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Arsenic Contamination in Asia

Biological Effects and Preventive Measures

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Editors

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Editors
Hiroshi Yamauchi
Department of Preventive Medicine
St. Marianna University School of Medicine
Kawasaki
Japan

Guifan Sun
Research Center of Chronic Diseases
and Environment
School of Public Health
China Medical University
Shenyang, Liaoning
People's Republic of China

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Preface

In the late 1970s, the contamination of air and water by various potentially toxic substances discharged as by-products of industrial activities was recognized as a serious social problem in more economically developed countries. However, this nonnatural environmental contamination was not a serious problem in most of Asia, as agriculture was the predominant industry and this area was not at this time undergoing heavy industrialization. However, unlike the contamination due to human activities, toxic substances can be deposited in air and water due to natural processes, and the finding of arsenic in the drinking water in many places in Asia is an excellent example. Indeed, geological findings have demonstrated that arsenic in ores that arise from the earth's magma have a complex distribution due to the activities of the crust. A trace amount of arsenic can leach from the underground ores and rocks into nearby rivers, leading to the sedimentation of inorganic arsenic in the watershed and estuary regions of these rivers. Consequently, for instance, the Ganges River and Mekong River which both originate in the Himalayas have some of the highest recorded arsenic contamination in Asia. Similarly in China, groundwater flowing through underground rock formations beneath mountains where the Great Wall of China was constructed is also heavily contaminated with arsenic. Thus, the large-scale chronic arsenic poisonings seen in Asia are not caused by arsenic emanating from industries but are the manifestation of a rare health hazard arising from inorganic arsenic found in nature.

As the population increased in various developing Asian countries in the 1970s, a switch in the use of surface water to groundwater was recommended as a preventive measure against diseases associated with surface water consumption. International organizations widely supported the large-scale placement of pump-type wells to afford a supply of clean water to regional residents. However, in doing so, the exposure to inorganic arsenic occurred, and this exposure continues even today, particularly in people who sank wells into the estuary sediment in search of groundwater. Unfortunately, these people did not understand how to properly conduct a safety risk assessment when using well water and lacked both the equipment and techniques to test for arsenic. In our previous epidemiological survey of chronic arsenic poisoning in China, it was impossible to assay arsenic at the survey sites

during 1996–2000; thus, we analyzed the test samples for arsenic after transporting them back to Japan. We believe that other cooperative surveys on people in other Asian countries likely had to be conducted in a similar manner. Chinese and Japanese researchers who participated in a field survey in China conducted from 1996 to 2017 are in charge of several chapters of this book and will discuss their issues and findings.

Epidemiological studies of chronic arsenic poisoning in Asian countries reveal serious health hazards in Bangladesh, Western India, and China. Severe keratosis is common on the palms, the soles of the feet, and elsewhere and occasionally results in skin cancer in some patients. As epidemiological surveys progressed, a relationship between exposure to inorganic arsenic and lifestyle diseases, such as diabetes and hypertension, was also revealed. New problems, such as influences on the next generation and brain dysfunction arising from the exposure of pregnant women and children to inorganic arsenic, have recently gained attention.

It is important to discuss how chronic arsenic poisoning has become a social problem in Asia. The general keratosis that develops on the palms and soles of the patients causes severe pain. Hand and foot pathology and pain make it difficult for patients to work, causing them and their family's economic difficulties. Additionally, cancer mortality arising from the exposure to inorganic arsenic, which has increased over time, has become a serious problem. Therefore, efforts are being made to reduce this risk by decreasing the level of exposure.

A new problem has arisen in several more economically developed countries in which the health hazards from arsenic exposure are not yet fully understood. The ingestion of $\omega - 3$ fatty acids (eicosapentaenoic acid and docosahexaenoic acid), via the consumption of fish and shellfish, is recommended as a preventive measure for lifestyle diseases for people who have generally improved living standards in advanced countries. However, marine organisms contain abundant arsenic compounds, although the potential toxicity of these organic arsenical compounds is poorly defined as partially because concern was focused on inorganic arsenic in seafood. However, recent research results indicate that the toxicity of organic arsenical compounds and their metabolites can be equal to or even higher than that of inorganic arsenic; the safety of seafood-derived organic arsenicals requires further scientific evaluation.

Thus, Asian residents who are exposed to inorganic arsenic or those who ingest organic arsenical compounds along with health-promoting substances live in distinctive areas with regard to "natural" overexposure to arsenic. They may also face unique and serious problems, such as arsenic-induced impact on the next generation and brain dysfunction, which will continue as long as pregnant women and children are exposed to inorganic or organic arsenical compounds. Potential future problems resulting from these phenomena include work loss and influence on social security expenditure, including medical welfare disbursements. Currently, there are no effective drugs or treatment methods for chronic arsenic poisoning, and exposures to high concentrations of arsenic occur all too frequently. We believe that research activities using new approaches, particularly regarding various preventive measures for this health hazard, are necessary for the study of arsenic toxicity.

This book discusses the relationships between exposure to inorganic arsenic and its biological influences elucidated from surveys of chronic arsenic poisoning in Asia; international research achievements are also considered. The book introduces proposed drugs aimed to prevent and/or improve disease states created by arsenic. In addition, we discuss the significance and status of developing elimination technologies for the detoxification of arsenic. We hope that the information provided in this book will help develop new innovations that will contribute to the prevention and amelioration of the influences of arsenic on health that are present in contemporary society.

Kawasaki, Japan Hiroshi Yamauchi

Contents

Chapter 1
Past and Current Arsenic Poisonings

Hiroshi Yamauchi and Ayako Takata

Abstract The health issues arising from the exposure to inorganic arsenic (iAs), an increasingly important problem in Asia, are remarkable because the source of the iAs is the natural environment and the number of people affected is in the order of tens of millions. In many cases 20–30 years have passed since the onset of exposure to high-level iAs, which raises concern about the imminent occurrence of excessive cancers related to this exposure, as this is the typical time frame for chemically induced cancer in humans. Studies conducted in Chile and Argentina, where oral exposure to iAs has a longer history than in Asia, have revealed a causal relationship between iAs and lung and bladder cancers. Furthermore, multiple studies have presented evidence that early life iAs exposures can influence the next generation by induction of tumors and brain dysfunctions. Follow-up surveys on subacute arsenic poisoning in infants that occurred about 50 years ago in Japan (approximately 12,000 victims, including 130 deaths) confirmed the manifestations of growth inhibition and central nervous system disorders. These findings over the last century provide essential information for the planning and implementation of future studies. Because it is necessary to intensify research on the influences of arsenic compounds on the next generation and on its impact on brain function, promotion of future studies involving collaboration across the fields of medicine, neurology, environmentology, pharmacy, nutritional science, and engineering is required.

Keywords Historical arsenic poisonings · Chronic arsenic poisoning · Inorganic arsenic contamination of drinking water · Inorganic arsenic contamination of food · Medicinal arsenicals

H. Yamauchi (✉) · A. Takata
Department of Preventive Medicine, St. Marianna University School of Medicine, Kawasaki, Japan
e-mail: hyama@marianna-u.ac.jp

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1.1 Introduction

Arsenic is one of the most well-known poisons in human history and was easy applied to murder by poisoning because of its physical properties, including being tasteless and odorless. Arsenic has been suggested to have been involved in the deaths of major historical figures such as Alexander the Great and Napoleon I. Although arsenic has been feared as a poison, it also has a long history of being used as a medicine. However, chronic arsenic poisoning developed as an adverse reaction to its use of medicinal arsenicals.

Among the various health problems associated with the exposure to arsenic in the twentieth century, development of cancer in copper smelters workers and those engaged in manufacturing of arsenic trioxide attracted early attention [1–3]. However, since the 1980s, chronic arsenic poisoning due to consumption of potable water naturally contaminated with inorganic arsenic (iAs) was also widely found in Asia, shifting the focus to environmentally caused chronic arsenic poisoning. The number of individuals excessively exposed to arsenic in Asia is speculated to be in the tens of millions. Serious health issues due to iAs exposure in Bangladesh, western India, and China have been detected, including skin cancer in some cases [4]. Furthermore, environmentally induced chronic arsenic poisoning occurred in Chile and Argentina as early as several decades before its occurrence in Asia. Indeed, an increasing number of lung and bladder cancer cases was already been confirmed in Argentina [5–8] and Chile [9–11] in association with arsenic exposure. The International Agency for Research on Cancer (IARC) has confirmed a causal relationship between the exposure to iAs and skin, lung, bladder, and liver cancers, for which the time of high-level iAs exposure was about 30 years [12]. In general, the total exposure time required from first exposure to tumor formation for human tumors associated with chemical insults is speculated to be approximately 30 years [12]. Given that 20–30 years on average have elapsed since the first exposures to high-level iAs in Asia, apart from in Taiwan, we consider it important to actively enhance the clinical diagnosis of cancer at sites typically associated with arsenic exposure in this geographic area as well as to conduct advanced studies using molecular biologic techniques to characterize the cancers as they are detected.

We expect that this book, titled *Arsenic Contamination in Asia: Biological Effects and Preventive Measures*, will aid in shedding light on the causes, consequences, and possible responses to this environmentally based chronic arsenic intoxication in Asia. We also hope the contents will help to stimulate important ideas for new study concepts and designs and define critical public health responses and possible administrative actions for this issue. We believe that, without exception, previous case studies on arsenic poisoning are helpful as references when clarifying current issues as well as issues that may arise in the future.

This chapter provides an outline of prior human arsenic poisoning cases arising from oral exposure. These cases are outlined here for the purpose of providing a historical perspective and to help in resolving current and future issues that will arise regarding arsenic poisoning.

1.2 Specific Historical Cases of Arsenic Poisoning

1.2.1 Arsenical Medicines

Arsenic compounds have a very long history of medicinal use worldwide. In Europe by the late 1700s, a major early medicinal arsenical had been introduced, namely, Fowler's solution, which was a solution of potassium arsenite and was in frequent use (Fig. 1.1) for the treatment of cancer, infections, epilepsy, asthma, and skin diseases such as psoriasis and eczema. Generally, this solution was orally used by diluting it in water, and the oral arsenic intake is estimated to be approximately 9 mg/day in average pharmacological use [13, 14]. Coincidentally, this arsenic intake is similar to the daily dose of arsenic trioxide (10 mg/day) [15], which is a currently used, medically accepted medicinal arsenical drug which has proven highly efficacious in the treatment of acute myelocytic leukemia. An interesting point in the medical use of Fowler's solution was that the daily consumed volume (and thus arsenic dose) was typically increased until the disease was completely cured. However, the medication would be stopped, at least temporarily, when eyelid swelling and/or severe gastrointestinal peristalsis occurred, which were considered to be limiting adverse reactions. Once these adverse reactions resolved, or at least diminished, therapy would again commence with a repeat of the same course of doses as from the onset of treatment. The long-term ingestion of Fowler's solution was found to be associated with pigmentary degeneration of the trunk and palm and with footpad keratosis and squamous cell carcinoma on the hands and feet [13, 14]. Interestingly, these

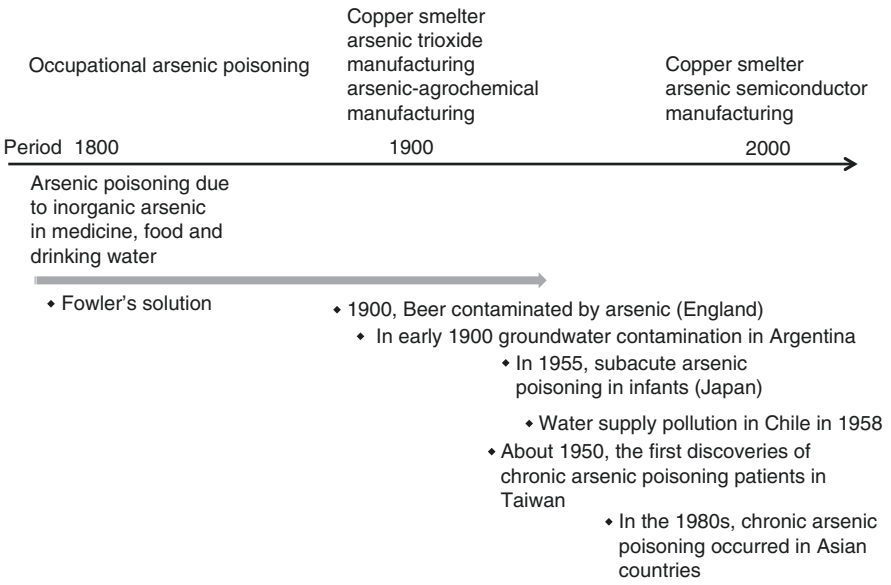

Fig. 1.1 Timing and historical causes of arsenic poisonings

findings are strikingly similar to lesions seen in patients with chronic arsenic poisoning in Asia. Fowler's solution was also used as a tonic, but demand for it gradually waned, and its use disappeared by the middle of the twentieth century.

1.2.2 Beer Contaminated by Arsenic

In 1901, Reynolds reported over 500 patients with chronic arsenic poisoning due to oral exposure [16]. This arsenic poisoning developed in residents of the central and northern parts of the United Kingdom in 1900 (Fig. 1.1) and was caused by beer contaminated with arsenic (likely arsenic trioxide), which the patients unknowingly consumed for several months. A notable achievement by Reynolds is that he differentially diagnosed the symptoms of alcoholism from those of arsenic poisoning. The symptoms of arsenic poisoning observed in the patients were initially gastrointestinal, followed by catarrhal (mucosal inflammation), peripheral nervous system, and cutaneous symptoms, in that order. Reynolds reported that the cutaneous symptoms included raindrop pigmentation changes and subsequent palm and footpad keratosis. These clinical findings are similar to those of arsenic poisoning due to consumption of Fowler's solution that was in common used in Europe and the United States from the eighteenth to mid-twentieth century. Thus, it is fascinating that the criterion for the diagnosis of arsenic poisoning was established over 100 years ago in the United Kingdom.

1.2.3 Dry Milk Contaminated with Arsenic

The Morinaga Milk arsenic poisoning incident that occurred in Japan in the 1950s could be the worst incident of poisoning from a food contaminated by arsenic in human history. However, this incident may not yet be correctly understood internationally because the situation and details of how the incident occurred have not been presented in English in the form of a report or a scientific research article.

In the Morinaga Milk arsenic poisoning incident in 1955, individuals from several cities in the western part of Japan, including Kyoto, Osaka, and Okayama, were affected (Fig. 1.1). The cause of the arsenic poisoning was the contamination of dry milk powder used for infants with iAs. Over 12,000 infants who ingested the iAs contaminated powdered milk after formulation developed subacute arsenic poisoning, and of these 130 died [17, 18]. The powdered milk contaminated with iAs was ingested on average for 3 months, and the estimated daily iAs intake per infant was 1.3–3.6 mg, which leads to an average total consumption of 90–140 mg of iAs. The primary initial symptoms presented as fever, vomiting, diarrhea, a sense of abdominal distension, hepatomegaly, cough, nasal discharge, conjunctivitis, and melanoderma. However, neurological manifestations likely would not have been easily identified because the patients were infants. Laboratory findings included anemia, decreased granulocyte count, electrocardiographic abnormalities, and a radiographic band-like shadow of the epiphysis of long

bones. A foundation was formed for the long-term support and clinical care of these patients. Results of a survey of patients in the 15th year after the poisoning event identified delayed growth, intellectual disability, central nervous system disorders such as epilepsy, hearing loss, and skin disorders such as melanoderma and keratosis [19, 20]. A survey in the 50th year post-poisoning indicated increased incidence of cancer and dementia. Moreover, it was revealed that the survivors of the arsenic poisoning in their infancy had a significantly higher risk of death from a neurological disease than the general population [21, 22]. We consider that the results of these follow-up surveys of the survivors of the subacute arsenic poisoning in infancy are a warning of the risks of human exposure to iAs in early life and would likely include exposures to infants and fetuses (i.e., in utero) via pregnant women. That this early life "pulse" exposure to arsenic in humans can have such dire consequences, like cancer, dementia, and epilepsy, decades later in life indicates arsenic may have a special negative affinity for developing systems, including the developing nervous system.

1.3 Chronic Arsenic Poisoning Arising from the Exposure to iAs in Potable Water

Large-scale chronic arsenic poisoning arising from the long-term ingestion of potable water naturally contaminated by iAs seems to have been detected first in areas of Argentina, followed by sites in Taiwan, Chile, and other Asian countries.

1.3.1 Chronic Arsenic Poisoning in Argentina and Chile

Interestingly, it has been reported that arsenic contamination of drinking water in Argentina and Chile is linked to cancer risk, among other adverse health effects (Fig. 1.1).

In Argentina, the contamination of well water by iAs from the environment was reported in Cordoba Province since the 1910s, and patients with chronic arsenic poisoning from this area have been identified, although sporadically. In this vast country, because underground water is the only water source, the use of wells is common. The average arsenic (inorganic arsenic) concentration in groundwater of Cordoba Province is reported to have been 178 μg/L. When the standardized mortality ratio was compared between all of Argentina and just Cordoba Province on the basis of studies conducted mainly by the US researchers since the 1990s, significant increases in cancers of the lung and bladder, among other sites, were observed associated with the exposure to arsenic, though there was association observed for skin or liver cancers [5–8].

A well-known case of mass chronic arsenic poisoning occurred in Antofagasta in Chile, which is the world's leading country for copper mining. In 1958, the source of tap water supplied to the residents of this city (with a population of approximately

300,000) was contaminated by mine drainage containing iAs. Because of the lack of an alternative water source due to the severe geographic conditions, this exposure to iAs continued until 1970 when water cleaning equipment was developed. In the 1970s, Chilean researchers reported the actual conditions of this chronic arsenic poisoning, in which the number of affected individuals was roughly estimated to be over 200,000. Subsequent studies, mainly by US researchers since the 1990s, revealed several relationships between exposure to iAs and causes of death. The cessation of iAs exposure significantly reduced mortality due to acute myocardial infarction. However, mortality due to lung and bladder cancers increased even after cessation of exposure. This indicates that the risk of cancer in individuals who have been exposed to chronically iAs is unlikely to decrease after cessation of exposure, at least at key sites [9–11].

1.3.2 Chronic Arsenic Poisoning in Asia

In the 1950s, chronic arsenic poisoning from iAs contamination in well water was discovered in the residents of southwest Taiwan (Fig. 1.1) [23, 24]. Some of these residents showed group of symptoms that formed a skin disorder in which the tips of the toes and fingers underwent a necrosis involving the microvasculature, leading to the diagnosis of would be called Blackfoot disease. However, Blackfoot disease has not been found in regions outside of Taiwan where chronic arsenic poisoning is endemic. Thus, Blackfoot disease has been suggested to be a pathological condition that is specific to Taiwan potentially because of how iAs interacts specifically with this local population or the possibility based in an interaction between iAs and local exposure to an ergot alkaloid [25, 26]. Its actual basis has never been definitively clarified. However, research in Taiwan has revealed a dose-response relationship between arsenic intake from well water and skin and bladder cancer. Because chronic arsenic poisoning by drinking water in this region was identified earlier than in other regions, it provided important foundational information for subsequent studies.

In the late 1970s, as the population increased in Asia, a switch in the use of surface water to groundwater was recommended as a measure to help prevent surface waterborne infections, and various international organizations supported the placement of pump-type wells on a wide scale to ensure the extensive availability of a clean water supply to local residents. However, a lack of a sufficient safety assessment of contaminants in well water, specifically iAs, subsequently led to the development of health issues. Thus, we must stress the importance of environmental risk assessments for researchers and administrators in the fields of environment science, public health, community medicine, and preventive medicine.

Hundreds of thousands of patients with chronic arsenic poisoning have been identified in Bangladesh [27, 28], western India [29, 30], China [31–35], and Nepal [36, 37] (Fig. 1.1). Moreover, although not showing signs of overt chronic arsenic poisoning, many more individuals from various countries have been identified that

consume water contaminated by iAs at a level much higher than the WHO's drinking water quality standard (10 μg/L), including Vietnam [38], Myanmar [39], and Cambodia [40], among others. However, the precise mechanism behind the contamination by iAs of groundwater that is tapped by wells remains unknown.

We propose a potential mechanism by which the iAs became available to intensively expose those in Bangladesh, western India, China, and Nepal that used groundwater for drinking. Accordingly, the actual source of iAs contamination in these regions, apart from China, was originally the presence of trace amounts of arsenic in the Himalayan mountains. Briefly, arsenic that seeped from the rocks in the Himalayas entered large rivers and then settled as sediments in the watersheds and estuaries of these rivers, which resulted in the exposure to iAs of individuals who sank wells into the sedimentary rock in search of underground water. On the other hand, in China, some of the underground water flowing through the rocks in the mountains where The Great Wall of China was constructed is contaminated by arsenic (Fig. 1.2). It is speculated that chronic arsenic poisoning developed there in individuals who consumed this contaminated well water. In any event, in the countries in which iAs contamination has been identified, various administrative procedures have been implemented to ensure compliance with the WHO's drinking water quality standards. However, although wells with extremely high iAs contamination can be easily closed, the rapid population growth and heavy demand for inexpensive

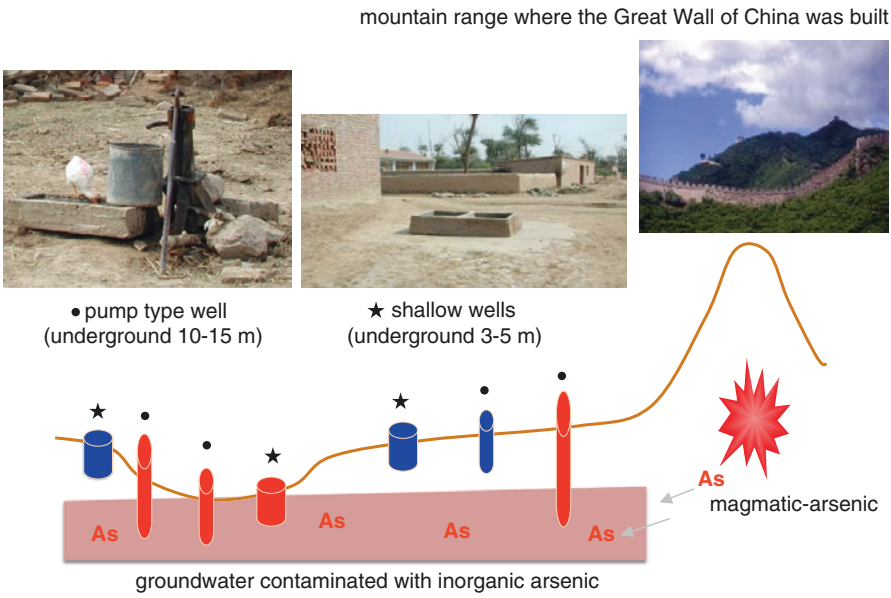

Fig. 1.2 The potential mechanisms behind the contamination of underground water by iAs in Inner Mongolia and Shanxi Province in China. Chronic arsenic poisoning occurred in people who used groundwater contaminated with iAs. For groundwater, iAs-contaminated and nonpolluted waters are clearly distinguished and have complicated distribution. The average of iAs concentration in 303 well waters in Baotou City, Inner Mongolia was 133 ± 199 μg/L

tap water in these developing countries make close all suspect wells an economically and technically difficult issue, and this severely hampers the complete avoidance of the use of well water contaminated with arsenic.

