be true. With the development of better biochemistry and genetics tools, it is becoming clear that necrosis can be a regulated form of cell death independent of apoptosis. Recently, Degterev et al. described a necrosis-like death pathway, which they termed "necroptosis," and identified Nec-1 as chemical inhibitor of this pathway, although the biochemical basis for these alternative morphological forms of cell death remains largely unknown [30].

The human neuroblastoma cell line SH-SY5Y is widely used as an in vitro model system for human neuronal cells. In the present study, we employed the well-

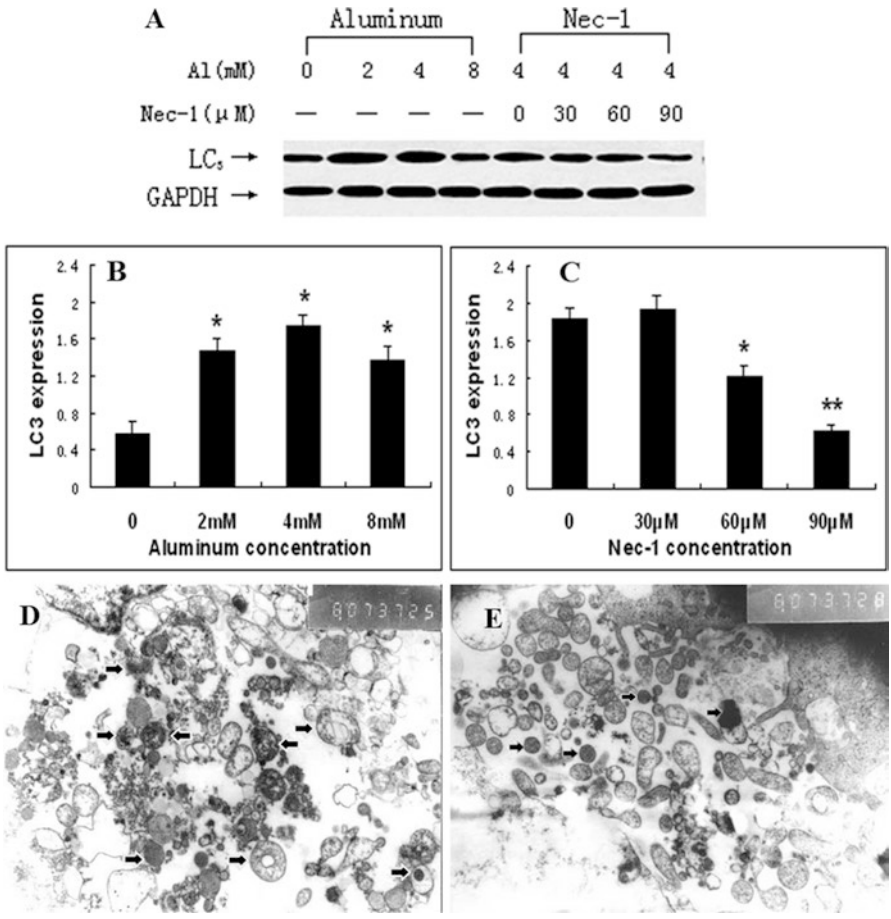

Fig. 8.9 LC3 expression and autophagosomes in cells treated with Al and Nec-1. Figures show the changes of subsequent autophagosomes and expression of their marker protein LC3 in cells treated with Al and Nec-1. The data shows that Nec-1 could significantly reduce the appearance of autophagosomes and decrease the expression of LC3. (**a**) Western blot bands of LC3 expression in 0–8 mM Al- and 0–90 μM Nec-1-treated cells. (**b**) LC3 expression in 0–8 mM Al-treated cells. (**c**) LC3 expression in 0–90 μM Nec-1- and 4 mM Al-treated cells. (**d**) TEM photograph of 4 mM Al-treated cells. (**e**) TEM photograph of 4 mM Al- and 60 μM Nec-1-treated cells. (cited from: Zhang et al. [32])

characterized SH-SY5Y cells treated with Al to find out if necroptosis is present in Al-induced neural cell death. Our results show that Nec-1 helped to ameliorate the necrosis-like changes in the Al-treated cells (Fig. 8.7). Furthermore, the cell viability assay confirmed Nec-1 concentration-dependent cell proliferation (Tables 8.1 and 8.2), which was consistent with the morphology of cells with H33258-PI double staining (Fig. 8.8). The results of cytometry showed that the increment of necrotic rates induced by Al could be depressed by Nec-1 (Tables 8.3 and 8.4) and so did the increment of MMPs, while ROS retained (Tables 8.5 and 8.6). The results were supported by the report of Degterev et al.'s [31].

Though necroptosis was reported as a caspase-independent cell death (22), the present data demonstrate that Nec-1 could take effect on caspase-3 activity through caspase-8 pathway. There was significant difference in caspase-8 activity between Al plus Nec-1-treated cells and that of cells treated with Al alone (Table 8.7). Besides, Nec-1 seemed to influence Al-induced autophagy as well, being expressed as reduced autophagosome numbers and lowered expression of its marker protein LC3 (Fig. 8.9).

It is also worth noticing that although efforts put forth on Nec-1 have not proposed to characterize cell death strictly in the precise terms of the measurable parameters, the effects of Nec-1 on inhibition of cell death and injury recovery have already been consistent in many areas of apoptosis inhibition [33], myocardial cell death and infarct size reduction [34], and cancer therapy [35, 36]. Therefore, the application of Nec-1 in our study not only suggests the existence of necroptosis in Al-induced cell death but also has physiological relevance and the potential for advancing therapeutic development. The stringent specificity of Nec-1 in inhibiting necroptosis prompted us to use it to explore the previously unknown role of necroptosis in vitro.

8.4 Al-Induced Neural Cell Loss and AD

Apoptosis and necrosis are the two major mechanisms of neuronal demise in the process of neurodegeneration. Apoptosis is a specific form of gene-directed programmed cell death (PCD), which removes unnecessary, aged, or damaged cells, and is characterized by distinct morphological and biochemical features. Necrosis, by contrast, is originally defined as a passive occurrence of cell death arising from spontaneous insults, e.g., stroke or trauma. Programed necrosis or necroptosis, a type of controlled cellular necrosis, has also been implicated in the process of neurodegeneration [37–39], but direct evidence has not been presented. We have reported previously that necroptosis is involved in Al-induced neuroblastoma cell death [32]. In this study, we try to elucidate whether necroptosis plays a critical role in Al-induced neurodegeneration. Given the similarity between neuroblastoma cells and neural cells, as well as the role of necroptosis in Al-induced neuroblastoma cell death, it is possible that necroptosis plays a critical role in Al-induced neural cell death. However, no studies have explicitly demonstrated that this is the case. Moreover, it is not known the biological effect of necrostatin-1 (Nec-1) as a specific inhibitor of necroptosis on neurodegeneration. It is our interest to investigate the inhibitory effect of Nec-1 on neurodegeneration and necroptosis in both in vitro and in vivo models.

From the historical coincidence of the first case report of AD with the boom of Al salt utilization as flocculation agent in drinking water treatment to the proven fact that, once absorbed, Al can be transported to the brain, there have been many attempts to hypothesis that a lifelong accumulation of Al in the brain may significantly contribute to the etiology of AD [40–42]. Furthermore, there is evidence that

Al is neurotoxic both in humans and in experimental animals. It has also been shown that Al salts administered intracerebrally or peripherally in rabbit, cat, mice, rat, and monkey induce the formation of neurofibrillary tangles, which are used as animal models of AD [27, 43–45]. In addition, animals chronically exposed to aluminum are associated with behavioral, neuropathological, and neurochemical impairments, among which neural cell death and deficits of learning and behavioral functions are the mostly evident [46]. This leads to a major conclusion that Al is one of the factors contributing to development of several neurodegenerative disorders, mainly AD. In the present study, Al-treated primary cultured neural cells and mice are used as neurodegenerative models, and the inhibitory effect of Nec-1 on Al-induced neurodegeneration has been investigated.

8.4.1 Methods

Al-exposed primary cultures from newborn mice cortical cells were separately treated with 3-methylamphetamine (3MA), benzyloxycarbonylvalyl-alanyl-aspartic acid (O-methyl)-fluoro-methylketone (zVAD-fmk), and Nec-1; the cell viability analysis was used to evaluate cell damage from apoptosis, necroptosis, and autophagy. Morphology of neural cells treated with 2 mM Al and 2 mM Al plus 60 M Nec-1 was examined by fluorescent microscope, and the cell death rates were quantified by cytometry. For the in vivo experiments, male ICR mice were microinjected with normal saline, 2 mM Al, and 2 mM Al plus Nec-1 at the concentrations of 2 mM, 4 mM, and 8 mM into the lateral cerebral ventricles. The Morris water maze task was performed in 20 days after intracerebroventricular injection, Nissl staining was used to demonstrate the loss of Nissl substance and the number of neural cells, and Western blot was used to analyze the expressing of cell death- and Alzheimer's disease-related proteins.

8.4.2 Results

8.4.2.1 Cell Viability Analysis In Vitro

The cell viability analysis was used to evaluate cell damage from apoptosis, necroptosis, and autophagy (Fig. 8.10). Various concentrations of zVAD-fmk, Nec-1, and 3MA were used to inhibit apoptosis, necroptosis, and autophagy, respectively, which were induced by Al. The results demonstrated a significant cell viability decrease induced by Al at the concentration range of 0–8 mM (A), based on which, 2 mM was selected as a standard Al treatment concentration. The viability of cells treated with 2 mM Al was significantly enhanced by Nec-1 at the concentration range of 0–135 M (B), zVAD-fmk at 0–160 M (C), and 3-MA at 0–3.5 mM (D). Based on the data, 60 M Nec-1, 100 M zVAD-fmk, and 2 mM 3-MA were chosen

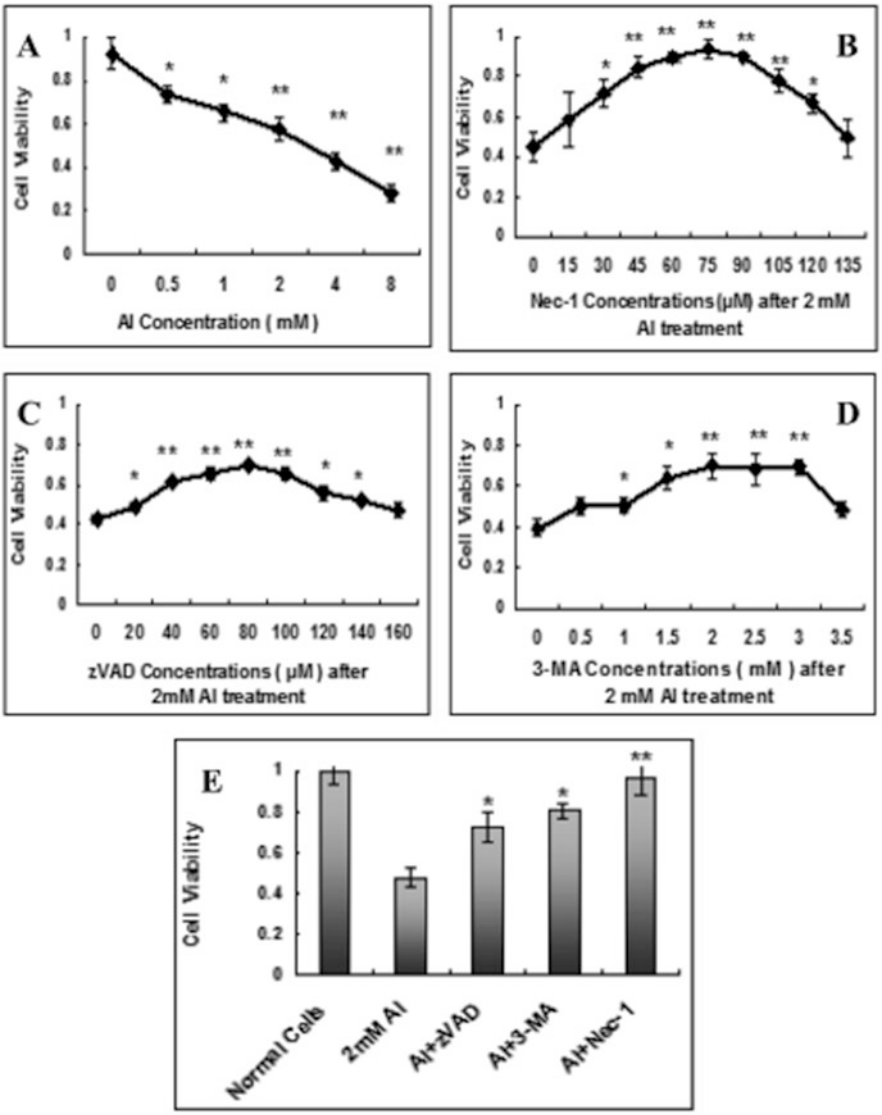

Fig. 8.10 Cell viability of Al-treated neural cells enhanced by Nec-1, zVAD, and 3-MA in vitro. Data demonstrated a significant decrease in cell viability of neural cells treated by Al at the concentration range of 0–8 mM (**a**), while the cell viability of 2 mM Al-treated neural cells increased significantly by administration of Nec-1 at the concentration range of 0–135 μM (**b**), zVAD at 0–160 μM (**c**), and 3-MA at 0–3.5 mM (**d**). The cell viabilities of neural cells treated by 2 mM Al plus Nec-1, zVAD, and 3-MA were compared with each other (**e**). *, compared with control, $P < 0.05$; **, $P < 0.01$. (cited from: Qinli et al. [47])

as the treatment dosages. The cell viabilities were compared among the groups (E). The results indicated that Nec-1, zVADfmk, and 3-MA enhanced cell viability significantly ($P < 0.05$, $P < 0.01$), of which Nec-1 improved cell viability to the greatest extent. Thus, Nec-1 played a dominant role in inhibiting Al-induced neural cell death and necroptosis.