In the Araihazar District of Bangladesh [41, 42], the Health Effects of Arsenic Longitudinal Study (HEALS) has been promoted by the Columbia University. This study was implemented as a prospective cohort study including patients with chronic arsenic poisoning and those with a high risk of exposure to iAs. To date this study has been providing a variety of interesting findings and will no doubt continue to in the future.

This book introduces diverse information regarding the clinical findings and biological impact of chronic arsenic poisoning in its several chapters, mainly on the basis of the findings of the studies conducted in Bangladesh and China.

1.4 Summary

Arsenic poisoning has arisen from the oral ingestion of arsenic when used as a medicine and when found as a contaminant in food or potable water, among other sources. In the majority of cases, these poisonings are due to inorganic arsenicals. Findings that associate chronic oral iAs poisoning predominantly with skin lesions, including cancers, were originally made over 100 years ago and remain valid today. Considering that studies conducted in Argentina [5–8] and Chile [9–11] have now also identified that lung and bladder cancers are also associated with the long-term oral exposure to iAs, it is necessary to conduct screening tests for these cancers, in addition to skin cancer, in any future populations that arise.

In the recent years, the issues of how exposure to iAs in utero or in early life adversely impacts that generation as it matures have become apparent, and exposure during these key times in development can cause debilitating brain dysfunction as seen in studies of chronic arsenic poisoning in Asia [43–46]. Indeed, growth inhibition and severe central nervous system disorders were clearly identified as the aftereffects of infant subacute arsenic poisoning in infants in Japan [19–22]. This makes it apparent that the developing brain is highly sensitive to arsenic. Health issues arising from iAs exposure in Asia are an issue for which early resolution should not be expected. Moreover, in the regions contaminated by iAs, which include densely populated Asian countries, pregnant women and infants are readily exposed to iAs. Imagination and innovation are required to determine how the brain function of these most vulnerable humans can be preserved from the exposure to perhaps even low-level arsenic. Currently, the costs of social welfare and medical care for affected individuals are enormous when compared with the past and represent an increased burden on individuals, society, public health administrations, and governments.

In future studies on arsenic, the establishment of ways to prevent the health impact linked to arsenic exposure will likely be a critically important subject. To resolve such issues, there is an urgent need to develop environmental awareness but also remediation and purification technologies via collaboration across diverse

fields like medicine, environment sciences, pharmacology, nutritional science, and engineering. Further, there is a need to elucidate the relationship between the exposure to arsenic and the host defense systems and their genetics as well as to develop drugs which help prevent, or at least mitigate, the chronic toxic manifestations of arsenic exposure. To this end, much more need to be elucidated with regard to molecular mechanisms of arsenic-induced diseases within specific target tissues or sites.

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Chapter 2
Arsenic Metabolism and Toxicity in Humans and Animals: Racial and Species Differences

Yayoi Kobayashi and Tetsuro Agusa

Abstract Susceptibility to the toxic effects of arsenic is influenced by an organism's capacity for arsenic metabolism. To fully understand this potential, the pathways and properties of arsenic species (trivalent, pentavalent, methylated, or thiolated) as well as glutathione conjugates in the animal or human body must be assessed. Notably, the metabolism of arsenic may comprise detoxification as well as bioactivation processes. Because of the large difference in arsenic methylation capacity and binding affinity toward red blood cell in different animals, an animal model for studies involving human arsenic metabolism and toxicity has not yet been fully established. Individual and ethnic variations in the arsenic methylation capacity of humans are likely explained by genetic polymorphisms of arsenic metabolic enzymes. In particular, the genotype of arsenite methyltransferase (AS3MT) can highly influence the susceptibility to arsenic methylation and thus may be useful to assess the risk of arsenic exposure in humans.

Keywords Detoxification · Bioactivation · Glutathione · Reduction · Oxidation · Arsenic methylation · AS3MT · Thiolated arsenicals · Animal species and racial differences · Single nucleotide polymorphisms (SNPs)

Y. Kobayashi (✉)
Center for Health and Environmental Risk Research, National Institute for Environmental Studies, Ibaraki, Japan
e-mail: kobayashi.yayoi@nies.go.jp

T. Agusa
Faculty of Environmental and Symbiotic Sciences, Prefectural University of Kumamoto, Kumamoto, Japan
e-mail: te-agusa@pu-kumamoto.ac.jp

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2.1 Metabolism of Arsenic in Mammals

Inorganic arsenic (iAs) is generally absorbed into the body via the respiratory tract, mouth, and skin. The absorbed arsenic is then transported to the whole body through blood flow and primarily distributed in the liver, kidneys, spleen, and lungs [1]. Arsenic is mainly metabolized in the liver and excreted in the urine as methylated arsenicals [2, 3] and accumulated in the hair and nails [4].

The classical metabolic pathway of arsenic is generally accepted to proceed by repetitive reduction and oxidative methylation [5] (Fig. 2.1a). The former is mediated by arsenic reductases such as glutathione S-transferase omega (GSTO), with glutathione (GSH) as a reducing agent [6]. In contrast, the latter is mediated by arsenic methyltransferase (AS3MT) and *S*-adenosyl-L-methionine (SAM), a methyl group donor, also with GSH [7]. During the reduction/oxidative methylation process, absorbed arsenate (iAs^{V}) is reduced to the more toxic arsenite (iAs^{III}), which is then oxidatively methylated to monomethylarsonic acid (MMA^{V}). In turn, MMA^{V} is reduced to monomethylarsonous acid (MMA^{III}), which is subjected to a second oxidative methylation generating dimethylarsinic acid (DMA^{V}). In some animal species, trimethylarsine oxide ($TMAO^{V}$) has been found in the urine [8–10], which results from DMA^{V} reduction to dimethylarsinous acid (DMA^{III}) followed by methylation to $TMAO^{V}$ [11]. In addition, alternative metabolic pathways for arsenic methylation have also been proposed [12, 13]. In both new pathways, trivalent arsenic compounds bind to cellular thiols such as GSH [12] or components of cellular proteins [13], after which the thiol-bound arsenic compounds undergo a sequential reductive methylation. Here, oxidation of these compounds forms corresponding

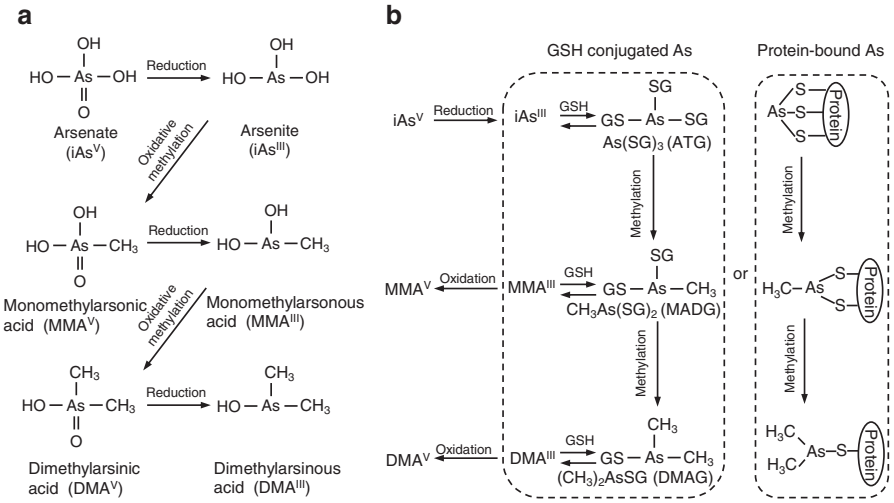

Fig. 2.1 Metabolic pathways for arsenic in mammals. (**a**) Generally accepted pathway as proposed by Challenger [5]. (**b**) Proposed pathways for arsenic methylation via GSH-conjugated arsenic by Hayakawa et al. [12] and via protein-bound arsenic by Naranmandura et al. [13]

pentavalent arsenicals. In particular, it has been suggested that the production of H_2O_2 by xanthine oxidase might constitute an important route for decreasing the toxicity of trivalent arsenicals by oxidizing them to their less toxic pentavalent analogues [14, 15].

GSH plays a key role in the metabolism of arsenic. It has been reported that As-GSH complexes such as arsenic triglutathione [$As(SG)_3$; ATG] and methylarsenic diglutathione [$CH3As(SG)_2$; MADG] were found in the bile of rats that had been administered iAs^{III} [16–20], as well as in the urine of gamma-glutamyl transpeptidase deficient mice that cannot metabolize GSH [21]. These As-GSH complexes are excreted in bile through the multidrug resistance protein 2/canalicular multi-specific organic anion transporter (MRP2/cMOAT) [16]. As ATG and MADG are chemically unstable in bile, they could easily be hydrolyzed to iAs^{III} and MMA^{III}, respectively [19]. In turn, it was reported that the concentration of both GSH and H_2O_2 was increased in bile following administration of iAs^{III} [22]. GSH stabilized these As-GSH complexes in bile by suppressing hydrolysis, whereas H_2O_2 oxidized ATG and MADG into the less toxic corresponding pentavalent arsenicals, iAs^{V} and MMA^{V}, respectively [22].

It is also necessary to consider arsenic compound metabolism by microbiota in the intestine. The reduction of iAs^{V} to iAs^{III} in rat [23] and methylation of iAs to MMA^{V} and DMA^{V} in the small intestine and cecum of mice and rats [23, 24], along with demethylation of DMA^{V} to MMA^{V} and iAs^{V} [25], have been reported. Moreover, thiolated methyl arsenicals, such as trimethylarsine sulfide ($[(CH_3)_3AsS]$; $TMAs^{V}$; previously M-1) and dimethylmonothioarsinic acid ($[(CH_3)_2AsS(OH)]$; $DMMTA^{V}$; previously M-2), were detected in the urine of rats after the administration of DMA^{V} [10, 26], which are produced in part through the action of intestinal bacteria [27, 28]. $DMMTA^{V}$ was also detected in the plasma of iAs^{III}-treated rats [29, 30], with *Escherichia coli* A3-6 associated with this thiolation being isolated from rat cecum contents [28]. $DMMTA^{V}$ has been found not only in rodent urine but also in human urine [31–33]. In addition, dimethyldithioarsinic acid ($[(CH_3)_2AsS(SH)]$; $DMDTA^{V}$; previously M-3) was found in the feces of rats that were consecutively administered DMA^{V} [27]. These thiolated arsenicals, $DMMTA^{V}$, $DMDTA^{V}$, and $TMAs^{V}$, have also been detected in both the urine and feces of rats after a single oral administration of DMA^{V} [10]. Monomethylmonothioarsinic acid ($[CH_3AsS(OH)_2]$; $MMMTA^{V}$) was detected in the urine of orally iAs^{III}-treated rats and hamsters [34].

In particular, the enterohepatic circulation is hypothesized to play a key role in the generation of these thiolated arsenicals. Comparing urinary excretion of arsenic after oral administration of iAs^{III} in male Sprague Dawley (SD) rats and Eisai hyperbilirubinuric (EHB) rats (with a deficiency of MRP2), significantly higher levels of $MMMTA^{V}$ and $DMMTA^{V}$ were detected in SD rats, whereas only a small amount of $DMMTA^{V}$ was detected in EHB rats [35]. However, it was also shown that $DMMTA^{V}$ was produced in rat liver [36] as well as in human red blood cells [37] in an in vitro study. In another study, $DMMTA^{V}$ could be produced from DMA^{III} by cellular fractions from mouse liver homogenates and by rhodanese from bovine liver with thiosulfate as a sulfur donor, with $DMMTA^{V}$ and DMA^{III} subsequently converted to DMA^{V} by monooxygenase [38] (Fig. 2.2).

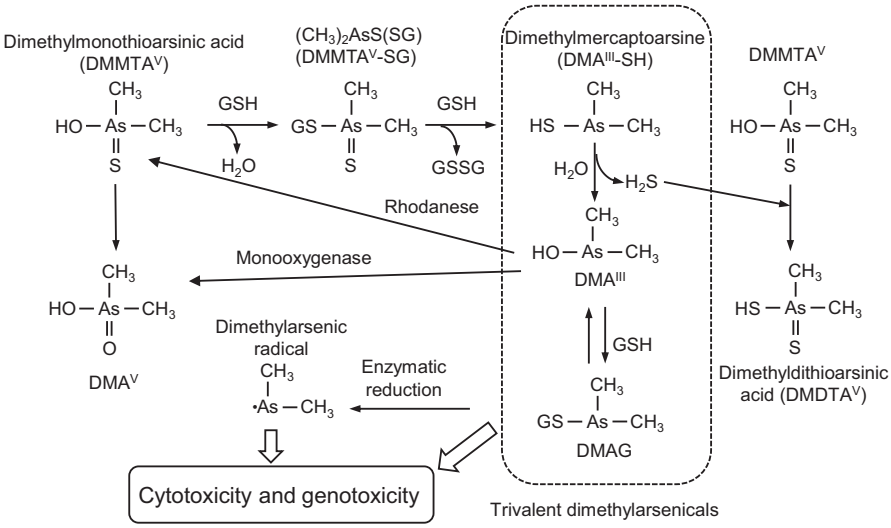

Fig. 2.2 Proposed reaction of DMMTA^{V} and GSH, and of the metabolic pathway of dimethyl arsenicals (Modification of the figure from Shimoda et al. [38] and Kurosawa et al. [73])

2.2 Species Differences

In general, laboratory animals are used to study the metabolism and toxicity of arsenic in vivo. Previously, rats were commonly used for arsenic metabolism studies because they metabolize arsenic in a manner similar to other species including humans, are easily available, are readily handled, and yield a sufficient amount of organs and biofluid for analysis. However, rats are currently recognized as a poor model for arsenic metabolism as they accumulate huge quantities of arsenic in red blood cells [39, 40] in a form in which DMA^{III} is bound to the reactive cysteine of hemoglobin [41]. Such accumulation suggests the likelihood of lower urinary excretion compared with other animals such as mice, rabbits, and hamsters [40, 42, 43]. In addition, biliary excretion of arsenic in rats also differs from that of rabbits and dogs; specifically, it has been reported that the excretion rate of rats was 37 times faster than that of rabbits and 800 times that of dogs [44]. In this regard, rabbits and hamsters appear to represent suitable animal models for arsenic metabolism in humans [15].

Species differences also exist in the methylation ability of arsenic, excretion rate, and ratio of MMA^{V} and DMA^{V}. Humans and common laboratory animals such as rats [45], mice [46], hamsters [47], rabbits [48], and dogs [49] are known to effectively methylate iAs to mono- and dimethylarsenicals. However, some animals are lacking this methyltransferase activity. Guinea pig [50]; great apes such as gorillas, orangutans, and chimpanzees; old world monkeys such as yellow baboons; new world monkeys such as owl monkeys, marmosets, and tamarins; and prosimians such as aye-aye, lesser bush baby, and slow loris do not have efficient methyltransferase activity [51]. However, these primates do exhibit iAs^{V} reductase activity.

2.3 Relationship Between Arsenic Metabolism and Toxicity

Although the liver is generally the main target organ for methylation of arsenic [52], this process also occurs in other organs. In an experiment examining the mRNA and protein levels of Cyt19 (current name AS3MT) and arsenic methyltransferase activity in the rat heart, brain, spleen, lung, liver, skeletal muscle, kidney, and testis, *CYT19* mRNA level was the highest in the liver followed by the testis, heart, brain, and kidney. In comparison, the mRNA levels in the spleen, lung, and skeletal muscle were low, whereas no clear band of *CYT19* mRNA could be detected in the testis. The protein level of Cyt19 was also highest in the liver, followed by the kidney, heart, and spleen, whereas protein levels in the brain, lung, skeletal muscle, and testis were low [53]. In addition, following incubation of rat tissue cytosol with iAs^{III}, SAM, and GSH in vitro, the generated methylated arsenicals were analyzed by high-performance liquid chromatography inductively coupled argon plasma mass spectrometry. The generation of MMA^{V} was highest in the liver; however, this form was not detected in the brain or muscle [53]. In another experiment examining the liver, kidney, testis and lungs of mice, the specific activity of the methyltransferase was found to be testis > kidney > liver > lung [46]. The discrepancy between these experiments might be due to the difference in animal species.

Pentavalent methylated arsenicals, MMA^{V} and DMA^{V}, comprise the major metabolites of inorganic arsenicals (iAs) in human urine [2]. Until the end of the twentieth century, methylation of arsenic had been considered as a detoxification process for toxic iAs because MMA^{V} and DMA^{V} were less acutely cytotoxic than iAs [54]. However, it is currently understood that the trivalent arsenic intermediates MMA^{III} and DMA^{III} are more cytotoxic than iAs [55–58]. It is also known that MMA^{III} and DMA^{III} exhibit genotoxicity [59–61] and stronger activity for antioxidant enzyme inhibition than iAs [62, 63]. These results argue that the methylation of arsenic functions as a bioactivation process rather than a detoxification mechanism. Significantly higher percentages of arsenic doses were retained in the liver, kidneys, urinary bladder, lungs, and heart in radioisotope-labeled iAs^{V}, [^{73}As]-iAs^{V}, —treated As3mt knockout mice than in C57BL/6 mice, and the clearance of arsenic in As3mt knockout mice was markedly slower than in C57BL/6 mice [64]. Together, these results suggest that further research is necessary to clarify the linkage between arsenic methylation and toxicity.

Although $DMMTA^{V}$ constitutes a pentavalent methylated arsenical, its uptake and distribution in the organs/tissues resembles that of DMA^{III} but not DMA^{V} in rats. Conversely, the distribution and excretion of $DMDTA^{V}$ were similar to those of DMA^{V} [65]. It has been reported that the cytotoxicity of $DMMTA^{V}$ was much higher than that of iAs^{III}, iAs^{V}, MMA^{V}, and DMA^{V} [66–70], with $DMMTA^{V}$ also exhibiting genotoxicity [66, 69]. This toxicity profile of $DMMTA^{V}$ is quite similar to that of DMA^{III} [69, 70], with GSH further increasing $DMMTA^{V}$ toxicity [66]. The cause of the high toxicity of $DMMTA^{V}$ toward human bladder cancer EJ-1 cells has been considered to reflect the production of reactive oxygen species (ROS), with $DMMTA^{V}$ suggested to generate ROS indirectly. Specifically, thiolated $DMMTA^{V}$ is converted to hydroxylated DMA^{V} after uptake into a cell, whereupon DMA^{V} is

reduced to DMA^{III} by GSH. ROS are generated via the redox reaction between DMA^{V} and DMA^{III} [70].

Although it is well known that trivalent arsenicals can easily bind to a thiol group, it was previously assumed that pentavalent arsenicals do not conjugate with thiol. Similarly, it has been reported that the reaction of DMA^{V} with GSH generates dimethylarsenic glutathione [$(CH_3)_2As(SG)$; DMAG], which are trivalent arsenicals, but not GSH-conjugated DMA^{V}. However, GSH-conjugated $DMMTA^{V}$ [$(CH_3)_2AsS(SG)$; $DMMTA^{V}$-SG], in which pentavalent $DMMTA^{V}$ was directly bound to the thiol group of GSH, was identified in DMA^{V}-treated cabbage [71]. In theoretical chemistry, $DMMTA^{V}$-SG may be promoted nonenzymatically under weakly acidic condition [72]. In another study, the stable complex of $DMMTA^{V}$-SG was produced by direct reaction of $DMMTA^{V}$ and GSH without reduction to DMA^{III} and DMAG [73] (Fig. 2.2). It is therefore plausible that clarifying the metabolism of $DMMTA^{V}$ may be important for elucidating its mechanism of toxicity. $DMMTA^{V}$ toxicity might be induced not only by its conversion to DMA^{III} but also to dimethylmercaptoarsine [$(CH_3)_2AsSH$; DMA^{III}-SH] [73] and/or dimethylarsenic radical [$(CH_3)_2As$] [38, 74] (Fig. 2.2). Thus, clarifying the metabolism of dimethylarsenicals is important for elucidating the toxic mechanisms of arsenic.

2.4 Variation in Arsenic Methylation Capacity in Humans

Urinary arsenic species (iAs, MMA^{V}, and DMA^{V}) has been used to assess arsenic methylation capacity in humans. This capacity influences the accumulation of arsenic compounds in the body followed by associated toxic effects. Accordingly, several epidemiological studies have reported that individuals with high DMA^{V} percentage (%) in urine excreted higher amounts of arsenic [75, 76]. Furthermore, high risks of arsenic-related diseases exist in populations having high MMA^{V} concentration or percentage (%) in urine [77–79].

Individual or ethnic differences exist in the metabolic capacity of arsenic. For example, in arsenic-contaminated groundwater areas, women in the Andes showed much lower MMA^{V}% in urine (0–11%) [80], whereas that of the northeastern Taiwanese population was high (26.9%) [81]. In addition, Chung et al. [82] found that arsenic methylation was more similar among relatives than among unrelated individuals. Because compositions of iAs, MMA^{V}, and DMA^{V} in human urine generally range from 10 to 30%, 10–20%, and 60–70%, respectively [83], the difference reported by previous studies [80–82] may be explained in part by the association of genetic variants in arsenic metabolism enzymes [84].

Among these enzymes, AS3MT, which catalyzes the transfer of a methyl group from SAM to trivalent arsenic compounds, plays a role in arsenic methylation [7]. Notably, several single nucleotide polymorphisms (SNPs) and variable number of tandem repeats (VNTRs) have been identified in the *AS3MT* gene [85, 86]. These genetic variations may modify the function and/or conformation of AS3MT, leading to differences in arsenic methylation capacity.

2.5 In Vitro Study on *AS3MT* Genotype and Arsenic Methylation

In the following discussion, SNP IDs based on the consensus sequence AY817668 and dbSNP rs# cluster ids listed in the NCBI Reference Assembly [86] are used as the notation of SNPs in *AS3MT*.

In vitro studies show an association of arsenic methylation with *AS3MT* genotype. To assess the association, Drobna et al. [87] treated human primary hepatoma ($n = 8$) with iAs^{III} and investigated the variation in arsenic compounds. This demonstrated that the liver of one donor exhibited higher methylation capacity than that of samples from other donors. In particular, the genotype of the novel donor for *AS3MT* M287T (*AS3MT* 14458; rs11191439) was heterozygote, providing the first suggestion of a significant relationship between arsenic methylation and *AS3MT* genotype. Wood et al. [85] later performed a functional analysis using COS-1 cells expressing the *AS3MT* genotypes Arg173Trp, Met287Thr, and Thr306Ile. These exonic SNPs were identified from DNA sequences of African-Americans ($n = 60$) and Caucasian-Americans ($n = 60$). These cells treated with arsenic showed significant variation in AS3MT enzyme activity and protein expression; those of Met287Thr mutant cells were high, whereas Arg173Trp and Thr306Ile showed the opposite trend. Furthermore, a reporter gene assay using several VNTRs in *AS3MT* revealed that the transactivation was cell line-dependent, and in that HepG2 cells, a shorter number of VNTRs increased the response.