8.4.2.2 Fluorescent Observation and Analysis on Neural Cell Death Rates In Vitro

Primary cultured neural cells were treated with 2 mM Al for induction of cell death and Nec-1 for inhibition of necroptosis (Fig. 8.11). Morphology of neural cells treated with 2 mM Al and 2 mM Al plus 60 M Nec1 was examined by fluorescent microscope (A-I), and the cell death rates were quantified by cytometry (J). AO/EB double-stained neural cells (controls) presented uniform green chromatin staining (A, D, G), while 2mMAl-treated cells displayed increasing number of apoptotic cells (orange stained) and necrotic cells (red stained) (B, E, H). Treatment with 2 mM Al plus 60 M Nec-1 reduced the number of apoptotic and necrotic cells (C, F, I). The cell death rates were compared (J), which indicated that 2 mM Al-treated neural cells displayed higher apoptosis and necrosis rates compared with those in controls. The necrosis rates decreased significantly when the neural cells were treated with 2 mM Al plus 30 M Nec-1 ($P < 0.05$) and with 2 mM Al plus 60–90 M Nec-1 ($P < 0.01$). Furthermore, we noticed that the apoptotic rate was reduced subsequently in 2 mM Al plus 90 M Nec-1-treated neural cells ($P < 0.05$).

8.4.2.3 Western Blot Analysis on Expression of Cell Death-Related Proteins In Vitro

Primary neural cells were treated in vitro with Al and Nec-1 as described in the Methods. Protein extracts from cortical neurons treated with 2 mM Al and 2 mM Al plus 30–90 M Nec-1 were analyzed by Western blot using anti-RIP1, active caspase-3, LC3-II, and NF-κB antibodies. We found that expression levels of cell death-related proteins in 2 mM Al treated group were decreased significantly, while 2 mM Al plus 30–90 M Nec-1 treatments resulted in their decrease ($P < 0.05$, $P < 0.01$). The reduced expression of RIP1 in primary neural cells treated with 2 mM Al plus 30–90 M Nec-1 manifested a significant role of Nec-1 in necroptosis that eventuated in neuronal death. In addition, the expression of NF-κB, a regulating protein for cell death, decreased significantly in 2 mM Al-treated neural cells but increased significantly in cells treated with 2 mM Al plus 30–90 M Nec-1 ($P < 0.05$, $P < 0.01$). Representative results from Western blot analysis are shown in Fig. 8.12.

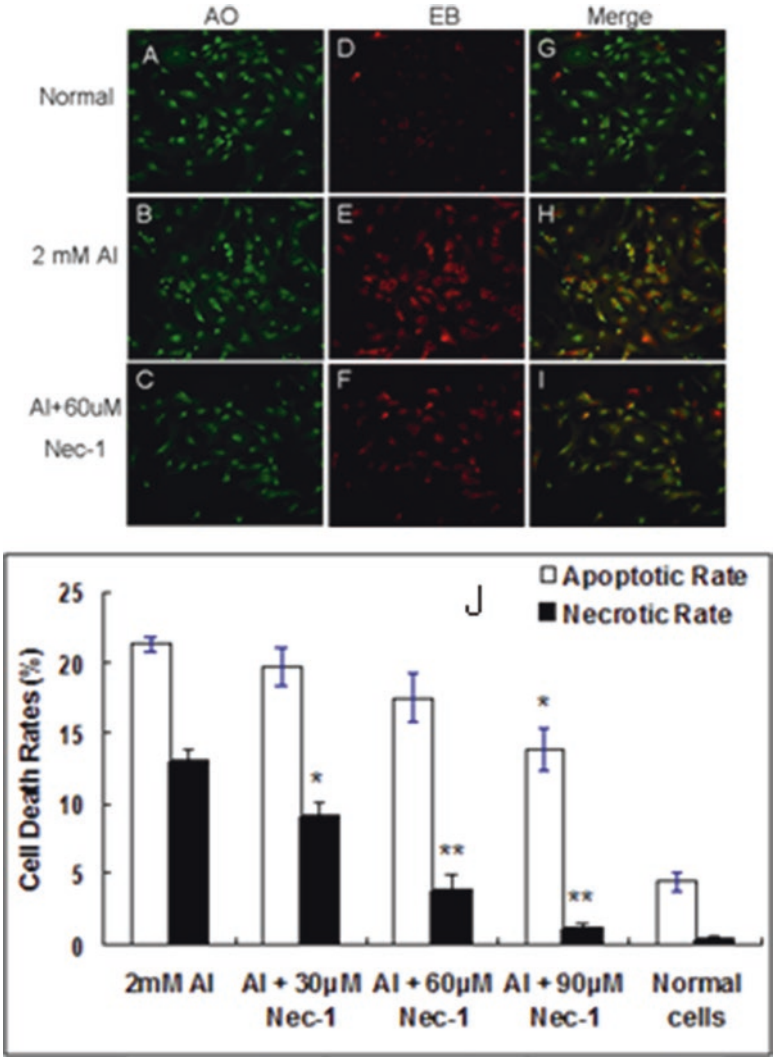

Fig. 8.11 Fluorescent observation and quantification of cell death in neural cells treated with Al and neural cells treated with Al plus Nec-1 in vitro. Normal cells (**g**) were stained green with AO (a) and EB (**d**); 2 mM Al-treated cells (**b**) displayed increasing red necrotic cells (**e**) and orange apoptotic cells (**h**). The cells treated with 2 mM Al plus 60 μM Nec-1(**c**) demonstrated reduction of necrotic cells (**f**) and apoptotic cells (**i**). The cell death rates measured by cytometry were compared among 2 mM Al and 2 mM Al plus 30–90 μM Nec-1-treated neural cells (**j**). There was a dose-dependent decrement of necrotic rates with the increase of Nec-1 concentrations in 2 mM Al-treated neural cells. (cited from: Qinli et al. [47])

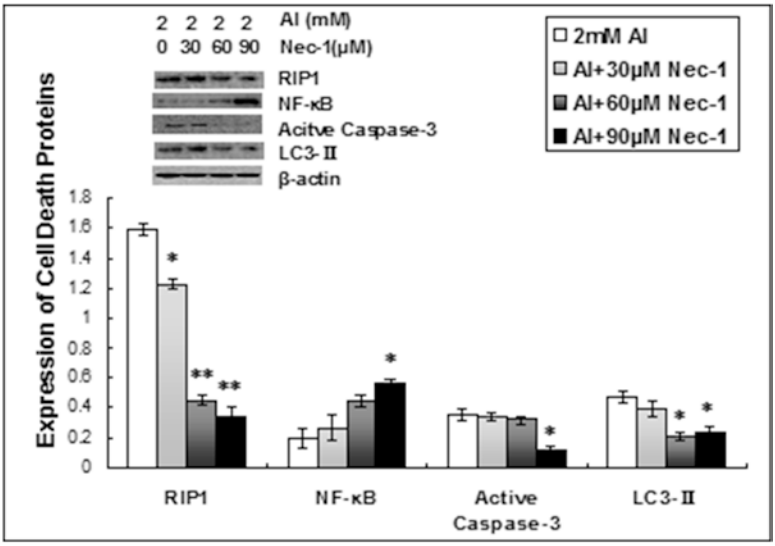

Fig. 8.12 Induction of cell death-related proteins in primarily cultured neural cells treated with 2 mM Al and 2 mM Al plus 30–90 μM Nec-1 in vitro. Representative Western blot analysis was performed in protein extracts prepared from 2 mM Al-treated cells at different Nec-1 concentrations as indicated on the top of the figure. ▲, compared with control, $P < 0.05$; *, compared with 2 mM Al group, $P < 0.05$; **, compared with 2 mM Al group, $P < 0.01$. The experiments were repeated four times from separate cell preparations, and similar results were observed during repeats of the experiment. (cited from: Qinli et al. [47])

8.4.2.4 Neural Behavioral Profile in Mice

The effect of Nec-1 administration on the swimming time for mice to reach the submerged platform was illustrated in Fig. 8.13. 2mM Al was used to induce degenerative neuronal cell death in mice, and 0–8 mM Nec-1 was administered to inhibit neurodegeneration. There was a marked increase in escape latency due to Al-induced memory deficits, while the 2 mM Al plus Nec-1-treated mice (2 mM, 4 mM, and 8 mM) showed a decline of escape latency and an enhancement of time in the target quadrant throughout the training period (A). Analysis of the training data by repeated measures indicated that escape latency and time in target quadrant differed significantly in Nec-1-treated neurodegenerative mice model in a concentration-dependent manner, when the swimming speed measurements were similar to overall sessions ($P < 0.05$, $P < 0.01$). A SNK post hoc test revealed that both the 4 mM and 8 mM Nec-1-treated mice had a significantly reduced swimming latency ($P < 0.01$) and increased time in the target quadrant ($P < 0.05$) compared with those treated with 2 mM Al only. However, when 4 mM Nec-1 was given to the mice at various time points (2 h, 4 h, 8 h) after Al treatment (B), there was no protective effect on learning and memory performance, the latency for finding the platform was longer ($P < 0.01$), and the time in the target quadrant was shorter ($P < 0.05$, $P < 0.01$) at various time points.