Recently, Li et al. [88] purified proteins of eight polymorphic AS3MT variants (rs201702937, H51R, a152g; rs80317306, C61W, t183g; rs112056792, I136T, t407c; rs35232887, R173W, c517t; rs370022454, W203C, g609t; rs139656545, R251H, g752a; rs11191439, M287T, t860c; and rs34556438, T306I, c917t) in humans and examined the respective arsenic methylation capacity. They found that all variant proteins exhibited decreased activity compared with wild type owing to lower affinity of iAs^{III} or SAM and lower stability. This result indicates a high risk upon arsenic exposure in the variant AS3MT carriers.

2.6 Human Case Study of *AS3MT* Genotype and Arsenic Methylation

The first study related to the association of AS3MT with arsenic methylation was reported by Meza et al. [89]. They analyzed urinary arsenic species and genotyped *AS3MT* in people drinking arsenic-contaminated water in Yaqui Valley, Sonora, Mexico, and found significant association of *AS3MT* 7395 (rs12767543), *AS3MT* 12390 (rs3740393), and *AS3MT* 35587 (rs11191453), which are located in the non-exonic regions, with DMA^{V}/MMA^{V} in the urine of children (7–11 years old) but not of adults (18–79 years old). These SNPs in *AS3MT* belonged to the same linkage disequilibrium (LD) cluster.

To better understand the relationship between *AS3MT* variants and arsenic methylation capacity, several human epidemiological studies have been subsequently conducted in arsenic-contaminated areas (groundwater and mining) as well as non-contaminated areas. These human case studies have suggested two SNPs, *AS3MT* 12390 (rs3740393) and *AS3MT* 14458 (rs11191439, Met287Thr), as likely influencing arsenic methylation, although inconsistent results were obtained regarding the association between arsenic metabolic capacity and *AS3MT* genotypes among entire ethnic groups. This indicates that these constitute ethnicity-independent SNPs influencing arsenic methylation.

AS3MT 14458 (rs11191439) is located in exon 9 of *AS3MT* and represents a non-synonymous substitution of an amino acid at codon 287 (T(ancestral)/C; Met → Thr). In vitro [85, 87] and human case studies [90–97] found that the C allele type in this SNP had a higher first methylation capacity than the T allele type. This genotype frequency was relatively low in Asians [98] (Fig. 2.3), indicating this as a genetically less sensitive ethnic group.

In contrast, *AS3MT* 12390 (rs3740393) is located in the intron 6 region of *AS3MT*. C allele carriers of this SNP among Argentines, Mexicans, Taiwanese, and Vietnamese showed high DMA^{V}/MMA^{V} levels in urine [90–92, 96, 99], indicating strong capacity for the second methylation. Notably, although *AS3MT* 12390 (rs3740393) comprises an LD cluster in these ethnicities, their respective cluster pattern differs. In particular, the C allele frequency of this SNP in Argentines (72%) is much higher than that in other ethnic groups [100] (Fig. 2.3). This specific allele distribution may explain the higher second methylation capacity as indicated by the urinary arsenic profile [80]. Consistent with this, Schlebusch et al. [101] found that populations having a high frequency of a C-T-A haplotype composed by *AS3MT* 12390 (rs3740393), *AS3MT* 14215 (rs3740390), and *AS3MT* 35991 (rs10748835) exhibited a higher DMA% and lower MMA% in urine than Native American and Peruvian populations did. Therefore, this protective *AS3MT* haplotype may have been obtained through natural evolutionary selection to resist arsenic toxicity over several thousand years.

Few studies are available, however, regarding the variant proteins of *AS3MT* 14458 (rs11191439) and *AS3MT* 12390 (rs3740393) at the levels of structure and function. For the SNPs in exon regions of *AS3MT*, Li et al. [88] purified eight AS3MT variant proteins including *AS3MT* 14458 (rs11191439) and showed that this exhibited lower catalytic activity than the wild type owing to decreased affinity for arsenic compounds, lower cabalistic rate, and lower stability. In comparison, *AS3MT* genotype variants in non-exonic regions (intron and noncoding regions) such as *AS3MT* 12390 (rs3740393) may indirectly affect arsenic methylation. For example, focusing on the splice variants of AS3MT and their function, Sumi et al. [102] identified two splice variants of AS3MT (exon 3 deletion mutation and exon 4 and 5 deletion mutation) from HepG2 cells and demonstrated that the AS3MT without exon 4 and 5 regions showed no functionality with regard to arsenic methylation.

To clarify the relevance of other AS3MT variants, increased numbers of subjects are required. Almost all human case studies relied upon small sample size, wherein

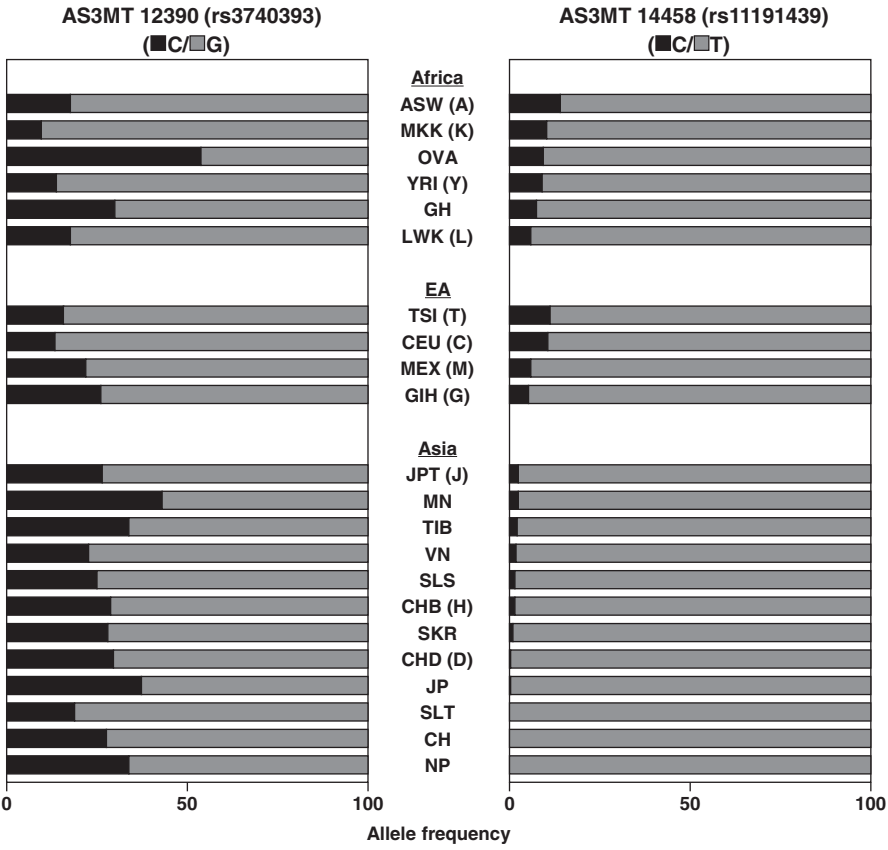

Fig. 2.3 Frequencies of alleles in *AS3MT* 12390 (rs3740393) and AS3MT 14458 (rs11191439) in various populations [111]. EA Europe and America; ASW (A) African ancestry in southwest USA; MKK (K) Maasai in Kinyawa, Kenya; OVA Ovambos; YRI (Y) Yoruban in Ibadan, Nigeria; GH Ghanaians; LWK (L) Luhya in Webuye, Kenya; TSI (T) Tuscan in Italy; CEU (C) Utah residents with Northern and Western European ancestry from the CEPH collection; MEX (M) Mexican ancestry in Los Angeles, California; GIH (G) Gujarati Indians in Houston, Texas; JPT (J) Japanese in Tokyo, Japan; MN Mongolians; TIB Tibetans; VN Vietnamese; SLS Sri Lanka-Sinhalese; CHB (H) Han Chinese in Beijing, China; SKR South Koreans; CHD (D) Chinese in Metropolitan Denver, Colorado; JP Japanese; SLT Sri Lanka-Tamils; CH Chinese; NP Nepalese

the statistical power to detect *AS3MT* SNP association may not always be sufficiently strong. Furthermore, other enzyme SNPs influencing arsenic metabolism such as glutathione *S*-transferases (GSTs) [103, 104] and methylenetetrahydrofolate reductase (MTHFR) [105] may also explain differences in susceptibility of arsenic methylation. Genome-wide association studies (GWAS) of large subject numbers using SNP arrays can be useful to screen for SNPs associated with arsenic methylation and their contribution. In particular, recent GWAS targeting arsenic-contaminated areas revealed the most significant association of arsenic methylation with SNPs in or near the *AS3MT* region [106–109].

Information of *AS3MT* SNPs may in turn be useful to assess the risk of arsenic exposure in humans. For example, arsenic trioxide (As_2O_3) has been used for the treatment of acute promyelocytic leukemia [110], although the side effects resulting from arsenic exposure is concerning. To decide the most effective dose of arsenic trioxide or the treatment interval, data on *AS3MT* SNPs influencing arsenic methylation may be valuable for individualized care. Further studies are therefore required to understand the relationship between arsenic methylation and *AS3MT* genotypes at the individual and population levels.

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Chapter 3
Arsenic Exposure and Reproductive Toxicity

Osamu Udagawa, Kazuyuki Okamura, Takehiro Suzuki, and Keiko Nohara

Abstract A number of epidemiological studies have indicated significant associations between maternal exposure to naturally occurring arsenic and adverse pregnancy outcomes, such as increased spontaneous abortion and infant mortality. Recent studies have also reported arsenic-associated male sexual dysfunctions, such as lower sperm quality. Animal studies suggest that a variety of cell types and systems are involved in such dysfunctions as the targets of arsenic. *In vivo* and *in vitro* experiments have shown that arsenic exposure causes defects in oocytes or embryos leading to embryonic growth retardation. Vasculogenesis in placentas and steroidogenesis in ovarian follicular cells are also implicated as the targets of arsenic. Animal studies in males have reported lower sperm quality and defects in the testis, epididymis, and Leydig cells, all of which are pivotal in spermatogenesis, are caused by arsenic exposure. Arsenic-induced changes in the levels of sex hormones in males have also been reported. As a background mechanism, an arsenic-induced increase in the levels of reactive oxygen species (ROS) is shared between males and females. In addition to infant adverse outcomes, late- or adult-onset outcomes of gestational arsenic exposure, which are not obvious at birth, are also reported by epidemiological and animal studies. Furthermore, multigenerational effects of gestational arsenic exposure have emerged as concerns.

Keywords Arsenic · Reproductive toxicity · Gestational exposure · Embryo development · Spermatogenesis · Late-onset effects

O. Udagawa · K. Okamura · T. Suzuki · K. Nohara (✉)
Center for Health and Environmental Risk Research, National Institute for Environmental Studies, Tsukuba, Japan
e-mail: udagawa.osamu@nies.go.jp; okamura.kazuyuki@nies.go.jp; suzuki.takehiro@nies.go.jp; keikon@nies.go.jp

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3.1 Introduction

Reproductive toxicity includes adverse effects on the reproductive ability of males and females, as well as adverse effects on the development of offspring. There has long been concern about the association of arsenic with pregnancy, since, for instance, arsenic compounds were frequently used for gestational antisyphilitic therapy [1] until the application of antibiotics. A number of epidemiological studies investigating the effects of environmental arsenic exposure worldwide have shown the correlation between maternal arsenic exposure and adverse pregnancy outcomes. Recent studies have also reported arsenic-associated male sexual dysfunctions. Animal experiments have revealed a variety of probable targets of arsenic in both female and male reproductive systems. This article reviews the effects of arsenic on both females and males and the possible background mechanisms. It also touches upon two recently emerging concerns: late-onset effects and multigenerational effects of gestational arsenic exposure.

3.2 Epidemiological Studies on Reproductive Toxicity of Arsenic Exposure

Humans are exposed to arsenic through ingestion, inhalation, or skin absorption. Drinking water is one of the major pathways of human exposure to arsenic. Arsenic in drinking water is almost all inorganic and stable, both in the forms of trivalent (arsenite; iAs^{III}) and pentavalent (arsenate; iAs^{V}) arsenicals [2].

3.2.1 Adverse Effects on Females

Various epidemiological studies have found significant associations between arsenic exposure and adverse infant outcomes, such as spontaneous abortion, low birth weight, and infant mortality [3–7]. The association between arsenic and adverse infant outcomes has been studied in Bangladesh, India, China, Chile, Taiwan, and the United States. In most of these epidemiological studies, arsenic levels in groundwater were measured. An epidemiological study in China demonstrated that maternal arsenic exposure is negatively associated with birth weight of males but not with that of females [6]. This example may suggest sex differences in susceptibility to arsenic during early stages of development.

3.2.2 Adverse Effects on Males

The association between arsenic exposure and male reproductive dysfunction is not well established. However, recently, epidemiological studies in China and Taiwan have shown that arsenic exposure from drinking water significantly causes low sperm quality and erectile dysfunction [8, 9]. Luteinizing hormone (LH) acts directly upon Leydig cells to stimulate testosterone production which is one of the most important sex hormones playing a key role in the maintenance of spermatogenesis and sexual function. Meeker et al. reported that an elevated arsenic level was inversely associated with LH in serum [10]. The decreased levels of sex hormone may result in low sperm quality and male sexual dysfunction.

3.2.3 Late-Onset Effects

In addition to infant outcomes, *in utero* and childhood arsenic exposure have been related to later adverse effects, including cancer. Epidemiological studies carried out in Antofagasta in northern Chile revealed that exposure to arsenic in drinking water during early childhood or *in utero* resulted in an increase of mortality in young adults from bladder cancer, laryngeal cancer, lung cancer, and chronic renal disease [11–13]. These early life exposures also resulted in increased chronic respiratory symptoms in children aged 7–17 years [14] and decreased lung function in adults [15]. Epigenetic modifications such as DNA methylation, histone modifications, and noncoding RNA expression are implicated in the etiology of various diseases including cancer and also in mediating early life environment impacts on later health (as described later in this chapter). Recently, global DNA methylation measurements have been started using genomic DNA extracted from blood of people living in arsenic-endemic areas [16, 17].

3.3 Animal Studies and Mode of Action: Female Reproductive Toxicity in Animals

3.3.1 The Transition of Understanding the Toxicity of Gestational Arsenic Exposure

Historically, anomalies such as neural tube defects (e.g., exencephaly), in addition to fetal death and growth retardation, were reported as one of the prominent toxicities of gestational exposure to arsenic compounds in animal studies [18, 19]. It should be noted that these results were observed using relatively higher doses of

arsenic, such as those causing some maternal mortality. Essentially, there are no convincing human data on malformations including neural defects [20].

Further animal studies have revealed that even repeated oral arsenite gavages or inhalations at a maternally toxic level (i.e., decreased maternal food intake; depressed maternal body weight) before and throughout the gestational period do not induce post-implantation loss nor fetal malformation in rats [21]. In mice and rabbits, a repeated gavage study of arsenate in accordance with the guideline of EPA conducted in the gestational period during the organogenesis (mice, gestational day (GD) 6–15; rabbits, GD 6–18) demonstrated that fetal resorptions and weight losses were observed only at doses that caused maternal mortality [22]. Given that spontaneous abortion and fetal weight loss are typically found to be associated with gestational arsenic exposure in human epidemiological studies, the development of rodent fetuses might be relatively less sensitive to arsenic.

3.3.2 Metabolic Properties of Arsenic in Relation to Gestation

In humans, ingested inorganic arsenic is sequentially biotransformed into monomethylated arsenic (MMA) and dimethylated arsenic (DMA). The prime mediator of the arsenic metabolism is the cytosolic enzyme, arsenic (+3 oxidation state) methyltransferase (AS3MT), which catalyzes the *S*-adenosylmethionine (SAM)-dependent methylation of inorganic arsenic [23]. Methylation of inorganic arsenic has long been considered to be a major process of detoxification of arsenic. In mice, approximately half of the given arsenic was detected as DMA at least 6 h after the intraperitoneal/gavage administration of arsenite to GD 18 dams [24]. In humans, methylation of arsenic is increased during pregnancy, and DMA is the major form transferred to a fetus [25]. In addition, a recent epidemiological study demonstrated that the AS3MT polymorphism of pregnant mothers carrying male fetus is associated with both the clearance rate of arsenic and birth weight [26]. Thus, it seems likely that maternal methylation capacity could be one of the determinants for the potential effects of arsenic on the conceptus. Actually, the no-observed-adverse-effect-levels (NOAEL) of MMA^{V} and DMA^{V} for developmental toxicity shown in rabbits were 7 mg/kg/day and 12 mg/kg/day, respectively [27]. These values are much higher, that means less toxic, than that of inorganic arsenate, at 0.75 mg/kg/day [22].

On the other hand, recent studies have reported that trivalent methylated arsenic, particularly MMA^{III}, is much more toxic than MMA^{V} or DMA^{V}, and even more toxic than iAs^{III} [28], indicating that methylation processes may not simply reduce the

arsenic toxicity. Therefore, further studies on methylation processes would be needed to understand the developmental toxicity of arsenic more in detail.

3.3.3 Mode of Actions and Perspectives

The developmental arrest of embryo is observed after exposure to arsenite *in vitro* such as in mice [29, 30]. Using *in vitro* mouse oocyte/preimplantation embryo culture systems, several insights on arsenite-induced decrease in embryonic growth have been reported as follows. Redox imbalance and metabolic abnormality of amino acids are suggested to be involved in the promotion of apoptosis and growth retardation in arsenite-exposed embryos especially at a lower dose [31]. Recently, knockdown of $p66^{Shc}$ (Src homology 2 domain-containing transforming protein A), a stress sensor for the reactive oxygen species (ROS), is demonstrated to rescue the arsenite-induced developmental arrest, thus emphasizing the contribution of ROS [32]. Another report demonstrated that chronic oxidative impact of arsenite damages telomere in mouse embryos, resulting in telomere attrition and chromosomal instability [33].

In addition to oocytes and embryos, arsenite targets multiple organs/tissues that are involved in female reproduction processes such as vasculogenesis in placentas [34] and steroidogenesis in ovarian follicular cells [35]. Thus, it should be important to find the direct molecular targets of arsenic to understand the mechanistic details on the arsenic-induced reproductive toxicity.

3.4 Animal Studies and Mode of Action: Male Reproductive Toxicity in Animals

3.4.1 Factors for Acquiring Fertile Spermatozoa

Formation of fertile spermatozoa is essential for male reproductive functions. There are two important phases to acquire fertile spermatozoa; one is spermatogenesis, and the other is maturation of spermatozoa. Those steps proceed in testis and epididymis, respectively [36]. In the testis, cell type-specific ablation studies showed that Leydig cells located in the interstitial spaces between seminiferous tubules and Sertoli cells in the seminiferous tubules (Fig. 3.1) have pivotal roles in spermatogenesis [37]. Hormones such as anterior pituitary-derived luteinizing hormone (LH)

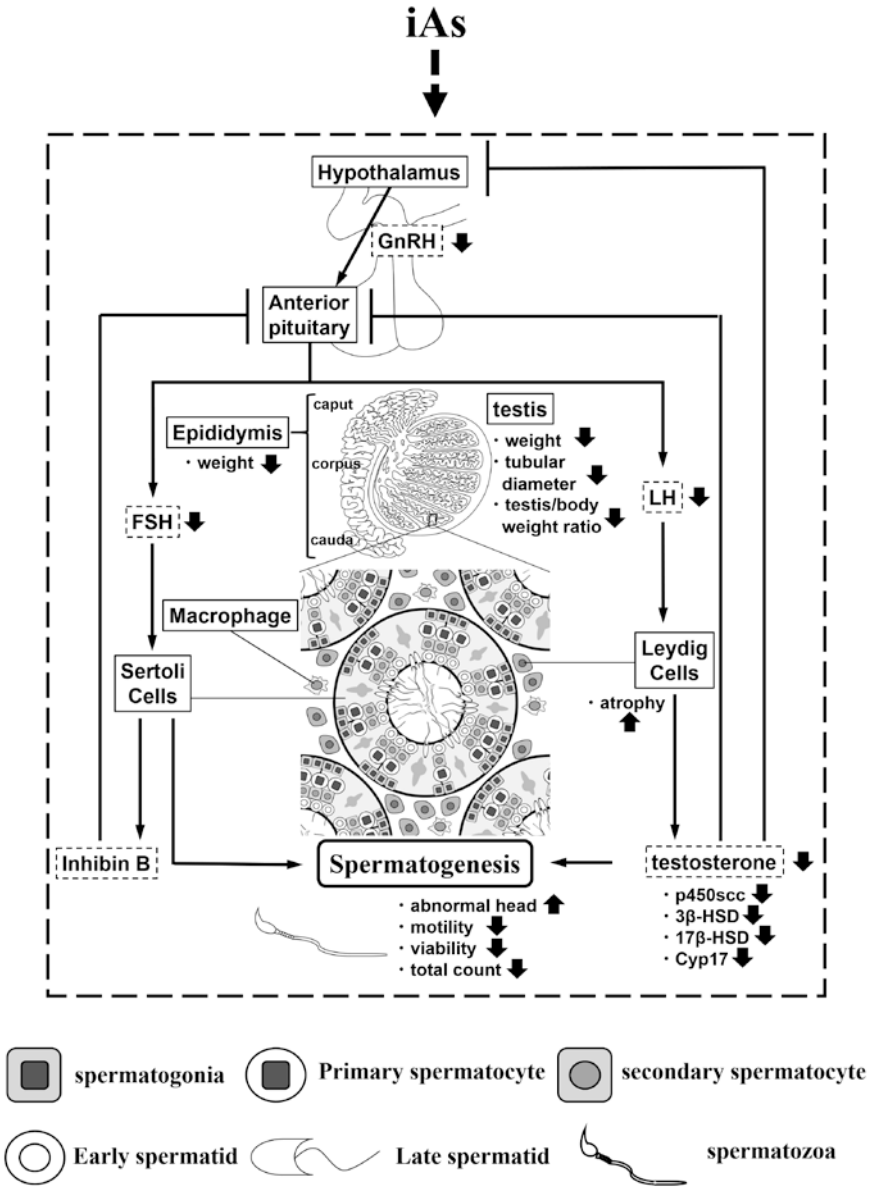

Fig. 3.1 Schematic of inorganic arsenic-induced male reproductive dysfunction

and follicle-stimulating hormone (FSH) stimulate Leydig cells and Sertoli cells, respectively, and testosterone produced by Leydig cells also has important roles for spermatogenesis [38].

3.4.2 Animal Studies of Arsenic-Induced Reproductive Toxicity (Fig. 3.1)

3.4.2.1 Sperm Parameters

Sperm parameters, such as number, motility, and morphology, were affected by inorganic arsenic exposure in mice [39, 40, 41]. Sodium arsenite exposure of mice via drinking water at a dose 534 μM for 35 days (one spermatogenic cycle) showed a significant decrease in sperm count and motility along with increase in abnormal sperm ratio in mice [42]. When the exposure time elongated to 365 days, at least 53 μM (4 ppm As) of arsenite exposure induced reduction of total sperm count and sperm motility [43]. These results suggested that chronic exposure of arsenite induces adverse effects even at relatively lower concentrations.