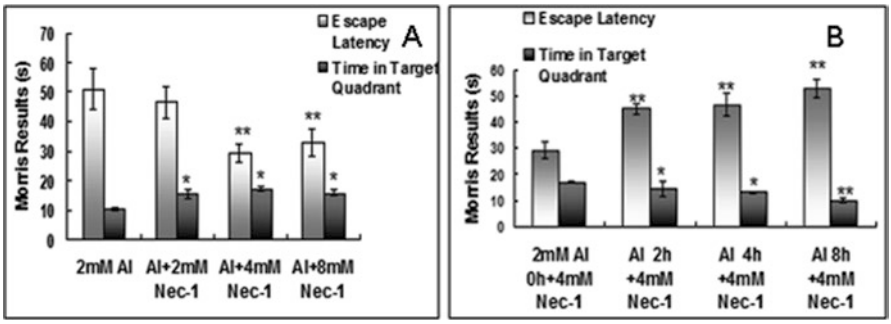

Fig. 8.13 Neurobehavioral alternations in mice exposed to Al and Nec-1 in vivo. There was a significant decrement of escape latency in Al plus 4 mM and 8 mM Nec-1-treated mice ($P < 0.01$) and a significant increment of time in target quadrant when treated with Al plus 2–8 mM Nec-1 ($P < 0.05$) (**a**). However, if the administration of Nec-1 was delayed 2–8 h after Al treatment (**b**), there were inversed trend in escape latency ($P < 0.01$) and time in target quadrant ($P < 0.05$, $P < 0.01$). Data were analyzed using ANOVA followed by SNK post hoc test. Compared with control, ▲, $P < 0.05$; ▲▲, $P < 0.01$; compared with 2 mM Al (**a**) or 2 mM Al 0 h + 4 mM Nec-1 (**b**), *, $P < 0.05$; **, $P < 0.01$. (cited from: Qinli et al. [47])

8.4.2.5 Nec-1 Decreased Neural Cell Death Induced by Al In Vivo

Two mM Al was injected into the mice brain as described in the Methods, and 0–8 mM Nec-1 was given by intracerebroventricular injection simultaneously with Al for 5 days. Representative Nissl staining in the CA3 area of hippocampus at the 20th day after termination of Al treatment is shown in Fig. 8.14. The Nissl stain for the coloration of the chromophile substance of the nerve cells has aided more than any other stain methods in throwing light upon changes of structure in the central nervous system. The neural cells in the controls showed an integrated and clear membrane and triangular-shaped and rich cresyl violet-stained Nissl bodies (A), while the Al-treated mice demonstrated vaguely outlined boundary and contracted neural cell size of neural cells with less Nissl bodies (B). However, Nec-1 treatment at different concentrations in mice helped the neural cells recover in a dose-dependent manner. It manifested that there were larger cell bodies with more Nissl substance and longer dendrites, while the Nec-1 concentrations increase (CE). The number of surviving neurons was decreased significantly in 2 mM Al-treated group as compared with that in controls ($P < 0.05$), whereas Nec-1 treatment at 4 mM and 8 mM concentrations significantly attenuated the neuronal loss induced by aluminum ($P < 0.05$) (F).

8.4.2.6 Expression of Cell Death-Related Proteins in Cortical Neural Cells In Vivo

Cerebral cortical neural cells were treated in vivo with Al and Nec-1 as described in the Methods, and the expressions of cell death-related proteins were shown in Fig. 8.15. Two mM Al enhanced protein levels of RIP1, active caspase-3, and

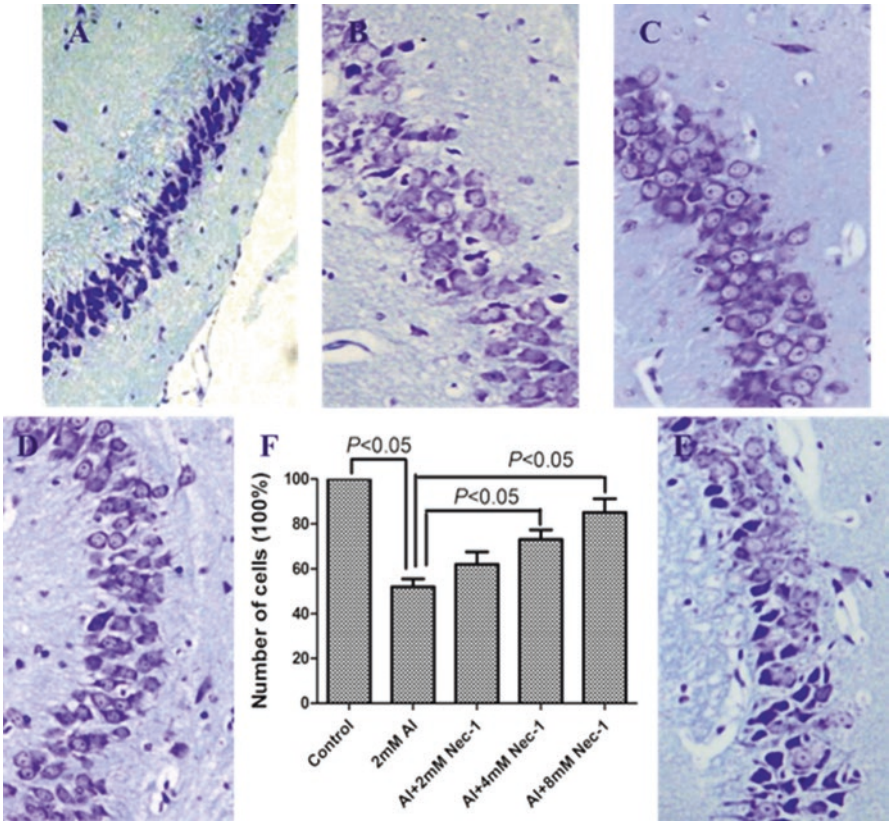

Fig. 8.14 Nissl staining of neural cells in hippocampal CA3 area treated with 2 mM Al and 0–8 mM Nec-1 in vivo (×400). Sequential morphological changes of cells treated with 2 mM Al plus 0–8 mM Nec-1 in hippocampal CA3 area were observed with Nissl staining. The pictures are photographs of hippocampal cells of normal control (**a**) and hippocampal neural cells treated with 2 mM Al (**b**), 2 mM Al plus 2 mM Nec-1 (**c**), 2 mM Al plus 4 mM Nec-1 (**d**), and 2 mM Al plus 8 mM Nec-1 (**e**). The number of surviving neurons was decreased significantly in 2 mM Al-treated group as compared with that in controls ($P < 0.05$), whereas Nec-1 treatment at 4 mM and 8 mM concentrations significantly attenuated the neuronal loss induced by Al ($P < 0.05$) (**f**). (cited from: Qinli et al. [47])

LC3-II, which are the marker proteins of necroptosis, apoptosis, and autophagy, respectively ($P < 0.05$, $P < 0.01$). However, the protein expression levels of RIP1, active caspase-3, and LC3-II were significantly downregulated in a concentration-dependent manner in the brains of mice treated with 2 mM Al plus 30–90 M Nec-1 ($P < 0.05$, $P < 0.01$). Of the three proteins, RIP1 manifested the maximal reduction. Since the upregulations of RIP1, active caspase-3, and LC3-II were associated with an increased cell death in the modes of necroptosis, apoptosis, and autophagy, we thus confirmed the possibility that, in this paradigm, Nec-1 could result in specific downregulation of RIP1 protein and subsequent downregulations of LC3-II and active caspase-3. However, the expression of NF-κB protein was not altered significantly after treatment with 2 mM Al and Al plus Nec-1.

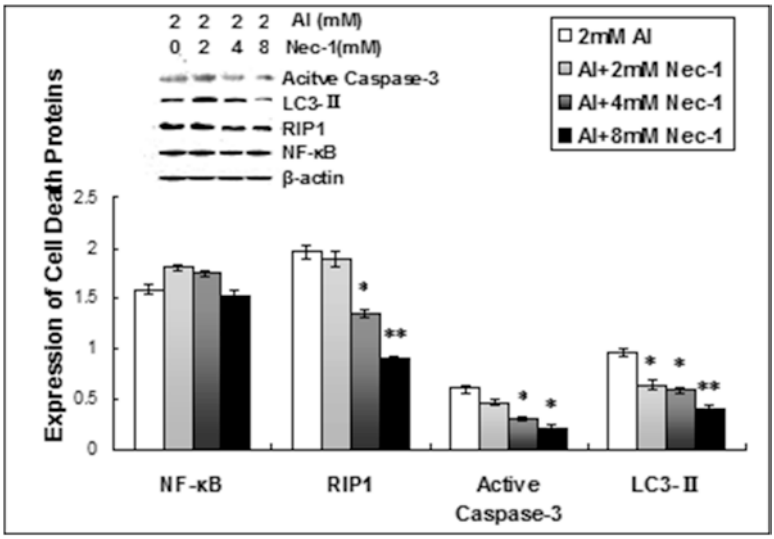

Fig. 8.15 Inhibition of cell death-related proteins with Nec-1 in cortical neural cells of mice in vivo. Western blot data demonstrated the inhibition of cell death-related proteins in cortical neural cells exposed to 2 mM Al plus 2–8 mM Nec-1. The involvement of RIP1, active caspase-3, and LC3-α was presented, which were recognized as important factors regulating necroptosis, apoptosis and autophagy, respectively, and so did NF-κB, which regulates repairing systems for damaged DNA. ▲, compared with control, $P < 0.05$; *, compared with 2 mM Al group, $P < 0.05$; **, compared with 2 mM Al group, $P < 0.01$. (cited from: Qinli et al. [47])

8.4.2.7 Expression of AD-Related Proteins in Mice

Mice treated with Al were treated in vivo to Nec-1 as described in the Methods, and the results from Western blot analysis of Al plus Nec-1-treated neural tissue treated in vivo using anti-mGluR2, mGluR5, and anti-Aβ and total tau are shown in Fig. 8.16. Protein extracts were prepared from Al-treated cells at different Nec-1 concentrations (2–8 mM) as indicated on the top of the figure. Samples were electrophoresed, transferred to nitrocellulose paper, and immunoblotted with mGluR2, mGluR5, Aβ, and tau antibodies. Cells treated with either 4 mM or 8 mM Nec-1 displayed reduction of mGluR2, mGluR5, Aβ, and tau protein levels ($P < 0.05$, $P < 0.01$), while very high protein expression was observed in Al-treated mice ($P < 0.05$, $P < 0.01$). Under similar experimental conditions, a higher concentration of Nec-1 was associated with potential increase in learning and memory functions. It is confirmed from this paradigm that Nec-1 could result in upregulation of mGluR2 and mGluR5, which provided evidence for improvement of learning and memory. Furthermore, we investigated the involvement of Nec-1in the expression of Aβ and tau, which are AD target proteins. We found that Nec-1treatments resulted in a decrease of Aβ and tau protein levels in neurodegenerative mice in a dose-dependent manner.

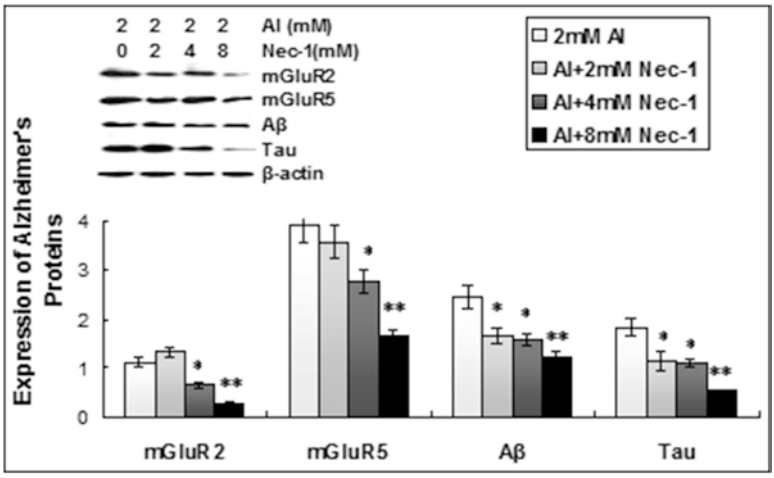

Fig. 8.16 Nec-1 reduced expression of AD-related proteins in vivo. The expression levels of the AD-related proteins were significantly decreased in the Al plus 2–8 mM Nec-1-treated neural cells than those treated with only Al; images were from one of the experiments; similar results were obtained in four different experiments from separate preparations. Compared with control, ▲, $P < 0.05$; compared with 2 mM Al-treated mice, *, $P < 0.05$; **: $P < 0.01$. (cited from: Qinli et al. [47])

8.4.3 Discussion

Progressive cell loss in specific neuronal populations associated with typical learning and memory dysfunction is a pathological hallmark of neurodegenerative disorders, especially in AD. However, the nature, time course, and molecular causes of cell death and their relation to behavioral alternations are still not fully understood. Based on recent data in human brain, as well as in animal and cell culture models, a picture is beginning to emerge, suggesting that in addition to apoptosis, other forms of programmed cell death may participate in neurodegeneration. It is reported that a new type of programmed necrotic cell death, necroptosis, might be involved in the process of neurodegeneration [38, 39, 48], and the link between Nec-1 and necroptosis has been the focus of extensive investigations during the last 5 years [37, 49, 50, 51].