3.4.2.2 Testis (Including Leydig cells and immune cells)

Arsenite exposure induced accumulation of arsenic in testis and reduction of testicular weight and tubular diameter in rodents [43, 44]. The Leydig cell atrophy was significantly increased by arsenite exposure in mice [45]. Also, the arsenite exposure significantly elevated volume of nuclear region of Leydig cells accompanied by a decrease in cytoplasmic volume, suggesting impairment of Leydig cell function [46]. The resident testicular macrophages, the largest population of immune cells located in the testicular interstitium, also play an essential role in the development of Leydig cells [37]. Recent studies reported that volumetric proportion of macrophages was increased by arsenic exposure [47].

3.4.2.3 Epididymis

The epididymis plays an important role in the maturation of spermatozoa including their acquisition of progressive motility and fertilizing ability [36]. The weight of epididymis was reduced by arsenite exposure via drinking water in mice [48].

3.4.2.4 Hormone (LH, FSH, Testosterone, and Gonadotropin-Releasing Hormone (GnRH))

Leydig cells are activated by LH secreted from the anterior pituitary gland and secrete hormones, mainly testosterone, into circulation. Spermatogenesis depends on the intratesticular testosterone [49]. The levels of plasma LH and testosterone were significantly decreased in mice and rats by arsenite exposure [50, 51].

FSH is the major endocrine regulator of Sertoli cell function [52]. Plasma FSH, LH, and testosterone were decreased by oral exposure of 5 mg/kg body weight/day sodium arsenite for 4 weeks in rats [53]. Subcutaneous exposure to 3 mg/kg body weight arsenite for 3 weeks induced significant decreases in plasma LH and testosterone, but not in plasma FSH in mice [50]. This result may indicate that the level of plasma FSH is less sensitive to arsenite than that of LH and testosterone, at least in mice.

Secretion of LH and FSH are regulated by GnRH from the hypothalamus [54]. Exposure to arsenite from GD1 until 120 days after birth induced significant reduction of GnRH in whole hypothalamus in rats [55].

3.4.3 Mode of Action

A number of studies indicate that the oxidative stress associates with arsenic-induced adverse reproductive effects. Exposure to arsenite causes a significant decrease in epididymal superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST) activity in rats as well as increase in the level of hydrogen peroxide (H_2O_2) and malondialdehyde, an index of lipid peroxidation in rats [56]. Actually, significantly lower testicular glutathione (GSH) levels were observed in mice exposed to arsenite [57]. Increases in the arsenic accumulation, protein carbonylation, and lipid peroxidation levels were also observed in mice exposed to arsenate [40]. Moreover, antioxidants such as vitamin C, $ZnCl_2$, plant extracts, and *N*-acetylcysteine alleviated the harmful effects of arsenite on sperm in rats and mice [58–60].

Following the oxidative stress, apoptosis was activated by arsenite exposure in mice and rats [61, 62]. In particular, the protein levels of Bad and cleaved-caspase 3 in testes were upregulated, and Bcl2 was downregulated by arsenite exposure [61]. At the same time, the protein levels of phospho-Erk, phospho-p38, and p65 were increased in testicular tissue. Those results suggested that MAPKs and NF-κB were activated by arsenite exposure in testes [61]. Proteomics analysis detecting MAPK and NF-κB pathway activation by arsenite exposure in rat testis supported the results as well [63].

Molecular mechanisms regarding arsenite-induced inhibition of testosterone synthesis have also been reported. The levels of mRNAs and their activities of the enzymes involved in testicular testosterone synthesis, such as 3β-hydroxysteroid dehydrogenase (3β-HSD), were significantly decreased after arsenite exposure in mouse testes [50, 53]. The arsenite-induced suppression of steroidogenic enzyme activities was ameliorated by simultaneous administration of human chorionic gonadotropin (hCG) [53]. Although, the regulation of mRNA level of 3β-HSD is

related to epigenetic modifications [64], the association with arsenite-induced reproductive dysfunction is still unknown.

3.5 Emerging Concerns About Health Effects of Arsenic Exposure

3.5.1 Late-Onset Effects of Gestational Arsenic Exposure and Involvement of Epigenetics

The major focuses of reproductive toxicity are on the male and female fertility and the conditions of newborn babies. On the other hand, as reviewed above, some epidemiological studies observed that gestational and childhood arsenic exposure associate with late-onset adverse outcomes, such as higher incidence of cancer [11]. Rodents, particularly mice, are much more refractory to arsenic carcinogenicity than human, and a limited number of studies succeeded in detecting tumor-augmenting effects of arsenic (reviewed in [65]). On the other hand, gestational exposure of C3H mice for only 10 days to arsenite increased tumor incidence in various organs including livers of their F1 offspring in late adulthood [66]. These findings suggest that developing fetus is highly sensitive to cancer-augmenting activity of arsenic. Outside the cancer-augmenting effect, exposure of pregnant mice to arsenite showed early onset of vaginal opening, significantly greater body weight gain, body fat content, and glucose intolerance in female offspring [67]. Exposure during gestation and continuous exposure to arsenite for male offspring on a Western-style diet exacerbated the diet-induced fatty liver disease in adulthood [68].

Those late-onset outcomes of developmental exposure might be one of the important issues of reproductive toxicity on which more attention should be paid. Epigenetics is considered to be an essential mechanism involved in such late-onset outcomes [69]. Epigenetics is a mechanism regulating gene functions by modifications of genome, such as DNA methylation, histone modifications, and noncoding small RNA expression [70]. Variety of chemicals including arsenic have been reported to affect epigenetic modifications [71, 72], possibly acting on the epigenetic enzymes and/or related molecules or as the results of changing cellular states, such as differentiation and proliferation. Waalkes and colleagues found decreased DNA methylation at estrogen receptor-α (ERα) promoter and ERα upregulation in the liver tissues of C3H mice gestationally exposed to arsenic, which suggested DNA methylation-dependent activation of ERα signaling pathway leading to tumor augmentation [73].

3.5.2 Multigenerational Effects of Gestational Arsenic Exposure

When pregnant mother (F0) is exposed to chemicals, the fetus (F1) and the fetus's germline that becomes F2 in the future can be also exposed (Fig. 3.2) [74]. Thus, gestational exposure is mutigenerational, potentially leads to multigenerational effects (Fig. 3.2). These effects may not have been argued as the issues of

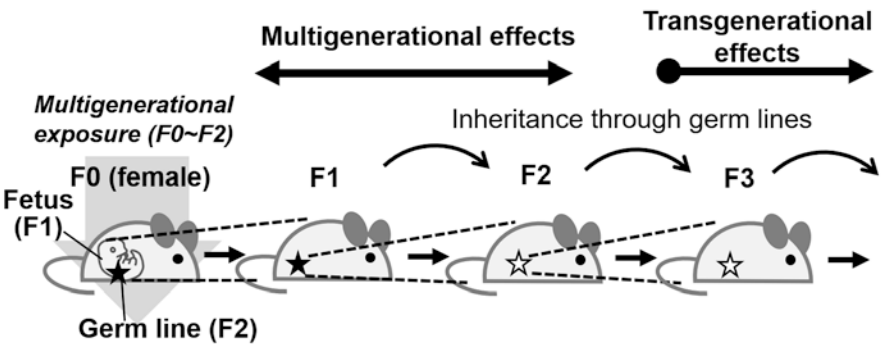

Fig. 3.2 Schematic of gestational exposure and multigenerational or transgenerational effects

reproductive toxicity; however they would be also ones since they are the outcomes of gestational exposure and are transmittable through germlines (sperm or egg).

Our recent study in the tumor-augmenting model in C3H mice gestationally exposed to arsenite revealed that the hepatic tumor incidence is increased not only in F1 males but also in F2 males by arsenic [75]. As a mechanism, epigenetic alterations are deeply involved in the multigenerational effects [74]. We have also identified several tumor-related genes whose promoter regions are altered in the levels of methylation, coordinately linking with gene expression changes in the arsenite-exposed F2 livers (unpublished data).

So far, no epidemiological studies on gestational exposure to arsenic have reported F2 or transgenerational effects. We would need to consider that such long-term studies following more than three generations are hard to be accomplished in humans. Suitable assessing methods, such as epigenetic markers detecting heritable changes, might be great help to investigate this issue.

3.6 Future Prospects

In endemic areas, the residents could be chronically exposed to naturally occurring arsenic more than a generation. For example, in Bangladesh, tube well installation since the 1970s unknowingly led to the use of arsenic-contaminated groundwater for more than 40 years. Epidemiological studies have been reporting associations with arsenic exposure and cancer as well as a variety of adult-onset lifestyle-related diseases, including metabolic dysfunctions and heart diseases. While embryos/fetuses might be more vulnerable to arsenic, the contribution of gestational exposure in the development of these dysfunctions is yet to be clarified.

Likewise, multigenerational effects of gestational arsenic exposure are not fully investigated in humans. Recently, transgenerational effects, in addition to multigenerational effects, of gestational exposure to environmental chemicals are among emerging concerns. In animals, transgenerational effects, such as the effects observed in the F3 offspring and later generations of gestationally exposed F0 moth-

ers, are transmitted between generations through germ cells in the absence of direct exposure [74] (Fig. 3.2). We are now facing these emerging concerns, and these issues would be deeply debated in future researches in the field of reproductive toxicology.

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Chapter 4
Characteristics and Health Effects of Arsenic Exposure in Bangladesh

Khaled Hossain, M. M. Hasibuzzaman, and Seiichiro Himeno

Abstract Arsenic is a potent environmental pollutant and a well-established human carcinogen. Contaminated groundwater is the main source of arsenic exposure in many countries, including Bangladesh. Rural people in Bangladesh depend almost entirely on hand-pumped tube well water, which has been heavily contaminated with arsenic. Considering the country's 125.5 million population in 1999, it has been estimated that more than half of the total population has been exposed to arsenic through drinking water resulting in a serious public health concern and a socioeconomic burden to the country. Chronic arsenic exposure is associated with skin lesions, cancer, cardiovascular diseases (CVDs), and other chronic diseases such as diabetes and respiratory dysfunctions. We conducted a series of epidemiological studies to quantitatively evaluate the arsenic-related organ and vascular dysfunctions and to explore the underlying mechanisms of arsenic-induced chronic diseases. We used three arsenic exposure metrics: drinking water arsenic concentrations as an external exposure marker and hair and nail arsenic concentrations as internal exposure markers reflecting long-term arsenic exposure at the individual level. Using these multiple arsenic exposure metrics (which showed significant correlations with each other), we investigated the dose-response relationships of these exposure markers with a variety of blood biochemical markers for organ dysfunctions, atherosclerosis, and cancer among study subjects recruited from arsenic-endemic and non-endemic areas in Bangladesh. Our results demonstrate that chronic

K. Hossain (✉) · M. M. Hasibuzzaman
Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi, Bangladesh
e-mail: khossain@ru.ac.bd

S. Himeno
Laboratory of Molecular Nutrition and Toxicology, Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima, Japan
e-mail: himenos@ph.bunri-u.ac.jp

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arsenic exposure can induce pro-inflammatory, pro-oxidative and pro-angiogenic microenvironments in the vascular system, leading to the development of CVDs as well as cancer. Further studies are required to elucidate the effects of neonatal and early-life arsenic exposure on later life in Bangladesh, where over 30% of the population is under 15 years of age.

Keywords Arsenic · Bangladesh · Cardiovascular diseases · Cancer · Endothelial dysfunction · Biochemical markers

4.1 Introduction

Arsenic, a metalloid, is a potent environmental pollutant and a well-established human carcinogen that is ubiquitously present in water, soil, and foods. The International Agency for Research on Cancer (IARC) has classified arsenic as a class I human carcinogen. Contaminated groundwater is the major source of arsenic poisoning in many countries, including Bangladesh. Foods grown in arsenic-affected areas, where contaminated groundwater is also used for irrigation, have become the second major route of arsenic exposure [1, 2]. Bangladesh is an agro-based country, and because of the difficulties in supplying safe and pathogen-free water from surface-water resources, groundwater has been extensively used for decades for drinking water and for food production to sustain the country's growing population. The groundwater polluted by naturally occurring inorganic arsenic has posed a severe threat to the public health in the country.

The rural population in Bangladesh almost entirely (97%) depends on tube wells for their drinking and household water [3]. According to a survey conducted by the Department of Public Health Engineering (DPHE) of the Government of the People's Republic of Bangladesh, and the British Geological Survey (BGS), arsenic was identified in the drinking water in 61 of the country's 64 districts [4]. The World Health Organization (WHO) has described the arsenic crisis in Bangladesh as "the largest mass poisoning of a population in history" [5, 6].

It was estimated that more than half of the total population (57 million among the 125.5 million people in 1999) in Bangladesh drank groundwater with arsenic concentrations >10 μg/L, the permissive limit set by WHO [4]. Chronic exposure to arsenic is linked to several adverse health conditions, including skin lesions, a variety of cancers, hypertension, several forms of cardiovascular disease (CVD), hepatic and renal dysfunctions, neuropathy, and cognitive dysfunctions [7–21]. According to the Bangladesh population census data of 2001 obtained by the Multiple Indicator Cluster Survey (MICS), 68,000 deaths per year were estimated to be attributable to arsenic poisoning [22].

Several studies have been conducted in Bangladesh to characterize and evaluate the detrimental health effects of this catastrophic pollution, but further studies are required to quantitatively evaluate the associations between the extents of arsenic

exposure at individual levels and the development of chronic diseases. For that purpose, we used three types of exposure metrics, i.e., the arsenic concentrations in drinking water, hair, and nails, and we examined the blood-circulating molecules related to chronic diseases. This chapter describes the characteristics and health effects of chronic exposure to arsenic in Bangladesh with special reference to the biochemical markers related to organ dysfunction, CVD, and cancer.

4.2 The Cause and Magnitude of Arsenic Contamination in Bangladesh

The presence of high concentrations of arsenic in tube well water in Bangladesh was first detected in 1993 by the DPHE in Chapai Nawabganj, a northwestern district of Bangladesh. Bangladesh has one of the largest deltas (the Bengal delta) in the world; it contains the Ganges-Brahmaputra-Meghna river system. The Himalayan orogenic belt is thought to be the source of the arsenic in the Bengal delta; arsenic-containing sediments from the orogenic belt are transported to the peripheral Bengal delta through the Ganges-Brahmaputra-Meghna river system and deposited along with sand and gravel. Over thousands of years, the deposition of these materials built up to create the land now known as Bangladesh. Finally, arsenic is released from the deposits to the circulating groundwater by complex geochemical processes [23].

Before the 1970s, the people of Bangladesh largely relied on the bacteria-contaminated surface water from ponds, rivers, and canals for their drinking and household purposes, resulting in high prevalences of cholera, dysentery, and other waterborne diseases. To protect the people from waterborne diseases, the United Nations Children's Fund (UNICEF) and the DPHE installed many tube wells. Approximately eight million tube wells have been drilled all over the country, and bacteria-free groundwater is currently being supplied to 97% of the rural people in Bangladesh. However, this great success turned into a nightmare in the 1990s when high concentrations of arsenic were found in the tube well water.

The DPHE and BGS survey data published in 2001 showed that the water from 27% of the tube wells (<150 m deep) contained arsenic at concentrations exceeding the Bangladesh standard for arsenic in drinking water (50 μg/L), and 46% of the wells had arsenic concentrations exceeding the WHO guideline value (10 μg/L) [4]. According to the survey report, approximately 35 million people were exposed to arsenic through drinking water above the Bangladesh standard, and 57 million people were exposed to arsenic above the WHO guideline. Moreover, alarmingly high concentrations of arsenic, the majority of which was found to be inorganic arsenic, have been observed in rice, vegetables, and other food items [24, 25]. Arsenic enters the food chains through the irrigation water, which is also derived from contaminated groundwater. The total amounts of arsenic intake among individuals living in arsenic-polluted areas may therefore be much higher than that estimated solely from drinking water.

4.3 Arsenic Exposure Markers at Individual Levels

Since contaminated groundwater is the major source of arsenic exposure worldwide, many studies on the health effects of arsenic have been using the levels of arsenic in drinking water as an exposure marker. In highly polluted areas, however, not only drinking water but also arsenic-contaminated foods contribute substantially to the consumption of arsenic among people. Thus, better and more reliable biomarkers are needed to elucidate the dose-response relationship between arsenic exposure (total intake) levels and hazardous health effects.

Several biological markers including blood, urine, saliva, hair, and nails have been used to estimate individual levels of arsenic exposure. Blood arsenic concentrations reflect short-term exposure, since arsenic in the blood is cleared through the kidneys quickly, i.e., within several hours [26, 27]. Urinary arsenic levels also reflect the recent exposure to arsenic because arsenic is metabolized rapidly in the body, and the metabolites are quickly excreted into urine. When individuals are chronically exposed to arsenic at a steady rate, urinary arsenic levels could be used for the estimation of chronic arsenic exposure. It is noteworthy that the quantities and qualities of arsenic compounds in urine are largely affected by the individual differences in hydration state, metabolic (methylation) ability, and food habits.

Inorganic arsenic is transformed into monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) through enzymatic reactions in the body. Along with inorganic arsenic, DMA and MMA are excreted into urine. Although a speciation analysis of urinary arsenic metabolites is a useful approach to evaluate the arsenic methylation ability and its relationship with arsenic toxicity, the rate of arsenic methylation may be influenced by a variety of factors such as arsenic exposure levels, smoking, sex, and age [28–30]. Among the populations who consume a large amount of marine fish and seaweeds, high concentrations of arsenobetaine and arsenosugars, which are readily excreted into urine, will disturb the evaluation of total arsenic concentrations in urine. Despite these limitations, many researchers use the urinary arsenic concentration as an exposure marker in part because urine samples can be noninvasively collected and readily processed for measurements.

Hair and nail arsenic concentrations represent relatively long-term exposure to arsenic. As hair grows approx. 1 cm/month, 2–3 cm hair samples from a subject's skull reflect the past several months of arsenic exposure. Nail arsenic levels have been shown to represent the exposure levels from several months to years [31]. Hair and nails retain high concentrations of arsenic due to their high contents of keratin, a protein containing a sulfhydryl group that can bind arsenic. The collection of hair and nails from humans is a noninvasive process, and the transportation and storage of hair and nail samples are easier and safer than those of blood or urine samples.

One of the disadvantages of using hair and nails is the possibility of external contamination. Hinwood et al. [32] reported a strong correlation between toenail arsenic concentrations and arsenic levels in both water and soil, while this association was less pronounced in hair [32]. On the other hand, externally deposited arsenic cannot be completely removed from hair by any variety of washing and rins-

ing techniques. Experimental evidence suggests that compared to hair, human nails are more resistant to external contamination. Agahian et al. [33] estimated that 98% of external arsenic was removed by washing fingernail samples exposed to particulate arsenic [33]. Similarly, Karagas et al. [34] indicated that <1% of the arsenic was detected even after the exposure of toenail samples to water arsenic for 15 h [34]. Karagas et al. [35, 36] reported that arsenic levels in toenails are a reliable long-term biomarker of arsenic exposure and reflect the intake of arsenic from drinking water at low levels to an extent greater than the urinary concentrations of total arsenic or each arsenic metabolite [35, 36]. Another study suggested that nail arsenic levels reflected the environmental arsenic exposure levels in a manner similar to that of urinary arsenic levels [37]. All of these findings support the notion that human nail arsenic concentrations are a reliable marker for chronic arsenic exposure.

We hypothesized that measurements of arsenic from multiple biological samples along with environmental samples such as drinking water can provide more accurate and reliable assessments of arsenic exposure levels in humans. Based on this hypothesis, we conducted a series of epidemiological studies using three exposure metrics: drinking water arsenic as an environmental or external exposure metric and hair and nail arsenic concentrations as internal metrics reflecting long-term exposure. Our studies revealed that the study subjects' hair and nail arsenic concentrations were significantly correlated with their drinking water arsenic concentrations (Fig. 4.1), which confirmed that water is the major source of chronic exposure to arsenic in Bangladesh. Our results also showed that hair and nail arsenic concentrations are significantly correlated with each other (Fig. 4.1). The strong correlations among the three exposure metrics provide reliability for the estimation of arsenic exposure levels when analyzing the dose-response relationships, with less bias due to the use of a sole exposure marker.

4.4 The Health Effects of Chronic Exposure to Arsenic

4.4.1 Skin Lesions

Skin lesions are the most common visible symptoms caused by chronic exposure to arsenic. Skin lesions usually develop 5–10 years after the start of persistent and chronic exposure to arsenic [38]. The common skin symptoms of chronic exposure to arsenic are hyperkeratosis and melanosis. Hyperpigmentation is manifested by raindrop-like spots, diffused dark and brown spots, and darkening of the skin on the limbs, chest, back, and abdomen, whereas keratosis is manifested by thickening of the skin of the palms of the hands or the soles of the feet, or small flanges that emerge as small corn-like elevations on the palms and soles. Benign or premalignant skin lesions can turn into malignant diseases with prolonged exposure to arsenic [7]. The major forms of arsenic-induced skin cancer are Bowen's disease, squamous cell carcinoma, and basal cell carcinoma [39–42]. Figure 4.2 provides photographs of some typical skin lesions taken by our research group.