In the present study, zVAD-fmk, 3-MA, and Nec1 were used as the inhibitors of apoptosis, autophagy, and necroptosis, respectively. Added to the media containing 2 mM Al-treated neural cells, which caused evidence of necroptosis, Nec-1was effective in inhibiting neural cell death (Fig.8.10). Fluorescence light microscopy with differential uptake of fluorescent DNA-binding dyes (AO/EB staining) demonstrated that the treatment with Nec-1 (60 M), on the other hand, attenuated neuronal death evident as judged by reduced red-stained necrotic cells. Cytometry in neural cells stained by annexin V-PI quantified the inhibition of Nec-1 on necrotic cell death (Fig. 8.11). There was a concentration-dependent effect of Nec-1 on down-regulating expression of necroptosis-specific RIP1 and upregulating expression of

NF-kB. LC3-II, which is reported as a subsequent protein of necroptosis, was also reduced hand in hand with the increment of Nec-1 concentration (Fig. 8.12). We also noticed that active caspase-3, a marker protein of apoptosis, was subsequently decreased as well, the molecular mechanism of which needs further investigation.

To examine the effect of Nec-1 on neurodegenerative animal model, the neurobehavioral alternations, neural cell loss, and expression of AD-related proteins were analyzed. Our results indicated that the poor neurobehavioral performance of Al-treated mice was significantly improved by Nec-1 in a dose-dependent manner. Different from the Al and Nec-1 simultaneously treated mice, the animals treated with Nec-1 after Al treatment for 2 h manifested a prolonged escape latency and less time in the target area, indicating the decreased learning and memory performance as measured by the MWM test; similar results were seen in animals treated with Nec-1 at 4 h and 8 h after Al treatment (Fig. 8.13). Thus, it was evident that administration of Nec-1 could reduce the adverse effect of Al on learning and memory abilities significantly when animals were treated with Al and Nec-1 simultaneously. However, if Nec-1 administration was later than Al treatment, its effect on improving learning and memory function was greatly diminished.

It is known that the upregulation of RIP1, active caspase-3, LC3-II, and NF-kB is associated with increased cell death through necroptosis, apoptosis, and autophagy, under similar experimental conditions. In the present study, the protein expression of active caspase-3, RIP1, LC3-II, and NF-kB was used to distinguish apoptosis, necroptosis, and autophagy. We evaluated the possibility that, in this paradigm, Nec-1could result in specific downregulation of RIP1 protein and subsequent downregulation of LC3-II in mice. The protein NF-κB, which is a regulator of DNA damage repairing systems, was significantly increased in vitro rather than in vivo. The present study had shown that altered protein expression of RIP1, active caspase-3, and LC3-II in Al-treated primary neural cells was associated with evidence of memory deficits induced by Al in mice (Figs. 8.14 and 8.15). We also demonstrated a significant decrease in mGluR2 and mGluR5 protein expression in the cortical tissues of Al-treated mice and the effect of Nec-1 treatment in attenuating this decrease (Fig. 8.16). Several studies had suggested an association between cortical mGluR2 and mGluR5 expression and memory performance, particularly in AD patients [52, 53]. Our study suggested a direct correlation between reduced mGluR2 and mGluR5 protein expression in the cortex and impaired cognitive performance induced by Al. Nec-1 not only ameliorated impaired cognitive performance but also increased mGluR2 and mGluR5 expression in the cortex.

It is believed that several pathogenic events might contribute, either directly or indirectly, to neurodegeneration, especially formation of Aβ-containing plaques and neurofibrillary tangles composed of tau aggregation. Although several kinases are capable of phosphorylating tau in vitro, it is not yet clear whether all of them participate in tau phosphorylation under physiological or pathological conditions in vivo [54]. In AD and related neurodegenerative disorders, the largest burden of tau

pathology (~95% of total tau by morphometric analyses) is found in neuronal processes known as neuropil threads or dystrophic neurites [55]. For the above reason, we chose total Aβ and tau as the markers of neurodegeneration induced by aluminum. We reported the significant higher expression of Aβ and tau in 2 mM Al-treated mice, which indicated the involvement of AD marker proteins (Fig. 8.16). We found that Nec-1 treatment resulted in a decrement of Aβ and tau protein levels in a concentration-dependent manner in the Al-treated mice. Nec-1, in addition to its use as a therapy agent for cell death, might therefore be of use in slowing the progression of the cognitive deficits associated with neuronal degeneration.

8.5 Conclusions

Although many in vivo and in vitro data are in favor of Al involvement in neurodegenerative processes, there is considerable evidence that very complex events may contribute to Al-induced neural cell death with possible repair mechanisms. In the present chapter, primary cultured neural cell death induced by Al, Al-treated human neuroblastoma cell death, and Al-treated mice were used as degenerative cell and animal models. The present data demonstrated that apoptosis and necrosis induced by Al are closely related to the degeneration of neural cell death, resulting in neurodegenerative cell loss. In addition, necroptosis as a newly discovered cell death pathway appears to be involved in Al-induced neuronal degeneration. To elucidate the underlined degenerative mechanism of the Al-induced cell death, small chemical probes for specific cell death pathways of apoptosis, necroptosis, and autophagy were used. The inhibited cell viabilities in Al-treated primary cultured neural cells and neurobehavioral changes in Al-treated neurodegenerative animal model were detected. The neurodegenerative cell viabilities induced by Al could be enhanced by 3-MA, zVAD-fmk, and Nec-1, of which Nec-1 improved the cell viability the most. Furthermore, the cell viability of neural cells treated with Nec-1 at various concentrations could be increased dose dependently. The results consisted of experiments in Al-treated degenerative animal models; the neurobehavioral function of mice treated by Al could be much ameliorated by Nec-1 than that of mice treated by zVAD-fmk. The results in vitro and in vivo indicated that necroptosis as a programed necrosis was involved in degenerative cell death apart from apoptosis and autophagy. Nec-1 can protect human neuroblastoma cells and neurons in primary culture from Al-induced neural cell death and improved cognitive performance in Al-treated mice, the effects of which were greater than those of zVAD-fmk and 3-MA.

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Chapter 9
Aluminum-Induced Electrophysiological Variation, Synaptic Plasticity Impairment, and Related Mechanism

Huifang Zhang

Abstract Aluminum, an environmentally abundant non-redox trivalent cation, has long been reported to alter blood-brain barrier and gets deposited in different regions of the brain. Many reports strongly indicated that Al had an adverse impact on the central nervous system (CNS), particularly on cognitive ability. Until now, studies in animal models and cell cultures have revealed that Al exposure results in altered behavioral performance and memory damage. The present paper reviews the scientific literature linking aluminum and the impairment of electrophysiological variation and synaptic plasticity. The focus is on the changes of electrical excitability, voltage-operated ion channels, and synaptic plasticity induced by aluminum. A detailed mechanism of the role of aluminum in hippocampal LTP which is the most widely studied example of synaptic plasticity is highlighted. Evidence revealed that glutamate-NO-cGMP, PLC, Ca^{2+}-CaM-CaMKII, MAPK, and Wnt pathway may be important in the mechanism underlying Al-induced long-term memory impairment. Further studies are required to establish the upstream activators and downstream effectors of these cascades and to answer how so many signaling cascades relate to the other signaling processes that might be involved in the Al-induced inhibition of synaptic plasticity.

Keywords Aluminum · Neurotoxicity · Electrophysiological variation · Synaptic plasticity

9.1 Aluminum Neurotoxicity

Aluminum (Al) is one of the most abundant elements on the earth's crust and gets an easy access to our body through the use of cooking utensils, deodorants, antacids, etc. [1]. Al has been reported to alter blood-brain barrier [2] and gets deposited in the cortex, cingulate bundles, corpus callosum, and hippocampus [3]. Many scientific studies have brought to light the potential toxicity of Al in experimental

H. Zhang (✉)
Department of Occupational Health, School of Public Health, Shanxi Medical University, Taiyuan, Shanxi, China

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animal models and in humans under different clinical conditions. In 2007, Paula P [2] reviewed the following pioneering studies on the human neurological effects of aluminum exposure:

1. The incidence of neurological symptoms and subclinical neurotoxic effects among miners treated with the prophylactic McIntyre powder and welding, pot-room, smeltery, and foundry workers chronically exposed to aluminum since 1962 [3]
2. The aluminum connection to dialysis encephalopathy since 1972 [6]
3. The possible role of aluminum in the etiology of neurodegenerative diseases, like Alzheimer's dementia since 1973 [4]
4. The onset of neurological symptoms following accidental ingestion of aluminum compounds since 1986 [5]
5. The occurrence of aluminum-related endemic neurodegenerative diseases [6]

These reports strongly indicated that Al had an adverse impact on the central nervous system (CNS), particularly on cognitive ability; it is, therefore, crucial and urgent to explore the mechanism of learning and memory impairment induced by Al.

In the present study, effect of long-term Al administration was assessed at behavioral, biochemical, and electrophysiological levels to investigate the possible pathophysiology associated with Al toxicity. Sethi et al. demonstrated that the spatial learning and memory abilities of both young and old Wistar rats were adversely affected by a 6-month duration of 50 mg/kg/day $AlCl_3$ supplied in drinking water [10]. Moreover, the neurotoxic effects of Al on mouse neurobehavioral profiles were confirmed [7], and neurotoxic symptoms were observed, which demonstrated that Al also impairs neurobehavioral function. In another study, the perinatal oral exposure of the dams to 300 or 600 mg/kg/day $AlCl_3$ resulted in significant and deleterious effects in the offspring on locomotor activity at postnatal day 22 (PD22), learning capacity at PD25, and cognitive behavior at PD30–36 [8].

Biochemical and microscopic studies have been performed on hippocampus to explain the possible reason behind behavioral alterations reported and observed in those studies. Al being an inert metal has been suggested to promote reactive oxygen species (ROS) formation and induce oxidative stress by inflicting damage to membrane lipid, proteins, and antioxidative enzyme defense system [9]. In vitro, $AlCl_3$ has been demonstrated to preferentially accumulate in cultured astrocytic cells [10]. The in vivo studies suggested that Al treatment causes changes like apoptosis [11], abnormal mitochondrial swelling, thinning of myelin sheath, cytoplasm with multivehicular bodies [12], and synaptic vesicle accumulation [13]. Until now, the effects of Al exposure have been hypothesized to occur through different mechanisms. Studies in animal models and cell cultures have revealed that Al exposure results in altered behavioral performance and memory damage.

9.2 Al-Induced Electrophysiological Variation

Previous reports strongly indicated that Al had an adverse impact on the central nervous system, particularly on the learning ability which includes important reactions for brain development such as the axonal transport, neurotransmitter synthesis, synaptic transmission phosphorylation or dephosphorylation of proteins, protein degradation, gene expression, and inflammatory responses [14]. Researchers concluded that learning-related cellular changes can be divided into two general groups: modifications that occur at synapses and modifications in the intrinsic properties of the neurons [15]. It is commonly agreed that changes in strength of connections between neurons in the relevant networks underlie memory storage; a great deal of evidence suggest that modifications in intrinsic neuronal cells may also account for learning-related behavioral changes. Long-lasting modifications in intrinsic excitability are manifested in changes in the neuron's response to a given extrinsic current which is generated by synaptic activity or via the recording electrode [16]. We know that Al exhibits in only one oxidation, Al^{3+}. It has a greater affinity toward negatively charged, oxygen-donor ligands. These possible characteristics of Al^{3+} make a strong bonding with enzymes and receptors that are involved in the neurotransmitter synthesis and thus affect the neurotransmitter content. Specifically, Al^{3+} also inhibits voltage-gated Ca^{2+} channels and neurotransmitter receptors and impairs synaptic transmission [17]. All of these Al^{3+} characteristics finally result into neurotoxicity and neurodegeneration and impair various brain functions related to learning and memory.

9.2.1 Aluminum Effect on Electrical Excitability

Learning-induced enhancement in neuronal excitability has been shown in hippocampal neurons following classical conditioning of the trace eyeblink response [8] and the Morris water maze task [9] and in piriform cortex neurons following operant conditioning [10]. Learning-specific modifications in neuronal excitability were shown also in cerebellar neurons [18] and in *Hermissenda* [19] after classical conditioning. In hippocampal and piriform cortex neurons, this enhanced excitability is manifested in reduced spike frequency adaptation in response to prolonged depolarizing current applications [8]. Neural electrophysiology is a science based on the changes of electrical activity of nervous system to study the activity of nervous system and the pathway of nerve impulse. Aluminum-induced electrophysiological alterations as well as cognitive deficit have been widely reported in many literatures. The normal functional development of brain networks requires neurotransmission, since it is an essential regulator of electrical phenotype. In 1968, Blaustein and Goldman [13] observed that aluminum significantly reduces the action potential evoked by a depolarizing current in the giant axon of the lobster. Here, excitability exhibited a marked reduction in early transient current accompanied by a reduction

in the steady-state current. Since action potentials are used extensively by the nervous system for communication between neurons, this early finding was the first evidence for impairment of neurotransmission by aluminum. Significant effects of aluminum on electrical properties of neurons were clearly shown in the study of M.M. Campbell. This research using the isolated central nervous system demonstrates toxicity at the cellular level. Extracellular application of Al (100 μM) led to membrane depolarization, bursts of action potentials, and action potential broadening [20]. In neurons aluminum exposure also seems to alter baseline synaptic transmission and drastically damage synaptic excitability [21].