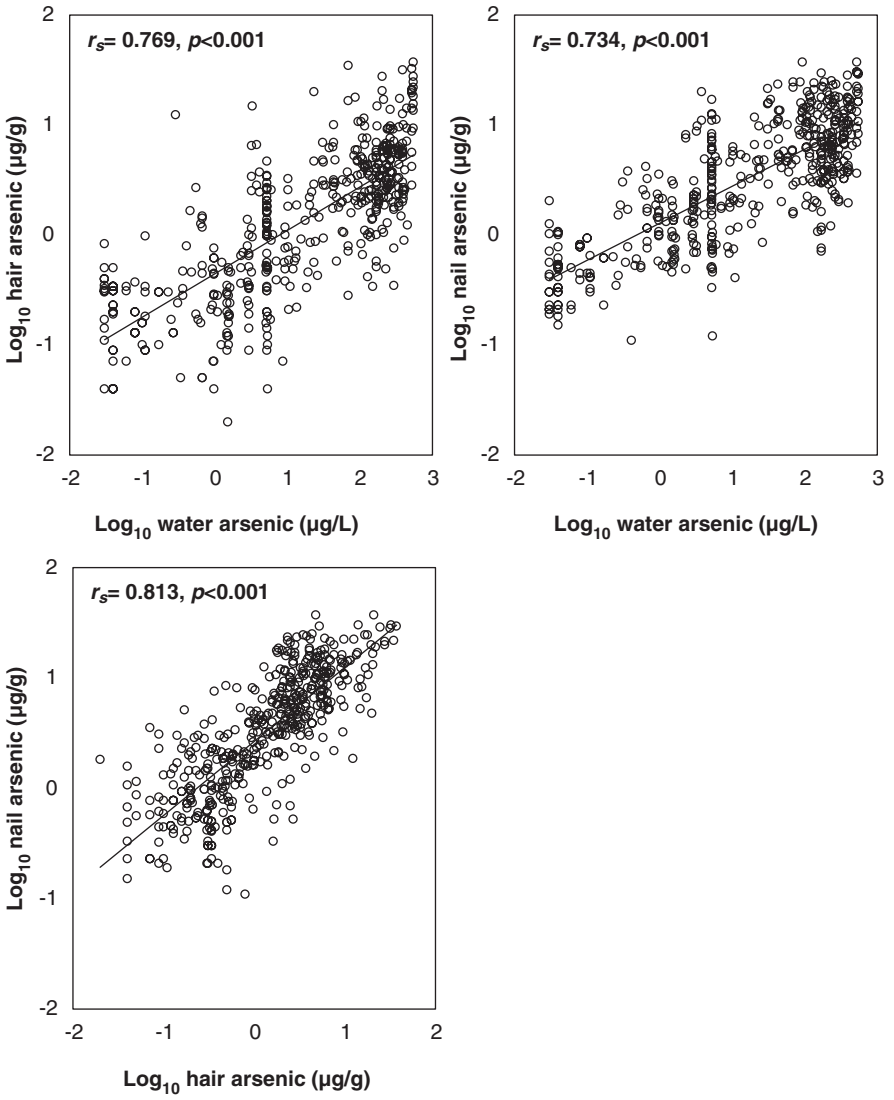

Fig. 4.1 Inter-relationships of arsenic exposure (drinking water, hair, and nail arsenic) metrics. Log_{10}-transformed values of arsenic concentrations were used. r_s and p-values were obtained using Spearman's correlation coefficient test. Total number of subjects = 483. Study subjects and their exposure levels were used from our previous study [58]

Some studies have shown that males are more susceptible to arsenic-induced skin lesions than females [43–47]. However, Tondel et al. [48] did not find any differences in the prevalence of skin symptoms between males and females [48]. The difference in arsenic methylation capacity between males and females may also be associated with skin lesions [8]. Polymorphism in genes related to the metabolism of arsenic has been reported to be associated with the risk of skin lesions [49, 50].

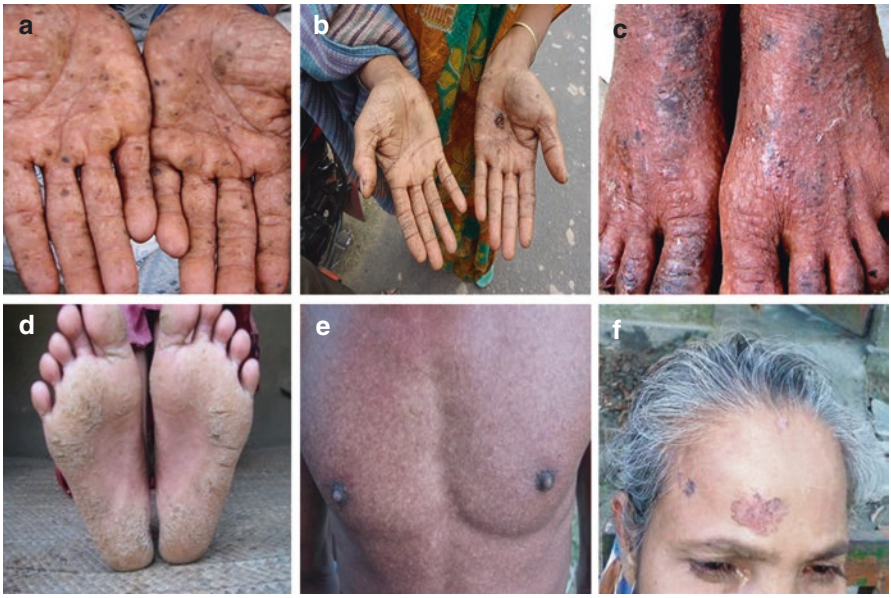

Fig. 4.2 Skin manifestations of chronic arsenic poisoning. (**a**) Palms and fingers: Punctate and diffuse keratosis. (**b**) Palms and fingers: Punctate keratosis on right hand and Bowen's carcinoma on the left hand. (**c**) Dorsum of foot: Diffuse and punctate pigmentation. (**d**) Sole: Severe punctate keratosis. (**e**) Chest: Diffuse pigmentation and punctate leukoderma. (**f**) Forehead: Multiple Bowen's disease

However, the precise roles of polymorphic genes in the development of skin lesions have not yet been established.

4.4.2 Dysfunctions of Internal Organs

In addition to the skin, internal organs such as the liver, the cardiovascular system, the nervous system, kidneys, and lungs are affected by chronic exposure to arsenic. After the discovery of arsenic pollution, extensive studies were conducted on the health effects of arsenic in endemic areas in Bangladesh, but little information has been obtained regarding the precise dose-response relationship between the levels of arsenic exposure and hazardous effects in internal organs. In 2007, we began to conduct population-based studies to explore the association between chronic arsenic exposure and circulating blood biochemical markers in respect to the functions of internal organs and vascular systems. We collected drinking water from tube wells and biological samples such as hair, nails, and blood from study subjects in villages in the northwest region (Kushtia, Chuadanga, and Jessore districts) of Bangladesh where moderate to severe arsenic pollution was identified. Drinking water and biological samples were also collected from subjects in a village in the northern region (Naogaon district) with no history of arsenic contamination.

We first measured the activity of lactate dehydrogenase (LDH) in serum [51]. We observed a dose-response relationship between LDH activity and the arsenic concentrations in the subjects' drinking water, hair, and nails. The elevation in serum LDH activity has been reported to be associated with renal and hepatic dysfunctions, myocardial infarction, hemolysis, and a variety of cancers [52–54]. If tissue damage occurs, LDH is leaked from the damaged tissue to the bloodstream. Thus, elevated serum LDH activity is a rather nonspecific indicator of organ damage. The positive association between chronic arsenic exposure and serum LDH activity led us to investigate the relationships between arsenic exposure metrics and organ-specific biomarkers.

The liver is the primary organ for arsenic metabolism, and hepatic disorders appear to be one of the major causes of arsenic-related mortality [18, 55]. In one of our studies, we examined three serum biomarkers of liver function: alkaline phosphatase (ALP), aspartate transaminase (AST), and alanine transaminase (ALT). The results of our analyses showed that the activities of these enzymes in serum were increased with the increasing concentrations of the subjects' drinking water, hair, and nail arsenic in a dose-dependent fashion [17]. In another study, we detected a dose-dependent decrease in plasma cholinesterase (PChE) activity in the high-exposure groups of each arsenic exposure metric [56].

There are three types of cholinesterase: red blood cell cholinesterase, called "true cholinesterase"; plasma cholinesterase (PChE), called "pseudocholinesterase"; and brain cholinesterase. Acetylcholine causes the stimulation of neurons, while cholinesterase causes the discontinuation of such stimulation by breaking down the acetylcholine. Cholinesterase-inhibiting chemicals cause a dysregulation of this balance, leading to neurotoxicity. Red blood cell cholinesterase is the same enzyme that is found in the nervous system, whereas PChE is made in the liver. PChE, also known as butyrylcholinesterase, has a broader range of esterase activity and hydrolyzes butyrylcholine, acetylcholine, and other aliphatic esters. PChE activity is known to be decreased by heart attack, liver dysfunction, cancer metastasis, muscular tremors, and neurological disorders [57]. The decreased PChE activity observed in our study may be one of the biological mechanisms through which arsenic exerts its neuro- and hepatotoxicity in humans [56].

4.4.3 Cardiovascular Diseases

Chronic arsenic exposure is associated with CVD morbidity and mortality. We and other groups have conducted extensive studies regarding CVDs among populations in Bangladesh [12, 15, 16, 30, 58–61]. Chronic arsenic exposure causes preclinical manifestations of atherosclerosis such as increased thickness of the carotid intima-media, hypertension, and QT interval prolongation [13, 30, 62]. Atherosclerosis is a fundamental biochemical event for all forms of CVD. The blood-circulating molecules including lipoproteins, inflammatory molecules, and adhesion molecules are involved in the development of atherosclerotic lesions, and the serum concentrations of these molecules can be used for the prediction of the risks of atherosclerosis and CVD [14, 63].

Alteration of lipid-related blood biomarkers such as triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein (HDL-C) have been recognized as indicators of the risk of CVD [64]. The oxidized form of LDL (Ox-LDL), rather than total LDL, has garnered great attention because of its critical role in the development of atherosclerosis, and the blood levels of Ox-LDL have been used as a potential marker for oxidative stress in vascular systems [65, 66]. Many studies have shown that the levels of HDL-C are inversely associated with the risk of atherosclerosis and that the protective roles of HDL-C against CVD are mediated through its ability to remove cholesterol from artery wall foam cells via "reverse cholesterol transport" [67, 68]. However, recent reports have suggested that multiple functions of HDL, including its antioxidant and anti-inflammatory activities, act against the formation of atherosclerosis [69]. Thus, the blood levels of Ox-LDL and HDL-C may reflect the balance between oxidative or inflammatory stress and antioxidant or anti-inflammatory protections in the vascular system [68].

To explore the relationship between chronic arsenic exposure and the risks of atherosclerosis and CVDs, we conducted a series of population-based studies in arsenic-endemic areas in Bangladesh [15, 16, 58–60]. We demonstrated that chronic exposure to arsenic is associated with increased levels of circulating Ox-LDL with a concomitant decrease in HDL-C levels [16]. The imbalance between the circulating pro-oxidative (Ox-LDL) and antioxidative/anti-inflammatory (HDL-C) molecules in an arsenic-endemic population may be the reflection of pro-atherosclerotic conditions in the vascular microenvironment.

We also measured the levels of C-reactive protein (CRP), which has been recognized as a potential predictor of CVD and as a factor in the development of a pro-inflammatory microenvironment toward the atherosclerosis [16]. The circulating CRP, as well as endogenously produced CRP, induces the release of pro-inflammatory cytokines from monocytes [70] and promotes the expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) in endothelial cells [71]. In addition, CRP accelerates the monocyte adhesion to endothelial cells and the uptake of Ox-LDL by endothelial cells via the activation of LOX-1, which is the receptor of Ox-LDL [72]. We observed the increases in the serum levels of ICAM-1 and VCAM-1 concomitantly with CRP in the people living in arsenic-endemic areas, which suggests that arsenic-induced inflammatory events are involved in the pathological activation of ICAM-1 and VCAM-1 in the endothelial cells. Endothelial dysfunction is a major pathophysiological mechanism that leads to the development of atherosclerotic diseases, and consequently, an increased risk of CVD [73, 74].

We and other groups have shown that the percentage of hypertensive individuals and the average levels of diastolic and systolic blood pressure are higher in highly exposed individuals in arsenic-endemic areas in Bangladesh [15, 16, 30, 58–60]. Endothelial dysfunction causes an imbalance between vasoconstriction and vasodilation and initiates increased endothelial permeability, platelet aggregation, leukocyte adhesion, and the generation of cytokines [75]. To explore the roles of arsenic exposure in endothelial dysfunction, we investigated the blood-circulating levels of several biomarkers related to endothelial dysfunction in individuals living in arsenic-endemic and non-endemic areas in Bangladesh [15, 60]. We observed that arsenic exposure metrics have positive associations with circulating markers of endothelial dysfunction

such as the serum levels of Big endothelin-1 (Big ET-1) and soluble thrombomodulin (sTM) [15, 60]. Big ET-1 is the precursor of endothelin-1, which is implicated in the development of hypertension through its potent vasoconstrictive activity. As endothelin-1 has a very short half-life in blood, the levels of Big ET-1, which has a longer half-life than endothelin-1, can be used as a surrogate marker for endothelin-1.

Thrombomodulin, a receptor for thrombin, is located ubiquitously on the endothelial cell surface of arteries, veins, capillaries, and lymphatics [76, 77]. As a proteolytic cleavage of thrombomodulin occurs upon the injury of the endothelial cell surface, the blood level of sTM is thought to be a promising biomarker for endothelial dysfunction and as a predictor of the risk of CVD [75]. Blood levels of both Big ET-1 and sTM were dose-dependently increased by arsenic exposure [15, 60]. Moreover, hypertensive individuals in arsenic-endemic areas had significantly higher levels of Big ET-1 and sTM compared to their normotensive counterparts, suggesting a relationship between the endothelial dysfunctions caused by arsenic exposure and hypertension [15, 60].

Uric acid is involved in the development of gout, but accumulating evidence has suggested that elevated levels of uric acid are also associated with the risk of CVD development [78, 79]. We observed that arsenic exposure is positively associated with plasma uric acid (PUA) levels [58]. Uric acid causes endothelial dysfunction by reacting with and removing nitric oxide (NO), thereby preventing vasodilatation of the endothelium. Decreased NO and increased reactive oxygen species may promote a pro-inflammatory state that causes endothelial dysfunction and contributes to atherosclerosis [80]. Uric acid can also produce CRP through the stimulation of vascular smooth muscle cells. Uric acid stimulates the production of monocyte chemotactic protein-1 (MCP-1), a key chemokine implicated in increased cell proliferation and the production of CRP as well. We showed that PUA levels are positively associated with diastolic and systolic blood pressure of human subjects, and we observed that the hypertensive subjects living in arsenic-endemic areas had significantly higher levels of PUA compared to the normotensive subjects. The elevated levels of PUA and its relation to hypertension in arsenic-exposed individuals in our study suggest that higher levels of uric acid may also be involved in arsenic-induced CVDs [58].

Thus, a variety of biochemical markers related to atherosclerosis, inflammation, oxidative stress, and vasoconstriction are imbalanced by chronic arsenic exposure in a dose-dependent manner. Our human studies for the first time have provided biochemical and quantitative evidence showing the relationship between arsenic exposure and endothelial dysfunctions leading to atherosclerosis and hypertension. Our findings are summarized in Fig. 4.3, which demonstrates the pathways from arsenic exposure to the development of atherosclerosis.

4.4.4 Cancer

Chronic exposure to arsenic is associated with a variety of cancers of the skin, liver, lungs, bladder, and kidneys. A study conducted by Chen et al. [9] predicted that the excess mortality in Bangladesh due to cancer of the lungs, liver, and bladder

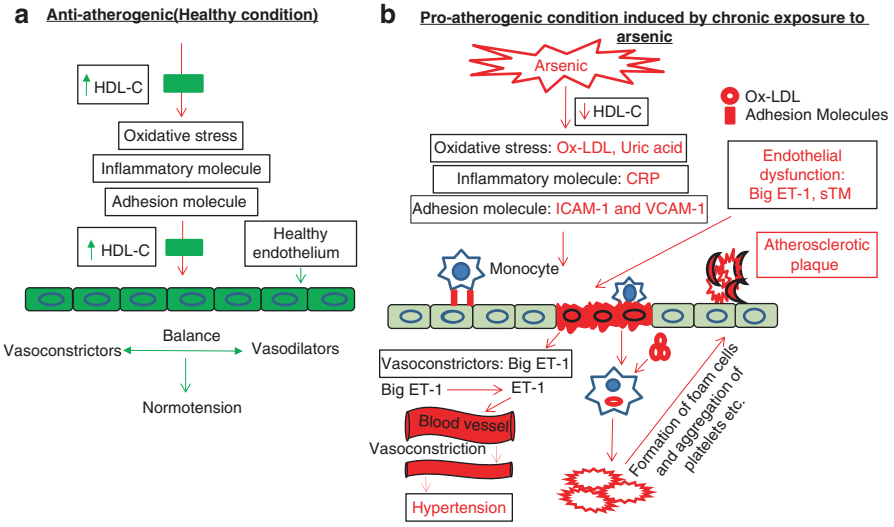

Fig. 4.3 Schematic presentation of the pro-atherogenic roles of arsenic. (**a**) In healthy condition, elevated levels of HDL-C inhibit the atherosclerotic process by inhibiting oxidative stress and the production of inflammatory and adhesion molecules. (**b**) Arsenic exposure induces a proatherogenic condition through increases in oxidative stress (measured by circulating ox-LDL and uric acid), inflammatory molecules (measured by circulating CRP), and adhesion molecules (measured by circulating ICAM-1 and VCAM-1) with the concomitant decreases in HDL-C levels, resulting in endothelial dysfunction (measured by circulating Big ET-1 and sTM). Endothelial dysfunction increases vasoconstrictors (measured by Big ET-1) that induce hypertension. Ox-LDL is taken up by monocytes that are recruited and migrated by adhesion molecules on the endothelial surface. Monocytes are converted into foam cells. Finally, foam cells with the aggregation of platelets form atherosclerotic plaques

resulting from exposure to arsenic in drinking water will eventually be doubled [9]. In epidemiological studies carried out in Taiwan and Chile, the associations between chronic arsenic exposure and cancer morbidity and mortality were well established [10, 81, 82]. However, arsenic has poor mutagenic activity, and the precise mechanisms of arsenic-induced carcinogenesis remain to be clarified.

Epigenetic changes are known to dysregulate gene expression, leading to the development of cancer and other diseases. Several studies were conducted to find the link between chronic exposure to arsenic and epigenetic changes [83–85]. However, the results of the studies are inconsistent, and no human studies have shown a direct link between arsenic exposure-related epigenetic changes and cancer. We conducted a study of subjects in Bangladesh and observed that chronic exposure to arsenic is associated with global DNA hypomethylation [85]. The degree of global methylation status was measured by the methylation of long interspersed nuclear element-1 (LINE-1) in blood leukocyte DNA. However, since arsenic induces carcinogenesis in the skin, liver, lungs, bladder, and kidneys, it remains unclear whether the global hypomethylation measured solely in the leukocytes can explain the arsenic-induced carcinogene-

sis in multiple organs. We have thus explored factors including angiogenesis that may affect cancer development and metastasis among arsenic-exposed populations in Bangladesh.

Angiogenesis is a pivotal step for the initiation, progression, and metastasis of cancer because tumor cells receive nutrients through angiogenesis. We investigated the associations between arsenic exposure metrics and several pro-angiogenic blood-circulating molecules including vascular endothelial growth factor (VEGF) and matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) [59, 86]. The serum VEGF levels were shown to be significantly increased with the increasing concentrations of the subjects' drinking water, hair, and nail arsenic concentrations. VEGF has been recognized as a marker of angiogenic activity for tumor progression [87]. In addition to tumor angiogenesis, VEGF is implicated in the pathophysiology of CVDs because of its ability to increase vascular permeability [88].

The serum levels of MMP-2 and MMP-9 are increased dose-dependently with arsenic exposure (59). MMPs degrade the extracellular matrix to facilitate the migration and recruitment of cancer and inflammatory cells [89]. Growing evidence suggests that MMP-2 and MMP-9 are deeply implicated in CVDs and cancers through vascular remodeling and the formation of new blood vessels (angiogenesis and arteriogenesis) [90–92]. When the tissues undergo vascular remodeling and abnormal angiogenesis under pathological conditions (particularly in CVDs and cancer), MMPs are markedly expressed, secreted, and activated. The positive association of arsenic exposure metrics with circulating levels of VEGF and MMPs observed in our studies suggest pro-angiogenic conditions among people who are chronically exposed to arsenic [59, 86]. Taking the above-described findings together, we here postulate that arsenic exposure induces pro-angiogenic and pro-inflammatory conditions in the vascular microenvironment, which may facilitate the development of both cancer and CVD.

4.4.5 Neonatal Exposure to Arsenic and Future Risks to Health

Arsenic can cross the placenta and affect the survival, growth, and functions of fetuses and neonates. It was observed that the fetal exposure levels of arsenic are equivalent to those of the mothers [93]. Epidemiological studies in Bangladesh have shown the associations between higher levels of arsenic and increased incidences of spontaneous abortion, stillbirth, infant mortality, low birth weight, and fetal growth retardation [94–99]. In utero or infant arsenic exposure was demonstrated to be associated with neurodevelopmental defects and cognitive dysfunction [21]. Evidence also suggests that neonatal or early-life exposure to arsenic is associated with dysfunction of immune and inflammatory pathways [100]. Maternal exposure to arsenic can increase the susceptibility of neonates to infections with pathogenic microorganisms [101]. A cohort study conducted in Bangladesh by Hawkesworth et al. [102] reported that higher in utero arsenic exposure was associated with increased blood pressure in 4.5-year-old children [102].

Several studies in northern Chile, where the contamination of tap water by high concentrations of arsenic occurred for a limited time in 1958–1970 but ceased after that time, reported the prolonged effects of early-life arsenic exposure on the health status of the adults in later life. The incidences of malignant and nonmalignant lung diseases, bladder cancers, and liver cancers in the adults who had been exposed to arsenic in early life were significantly higher than those of the nonexposed population even 20–40 years after the high exposures to arsenic had ceased, suggesting that humans are extraordinarily susceptible to early-life arsenic exposure [82, 103].

Fetal and neonatal exposure to arsenic is also associated with epigenetic changes that may affect an individual's susceptibility to a variety of diseases in later life. Approximately 34% of the entire population of Bangladesh is under 15 years of age, and many of these individuals may have been exposed to arsenic in utero and during infancy. More studies are required to clarify the effects of neonatal and early-life arsenic exposure on later life.

4.5 Future Perspectives on the Health Effects of Chronic Arsenic Exposure

The roles of arsenic exposure in the development of common or lifestyle-related diseases are not yet known clearly. Recent studies have indicated that chronic arsenic exposure is associated with lifestyle-related diseases such as metabolic syndrome, diabetes, and respiratory complications. However, the results of those studies are not consistent. Compared with the skin symptoms, which are visible and specific to arsenic exposure, it is difficult to evaluate the excess risks of hypertension, diabetes, and other common diseases (which are caused by multiple and nonspecific factors in addition to arsenic exposure). To overcome this problem, large-scale epidemiological studies with reliable quantification metrics of arsenic exposure levels are required. Growing evidence also suggests that immune dysregulation is deeply implicated in several chronic inflammatory diseases such as asthma, CVD, and cancer as well as the higher susceptibility to pathogens in infants. However, insufficient attention has been paid to this topic. Further studies are required to unveil the roles of arsenic exposure in immune dysregulation in the development of chronic diseases. It is also necessary to elucidate the effects of early-life arsenic exposure on the development of diseases in later life, particularly in Bangladesh, where a high percentage of the population is children.