9.2.2 Aluminum Effect on Voltage-Operated Ion Channels

At every instant, the distribution of ions across the cell membrane and the permeability of the membrane to these ions eventuate the electrical activity recorded from each neuron. Voltage-operated calcium channels are particularly significant in neurotransmission, since the influx of calcium ions through these channels triggers a series of events, which ultimately results in the release of neurotransmitters into the synaptic cleft. It has been suggested that aluminum acts as an inhibitor of Ca^{2+} influx through channels [22]. In fact, aluminum reduces the maximal velocity of Ca^{2+} influx rate and shifts the extracellular Ca^{2+} concentrations required to achieve maximal Ca^{2+} uptake toward higher values [23]. Aluminum effect on voltage-operated channels among N-, L-, and T-calcium currents shared the characteristics of the low effective concentration, the use dependence, and the specificity and irreversibility. Moreover, in hippocampal CA1 pyramidal neurons, the Ca^{2+} current activated by high-voltage stimulation appears to be blocked by low concentration of aluminum [27]. In vivo intraperitoneal injection of aluminum lactate to rats for a period of 4 weeks also produces a significant decrease in Ca^{2+} uptake via voltage-operated calcium channels [24].

9.2.3 Aluminum Effect on Synaptic Plasticity

Learning and memory are believed to arise from long-term changes in synaptic strength, in which repeated or continuous synaptic activation takes place, causing the alteration of the structure of the synapse itself. Activity-dependent change in synaptic strength is largely recognized as a mechanism associated with spatial learning and memory in the hippocampus, fear memory in the amygdala, task memory in the cortex, and learning in the cerebellum. The mechanism underlying this process is known as synaptic plasticity, which involves variations in synaptic transmission efficiency [16]. The processes involved in synaptic plasticity have drawn wide interests from neuroscientists over the last two decades. Synaptic plasticity expresses multiple forms dependent on the expressing brain region and neuron type.

The mechanisms expressing sites and expressing targets are different among the different forms of synaptic plasticity. The most studied form is hippocampal N-methyl-d-aspartate receptor (NMDAR)-dependent synaptic plasticity because of the importance of hippocampus in the memory storage and retrieval and the extensive experimental investigations in this area. For this particular form, synaptic plasticity is shown by the bidirectional modifications in the postsynaptic response following electrical stimulations. The postsynaptic response is represented by the magnitude of the postsynaptic receptor-mediated current (EPSC) in vivo [16].

The major postsynaptic receptors in hippocampus are glutamatergic receptors, including NMDAR, A-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPAR), and metabotropic glutamate receptors (mGluRs). Further, these receptors have critical roles in the emergence of synaptic plasticity [25]. Durable synapse modification in response to neuronal stimulation can result in enhanced or reduced synaptic strength and is known as long-term potentiation (LTP) or long-term depression (LTD), respectively. LTP and LTD are experienced by excitatory synapses in response to glutamate. Glutamate binds notably to NMDARs, AMPARs, and mGluRs and triggers an increase in calcium in the stimulated spine. NMDAR-dependent LTP and LTD are two important types of synaptic plasticity [16]. As reviewed in other papers, the emergence of NMDAR-dependent synaptic plasticity is a Ca^{2+}-driven process triggered by the activation of NMDAR. The Ca^{2+} influx following NMDAR activation activates a number of synaptic proteins, including adenylyl cyclase (AC), protein kinase C (PKC), protein kinase A (PKA), Ca^{2+}/CaM-dependent protein kinase II (CaMKII), calcineurin (PP2b), and protein phosphatase1 (PP1). These proteins interact and cause alterations on AMPAR as well as AMPAR-related proteins [26]. The details of the specific changes during LTP and LTD are explained in Table 9.1. The most widely studied example of synaptic plasticity is hippocampal LTP, which is induced by a brief, but intense, stimulus, resulting in synaptic strengthening [16].

Epidemiological surveys and animal studies have indicated that Al can cause cognitive dysfunction and learning and memory impairment [27, 28], which suggests that synaptic strength may be affected by aluminum. Electrophysiological recording techniques have been also applied to gain insight into the mechanisms underlying the deleterious action of aluminum on these advanced processing functions of the brain. Aluminum exposure reduces the range of synaptic plasticity, since it depresses high-frequency electrical evoked long-term potentiation (Table 9.2).

9.2.4 The Cell Signal Pathways and Aluminum Effect on Synaptic Plasticity

Neurobiological basis of LTP in the hippocampus is the changes in neurons that is synaptic plasticity, the material basis related to the changes of protein and gene in neurons and synapses [34]. It is well known that the formation of long-term memory

Table 9.1 Induction of NMDAR-dependent synaptic plasticity

Model	Basal transmission	Stimulation parameters	Change of transmission after the stimulus
LTP	Low intracellular Ca^{2+} level (around 0.1 uM)	High-frequency stimulation (100–200 Hz)	High intracellular Ca^{2+} elevation (more than 10 uM)
	Basal phosphorylation of S845 of AMPARs		Enhanced phosphorylation of S845 and exocytosis of AMPAR
	Very low phosphorylation of S831 of AMPARs		Enhanced phosphorylation of S831 and unitary AMPAR channel conductance
			Increased number of AMPAR in PSD;
			Increased AMPAR peak current and synaptic strength
LTD	Low intracellular Ca^{2+} level (around 0.1 uM)	Low-frequency stimulation (1 Hz)	Moderate intracellular Ca^{2+} elevation
	Basal phosphorylation of S845 of AMPARs		Enhanced dephosphorylation of S845 and endocytosis of AMPAR
	Very low phosphorylation of S831 of AMPARs		No significant change in phosphorylation of S831 of AMPARs
			Decreased number of AMPAR in PSD
			Decreased AMPAR peak current and synaptic strength

requires new gene transcription and subsequent new protein synthesis in the CNS, similar to the maintenance of LTP [35, 36]. The events of LTP, as known for NMDA receptor-dependent LTP, include series processes but not limited to postsynaptic NMDA receptor activation, postsynaptic calcium influx increase, and activation of several protein kinases, such as CaMKII, PKC PKA, and ERK [37]. The AMPAR is essential for brain function and plays an important role in changes in synaptic strength and connectivity [38]. During synaptic plasticity, changes in the content of AMPAR have been well demonstrated [43–45]. Recent studies have also shown that the activation of some signaling pathway in the hippocampus of rats plays a key role in long-term memory. It is reasonable to consider that the following signaling pathways may also be important in the mechanism underlying Al-induced long-term memory impairment.

9.2.4.1 Glutamate-NO-cGMP and Aluminum Effect on Synaptic Plasticity

The neurotransmitter glutamate activates NMDA receptor and then postsynaptic calcium influx increase through coupling calcium channels. The calcium ion activates nitric oxide synthase and catalyzes the synthesis of NO (nitric oxide), then NO activates guanylate cyclase to produce cGMP (cyclic guanosine monophosphate)

Table 9.2 Aluminum effect on synaptic plasticity

Experiment model	Effect	References
Wistar rat 7–8 weeks old (hippocampal slices)	0.68 μg/ml Al attenuated TEA LTP, while a complete block of long-lasting potentiation was obtained for 2.7 μg/ml Al	[29]
Wistar rat (80–100 days old)	Al reduced the amplitudes of both EPSP LTP (control, 132 ± 7%, $n = 7$; Al-exposed, 115 ± 10%, $n = 8$, $P < 0.05$) and PS LTP (control, 242 ± 18%, $n = 7$; Al-exposed, 136 ± 7%, $n = 8$, $P < 0.01$) significantly	[30]
Wistar rat (80–100 days old)	Aluminum exposure from parturition throughout life caused the greatest impairment of the range of synaptic plasticity	[31]
Great pond snail (right parietal dorsal 1 neuron)	Extracellular application of Al (100 μM) led to membrane depolarization, bursts of action potentials, and action potential broadening	[20]
Wistar rat (120–150 g) granule cell layer of dentate gyrus (freely moving animal with implanted electrodes); hippocampal (CA1) slices (transverse; 450 um)	Acute Al infusion at 0.68 and especially 2.7 μg/ml Al leads to a reduction in LTP, and the potentiation declined to baseline within 2 h. In chronic animals their neuronal responsiveness was reduced, and in 30% of the rats, the PS was completely lost. High-frequency tetanization failed to induce LTP	[21]
SD rats (intraperitoneal injection for 8 weeks)	Al suppressed in vivo LTP and damaged spatial learning and memory capacities	[32]
SD rats (intraperitoneal injection for 8 weeks; via intracerebroventricular injection for 5 min)	Acute Al treatment produced dose-dependent suppression of LTP in the rat hippocampus	[33]

which finally plays a biological effect [39]. Canales [40] cultured neuron cells of 8–13 days by culture medium containing 50 umol/L aluminum chloride and found that contents of cGMP activated by glutamate decreased by 77%, and NO-cGMP glutamate signal transduction was severely damaged. In addition, aluminum also blocks this signal transduction by interference expression of NMDA receptor [41] and Ca^{2+} [42], then the normal function of nerve cells was affected, and the motion performance and spatial memory's function of mice were damaged.

9.2.4.2 PLC Signaling Pathway and Aluminum Effect on Synaptic Plasticity

The muscarinic receptors which are abundant in the hippocampus activate phospholipase systems (including PLAl, PLA2, PLC, PLD) and catalyze the formation of PIP2 through coupling the G protein. PIP2 is cleaved to form 1,4,5- IP3 and DAG. Water-soluble IP3 is released into the cytoplasm; then Ca^{2+} is released from the calcium pool to regulate calcium- and calmodulin-dependent enzymes and other

channels. DAG activates the membrane PKC. M1 receptor and the activity of GTP were significantly inhibited in hippocampus and cerebral cortex [50, 51]. Another research reported that the phosphorylation of phosphatidylinositol was inhibited when the rats experienced long-term exposure of drinking aluminum salt. Aluminum can decrease the content of PIP2, and IP3 in brain tissue also affects the expression and activity of PKC [43]. The signal molecules in PLC signaling pathway are not normally expressed or activated by aluminum, leading to the disorder of PLC signaling pathway, which makes damage of LTP.

9.2.4.3 Ca^{2+}-CaM-CaMKII Signaling Pathway and Aluminum Effect on Synaptic Plasticity

Ca^{2+}-CaM-CaMKII signaling pathway in the hippocampus of rats plays a key role in long-term memory. Aluminum is an antagonist of enzymes containing calcium and magnesium which can be replaced by aluminum. The activity of ATP enzymes such as calcium-dependent protein kinase was eventually inhibited [44]. Morae's study found that aluminum competitively combined with calcium channel in the period of rapid flow of calcium ions in the competition, to prevent the influx of calcium ions [45]. Wang [42] found that aluminum inhibits the expression of CaMKII in the mouse brain and Ca^{2+}-CaM-CaMKII signal transduction. At the same time, calcium is a second messenger and participates in the regulation of cellular process. Once the cells are exposed to aluminum, cytoplasmic calcium homeostasis will be disturbed, the normal conduction pathway will be affected, and thus learning and memory will be impaired.

9.2.4.4 The MAPK Pathway and Aluminum Effect on Synaptic Plasticity

There are four subtypes of MAPK, namely, extracellular signal-regulated protein kinase (ERK), p38 mitogen-activated protein kinase (p38 MAPK), c-Jun N-terminal kinase (JNK, also known as stress-activated MAPK), and ERK5. After the phosphorylation of MAPK, MAPK can enter the nucleus and phosphorylate nuclear transcription factors, leading to the expression of downstream target genes and the synthesis of new proteins [46].