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Chapter 5
Field Researches on Chronical Arsenic Poisoning in Inner Mongolia, China

Takahiko Yoshida, Guifan Sun, Jungbo Pi, Xin Li, Bing Li, and Hiroshi Yamauchi

Abstract Chronic arsenic (As) poisoning in China as endemic disease observed in local residents is summarized with reference to the field research reports. The importance of choosing the appropriate parameter for As exposure based on the property of each field in the analytical research on the relationship between As exposure level and As-related diseases is discussed. Our field cohort study of chronic waterborne As poisoning established in Inner Mongolia is introduced as one of the leading models demonstrating that it is possible to evaluate the improvement of As-related diseases including skin lesions and to validate the attenuation of carcinogenesis by the mitigation of As exposure. Our cohort study reveals that the improvement of skin lesions due to chronic As poisoning occurred in the early phase as 1 or 2 years after the mitigation of As exposure, and then slow alleviation continued for

T. Yoshida (✉)
Department of Social Medicine, Asahikawa Medical University, Asahikawa, Hokkaido, Japan
e-mail: tyoshida@asahikawa-med.ac.jp

G. Sun · B. Li
Research Center of Chronic Diseases and Environment, School of Public Health, China Medical University, Shenyang, Liaoning, People's Republic of China
e-mail: gfsun@cmu.edu.cn; bli10@cmu.edu.cn

J. Pi
Department of Environmental Toxicology, School of Public Health, China Medical University, Shenyang, Liaoning, People's Republic of China
e-mail: jbpi@cmu.edu.cn

X. Li
Department of Occupational Health, School of Public Health, China Medical University, Shenyang, Liaoning, People's Republic of China
e-mail: lixin@cmu.edu.cn

H. Yamauchi
Department of Preventive Medicine, St. Marianna University School of Medicine, Kawasaki, Kanagawa, Japan

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long periods. On the other hand, after long, more than 25 years As exposure period, and even if the exposure had mitigated, the number of As-induced malignancy including skin cancer is expected to rise. So for well-directed screening to find the As-related malignancy cases, the use of appropriate parameters for identifying high-risk subjects is needed.

Keywords Arsenic poisoning · Parameters for As exposure level · China · Gangfangying village · Mitigation trial · Improvement of skin lesions · Carcinogenesis

5.1 History and Current Situation of Arsenic Poisoning in China

In this chapter, we summarize chronic arsenic (As) poisoning in China as endemic disease observed in local residents but exclude the cases that occurred in the industrial workers or in neighborhood residents at industrial contamination sources.

5.1.1 Old Arsenic Poisoning Cases Observed in Taiwan

In the southwestern coast of Taiwan, Blackfoot disease (BFD) had been known as endemic disease with peripheral circulatory deficit from the early twentieth century. BFD was characterized by signs of progressive arterial occlusion with typical gangrenous lesions in the lower extremities of patients [1–5]. Epidemiologic studies carried out in mid-twentieth century revealed co-occurrence of BFD and As-related skin lesions such as dyspigmentation and hyperkeratosis [6]. Since dose-response relationship between the prevalence of As-related skin lesions and the consumption of inorganic As (iAs) from the artesian wells was observed in BFD endemic area, As is thought to contribute to the cause of BFD. Although disturbing of peripheral blood circulation had been reported in several field researches in other countries [7], BFD has not been observed in other area of the world. Several explanations were proposed about confounding factors, and then humic substances were suggested as possible causes of BFD [3]. Since exposure history of As in Taiwan is relatively long, the malignancy cases had been reported [8–13]. Those researches reported that the duration for developing cancers was on average more than 10–30 years. In the study [12], the duration of As exposure, concentration of As, and multiplication of both are indicated as the index correlated with prevalence of skin cancer. Significant increases of lung and urothelial neoplasm were also found in the patients with As-related skin lesions [9]. Dose-response relationships were

observed between SMRs of the cancers of skin and several internal organs and BFD prevalence rate [8].

5.1.2 History of Waterborne As Poisoning in Mainland China

In late twentieth century, As poisoning cases caused via drinking water had been reported in mainland China [14–19]. The first prevalent case was discovered in Kuitun, Xinjiang Province, in 1983 [18, 20, 21]. After 1989, another severe epidemic region of As poisoning had been reported starting from Inner Mongolia and following Shanxi Province. The regions in Inner Mongolia and Shanxi provinces are of the same geographic formation: located on the watershed alluvial plain of Yellow River [14]. Based on those data, prediction model for exposure to As-rich groundwater was proposed [22].

The purpose of international recommendation to shift drinking water sources from surface or shallow well to deep well was to avoid pathogenic organism contamination [23]. Additional trigger of arsenic poisoning was intentional changing of water source to avoid fluoride poisoning, since surface or shallower well water contained fluoride at high concentration in certain area of mainland China, such as Inner Mongolia [24]. Unfortunately some deep wells were contaminated with high concentration of As, and what is worse, no adequate water quality safety check was carried out before using its water for drinking or cooking.

Since the As exposure history in mainland China via drinking water is not long, the reports of malignancy cases are few [9] except specific endemic disease area with skin lesions of unknown causes which are lately identified as As caused. The residents continuously drank local shallow well water contaminated with high As for a long period, and they developed skin lesions [25].

5.1.3 Arsenic Poisoning Due to Coal Burning in Mainland China

From the 1970s, in some areas of Guizhou and Shanxi provinces, a different type of As poisoning due to burning of As-rich coal had been recognized [16, 26–28]. Residents in those area used outcropped As-rich coal for cooking, drying crops including chili or corn, and heating in the open-pit stove without proper ventilation system such as chimney. Indoor air was polluted, and As fume was deposited on the surface of crops becoming exposure source. The estimated sources of As exposure were from As-deposited foods (50–80%), air (10–20%), water (1–5%), and direct contact to As-rich coal [26]. The level of As exposure in the area is relatively high, and the prevalence of skin lesions of the residents is high to such an extent that

progression to skin ulceration leading to skin cancers is observed as well as cases of lung cancers [29]. Contribution of As-polluted air is estimated to be relatively large, since the retrospective research revealed that stopping the use of As-rich coal and chelation therapy to the patients improved their skin lesions remarkably. The relatively long exposure history of airborne As poisoning in that area may be the reason for detection of malignancy cases compared to waterborne As poisoning area [10].

5.2 Field Research on Waterborne As Poisoning to Analyze the Dose-Response Relationship Between As Exposure and As-Related Diseases

Many cross-sectional field researches had revealed that the prevalence of skin lesions including skin cancer and internal malignancies showed significant dose-response relationship with As exposure level in the waterborne As poisoning area [30]. We suggested that the appropriate parameter for As exposure had to be chosen based on the property of each field in the analytical research and on the relationship between As exposure level and As-related diseases. We observed increase of prevalence in the skin lesions even at the exposure levels in the range of 0.005–0.01 mg/L As of the drinking water. On the basis of summarized data accumulated until around 2000, we alerted to the possibility of the increase in occurrence of malignancy cases within the next decades and to the necessity of careful observation in those epidemic areas to search malignancy cases and make possible countermeasures [30].

5.3 Trial of Mitigation as Countermeasure to the Waterborne As Poisoning in Mainland China

Avoiding to use the water with high concentration of As for drinking or cooking is common and a basic strategy to prevent not only the occurrence of new As poisoning cases but also the symptom exacerbation of existing As poisoning in patients. Therefore, searching the alternative safe water source becomes the priority concern of public health in the endemic areas.

After the nationwide survey of waterborne chronic As poisoning in China and followed spreading of pipeline water services with lower As water source by local governments, no new occurrence of As poisoning cases are expected, and the interest of public health had shifted to the treatment of existing patients. Since hyperkeratosis in palms or soles disturbs the farming works and walking of patients, the mitigation of As exposure is expected to improve the symptoms and QOL of them. Furthermore the occurrence of malignancies among them is not only an individual threat to their life but also a burden on the community by the increased medical cost. So early detection and early treatment are desired; however, those evidences

had not been checked thoroughly enough. Additionally there is no data on the carcinogenetic outcome of As-poisoned patients by the mitigation or treatment.

Around the time of designing our study, we got the information that a farm village Gangfangying in Inner Mongolia scheduled to start the new pipeline water service in September 1999. The implementation of mitigation in Gangfangying was relatively early case in mainland China. So we set the prospective cohort study field in Gangfangying. In late July 1999, we conducted a field research in Gangfangying as the baseline study of following research to evaluate the effect of mitigation of As exposure via drinking water by changing water source.

5.4 Advantage of Gangfangying for the Interventional Field Research to Confirm the Improvement of Skin Lesions and Prediction of Carcinogenesis by Mitigation

The initiation, duration, and concentration of As exposure were assessable in each individual of our cohort study in Gangfangying where we set the field cohort in 1999. The obtained information were necessary for evaluating the improvement of health disorders in the As-related disease subjects by the mitigation. So our cohort study appears to have several advantages to be the leading model for providing the evidence to promote the mitigation of As exposure and the closely focused survey to find the occurrence of malignancy cases in other areas.

5.4.1 Current Basic and Historical Information of Gangfangying

Gangfangying is one of the 47 villages of Baotou city located in the eastern suburb of this city in Inner Mongolia and known as an As endemic area. Main occupation of the villagers was farming; main crops were wheat, corn, and sunflower. The registered population in 1999 was approximately 2080, representing 480 households, and the number of inhabitants living there was approximately 1300, due to schooling or working outside of village. There were about 338 active wells, personal pump tube wells with 15–25 m depth, and 1 public open well with 3 m depth in the village. In the past, villagers used six public open well (shallow well) located in public space. Only one open well (named North public well) remained active, and others were abandoned with plugging by July 1999. From 1979, the village households gradually changed their drinking water source from the open wells to pump tube wells (deep well) established in the property yard of each house for their convenience and hygienic safety purpose, depending on the increase of their incomes. Forty-six households did not have own family pump well and obtained drinking water from neighborhood wells.

The first case of chronic As poisoning of the village was recognized when a woman having typical skin lesions relating to As visited a city hospital with her another health problem in 1996. Subsequently, the center of disease control (CDC) of local government of Baotou city conducted a spot survey of As concentration from well water and a small epidemiological study, then announced an outbreak of waterborne As poisoning in the area.

Based on our study, As concentration of water from 338 pump tube wells varied between 0.000049 and 1.79 mg/L, with average 0.126 mg/L and standard deviation 0.192 mg/L, mean 0.04 mg/L, first quartile 0.00923 mg/L, and third quartile 0.196 mg/L and from an open well was 0.00068 mg/L. Two hundred and forty-one well exceeded the 0.01 mg/L WHO standard for drinking water quality, and 158 wells exceeded 0.05 mg/L, the Chinese standard at the time. Major species of As was pentavalent inorganic iAs, and no organic arsenic forms were detected.

The As exposure level of the villagers was assumed to vary according to the As concentration in their drinking water, in other words depending on the well. As we summarized in our paper [30], among the parameters for As exposure, average or cumulative As exposure calculated from As in the drinking well water are the more suitable indexes for Gangfangying.

Figure 5.1 shows the changes in yearly average concentration of As in well water that had been consumed by 92 participants from 1980 to 1999. In this research, As concentrations of current (in 1999) water from each wells were substituted for the

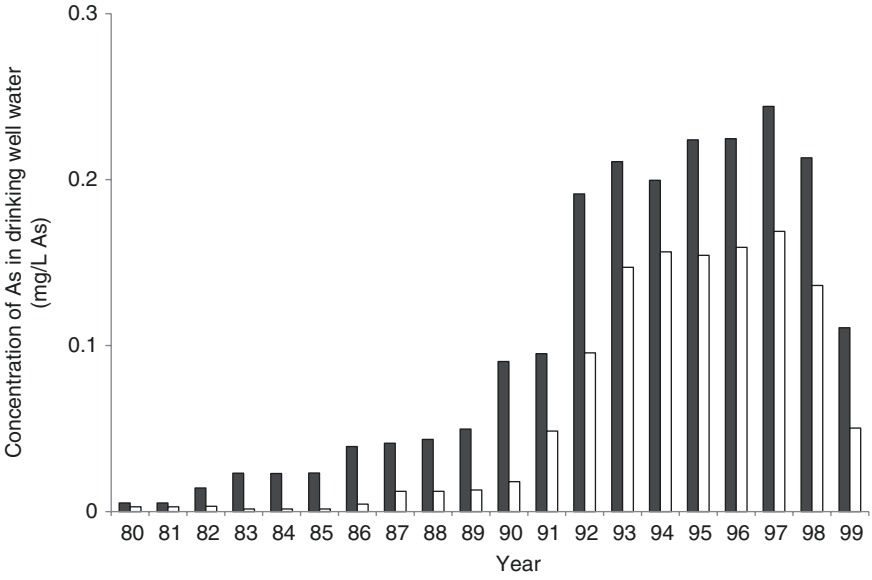

Fig. 5.1 Changes in average concentration of As in drinking water of the Gangfangying villagers from 1980 to 1999, before the starting of mitigation. Data is shown as mean consumed by male ■ and female □, respectively. Number of the male was 42 and female was 40–50, respectively. The increase in the number of female participants was due to coming from another village for mainly marriage

water consumed in the past, on the assumption that As concentration in well water had been stable. The average concentration of As of the well water consumed by the participants gradually increased through 1991, and exceeded 0.1 mg/L in 1992, and the high exposure level continued till 1998. Then it started to decrease from 1998. It was clearly observed that some of the participants with skin lesions changed their water source to neighborhood wells owned by villagers without skin lesions. It might have been influenced by notification of the outbreak of waterborne As poisoning in the area by local government after the find of the first As poisoning case in 1996. The As exposure level of male was higher than that of female, although the trends of As exposure level of both gender were similar.

5.5 Cross-Sectional Study in Gangfangying as Baseline Study of Cohort Research

One hundred and thirty-two villagers visited the reception desk as respond to the announcement asking voluntary participation on the research and were issued identification data (ID) numbers during 3 days in the late July 1999. This was a baseline study for cohort research. Among 132 registered villagers, the young subjects (under 20 years old, $n = 18$) were excluded from the analysis, because of their different appearances of skin lesions and different trends of As methylation capacity from those of adults (data not shown). The final maximum effective number of subjects was 98 (43 males and 55 females; 21–70 years old, mean age 40.1 ± 9.9 years old). The number of subjects in each analysis was different since the effective combination was restricted by the minimum data number of items. This research was approved by the Institutional Review Board of Tokai University and Asahikawa Medical University for the medical ethics (Tokai Univ. 12/09/1996, Asahikawa Med. Univ. 28/02/2001 No.57).

5.5.1 Analysis of Suitable Parameters of As Exposure Level in Gangfangying

Distribution of each grade of skin lesions based on gender is shown in Table 5.1. Male participants showed the trend to have higher grade in each type of skin lesions than female. Their principal skin lesions were hyperkeratosis on palms and/or soles and dyspigmentation mainly in trunk skin.

We analyzed what is the suitable parameter of As exposure level for the evaluation of the relationship between As exposure and the presence of skin lesions in Gangfangying (Table 5.2). The current water As concentration did not have significant correlation with the presence of skin lesions. The reason for this is understandable as follows below; a small number of participants especially those having skin lesions changed their water source to safer estimatedly low As wells as mentioned above (Fig. 5.1). Average As concentration of water for stretching back to 5, 10, 15,

Table 5.1 Distribution of each category of skin lesions based on gender

Skin lesions	Category[a]	Male	Female
Hyperkeratosis on palm	None	20	36
	Mild	7	10
	Moderate or severe	14	1
Hyperkeratosis on sole	None	24	40
	Mild	7	1
	Moderate or severe	10	6
Dyspigmentation in skin	None	27	45
	Mild	3	1
	Moderate or severe	11	1

[a]Each skin lesion is categorized as "None", "Mild", and "Moderate and Severe"

and 20 years from the time of study July 1999 showed significant correlation with the presence of skin lesions. Since the period of high As exposure level was short and was limited only between 1990 and 1999 in Gangfangying, parameters based on the length of period did not show positive length-response relation on the presence of skin lesions. Parameter of near 5 years to study point (average of 1995–1999) showed significantly larger OR than other durations. The OR observed in hyperkeratosis on palms or soles and dyspigmentation in the skin were almost similar. Although parameter of accumulative As amount considering the exposure length is one of the choices, there was no good relation between it and the presence of skin lesions (data not shown). It seems to be a less useful parameter for the cases of short As exposure period such as in Gangfangying.

Biological parameters for As exposure levels such as current As amount in the blood, urine, and hair showed significant correlation with the presence of skin lesions, but the relationships were not clear when comparing to past exposure parameters. It is corresponsive to the lack of significant correlation in the presence of skin lesions with the parameter of current water As concentration. As mentioned above in the observation of changes in average concentration of As in drinking water of participants, certain proportion of participants had changed their water sources to the wells with lower concentration of As. There was a possibility that severalty of skin lesions had changed during their autonomous mitigation by changing the water sources. So we concluded that average As concentration of drinking water near 5 years to study time point (average of 1995–1999) is the suitable parameter to analyze the dose-response effects of As exposure on health disorders in Gangfangying.

5.5.2 Characters of Skin Lesions and Findings in Gangfangying Relating to As Exposure

Finally using the parameter of average As concentration of drinking water near 5 years to study time point, we confirmed the male dominant gender difference but no difference in age among adults in skin lesions, the involvement of high concentration of blood MMA relating to higher first and lower second methylation of As

Table 5.2 Relation between parameters of As exposure level and presence of skin lesions[a] in Gangfangying

	Hyperkeratosis on palms					Hyperkeratosis on soles				Dyspigmentation in skin			
				95% CI				95% CI				95% CI	
Parameter of As exposure	*n*	OR[b]	*p*	Lower	Higher	OR	*p*	Lower	Higher	OR	*p*	Lower	Higher
Current As conc. of water (mg/L) (in five categories[c])	85	1.083	0.532	0.843	1.393	0.972	0.846	0.727	1.299	1.374	0.087	0.954	1.979
Ave. As conc. of water between 1995 and 1999 (mg/L) (in five categories[c])	85	1.906	0.0004	1.330	2.733	2.031	0.001	1.345	3.067	2.040	0.004	1.248	3.337
Ave. As. Conc. of water between 1990 and 1994 (mg/L) (in five categories[c])	85	1.619	0.006	1.152	2.274	1.348	0.099	0.945	1.921	1.631	0.029	1.052	2.527
Ave. As. Conc. of water between 1990 and 1999 (mg/L) (in five categories[c])	85	1.616	0.001	1.225	2.133	1.515	0.010	1.105	2.076	1.665	0.010	1.129	2.455
Ave. As. Conc. of water between 1985 and 1999 (mg/L) (in five categories[c])	85	1.259	0.0002	1.117	1.419	1.414	0.029	1.037	1.928	1.252	0.007	1.065	1.473
Ave. As. Conc. of water between 1980 and 1999 (mg/L) (in five categories[c])	85	1.243	0.0002	1.108	1.394	1.491	0.012	1.092	2.036	1.243	0.005	1.066	1.449
Current As amount in blood (IMD[d]) (μg/dL) (in five categories[c])	97	1.157	0.257	0.899	1.488	1.165	0.287	0.879	1.544	1.590	0.018	1.081	2.339
Current As amount in urine (IMD[d]) (μg/g.Cr) (in five categories[c])	75	1.200	0.182	0.918	1.570	1.413	0.049	1.001	1.994	2.305	0.004	1.303	4.078
Current As amount in hair (Total As) (μg/g) (in five categories[c])	77	1.447	0.007	1.108	1.889	1.590	0.005	1.147	2.205	1.847	0.007	1.184	2.882

[a]Each skin lesion was categorized as "None", "Mild", and "Moderate and Severe"
[b]Odds ratios was calculated by ordinal logistic regression analysis with adjustment for age and gender
[c]Each parameter data was not normal distributed. Therefore, the numerical value of each parameter was divided into five categories with close equally
[d]IMD = iAs + MMA + DMA

on the presence of skin lesions, occurrence of skin lesions even at the exposure levels in the range of 0.005–0.01 mg/L As of the drinking water [30], decrease in urinary excretion of cyclic guanosine monophosphate (cGMP) [31], elevated 8-hydroxy-2′-deoxyguanosine (8-OHdG) in urine and lower glutathione-SH (GSH) in the blood among children [24], etc. (data not shown).

5.5.3 Detailed Analysis of As-Related Health Disorders Reported by Field Research in China

Confounding factors between As exposure level and As-related skin lesions such as gender and methylation capacity to As were reported based on the field research in China even in the coal-burning endemic area and BFD endemic area [32]. Although among them the correlation with the presence of skin lesion was dominant [33–39], other correlations were also reported. Elevation of blood pressure and hypertension [39–41] and related atherosclerosis and peripheral vascular disease [3, 42] is highly reported. Other correlations with 8-OHdG levels [43], reduction of GSH and superoxide dismutase (SOD) [44], developmental delay in preschool children [45], disruption of endocrine systems [46], and unexplained male infertility [47] were also reported.

5.6 Follow-Up Study in Gangfangying After the Initiation of Mitigation

Public pipeline water supply service started in September 1999. The pipeline water (concentration of As; 0.037 mg/L) was obtained from the electric pump well at 10 km away from Gangfangying. We started the follow-up study in Gangfangying based on the basic research in the late July 1999. We had field researches in 6 months, 1 year, 5 years, 10 years, 15 years, and 20 years after the initiation of mitigation. In August 2000, we had relatively large survey as 1-year follow-up study including collecting of biological samples. Another time, we focused on the observation of skin lesions with comparison of photographs taken previously.

We analyzed the biological exposure monitoring to confirm the decline of arsenic exposure level by the mitigation of As exposure at 1 year after the initiation of pipeline water service. Figure 5.2 shows the comparison of the amount of As in urine as IMD. Concentration of IMD in urine from participants was reduced at 1 year after the initiation of mitigation. That means the mitigation by pipeline service with lower As concentration well water was effective to reduce the As exposure level of the residents in Gangfangying.

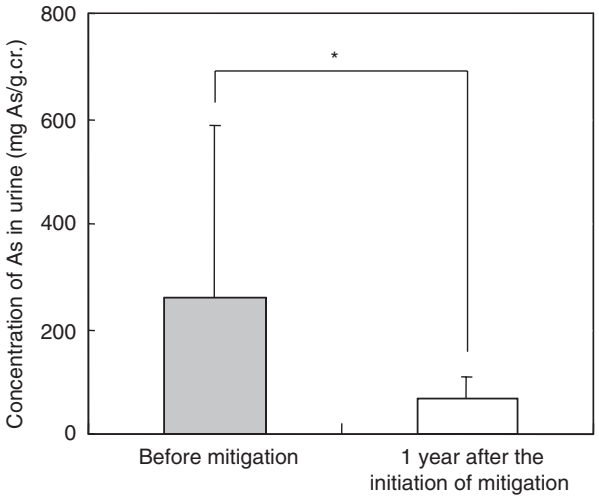

Fig. 5.2 Comparison of the amount of the As in urine as biological exposure monitoring. Arsenic is measures as inorganic arsenic (iAs), monomethyl arsenic (MMA) and dimethyl arsenic (DMA). Data is shown as mean ± SD of IMA = iAs + MMA + DMA. Significance of concentration of IMD between before and after mitigation was tested by paired *t*-test ($p < 0.001$)

5.7 Short Period Follow-Up Study in Gangfangying

5.7.1 Changing of Skin Lesions by the Mitigation Observed in Short Period

We examined the changing of skin lesions in each subject carefully by clinical examination with comparison between on-site observation and previously taken photographs and written descriptions using the delicate grading scale of skin lesions (Fig. 5.3). We developed the grading scale of skin lesions based on our experience in As poisoning endemic areas. It is shortly as below; skin lesions (hyperkeratosis on palms and soles or dyspigmentation in skin) are graded with combination of two metrics: one is the factor of severity grade with characteristic aspects and the other is the dimensional factor with spreading of lesions. Firstly, skin lesions are rated into four grades in the severity: none, mild, moderate, and severe, respectively. The cases of mixed skin lesions (exhibiting different grades) are judged to higher grade. Then, skin lesions are rated with its distribution: none, localized, wider, and extending into the rare region. Finally skin lesions are graded into ten classes.