In recent years, many scholars have shown that the small GTPase RAS signaling pathway plays important roles in LTP and in formation and the consolidation of memories in the brain [47]. Appropriate activation of the Ras/extracellular signal-regulated kinase (ERK) protein signaling cascade within the brain is crucial for optimal learning and memory. The Ras GTPase-activating protein (RasGAP), which attenuates Ras/ERK signaling by converting active Ras, is bound to guanosine triphosphate, activating Ras into inactive Ras, and is bound to guanosine diphosphate, inactivating Ras. Then ERK is transferred to the nucleus to phosphorylate transcription factor, such as CREB which plays a biological effect in LTP [48]. A study [49] which is carried out by long-term consumption of aluminum-containing

food to mice found that protein and mRNA levels of Ras in neurons were increased; at the same time, the protein and mRNA levels of rafl, ERK2, and CREB were hindered by aluminum. Aluminum affects the brain information storage and memory via Ras/ERK signal transduction [50]. Our previous study [51] found that with the increasing aluminum dosage, a gradually decreasing RAS activity of the rat hippocampus was produced after gradually suppressing on LTP; the RAS→PI3K/PKB→GluR1 S831 and S845 signal transduction pathway may be involved in the inhibition of hippocampal LTP by aluminum exposure in rats.

9.2.4.5 Wnt Pathway and Aluminum Effect on Synaptic Plasticity

As we know now, Wnt1, Wnt3a, Wnt7a, and Wnt8 bind the receptor Frizzled and the LRP5/6 co-receptors, activating the Wnt/ß-catenin [52]. Both Fz and LRP5/6 recruit the protein disheveled (Dvl) usually by phosphorylation, which oligomerizes in the plasma membrane forming a platform for the allocation of the scaffold protein Axin and the glycogen synthase kinase-3ß (GSK-3ß) [53, 54]. The phosphorylation of LRP5/6 causes the inhibition of GSK-3ß and adenomatous polyposis coli (APC). The consequence of this inhibition is the cytoplasmic stabilization of ß-catenin which enters the nucleus and regulates the transcription of Wnt target genes [55]. The activation of Wnt signaling increases synaptic transmission and facilitates LTP in hippocampal brain slices and in cultured neurons, suggesting a key role for Wnt signaling in the regulation of synaptic plasticity [56, 57]. Studies have shown that long-term exposure to aluminum environment could increase the activity of GSK-3ß and then inhibit the signal transduction [58]. Our previous study found that Al-induced LTP impairment might be related to the activation of GSK-3ß [59]. Researchers found that in PC 12 cells treated with aluminum maltolate,contents of Wnt3, DVL, and ß-catenin were decreased and finally Wnt/ß-catenin pathway was weakened [60].

9.3 Conclusion and Future Perspectives

As we know now, many potential new pathways provide strong evidence that Al exposure impairs synaptic plasticity. There are many questions on synaptic functioning that may be critical for the aluminum effect on synaptic plasticity:

1. How would the local dynamics of synaptic proteins of synaptic plasticity be impacted by Al? This problem deals with the colocalization of synaptic proteins mediated by AKAPs and spatial movement of synaptic proteins among synaptic compartments. Hence, the models of this category need to be developed based on the previous model findings.
2. How can Al induce the structural changes in PSD (so-called structural switch) and last for a long period and then damage the emergence of the outlasting LTP?

3. Why are there so many signaling cascade participants in the process of damage induced by Al? The cooperation between neurons and synapses is critical to understand the behavior of brain functions. Further studies are required to establish the upstream activators and downstream effectors of these cascades and to answer how so many signaling cascades relate to the other signaling processes that might be involved in the Al-induced inhibition of synaptic plasticity.

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Chapter 10
Cross Talk Between Aluminum and Genetic Susceptibility and Epigenetic Modification in Alzheimer's Disease

Ruifeng Liang

Abstract This chapter primarily focuses on two key aspects related to aluminum neurotoxicity and its mechanism in Alzheimer's disease (AD), which are genetic susceptibility and epigenetic modification. The toxicity of aluminum has been confirmed from plant experiments, animal experiments, in vitro experiments, and epidemiological studies. However, the mechanisms underlying this phenomenon have largely remained elusive. Furthermore, there are more and more genetic factors that have been found to be strongly implicated for causing or increasing the risk of AD development and have been proved to be associated with the neurotoxicity of Al and play a significant role in the initiation and progression of AD. Epigenetics provide a bridge between genes and environment to improve our understanding on the etiology of AD. Al can modify the epigenetic status by DNA methylation, histone modifications, and noncoding RNAs and might thereby contribute to the pathophysiology of AD. However, very little is known about exact epigenetic patterns in AD.

Keywords Aluminum · Alzheimer's disease · Genetic susceptibility · Epigenetic modification

10.1 Introduction

Aluminum (Al) is the most abundant metal and the third most abundant element in the Earth's crust [11, 28, 32]. At first people believed that Al is a rather inert and insoluble metal and can be effectively excluded from the biosphere [27]; Al does not possess a significant health hazard [10]. Therefore, the health effects of Al do not cause enough attention, and aluminum compounds were widely used in water and food processing. Furthermore, due to the special physical and chemical properties of Al, such as silvery-white, soft, low density, nonmagnetic, and ductile, additionally, the constant increasing occurrence and enlarging scope of acid deposition, Al is already ubiquitous in daily living conditions and in occupational environments, and

R. Liang (✉)
Department of Environmental Health, Shanxi Medical University, Taiyuan, The People's Republic of China

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a lot of aluminum compounds are widely used in a soluble form [35, 102], which increases the risk of Al entering the body and accumulating in the body. Nowadays, the geochemical cycle for Al has now become a biogeochemical cycle primarily through interference due to human activities either indirectly, for example, the acidification of catchments by acid deposition of anthropogenic origin, or directly by the extraction of Al from its inert ores [27]. Lots of studies have confirmed that Al can enter the human body through a variety of approaches, such as the environmental exposure, diet, drinking water, beverages, or medicines. Some epidemiological investigations and animal experimental studies have demonstrated that Al is difficult to eliminate from the body, and long exposure to Al leads to the accumulation of Al in the body [24, 125, 126]. With increasing exposure to Al, the risk of Al accumulation in human body is increasing. However, Al is a nonessential element for the human body and does not have any physiological functions or clear physiological action [61]. Therefore, it is very important for us to recognize the toxicity of Al. At present, Al has become a global public health problem [87]; of all, the neurotoxic effects of Al, particularly the role of Al in Alzheimer's disease (AD), have attracted much attention and is of continuing interest. Some epidemiological investigations and animal experimental studies have demonstrated that Al causes severe brain damage and cognitive impairment [34, 123, 125]. Given that it is highly probable that the use of Al will increase in the future [28], the etiologic mechanism researches of Al in the development and progression of AD will be more important.

AD is the most common progressive, irreversible neurodegenerative disease (ND) in the elderly. The main clinical features of AD are progressive impairments of memory, judgment, decision-making, orientation to physical surroundings, and language, and the typical pathological hallmarks of AD are the presence of extracellular senile plaques (SPs) containing the amyloid protein (Aβ) and neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau protein in the brain, and irreversible loss of neurons, particularly in the cortex and hippocampus [41, 79, 127]. With the increase of average lifespan of human population, AD is progressing rapidly and has become the major public health problem in the industrialized world [83]. Nowadays, AD has been the most common form of dementia in the elderly [1, 113, 115] and accounts for around 70% of dementia cases [115, 127]. Furthermore, its prevalence will increase significantly in the coming decades [113]. Despite improvements in knowledge and understanding, there are currently no effective approaches to prevent, cure, or even slow down the progression of AD [3, 111], because its exact etiology and pathological mechanisms remain to be determined [32, 60, 62, 79, 82]. Therefore, understanding AD etiology will be critical to effectively diagnose and treat the disease, and the relevant research of the causes and mechanisms of AD has become a hot point in the world.

ND is a complex disorder caused by the convergence of genetic and environmental factors in aging. In general, none of these factors has complete penetrance, and only the combination of some of them leads to the onset of the disease [90]. Same with other ND, AD is being revealed as multifactorial in nature. These genetic and environmental factors, gene interactions, and unhealthy active lifestyles may be involved in the occurrence and progression of AD [26, 79, 84]. Genetic factors seem to play a more significant role in the onset of the rare early-onset form of AD

(EOAD, onset <65 years) [56]. The late-onset Alzheimer's disease (LOAD, onset ≥65 years) is the more common form of AD, accounting for 90–95% of cases, and occurs sporadically, and risk is determined by complex underlying mechanisms [67]. As Sanchez-Mut has argued that, although several genetic alterations have been associated with AD, the vast majority of AD cases do not show strong genetic underpinnings and are thus considered a consequence of nongenetic factors [89]. There is evidence from studies about geographical variation in dementia rates found that environmental risk factors may be important in the pathogenesis of dementia [86]. A strong positive correlation between the level of Al in drinking water and the incidence of AD in the United Kingdom, Canada, Norway, and France has been reported. Even in sporadic AD, it is expected that genetics, interacting with environment, are strongly involved. However, the epidemiologic study of identical twins discovered that the discordant for the expression of AD in monozygotic co-twins is most likely due to the differences in respective environmental exposure [77]. Other epidemiological studies for AD, based on identical and nonidentical twin pairs, have consistently shown that the etiology of AD has significant environmental and genetic components [103]. Families and groups of individuals exhibit marked differences and heterogencity in histological features and patterns of progression characterized in AD, which are revealing distinct contributions of genomic/epigenomic and environmental factors in different cases.

Many in vitro and animal studies have identified the toxic effects of environmental factors to AD. Of all, metal neurotoxicants is one of the main environmental risk factors of AD [103]. Al is one of the most commonly toxic metals [29, 35, 50, 93, 125]. Many studies have suggested that Al is not only putatively regarded as an environmental neurotoxicant but also one of the most important environmental risk factors for AD [25, 30, 33, 34, 99, 124–126]. The analysis of brain autopsy samples from AD patients has also indicated that excessive Al exposure may contribute to the beginning and/or progression of AD [11, 29, 30, 93, 122]. Moreover, chelation therapy to reduce the Al burden in AD patients has been reported as beneficial [35]. Therefore, considerable evidence supports the possibility that AD is a form of chronic Al neurotoxicity that occurs in humans [103]. However, other studies failed to demonstrate the association or found that the associations between chronic exposure to Al and AD are not consistent, which is possibly due to differences in study populations, levels of Al exposure, or study designs. In a word, the role of Al in AD has been given a lot of attention, but the connection between AD and Al still exists with controversies [6, 62, 120]. Therefore, the exact relationship between Al and AD or the cause mechanisms of Al in AD has been the subject of scientific debate [62].

10.2 Aluminum and AD

Al exposure is considered to be one of the potential environmental risk factors for the pathogenesis of AD [32, 55] since 1973 when it was first shown that gray matter from brains of AD patients contains more Al than that of non-demented age-matched

controls [21]. The subsequent studies have shown that Al^{3+} can enter the central nervous system (CNS) [10]. Importantly, more Al enters the brain than can exit, resulting in a net increase in the brain's Al content, and Al particularly accumulates in the large highly active neurons of brain regions most prone to damage in AD [104]. Furthermore, a lot of studies demonstrate that Al levels are significantly elevated in serum and CSF of patients with AD [101].

Most of animal experiment studies have also demonstrated that these impairments caused by sub-chronic or chronic exposure to Al are similar to those impairments of AD [6, 10, 102, 109, 116], which include neuropathological, neurochemical, neurophysiological, and neurobehavioral changes [18]. Furthermore, the amounts of Al the animals consumed correlate positively with their serum Al levels [58, 103], and the degree of damage is correlated to the exposure dose of Al. Epidemiological studies found that the concentrations of Al in these EOAD brains are unlikely to be benign and are indeed highly likely to have contributed to both the onset and the aggressive nature of any ongoing AD in these individuals. Al has also been shown to be present in brain tissue in LOAD [68, 79, 127]. These studies lend support to the recent conclusion that brain Al will contribute toward all forms of AD under certain conditions [30].

These causality analyses have evidenced the hypothesis that AD is a human form of chronic Al neurotoxicity, in particular, focusing on chronic Al intake. Of all, high consumption of Al from drinking water may be a risk factor for AD [33, 85]. The most common form of human exposure to Al is by way of the gastrointestinal tract [10], and the absorption of Al occurs across in all parts of the intestine including the colon [112].

It is believed that the neurotoxicity of Al is likely to be the result of a combination of several mechanisms, including oxidative stress [15, 55, 74]; induction of apoptosis and neuronal damage [48]; leading to inflammatory reaction [122, 124]; cholinergic neuron dysfunction; increasing glycation end product formation; reducing neurotransmitter biosynthesis [87]; abnormal Aβ synthesis and metabolism, for example, the overexpression of amyloid precursor protein (APP) [105] and its secretases, such as β-secretase and γ-secretase, decreasing significantly the expression of ADAM9, ADAM10, and ADAM17; and the formation of amyloids deposition [58, 109] and amyloid neurotoxicity [17, 43, 45]; affecting other enzymes and biomolecules related to neurotoxicity and AD [122]; the neuronal and synaptic ultrastructure changes; and the impaired L-LTP of the hippocampus [125].

10.3 Al and Genetic Susceptibility in AD

Most neurodegenerative diseases are now thought to arise from a combination of genetic and environmental factors. In general, none of these factors has complete penetrance, and only the combination of some of them leads to the onset of the

disease [90], and it is supposed that individual genetic characteristics modulate environmental exposures. Twin studies offer a special design for teasing apart the relative importance of genetic and environmental influences [37]. Concordance studies on identical versus nonidentical twin pairs indicate that the etiology of AD is multifactorial with both environmental and genetic susceptibility factors [72]. A nationwide Finnish twin cohort study showed more than two-thirds (68.7%) of identical twin pairs were discordant for AD, indicating a significant environmental component for AD causality [76]. It has been suggested that various environmental factors are most likely causes of sporadic AD patients [51]. Other twin-based studies have consistently realized that AD has mixed causality with significant environmental and genetic components [70]. Our team considered also that environmental factors (such as Al, diet, and possibly viral infections), combined with genetic factors, may play a very important and controllable role in the development of AD, especially in neurobehavioral changes and neural cells loss [124]. Furthermore, there are more and more genetic factors found to be strongly implicated for causing or increasing the risk of AD development [114], for example, these mutations in APP, PSEN1, and PSEN2 genes might directly affect Aβ production or cleavage [84], leading to neuronal apoptosis and dementia [4]. However, with the role of genetics, the environmental influences, and the disease heritability, these interactions in AD remain poorly understood [4]. Therefore, the study of gene-environment interactions in AD is essential to predict disease risk in asymptomatic individuals [91].

The association of Al and AD has a significant history, and yet there remains no consensus as to a role for this known neurotoxin in the disease [20, 29]. A key gap in understanding of the role of Al in the pathological features of AD remains whether Al participates in the pathogenesis, or if plaques and tangles simply accumulate the metal due to increased affinity [16], or probably because of the multifactorial and highly variable presentation of the disease [20]. The present studies have indicated that a small amount of Al exposure has the potential to redirect APP cleavage from its non-amyloidogenic pathway (forming sAPPα) to its amyloidogenic pathway (forming Aβ). The susceptible rats chronically exposed to Al by drinking water or food lead to a slowly progressing cognitive function impairment [102].

Several genes on different chromosomes could be involved in AD's onset. Recently, the genetics of AD have been explored with increasing scope and intensity, revealing that while only a portion of AD is familial, genetics play a strong role even in the common, apparently sporadic cases of AD. Initially, autosomal dominant forms of AD were discovered in genes that are now considered central to the pathogenesis of the disease. Genetic studies have identified novel targets for the development of pharmaceuticals which modulate the influence of low-risk genetic factors [84]. Therefore, genetic testing should be important to understand the mechanisms and pathways leading to neurodegeneration and disease symptoms. Mouse models that express identified human mutations give rise to these pathological hallmarks of AD. In the case of AD, some genetic mutations are associated with both the expression and metabolism of APP [68]. The mutations in the presenilin 1 (PSEN1), presenilin 2 (PSEN2), and APP gene are the well-known genetic cause of familial AD (FAD), and the apolipoprotein E (APOE) ε4 allele has high

susceptibility to developing sporadic AD (SAD) [114]. However, the APP gene, PSEN1 gene, and PSEN2 gene which all encode proteins involved in APP breakdown and Aβ generation have been firmly implicated in the pathophysiology of EOAD. AD-linked mutations in these three genes exhibit high penetrance (>85%) [79]. Mutations in 21 other genes and an 18q deletion syndrome have also been reported to be associated with tau pathology reminiscent of AD [98]. The genomic susceptibility and mechanisms leading to (or accompanying) the impairment of the central APP processing and tau networks are widely accepted as major contributors to the diseased state.

Two polymorphic sites, located at codon 112 and 158, have been described in the human apolipoprotein E (APOE) gene. At least three main variations of the APOE gene have been identified, called ε2, ε3, and ε4 alleles [94]. ε3 is the most common variant (77%), while ε2 (8%) and ε4 (15%) alleles have been detected less frequently [4]. Among them, APOE allele ε4 is the strongest and most consistent risk factor of AD and extensively influences the clinical manifestations of AD, as well as neuropsychiatric symptoms [79, 84, 115].

When studying the pathogenesis of AD, the interaction between genetic factors and Al should be taken into account. The APPswe (Tg2576) transgenic mice that were orally exposed during 14 months to Al lactate (1 mg of Al per g of chow) are more sensitive to the worsening effects of Al in spatial learning [22]. Amyloidosis via Aβ production and accumulation is a central pathology in AD, and the Aβ pathway is further influenced by genetic and epidemiological risk factors than others [84]. In the brain of transgenic mice that overexpress APP, Al exacerbates oxidative stress, Aβ deposition, and SPs' formation [73]. The SPs' formation occurred earlier and in appreciably larger amounts in brains of Al-exposed transgenic mice than in brains of a transgenic cohort without Al supplementation. APP/tau triple-transgenic (3xTg-AD) mice are reported to have higher Al levels in their brains than controls, even without Al supplementation [23]. When male wild-type mice (C57BL/6 J strain) and male APP/PS1 transgenic mice, which expressed both the Swedish double mutations of APP (K595N/M596L) and mutant PS1 (Hu PS1 deltaE9), were microinjected separately into the left lateral cerebral ventricle with 2 μl of either modified artificial cerebrospinal fluid or $AlCl_3$ solution (l μg element Al in 2 μl ACF, pH 6.8) once a day for five successive days, the decrease of cognitive ability and neural cell loss in APP/PS1 transgenic mice exposed to Al was shown more extensively than those in APP/PS1 transgenic mice alone and wild-type mice exposed to Al alone. These findings indicate that there is a close relationship between overexpression of APP and PSEN1 genes and Al overload. The above research also suggested that APP/PS1 TG mice exposed to Al have potential value for improving AD models [124]. Chronic oral ingestion of Al may more strongly promote tau aggregation, apoptosis, and neurological dysfunction in tau transgenic mice than in wild-type mice [71].

10.4 Al and Epigenetic Modification in AD

It is increasingly acknowledged that epigenetic phenomena may be a crucial component in the development of complex brain disorders [107]. It is known that genetic and nongenetic factors contribute to the development of AD. However, rare mutations in three genes – APP, PSEN1, and PSEN2 – are associated with 1% of AD, and other frequent genetic variants such as APOE ε4 can account for up to 20% of total cases of the disease. In total, the heritability for AD is estimated to explain between one-half and two-thirds of total AD cases. The other third/half being attributable to nongenetic risk factors and epigenetics provides a means by which environmental factors such as diet, hazardous exposures, and life events can influence gene expression. These nongenetic risk factors in which epigenetic mechanisms are supposedly involved, which combine genetic and environmental risk factors in an epigenetic pathway, suggest that AD risk is established during early life [52, 53]. In addition, epigenetic modifications can mimic, exacerbate, or even cause genetic mutations. Furthermore, the involvement of epigenetic mechanisms in the developing memory formation either under pathological or physiological conditions has become clear. Therefore, epigenetic mechanisms are known to alter gene expression or cellular phenotype in a heritable manner and allow for the integration of long-lasting nongenetic inputs on specific genetic backgrounds. In a word, epigenetic mechanisms may provide a point of intersection for the diverse risk factors and pathophysiologic processes of AD [66]. However, research over the years has shown that epigenetic mechanisms of AD are not well understood.

The epigenome is responsible for the molding and the three-dimensional structure of the genomic material in the cell nucleus. Epigenetic alterations represent a sort of functional modifications related to the genome that is not responsible for changes in the nucleotide sequence [89]. Therefore, the epigenetics represents the heredity of changes in phenotype that are independent of altered DNA sequences [51, 75] and are transmitted from one generation to another [14, 51]. It is these types of processes that play an important role in making a combination of genetic and environmental factors for effecting long-term adaptive changes in expression of genes. It can be concluded that genome-environment interactions are mediated by epigenetic mechanisms [75]. Epigenetic modifications seem to have a special relevance in the nervous system [89]. Previous studies have indicated that epigenetic mechanisms provide a bridge between genes and environment by which environmental events can be translated to the cellular and molecular level and may help to improve our understanding on the etiology of complex diseases, AD, and PD [47].

Epigenetic status can be modified by environmental exposures such as nutrition, social status, chemical and emotional environment, pregnancy conditions, infertility, contraception, and different modalities of pharmacological intervention [14]. Further studies found that environmental factors may exert their influence via epigenetic

changes to DNA and other changes to gene expression [84], and alterations of epigenetic states play essential roles in protecting organisms from environmental stresses [5]. From the foregoing, AD is a complex disease caused by environmental, genetic, and lifestyle factors. Furthermore, several epidemiological and clinical features of AD suggest an epigenetic contribution to etiology [60]. Therefore, environmental toxins associated with AD can modify the epigenetic makeup and might thereby contribute to the pathophysiology of AD. Compared to genetic and environmental causes, epigenetic factors are probably much more suited to explain the observed anomalies in LOAD as aberrant epigenetic patterns may be acquired during many developmental stages [107]. Cacabelos R also found that AD-related genes exhibit epigenetic changes, and epigenetics might exert a pathogenic role in dementia [17]. It is known that epigenetic mechanisms participate in the processes of learning and memory formation. Wang et al. also considered that AD patients may undergo an enhanced epigenetic drift or alternatively their epigenomes were already at an advanced level of abnormality earlier in life, for example, due to the influence of environmental factors, transgenerational effects, or disruption of the epigenetic machinery [107]. A growing body of evidence indicate that epigenetic pathways could be involved in the pathogenesis of AD [84]. Recently, a growing number of epigenetic alterations in AD have been described, and these mutations of several enzymes that are associated with epigenetic machinery in neurodegenerative processes are altered in AD [89]. Furthermore, epigenetic modifications are reversible and can potentially be targeted by pharmacological intervention. Although lots of studies have pointed that epigenetic mechanisms could play a role in AD [64], very little is known about exact epigenetic patterns in AD and other neurodegenerative disorders [107], and a comprehensive assessment of the role of epigenetic mechanisms in the development of AD has not yet been done. Therefore, it is very important to know more about the epigenetic patterns of the genes involved in AD pathogenesis to understand the mechanisms that regulate gene function and to potentially enable pharmacological intervention on the epigenetic level [107].

Epigenetics can provide a mechanistic explanation that might offer unique opportunities to increase our understanding of such disorders [97, 100]. The primary mechanisms of epigenetic processes include DNA methylation, histone modifications (acetylation, phosphorylation, methylation, ubiquitination, ADP ribosylation, and sumoylation), and noncoding RNAs [14, 38, 60, 75]. DNA methylation and histone modifications are the most intensively studied among the major epigenetic modifications and have been reported to play a role in AD [111]. Current research suggests that the Al-dependent alteration of methylation status in DNA and histone actually occurred in some genes, but not all genes.