We compared the skin lesions at 1 year after the initiation of mitigation with those before the mitigation based on the previous photographs and description records of each participant with no distinction of the subjects who had already and

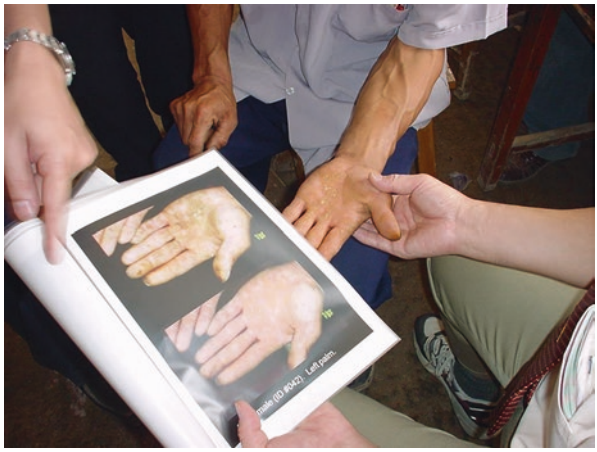

Fig. 5.3 Clinical examination follow-up of skin lesions at 1 year after the initiation of mitigation. Clinical examination on skin lesions had been done carefully with comparison between previous photographs (in here just before and half year after the initiation of mitigation) and description of characters

continuously been in the autonomous remediated situation from other subjects. The trends of improvement of skin lesions were unclear (data not shown). As mentioned above, many participants including the ones with skin lesions changed their water source to lower As well water. So we separated the subjects into two groups based on the situation of As exposure: a group which had autonomously reduced the As exposure level before the mitigation ($n = 49$) and the other group which had continued exposure to As until the initiation of the mitigation ($n = 18$). The latter group showed higher improvement ratio of skin lesions than the other (Fig. 5.4, right side panels). It means the improvement effects on skin lesions were more visible in the group which had been continuously exposed to As until its mitigation, since other groups who had reduced the As exposure level before the investigation had improved already their skin lesions in certain degree by the start of observation. Furthermore, it suggested that the skin lesions were remedied in the early phase as 1 or 2 years and the pace of further improvement might be tardy. However, the condition in a part of subjects had worsened after the mitigation of As exposure. It might be thought that accelerated progression of skin lesions would continue for a while even under the mitigation of As exposure level and would overlap with the improvement trend until the reversion vector would be dominant. Those speculations drawn from the observation in a 1-year follow-up study had been confirmed at 5-year follow-up study in October 2004 [24]. Although the skin lesions of subjects had remedied in certain degree within 1 year by the mitigation, most of their skin lesions stayed unchanged in the following 4 years. A few cases with mild hyperkeratosis on palms were improved, however in two subjects were even deteriorated. There was no question as to the importance of stopping the further As exposure to prevent the occurrence of new As poisoning patients, but the challenge remained in the improvement of As poisoning.

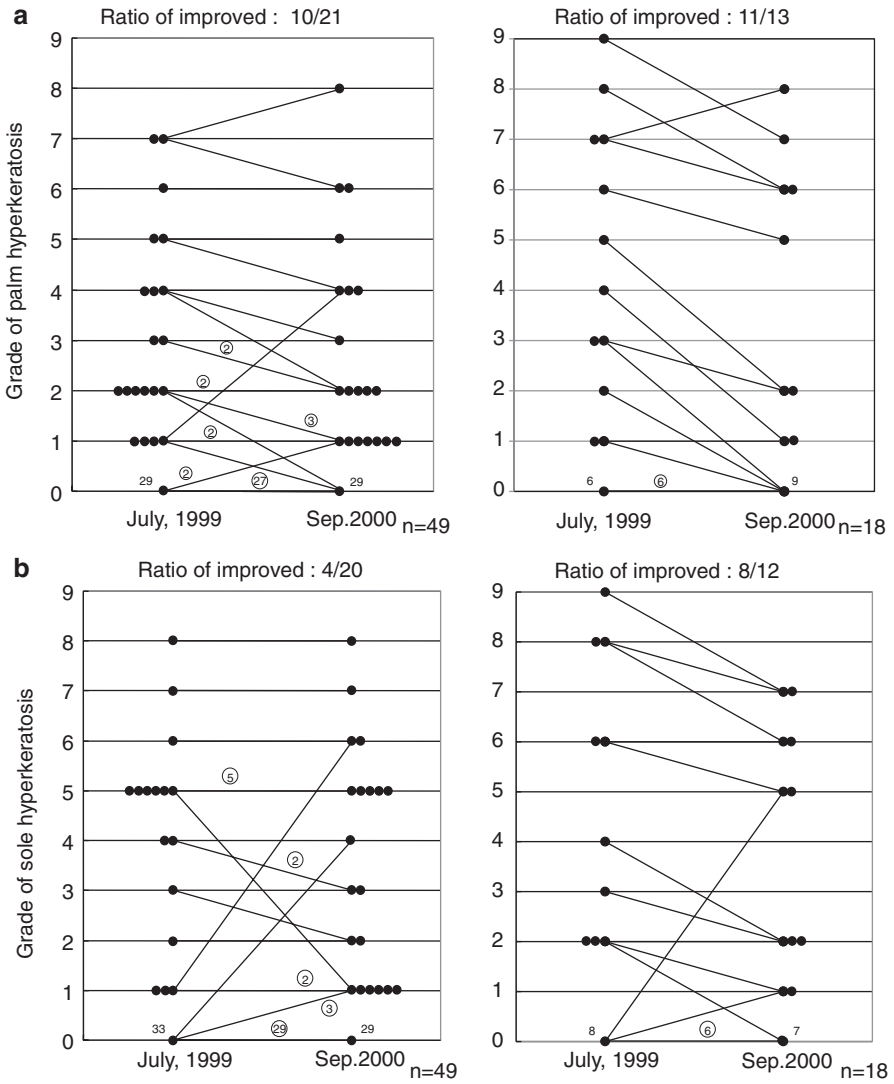

Fig. 5.4 Changes of skin lesions by the mitigation in each subject divided by As exposure situation just before mitigation. Each dot means one subject. Number near dot in grade '0' means the number of subjects with hidden dots. In the individual's scale the lines tie the grade of skin before and 1 year after the initiation of mitigation. Circled number on the lines means the number of cases showing same change and lines without indicated number represent one case

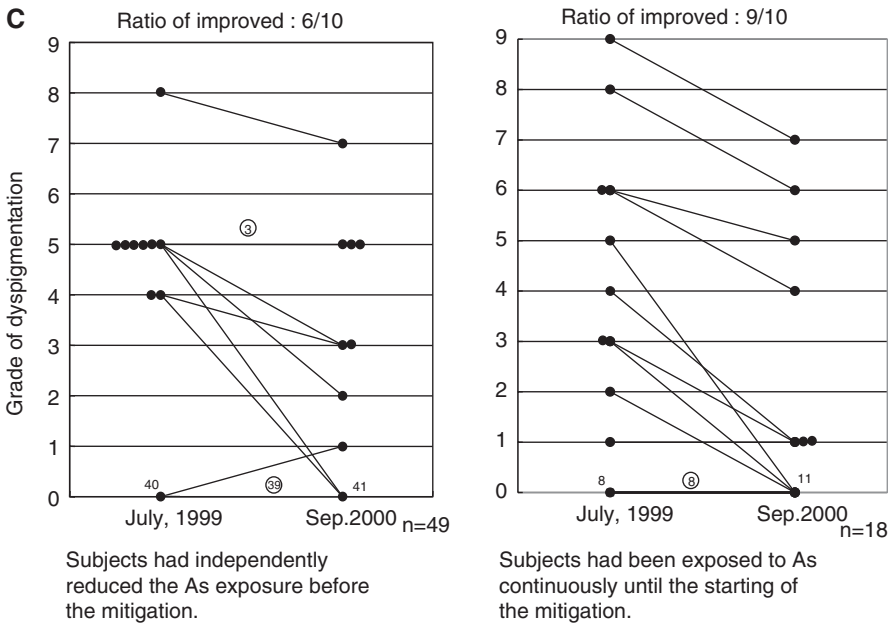

Fig. 5.4 (continued)

5.7.2 Changes in Other Parameters by the Mitigation Observed in Short Period

We also observed the effect of the mitigation as the reversion of decreased urinary excretion of cyclic guanosine 3,5-monophosphate (cGMP) and the recovery of peripheral vascular response to cold stress [31]. And improvement of increased 8-hydroxy-2′-deoxyguanosine (8-OHdG) excretion into urine was also observed [24].

The difference of the improvement by mitigation observed between skin lesions and peripheral blood circulation in the Gangfangying cohort study might be explained with the different mechanisms of those health disorders. Affection of peripheral blood circulation by As is a functional disorder and caused by disruption of nitrogen monoxide (NO) synthesis in arterial endothelium. The produced NO in arterial endothelium acts as a dilator on the arteries by relaxation in tension of the vascular smooth muscle. This mechanism is simple and reversible in short period. On the other hand, As-induced skin lesions are organic disorders and may be formed in multiple-step mechanism cascades involving several kinds of cells and come into existence in a significantly long period. While there is shifting to the alleviating

situation by the As mitigation, the process of formation may not be stopped or reversed quickly and in some instances might even progress. So the improvement of skin lesions may be prolonged.

5.8 Long Period Follow-Up Study After the Initiation of Mitigation in Gangfangying

We had 10 years and 15 years follow-up study at Gangfangying in February 2010 and September 2016, respectively. In February 2010, we followed up 15 subjects who were recorded at the baseline study, except four subjects without any skin lesions at the baseline study and also 10 years after. Among 11 subjects, the skin lesions of ten subjects were improved even in the cases whose skin lesions were unchanged for a while (date not shown). Although the number of Gangfangying residents had decreased, we diagnosed seven subjects and found four subjects were improved from baseline in the 15 years follow-up study in September 2017 at their skin lesions (data not shown). The number of residents was 2080 in July 1999 but decreased to around 600. The decrease of the population was due mainly to emigration out to city area. According to the report from the village physician, 2–3 residents died every year by some diseases. It became apparent that some degree of improvement of skin lesions of chronic As poisoning will occur in the early phase less than a year after the mitigation of As exposure, and then slow alleviation will continue for long periods. However, it is a little bit luck for the patients; it is a pity that they should have to put up with lower QOL for long years. So some kind of countermeasures for enhancing the alleviation of chronic As poisoning should be considered and implemented.

It should be noted that we found three cases having Bowen's disease and skin cancer among the participants of the cohort in Gangfangying in September 2016. They were two females and one male. Additionally some kinds of malignancy cases were reported by the village physician, but he did not mention the occurrence of skin malignancy. Because of the slow worsening of skin lesions, the actual number of skin malignancy cases may be larger.

5.9 Cancer Survey in the Epidemic Area of As Poisoning

In Gangfangying, more than 25 years has passed from the time when average concentration of As in the well water consumed by cohort participants exceed 0.05 mg/L. It is said the latency period in human for carcinogenesis of iAs is 20–30 years. So the time has come when the number of As-induced malignancy including skin cancer is expected to increase in Gangfangying, the representative area of waterborne As poisoning in China.

5.9.1 Past Field Research on the As-Induced Malignancy in China

Several As poisoning area known as endemic diseases epidemic areas in China have long As exposure history. Therefore, As-related malignancy cases had been reported from those fields, waterborne-type As poisoning area from Taiwan [12] or coal burning-type As poisoning area from Guizhou [26, 29].

In ecologic studies, participants are generally treated as groups, and the exposure level of each group is usually represented by a single indicator, mostly the mean exposure level. Primary studies of the association between As exposure level and malignancy including skin cancer from Taiwan were also performed by using the mean As concentration and ecological data of cancer prevalence in each endemic area. For example, Hsueh et al. analyzed the multiple risk factors associated with As-induced skin cancer using previously reported As concentration of each village as mean value [12]. Although they revealed dose-response relations between skin cancer prevalence and As exposure level, and also the confounding factors influencing the association, chronic liver diseases and malnutrition, it might have been possible to analyze more accurate risk assessment if they could use individual exposure levels. Guo et al. examined the association between As in drinking water and incidence of skin cancer using dummy variable data instead of the represented mean As exposure data of each group in a model ecological study, and it made possible to have more accurate risk assessment. So they concluded that using multiple variables is recommended for description of the exposure status in ecologic studies [13]. Their recommendation encourages our Gangfangying cohort for searching index to estimate future carcinogenesis among the As-poisoned subjects. We stress again the importance of selection of appropriate parameter for any exposure substances in the field research based on their characteristic.

The follow-up study reported from the coal burning-type As poisoning area showed the significantly increased mortality due to lung cancer, non-melanotic skin cancer, and liver cancer as top three malignancies among As-poisoned patients in Guizhou [29]. The authors evaluated the As exposure level only by the severity of skin lesions as severe, medium, and mild. From this type of research, further analysis to confounding factors is difficult.

So cohort study and the regression analysis using the data of individual As exposure level, amount of exposure at time and persisting period with initiation and termination time points, and the data of individual presence of malignancy including skin cancers are expected.

5.9.2 Field Research for Mechanisms of As Carcinogenesis

Some field researches concerning to the mechanisms of As carcinogenesis in the epidemic area of As poisoning in China had been reported. The possible mechanism of As carcinogenesis is proposed as below; increased reactive oxidants and

the decreased antioxidant capacity in peripheral blood owing to persistent oxidative stress were induced by long-term As exposure [48]. Recently many researchers aimed to identify the responsible genes for As carcinogenesis in the biological samples obtained from residents in epidemic area of As poisoning. Increasing association of As exposure with mutations at the Glycophorin A locus was observed in the patients with skin cancer in Guizhou and proposed to be a biomarker for As carcinogenesis [49]. Mo et al. reported that human telomerase reverse transcriptase (hTERT) mRNA expression levels in blood cells were significantly associated with As concentrations of water and also severity of skin hyperkeratosis among Inner Mongolia residents [50]. They discussed that the expressions of hTERT observed in the residents were the results of DNA injury induced by As and the possible indicator for the existence of a tumor promoter in human carcinogenesis. Hsu LI et al. found the skin cancer potential role in microsomal epoxide hydrolase (EPHX) Tyr113His, xeroderma pigmentosum group D (XPD) C156A, and glutathione S-transferases (GSTs) T1 null genotypes by logistic regression analysis on the residents of As poisoning area in Taiwan [51]. Zhanga et al. reported that p15INK4b hypermethylation and gene deletion occurred at higher As poisoning cases with skin cancer in Guizhou [52]. Those reports were based on the cross-sectional study, and observed genetic disruption might be a possible parameter to estimate skin cancer and other malignancies in the future among As poisoning patients.

5.9.3 Trials to Establish the Useful Parameters for Identifying High-Risk Subjects Developing Malignancy

Currently to identify the highly As exposing/exposed subjects is the priority by cross-sectional field survey not only in China but also in the world. But the number is estimated to be too numerous for the implementations in whole size of the survey. Selection strategies of high-risk subjects have to be considered for reduction of effort, time, and cost. So the useful parameters for identifying high-risk subject who will fall to malignancy in the future among As exposed are needed. Some trials done in the field of China for the purpose are shown below.

Since the methylation capacity of As in individual is associated with the development of skin lesions, it may be one of the candidates of predictive parameter. Several trials had been performed as field research to evaluate the usefulness as predictive parameter in the epidemic area of As poisoning. Hsu et al. reported prediction of malignancy based on the community-based prospective cohort study among the residents in Taiwan, hyperkeratosis and skin cancers to predict risk for subsequent internal malignancy, especially skin cancer for lung cancer and urothelial carcinoma, and hyperkeratosis for lung cancer, respectively [9]. Gou et al. applied proteomics technology for searching the biomarker candidate of early diagnoses in high-risk populations [53]. Downregulation of cadherin-like transmembrane glycoprotein and desmoglein 1 (DSG1) and upregulation of keratin 6c (KRT6C) and fatty

acid binding protein 5 (FABP5) were estimated to contribute to proliferation or differentiation of keratinocytes. Those may be good parameters for prediction of skin cancers, but necessity of samples from palms and foot soles remains an issue.

5.10 Future Aspects

Although it is difficult to evaluate individual exposure doses from current time to extend back to the past, we should make every possible effort to estimate the occurrence of health disorders especially carcinogenesis in the field research. In our research fields of Gangfangying has an advantage to estimate the individual As exposure level for lifelong by hearing the usage history of well for water consumption. More than 20 years has passed since the start of our research, and during this period, the population of villagers had decreased from 2080 to around 600. Accordingly the number of the participants of the cohort in Gangfangying also decreased. But this is the time when malignancy including skin cancer starts to show up. We are planning to have a resurvey for adding the participants to the cohort. It may be possible to estimate the individual As exposure level for lifelong by the hearing of usage history of well for water consumption with the assumption that concentration of As in water from each well had been stable and the memory of the residents is good enough. In September 2016, we confirmed three skin malignancy cases. If the number of participants whose As exposure level is determined will be increased, more accurate estimation of skin cancer risk may be possible. And the study searching the predicting parameters of malignancy and confounding factors contributing the carcinogenesis may be also possible. We would like to develop the cohort study in Gangfangying to be a useful model and possibly apply to other chronic As exposure areas.

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Chapter 6
Arsenic Exposure and Lifestyle-Related Diseases

Yuanyuan Xu, Jingqi Fu, Huihui Wang, Yongyong Hou, and Jingbo Pi

Abstract Arsenic is a naturally occurring toxic metalloid within the Earth's crust. It is found primarily in drinking water and food. Chronic exposure to high levels of arsenic is associated with a wide range of human diseases including typical skin lesions (hyperpigmentation, hypopigmentation, and keratosis), cancer, diabetes, cardiovascular disease (CVD), neurocognitive outcomes, etc. In this chapter, we will introduce the evidence indicating association between arsenic exposure and increased risks of the lifestyle-related diseases [cancer, type 2 diabetes (T2D), and CVD], including epidemiological studies and animal studies. Current understanding of the mechanisms underlying these diseases and arsenic exposure will also be reviewed.

Keywords Arsenic · Cancer · Diabetes · Cardiovascular disease

6.1 Arsenic and Cancer

Arsenicals were firstly reported to cause human cancer in the late 1800s, when secondary skin cancers were found in patients using arsenic medicinals [1]. The International Agency for Research on Cancer (IARC) linked inorganic arsenic (iAs) exposure to human cancer in one of its earliest published monographs, and even then it was considered that there was compelling evidence supporting iAs as a human carcinogen [2]. After almost 40 years of extensive study, arsenic and arsenic compounds now are considered as known human carcinogens, and there is

Y. Xu · J. Fu · H. Wang · Y. Hou · J. Pi (✉)
School of Public Health, China Medical University,
Shenyang, Liaoning, People's Republic of China
e-mail: jbpi@cmu.edu.cn

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sufficient evidence for the lung, skin, and urinary bladder as cancer target sites and the kidney, liver, and prostate possible targets [3]. In Asia, the epidemiological evidence for arsenic and cancer risk mainly comes from populations in areas of endemic exposure to arsenic in the drinking water and, to a lesser extent, in populations occupationally exposed to mixed agents that include arsenic by inhalation. Compelling studies from early-life arsenic exposure in rodents showing dramatic cancer susceptibility together with experiments showing cellular malignant transformation in vitro have added more evidence for arsenic carcinogenesis and provide information on mechanisms involved in this process.

6.1.1 Epidemiological Studies of Arsenic and Cancer

6.1.1.1 Arsenic and Lung Cancer

In 1959, a short-duration arsenic poisoning incident occurred in the town of Nakajo, Japan. Lung cancer mortality and survival studies performed on these arsenic-exposed and arsenic-unexposed subjects from 1959 to 1992 found that males had a significantly higher rate of lung cancer and expected mortality [4]. Similarly, a lung cancer screening study of radon- and arsenic-exposed tin miners in Yunnan, China, found that exposure to radon and arsenic increased the risk of lung cancer [5]. Most studies of miners with high exposure to dust at Chinese tin mines have shown that an increased risk of lung cancer was related to cumulative dust exposure, duration of dust exposure, cumulative exposure to arsenic, and co-exposure to tobacco smoking [6–8]. In Taiwan, the standardized mortality ratio (SMR) and cumulative mortality rate were significantly higher in residents of blackfoot disease-endemic areas for lung cancer than in the general population [9–12]. In Bangladesh, a case-control study found an elevated risk for lung cancer in male smokers consuming tube well water with arsenic levels between 101 and 400 μg/L [13]. However, in non-smokers, the lung cancer risk did not increase with increasing arsenic exposure [13]. Ecological studies and cohort studies in Taiwan showed that the occurrence of lung cancer was highly correlated with arsenic concentrations in drinking water and reported significant dose-response relationships between the level of arsenic in drinking water and the risk of lung cancer death [10, 11, 14–16], which was elevated further among cigarette smokers [17, 18]. According to an ecological study in Suzhou, China, there was a significant correlation between lung cancer and soil arsenic concentrations in general population [19]. In Niigata Prefecture, Japan, the occurrence of lung cancer was highly correlated with the level of arsenic in drinking water [18]. Among the three major cell types of lung cancer, squamous cell carcinoma appeared to be associated with arsenic levels in drinking water [20]. However, the association between adenocarcinoma and arsenic exposure through inhalation appeared to be stronger than squamous cell carcinoma [21]. Details of these studies are summarized in Table 6.1.

Table 6.1 Summary of epidemiological studies on arsenic exposure and lung cancer

First author	Location	Exposure route	Subjects	Type of study	Reference
Mc Laughlin	China	Occupation (29 mines)	316 male with lung cancer and 1,352 controls	Nested case-control	[5]
Qiao	Yunnan, China	Occupation (Gejiu tin mine)	8,346 miners with 243 cases	Cohort	[6]
Chen	Southern China	Occupation (4 tin mines)	130 cases and 627 controls	Nested case-control	[7]
Chen	China	Occupation (29 mines)	518 male with lung cancer and 1,884 controls	Nested case-control	[8]
Chen	84 villages, Taiwan, China	Drinking water	332 men and 233 women	Ecological	[9]
Chen	4 townships, Taiwan, China	Drinking water	76 cases and 368 controls	Nested case-control	[15]
Chen	Taiwan, China	Drinking water	Residents in survey area	Ecological	[10]
Chen	Taiwan, China	Drinking water	457 black-foot disease patients with 28 cases	Cohort	[10]
Wu	42 villages, Taiwan, China	Drinking water	147 men and 121 women	Ecological	[11]
Chen	314 precincts or townships, Taiwan, China	Drinking water	Residents in survey area	Ecological	[16]
Tsuda	Niigata Prefecture, Japan	Drinking water	113 residents with 9 cases	Cohort	[18]
Chiou	4 townships, Taiwan, China	Drinking water	263 residents with 9 cases	Cohort	[14]
Tsai	4 townships, Taiwan, China	Drinking water	669 men and 471 women	Ecological	[12]
Nakadaira	Niigata Prefecture, Japan	Drinking water	86 cancer patients	Cohort	[4]
Chen	Taiwan, China	Drinking water	10,591 residents with 139 cases	Cohort	[17]
Mostafa	Bangladesh	Drinking water	7,286 subjects (lung biopsy) with 4,811 cases	Cohort	[13]
Chen	Taiwan, China	Drinking water	8,086 residents with 178 cases	Cohort	[20]
Kuo	243 townships, Taiwan, China	Drinking water	11.4 million residents with 8,353 cases	Cohort	[21]
Chen	3 towns, Suzhou, China	Soil	46,675 cancer cases with 15,691 lung cancer cases	Ecological	[19]

6.1.1.2 Arsenic and Skin Cancer

The skin is exposed to arsenic via medicinals containing arsenicals, arsenical pesticide residues, and arsenic contaminated wine or drinking water. Skin cancer was the first type of cancer linked to arsenic exposure. Arsenic-induced skin cancers are typically squamous cell carcinomas arising in keratoses (including Bowen disease) or multifocal basal cell carcinomas [22].