DNA methylation, the addition of a methyl group to the 5’ position of cytosine in a dinucleotide CpG site, is a stable and self-perpetuating regulator of cellular identity through the establishment and propagation of persistent, heritable changes in gene expression across cell divisions [8]. CpG islands are extended regions of cytosine and guanine repeats in the promoter region of many mammalian genes [78]. Around 95% of these dinucleotides of CpGs are scattered through all the genome without showing any type of aggregation, and the remaining tend to accumulate in

CpG islands. The direction of association between DNA methylation and gene expression depends on where within the gene sequence the methylation occurs. DNA methylation in the promoter region of the gene downregulates its expression, whereas higher methylation in the gene body may promote the expression of the gene [10]. DNA methylation in the promoter region of a gene has been associated with decreased transcriptional activity. Whenever methylation in DNA occurs in the promoter region, transcriptional levels get affected mainly in two ways: (1) transcription factors are unable to bind to the promoter regions of a gene due to the methylation of DNA in that region, and (2) methyl-CpG-binding proteins (MBDPs) bind these methylated DNA sequences [75]. Importantly, some studies discovered differentially methylated CpGs outside of well-established AD genetic risk loci, highlighting the potential utility of epigenome-wide association studies (EWAS) in the characterization of novel genes and pathways underlying disease processes [110]. Recent studies highlight the importance of epigenetic modifications occurring outside of promoter CpG islands; in fact functionally relevant epigenomic variation may primarily occur at non-promoter CpG islands, low CG-content promoters, and the gene body [64].

DNA methylation is thought to be a very important modification in epigenetic regulation and has been studied intensively for the past several decades [89]. DNA methylation is involved in multiple neurodevelopmental and neurodegenerative disorders and could play important roles in the pathogenesis of AD [64]. Several lines of evidence point to the influence of DNA methylation in AD pathogenesis [110], including direct connections between AD and DNA methylation that have been observed both globally and at specific loci. The most evidence that AD is associated with epigenetic changes is global hypomethylation in AD [54]. Furthermore, it seems that at least three classical AD-associated genes are not epigenetically dysregulated in AD at the DNA methylation level, which might indicate that DNA methylation changes do not play a role in AD or that genetic and nongenetic forms of AD might be the results of alterations in a different subset of genes [89]. Al decreases the methylation level of pectin and consequently results in higher Al binding in the cell wall [95]. Wang also thought that there is a genome-wide decrease in DNA methylation in AD [108]. Our previous epidemiological research also showed that the Al-exposed workers had higher serum Al concentration and lower global DNA methylation [117]. Genes can be switched *on* and *off* by controlling the DNA methylation of their CGIs [89]. Many pathogenic genes (APP, PSEN1, APOE, BACE) in AD and other AD-related susceptibility genes contain methylated CpG sites in their promoter regions [108]. The promoter region of the APP gene is hypomethylated, with this contributing to a potential enhancement of Aβ production. An epidemiological study of aluminum workers showed that reduced methylation of the promoter region of APP gene may be associated with increased serum aluminum level, and downregulated methylation of the promoter region of APP gene may accelerate APP gene transcription [119]. Animal experiment also found that aluminum chloride might cause APP promoter methylation decline, which affect the APP mRNA, increase APP expression, and result in Aβ deposition in the hippocampus in male SPF grade SD rats [118]. Furthermore, in vitro hypomethylation of PSEN1

increased the cleavage of APP and the production of Aβ in a neuroblastoma cell line [36]. However, a sign of potential deregulation of the epigenomic machinery in the brains of affected individuals may be the observation that aberrant DNA methylation patterns are not uniform and occur either as demethylation or as de novo methylation [107]. Al stress resulted in demethylation and de novo methylation in both tolerant and non-tolerant triticale lines; however, de novo methylation of CHG sequence was affected in tolerant lines but not in non-tolerant lines [5]. Recent evidence shows that AD patients have an elevated DNA methylation state of repetitive elements [9]. Furthermore, some authors have reported no relevant changes in APP methylation, with an epigenetic drift in AD samples [107].

Learning and memory can be broadly defined as lasting alterations of a behavioral output produced in response to a transient environmental input [96]. In order for a transient stimulus to induce a lasting change in behavior, cells must undergo a complex set of stimulus-specific cellular and molecular changes that will consolidate a memory into an everlasting trace [128]. This epigenetic process of gene expression is critical to learning and memory. One of the most important findings that support the importance of epigenetics in the functioning of the brain has been the discovery that neuronal activity per se modifies DNA methylation and histone modifications patterns [128], which have emerged as important regulators of the memory process [128]. Our previous research showed that mild cognitive impairment (MCI) was significantly associated with global DNA methylation in blood of a total of 366 Al-exposed workers [117], and aluminum maltolate may induce learning and memory impairment and decrease genome-wide methylation rate in rats [121].

DNA methylation involves the transfer of a methyl group to the carbon-5 position of cytosine to produce 5-methylcytosine (5mC). Despite the clear alteration of DNA methylation observed in AD, whether and how 5hmC is involved in AD pathogenesis still remain largely unknown [92]. Mastroeni and colleagues report that global levels of 5mC and 5-hydroxymethylcytosine (5hmC) are significantly lower in neurons in the entorhinal cortex in AD patients compared to non-demented elderly controls [64]. However, the study conducted in 60 truck drivers and 60 office workers in Beijing have found that exposure to ambient PM_{10} and personal $PM_{2.5}$ and its elemental components (potassium, sulfur, iron, silicon, aluminum, zinc, calcium, and titanium) affects blood DNA 5hmC over time, but not 5mC [88].

DNA methyltransferases (DNMTs) responsible for the methylation process have been shown to catalyze the transfer of a methyl group to single-stranded DNA using S-adenosyl methionine as the methyl donor [7, 64]. The recognition sequence for the mammalian DNA methyltransferase is relatively invariant, with nearly all cytosine methylations occurring on CpG. There are four known active DNA methyltransferases in mammals, DNMT1, DNMT2, DNMT3A, and DNMT3B. DNMT1 and DNMT3A are the main enzymes in mammalian brain. DNMT1 has been reported to be a key player in maintaining methylation in somatic cells, and loss of this enzyme has been shown to lead to nuclear disorganization, increased histone acetylation, and apoptosis.

Besides, Al^{3+} modulates methylation reactions, including DNA methylation in SH-SY5Y human neuroblastoma cells by inhibited insulin-like growth factor-1(IGF-1) and dopamine-stimulated methionine synthase (MS) activity, as well as

folate-dependent phospholipid methylation, via a PI3-kinase- and MAP-kinase-dependent mechanism [106].

However, the methylation especially in DNA was very complex, and the alterations seemed not to simply follow whether the target gene was Al-induced type or Al-repressed type [5, 31]. Therefore, the development of a clear mechanistic understanding of the mechanisms underlying Al neurotoxicity remains elusive [10].

Epigenetic regulation via the posttranslational modification of histone proteins is another essential cellular mechanism regulating gene expression, with a spectrum of distinct histone modifications acting to dynamically alter chromatin structure and influence transcription [36]. The alkaline histones are abundantly found in eukaryotic cell nuclei, and they are chief protein components of chromatin. Histones can undergo posttranslational epigenetic modification by acetylation, methylation, phosphorylation, ubiquitination, or sumoylation [78]. Histone acetylation and deacetylation regulate gene transcription by altering the chromatin structure and the accessibility to transcription factors. Nucleosomes are efficient DNA-packaging units. The fundamental protein unit of the nucleosome is the histone dimer, a simple α-helical domain possessing a highly basic, curved surface that closely matches the phosphate backbone of bent duplex DNA [75]. Two copies each of histone heterodimer, H3/H4 and H2A/H2B, form a histone octamer that is wrapped with approximately 146 bp of duplex DNA in a left-handed spiral [81]. Histone H3 and H4 acetylation have been demonstrated to be markers of an "open" configuration of chromatin [114]. Nucleosomes are not only influenced by DNA methylation and sequence context but also primarily regulated by posttranslational modifications that tend to occur in the N-terminal tail of histone proteins [12]. In the nervous system, histone acetylation has been unequivocally associated with facilitating learning and memory [39]. It is becoming more evident that histone acetylation plays a key role in the etiology of AD [114]. Accumulating evidences in vivo and in vitro support the contention that histone modification and dysfunction are associated with the etiology of AD [114]. Acetylation of histones is generally characterized by an elevation in gene expression; conversely, deacetylation is associated with a decrease in gene expression, which is a commonality in AD [2].

Histone acetylases and histone deacetylases (HDACs) are the well-known covalent enzymes that modify the reversible acetylation of lysine residues in histone amino-terminal domains [40, 114]. The most compelling evidence on the role of epigenetics on AD comes from the results of treatment of AD patients with inhibitors of HDAC [111]. Some studies have found that a decrease in histone acetylation has been observed in animal models of AD due to increased histone deacetylase (HDAC)-2 [40]. Some recent studies indicate that HDAC inhibitors are neuroprotective by regulating memory and synaptic dysfunctions in cellular and animal models of AD, while on the other hand, increase of histone acetylation has been implicated in AD pathology [114]. Histone acetyltransferase p300 plays a critical role in controlling the expression of AD-related genes through regulating the acetylation of their promoter regions, suggesting that p300 may represent a novel potential therapeutic target for AD [59]. Some studies have found that histone phosphorylation exists in brain tissue from AD patients [78]. In AD, however, the roles of these enzymes are controversial.

Noncoding RNAs (ncRNAs) are produced by the cells having regulatory functions [75]. ncRNAs, including microRNAs (miRNAs), have emerged as a major class of regulatory molecules involved in virtually all physiological and disease states [63]. miRNAs, mainly located in the intergenic region or intron reverse repeated region, are a class of noncoding small RNAs composed of 20–23 nucleotides in length and work in posttranscriptional gene regulation by either targeting messenger RNAs (mRNAs) for degradation or by inhibiting the translation of mRNAs. miRNAs can reduce protein translation by binding to the complementary mRNA sequence [84] and can promote the cleavage of target mRNAs. miRNA plays a pivotal role in the development of ND including AD. The miRNAs are attractive molecules to utilize as one of the blood-based biomarkers for ND such as AD because miRNAs are relatively stable in biofluid, including serum or plasma [44]. It is recently demonstrated that miRNAs are involved in the responses to heavy metal stresses in plant. By guiding posttranscriptional cleavage or translational suppression or DNA methylation, miRNAs negatively regulate the expression of target mRNAs [49]. The modulation of miRNA expression can be implicated in Al toxicity and Al tolerance in plants [57]. It has been proven that Al can trigger broad changes in miRNA expression in rice roots [57]. Therefore, miRNAs may be one of the molecular mediators associated with responses to Al stress in plants. The regulatory roles of miR319, miR390, miR393, miR319a.2, and miR398 in Al stress signaling network have been identified. A study about the effects of aluminum oxide nanoparticles on the growth and development in tobacco shows that with the concentrations of aluminum, oxide nanoparticles increase; the root length, the average biomass, and the leaf count of each tobacco seedling decreased; and the expression profile of certain miRNAs was significantly upregulated [13].

In short, although some epigenetic studies on Al neurotoxicity have been reported, there remains much uncertainty as to the complete role for DNA methylation and other forms of epigenetic regulation to changes in gene expression associated and caused by AD [84].

10.5 Al and Genetic Susceptibility and Epigenetic Modification in AD

Given the above, AD is one of the most prevalent causes of human deterioration in modern society affecting old people. Genetic and environmental factors contribute to the initiation and progression of the disease [22]. Al has been proposed as a potential environmental risk factor to develop AD [22], and some genetic polymorphisms predispose to a greater susceptibility to its adverse effects [79]. Epigenetic mechanisms seem to play an ubiquitous role in the establishment of lasting neural and behavioral modifications in response to environmental stimuli [128]. Tg2576 mice fed for 210 days with rodent chow supplemented with Al lactate at 11 mg/g of food have impaired spatial learning than previous research [80]. Epigenetics

provide a bridge between genes and environment to improve our understanding on the etiology of AD [47]. A study found that in the case of dietary depletion, the PSEN1 promoter can become hypomethylated in TgCRND8 – harboring the Swedish and V717F Indiana APP mutations [19] – and APPswe/PS1dE9 AD models [46]. Furthermore, epigenetic processes integrate abundant signals of genetics and environment into phenotypic outcomes [42]. However, it is hard to draw any conclusions about specific AD-associated epigenetic changes from the limited existing literature [60]. Therefore, there has been increasing interest in the role of epigenetic mechanisms in the interaction between the genome and environment in AD [60, 65].

10.6 Summary

To date, substantial progress has been made over the past few decades in understanding neurotoxicity of Al and its role in AD. The roles for Al, genetic, and epigenetic factors in AD risk have been identified, and their interrelationship has been discussed. However, our knowledge of these risk factors is still incomplete and needs consistent evidence. Despite considerable speculation about the role of epigenetic dysfunction in AD, this is a relatively nascent area of the role of Al in AD.

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