In Asia, most studies on skin cancer were conducted in southwestern Taiwan, China. An early ecological study [23] showed that an eight-fold increase in the prevalence of skin cancer lesions occurred in groups exposed to the higher levels (> 600 μg/L) of arsenic compared to the lower levels (< 300 μg/L) of arsenic in the artesian wells [23]. The ecological studies in Taiwan, China showed a clear dose-response relationship between the average level of arsenic in the drinking water and skin cancer mortality [9–12, 16]. In addition, an association between arsenic exposure and increased risk of cancerous skin lesions has been seen in Bangladesh, Mainland China, and India [24–26].

In a retrospective cohort study of blackfoot disease patients in Taiwan, China, an SMR of 28 (95% CI: 11–59) for skin cancer deaths was reported [10]. Another cohort study in southwestern Taiwan, China found an incidence rate of 14.7 cases of skin cancer per 1,000 person-years [27]. These risks were significantly linked to duration of living in the endemic area with blackfoot disease, the duration of consuming arsenic-containing well water, the average arsenic concentration in water, and the index for cumulative exposure to arsenic. Similar findings were obtained from a nested case-control study [28]. One cohort study evaluated the prevalence and multiple risk factors for arsenic-induced skin cancer among 1,081 interviewed subjects. The overall rate of skin cancer was as high as 6.1% and showed a dose-response relation with chronic arsenic exposure. Liver function and nutritional status may also affect the metabolism of iAs and subsequent development of skin cancers.

Skin cancer due to long-term arsenic exposure via drinking water has also been seen in Mainland China, including in Yunnan Province [29] and Inner Mongolia. In Guizhou Province, China, studies found that utilizing coal containing high arsenic for cooking and heating caused skin cancer [30].

In Bangladesh, arsenic in drinking water is a widespread concern. Arsenic contamination of groundwater was not reported until after at least a decade from 1993. In six districts of West Bengal [31], at least 800,000 people from 312 villages in 37 blocks were drinking contaminated water, and more than 175,000 people were shown to have arsenical-associated skin lesions, a clear later-stage manifestation of arsenic toxicity. A case-control study reported that arsenic ingestion caused these skin lesions [32].

A recent community-based prospective study including 378 skin cancer cases and 242 controls studied the link between arsenic-induced skin lesions and subsequent internal cancers [33]. It suggested that patients with hyperkeratosis who had been exposed to arsenic should cease smoking. Details of these studies on skin cancer and arsenic exposure are shown in the Table 6.2.

Table 6.2 Summary of epidemiological studies on arsenic and skin cancer

First author	Location	Exposure route	Subjects	Type of study	Reference
Tseng	Southwest Taiwan, China	Drinking water	40,421 inhabitants with 428 case	Ecological	[23]
Chen	Taiwan, China	Drinking water	69,216 residents with 162 cases	Ecological	[10]
Wu	42 villages, Taiwan, China	Drinking water	125 men with 19 cases and 91 women with 17 cases	Ecological	[11]
Chen	314 precincts/ townships, Taiwan, China	Drinking water	162,500 residents with 30 cases	Ecological	[16]
Guo	243 townships, Taiwan, China	Drinking water	11.4 million residents with 952 cases and 595 women	Ecological	[34]
Tsai	4 townships, Taiwan, China	Drinking water	65,592 residents with 66 men and 68 women	Ecological	[12]
Guo	243 townships, Taiwan, China	Drinking water	11.4 million residents with 1,415 men and 954 women	Ecological	[35]
Chen	4 townships, Taiwan, China	Drinking water	87 residents with 7 cases	Cohort	[10]
Hsueh	3 villages, Taiwan, China	Drinking water	654 subjects with 155 cases and 61 controls	Cohort	[27]
Hsueh	3 villages, Taiwan, China	Drinking water	295 cases and 4,745 controls	Nested case-control	[28]
Yu	Southwest Taiwan, China	Drinking water	26 cases and 26 controls	Case-control	[36]
Chen	Southwest Taiwan, China	Drinking water	76 cases and 224 controls	Case-control	[37]
Qiao	Yunnan, China	Occupation (Gejiu tin mine)	8,346 miners with 243 cases	Cohort	[6]
Tondel	Bangladesh	Drinking water	1,481 subjects with 430 cases	Cross-sectional	[24]
Hsu	4 townships, Taiwan, China	Drinking water	2,447 residents with 378 cases	Cohort	[33]
Milton	Bangladesh	Drinking water	44 cases and 125 controls	Case-control	[32]
Li	Yunnan, China	Drinking water	25 subjects with 8 cases	Cohort	[29]

6.1.1.3 Arsenic and Liver Cancer

Many studies have evaluated the relation between liver cancer and arsenic in drinking water [9–12, 16, 38]. A case-control study investigating liver cancer risks was conducted in four townships of southwestern Taiwan, China. There was a time-response relationship between the consumption of arsenic in well water and risk of liver cancer when adjusted for cigarette smoking, habitual alcohol use and tea drinking, as well as consumption of vegetables and fermented beans [15]. However, a relationship between arsenic exposure and an elevated risk of liver cancer was not found in the studies from South America. The diffent results suggest that varied lifestyle components impact this cancer site with arsenic between Taiwan and South America. Based on rodent evidence, if people have early-life exposure to arsenic, they will be more vulnerable to arsenic poisoning and have a higher risk to develop liver cancer [38, 39]. However, young children consume more water relative to their body weight, which may also contribute to their appearing higher susceptibility to arsenic toxicity. Researchers in Taiwan, China assessed dose-response relationships between arsenic exposure in drinking water or urinary arsenic metabolites and the mortality of cause-specific cancer. The SMRs for liver cancer were significantly higher in arseniasis-endemic areas [40]. A recent study showed that male mortality rates of liver cancer are clearly associated with arsenic exposure in Suzhou, China [19]. Details of these studies concerning arsenic and liver cancer are shown in Table 6.3.

Table 6.3 Summary of epidemiological studies on arsenic and liver cancer

First author	Location	Exposure route	Subjects	Type of study	Reference
Chen	Taiwan, China	Drinking water	789 subjects with 17 cases	Cohort	[10]
Tsuda	Niigata Prefecture, Japan	Drinking water	443 subjects with 2 cases	Cohort	[18]
Nakadaira	Niigata Prefecture, Japan	Industrially contaminated well water	86 subjects with 1 case	Cohort	[41]
Chen	Taiwan, China	Drinking water	65 cases and 368 controls	Case-control	[15]
Chen	Taiwan, China	Drinking water	Residents in survey area	Ecological	[16]
Tsai	Taiwan, China	Drinking water	20,068 subjects with 786 cases	Ecological	[12]
Chen	4 towns, Taiwan, China	Drinking water	120,607 subjects with 451 cases	Ecological	[9]
Wu	42 villages, Taiwan, China	Drinking water	174,945 subjects with 174 cases	Ecological	[11]
Chen	Suzhou, China	Drinking water	46,675 subjects with 10,076 cases	Ecological	[19]
Chen	Suzhou, China	Drinking water	46,675 subjects with 10,076 cases	Ecological	[39]

6.1.1.4 Arsenic and Urinary Bladder Cancer

In Taiwan, China, the relation between urinary bladder cancer and arsenic in drinking water was evaluated in various studies. A dose-response relationship between the arsenic level in well water and risk of developing bladder cancer among both men and women were reported. An elevation of mortality for bladder cancer during various times between 1971 and 1994 was also found in these studies [9–12, 16]. Another study in Taiwan, China found that there is a comparable risk of bladder cancer in arsenic-exposed population based on incidence records [38]. A case-control study from the blackfoot disease-endemic area in Taiwan, China found an increasing trend in odd ratios with the increased consumption of well water containing arsenic based on location of residence and certificates [15]. The highest risks of bladder cancer were seen for population exposed to high arsenic levels for over 40 years, with an odd ratio of 4.1 in a multivariate analysis after adjusting smoking and other factors. In another case-control study that included analysis of arsenic species in urine samples, a higher bladder cancer risk associated with arsenic exposure was found among persons with higher MMA^V to DMA^V ratios, or alternatively, with a higher percentage of MMA^V [42–46]. Cohort studies conducted in southwestern and northeastern Taiwan, China, as well as in Japan, found that bladder cancer risk following long-term exposure to arsenic in the drinking water showed a dose-response relationship with exposure levels [10, 14, 18, 47]. In a recent study from Taiwan, China, SMRs for bladder cancer were found significantly elevated in arsenic-endemic areas with Cox proportional hazard models [40]. Details of studies linking arsenic exposure and bladder cancer are shown in Table 6.4.

Table 6.4 Summary of epidemiological studies on arsenic and bladder cancer

First author	Location	Exposure route	Subjects	Type of study	Reference
Chen	4 towns, Taiwan, China	Drinking water	120,607 subjects with 332 cases	Ecological	[9]
Wu	4 towns, Taiwan, China	Drinking water	174,945 subjects with 181 cases	Ecological	[11]
Chen	Taiwan, China	Drinking water	Residents in survey area	Ecological	[16]
Chiang	Taiwan, China	Drinking water	2,135 subjects with 140 cases	Ecological	[38]
Guo	Taiwan, China	Drinking water	11,400,000 subjects with 1,962 cases	Ecological	[48]
Tsai	Taiwan, China	Drinking water	20,068 subjects with 500 cases	Ecological	[12]
Chen	Taiwan, China	Drinking water	789 subjects with 15 cases	Cohort	[10]
Chiou	Taiwan, China	Drinking water	2,293 subjects with 29 cases	Cohort	[14]
Chiou	4 towns, Taiwan, China	Drinking water	8,102 subjects with 10 cases	Cohort	[47]
Tsuda	Niigata Prefecture, Japan	Drinking water	443 subjects with 2 cases	Cohort	[18]

Table 6.5 Summary of epidemiological studies on arsenic and kidney cancer

First author	Location	Exposure route	Subjects	Type of study	Reference
Chen	84 villages, Taiwan, China	Drinking water	42 men and 62 women	Ecological	[9]
Chen	Taiwan, China	Drinking water	Residents in survey area	Ecological	[10]
Chen	Taiwan, China	Drinking water	457 Blackfoot disease patients with 3 cases	Ecological	[10]
Wu	42 villages, Taiwan, China	Drinking water	26 men and 33 women	Ecological	[11]
Chen	314 precincts/townships, Taiwan, China	Drinking water	Residents in survey area	Ecological	[16]
Guo	Taiwan, China	Drinking water	11.4 million residents with 726 cases	Ecological	[48]
Tsai	4 townships,Taiwan, China	Drinking water	94 men and 182 women	Ecological	[12]
Chen	3 towns, Suzhou, China	Soil arsenic	46,675 cancer cases with 351 kidney cancer cases	Ecological	[19]

6.1.1.5 Arsenic and Kidney Cancer

Ecological studies have shown that kidney cancer is highly correlated with consumption of arsenic in drinking water [48]. Chen et al. found that both SMR and cumulative mortality rate for kidney cancer were significantly higher in blackfoot disease-endemic areas compared with the general population in Taiwan, China [9–12]. Ecological studies and the cohort studies in Taiwan showed that the occurrence of kidney cancer was highly correlated with the level of arsenic in drinking water. In addition, there was a significant dose-response relationship between kidney cancer mortality and the level of arsenic in drinking water [10, 11, 16]. One study indicated that there was an increase in age-adjusted mortality for kidney cancer per 100,000 person-years for every 0.1 ppm increase in arsenic level of well water that was 1.1 in males and 1.7 in females [16]. The carcinogenicity of arsenic may be cell type specific. There is also evidence suggesting no relationship between high arsenic levels in drinking water and renal cell carcinomas or nephroblastomas [48]. Details of studies on arsenic exposure and kidney cancer are shown in Table 6.5.

6.1.1.6 Arsenic Exposure and Prostate Cancer

Most studies in the area of endemic blackfoot disease on the southwest coast of Taiwan, China analyzed prostate cancer mortality in relation to levels of arsenic in well water [9–12, 16]. All studies reported a significant dose-response relationship between the prostate cancer mortality and the level of arsenic in drinking water. One

Table 6.6 Summary of epidemiological studies on arsenic and prostate cancer

First author	Location	Exposure route	Subjects	Type of study	Reference
Chen	42 villages, Taiwan, China	Drinking water	69,216 residents with 81 cases	Ecological	[10]
Wu	42 villages, Taiwan, China	Drinking water	49 men of deaths with 9 cases	Ecological	[11]
Chen	42 villages, Taiwan, China	Drinking water	162,500 residents with 8 cases	Ecological	[16]
Tsai	4 townships, Taiwan, China	Drinking water	65,592 residents with 48 cases	Ecological	[12]

study showed that the mortality of prostate cancer declined gradually following water-source replacement and the withdrawal of arsenic exposure from artesian well water. Moreover, mortality of prostate cancer declined gradually after the improvement of drinking water supply system [49]. Details of studies of arsenic exposure and prostate cancer are shown in the Table 6.6.

6.1.2 Arsenic-Induced Cancer in Experimental Animals

In recent years, researchers explored the carcinogenic effects of arsenic in animal studies by using exposure to iAs or methylated arsenic metabolites combined with/without cancer promoters. Successful carcinogenic animal studies with arsenicals now include human relevant routes like oral exposure in drinking water, inhalation exposure, and exposures during highly sensitive life stages, such as early-life exposure.

6.1.2.1 Oral Exposure

Drinking water is the most common route of arsenic exposure in humans [22, 50]. There have been several studies investigating effects chronic oral exposure to arsenicals on cancer risks in rodents. These include oral exposure to iAs or DMA^V in mice or rats.

In strain A/J mice, exposure to DMA^V in drinking water for 50 weeks increased the incidence and multiplicity of lung adenoma or carcinoma [51]. The similar effects were found in mutant $Ogg^{-/-}$ mice [52]. In male F344 rats, oral administration of trimethylarsine oxide in drinking water for 2 years caused a significant increase of benign liver tumors [53]. In another study in rats, *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine in the drinking water combined with four levels (10, 25, 50, and 100 ppm) of DMA^V for 32 weeks resulted in an increase in urinary bladder hyperplasias, papillomas, and carcinomas, while the arsenical treatment alone had no effect [54]. An increased incidence of tumors of urinary bladder

was found in another study in rats, in which they were given an initial pretreatment with a mixture of organic carcinogens, no treatment for 2 weeks, and then DMA^V in the drinking water for 24 weeks [55]. Similarly, a study in Wistar rats found that renal tumors increased after exposure to drinking water containing 160 ppm of sodium arsenite combined with diethylnitrosamine [56].

6.1.2.2 Inhalation Exposure

There are no available studies for cancer induced by typical inorganic arsenicals (e.g., sodium arsenite or arsenate) in rodents via inhalation [50, 57, 58]. However, there is clear evidence for carcinogenicity of gallium arsenide after inhalation in rodents provided by the National Toxicology Program, USA. Inhalation of gallium-arsenide particulate for 2 years caused an increase in lung tumors and adrenal medullary pheochromocytomas in female rats, while lung tumors were not seen but dose-related increases in atypical hyperplasia of alveolar epithelium occurred with inhalation of gallium arsenide in male rats [59]. Another study in male and female Swiss mice, which were treated with sodium arsenate at multiple doses, provided no evidence of elevated tumor incidence [60].

6.1.2.3 Intratracheal Instillations

Repeated weekly intratracheal instillations of calcium arsenate in hamsters caused 15% early mortality and induced lung adenomas when assessed over their life span [61]. Another similar designed study found that intratracheal instillations of calcium arsenate induced an increase in lung adenoma, as well as formation of combined lung adenoma and carcinoma over the lifetime of hamsters [62]. However, in these intratracheal instillation studies, there was severe general toxicity, together with lung pathology indicative of heavy lung overload of the test compound making their relevance to a specific carcinogenic process for arsenic improbable. Any attempt to explore mechanisms of arsenic carcinogenesis using this technique of arsenic exposure would have serious and numerous drawbacks.

6.1.2.4 Early-Life Arsenic Exposure and Adulthood Cancer

Exposure to adverse factors in early life is closely related to disease susceptibility in later life [63]. Arsenic, when women are exposed to it during the maternal period, has the ability to cross the placental and the blood-milk barrier and enter offsprings in early life, thus affecting the susceptibility to diseases, such as cancer, in offsprings when they mature [64].

Many studies used inorganic arsenicals for transplacental exposure followed by some non-arsenical given after birth in the offspring to promote tumor formation in adulthood. Michael Waalkes' group has done several studies with this design. In one study, pregnant C3H mice were exposed to sodium arsenite in drinking water, with/without 12-O-tetradecanoylphorbol-13-acetate (TPA) applied to the skin of offsprings after weaning. Increased incidences of liver adenoma and/or carcinoma and adrenal cortical adenoma in male offsprings compared with arsenic exposure alone were found [65]. In a similar study, C3H mice were exposed to sodium arsenite (85 ppm) in early life. Adult male offsprings showed increased formation of hepatic tumors which often harbored activating Ha-ras mutation [66]. Another study of this group found that transplacental exposure to arsenic combined with a second exposure to diethylstilbestrol or tamoxifen in offsprings of CD1 mice caused an increased incidence of tumors in the ovary, uterus, and adrenal cortex [67, 68].

The biomethylated product of iAs, DMA^V, is known as a tumor promoter of various non-arsenical carcinogen. Pregnant CD1 mice were provided with drinking water containing iAs (85 ppm). After weaning, the male offsprings were given DMA^V (200 ppm) in drinking water for up to 2 years. The iAs-DMA^V combined group showed an increased incidence of renal cell carcinoma compared with iAs or DMA^V alone. iAs alone, DMA alone, and combined groups all showed increased formation of lung adenocarcinoma and adrenal adenoma compared to control mice [69].

In conclusion, abundant evidence from animal experiments indicates that early-life exposure to arsenic increases incidence and various human relevant cancers in adulthood of offspring, including in the liver, lung, skin, bladder, and kidney [70, 71], which are also carcinogenic targets of arsenic found in humans.

The mechanism underlying increased cancer susceptibility due to early-life arsenic exposure is not clear yet. Alteration in epigenetics (such as DNA methylation, noncoding RNA expression, histone modification, etc.) is considered to play an important role [63, 72]. Additionally, adulthood cancers induced by early-life exposure would require a long-lived target cell population that retains the capacity for self-renewal even after the initial lesion, such as a stem cell population. Stem cells may be very sensitive to transplacental carcinogenesis based on their abundance and high activity in early life. Thus dysregulation of stem cells is proposed as a key early event in arsenic carcinogenesis [60, 64].

6.1.3 Mechanisms of Arsenic-Mediated Carcinogenesis

The mechanisms by which arsenic causes cancer remain complex and unclear. However, several mechanisms have been proposed, including oxidative stress, changes of the epigenome, chromosomal aberrations, and micronuclei formation. We will mainly discuss these aforementioned ways to introduce an overview of the mechanisms by which arsenic causes cancer.

6.1.3.1 Oxidative Stress

Oxidative stress is considered as an important mechanism in arsenic toxicity and probably arsenic carcinogenesis. iAs and its metabolites generate excessive reactive oxygen species (ROS), which damage structural integrity of DNA. It has been reported that biomethylation of iAs can produce single- and double-stranded DNA breaks through the formation of ROS, which lead to mutations and instability of mitochondrial DNA (mtDNA) [73]. In addition, ROS interfere with the permeability of the mitochondrial membrane resulting in aberrant expression of apoptosis-related genes [74]. However, recent studies *in vitro* suggest that chronic exposure to an environmental relevant dose of arsenic induces an adaptive antioxidative response [75–77]. This response is indicated by elevated expression of antioxidants and reduced levels of ROS, which may be mediated by constitutive activation of the master transcription factor in antioxidant defense, nuclear factor E2-related factor 2 (NRF2).

6.1.3.2 Epigenetic Features in Arsenic-Induced Cancer

In addition to oxidative stress, epigenetic regulation, including DNA methylation, histone posttranslational modifications (PTMs), and noncoding RNAs, alters gene expression profiling, which makes genome more vulnerable and unstable toward cancer risk. After exposure to low doses of iAs, the expression of DNA methyltransferase (DNMT) is reduced, resulting in a reduction of methylation at the target sites [78]. Histone PTMs can change the chromatin structurally and functionally and thereby alter gene expression. It has been reported that arsenic exposure resulted in global changes in histone PTMs via increasing H3K4me3, H3K9me2, and H3S10, increasing H3 and H4 pan-acetylation levels, and decreasing H3K27me3 [79–84]. Additionally, several *in vitro* and epidemiological studies demonstrated that arsenic altered the expression of microRNAs, such as miR167, miR319, and miR854, another epigenetic mechanism of gene regulation that affects development, growth, and stress response [85].

6.1.3.3 Chromosomal Alterations and Micronuclei Formation

Chromosomal instability may also play roles in the carcinogenicity of arsenic via large-scale chromosomal aberrations. Arsenic-induced chromatin instability results in the formation of centromeric chromosomes or centromere fusion between two chromosomes [22]. On one hand, the fusion that occurs at the end of a chromosome may result in the formation of a loop structure or participate in the exchange of abnormal sister chromatids [86]; on the other hand, the fusion of two chromosomes in its centrosome may result in inappropriate chromosome segregation leading to aneuploidy or micronuclei formation [22, 87].

6.2 Arsenic Exposure and Diabetes

Type 2 diabetes (T2D) is a metabolic disease which is attributed to a combination of genetic, environmental, and lifestyle factors. Arsenic exposure is currently considered as one of the environmental risk factors for T2D. There is compelling evidence that oxidative stress plays an important role in the pathogenesis of T2D: insulin resistance and pancreatic β-cell dysfunction. In this section, we summarize the critical findings on the potential mechanisms of how arsenic exposure induces T2D. In light of the pathogenic role of oxidative stress in T2D and the potent effect of iAs exposure on redox homeostasis, we highlight the role of oxidative stress and persistent antioxidant response in the pathogenesis of β-cell dysfunction and insulin resistance.

6.2.1 Arsenic Exposure, Oxidative Stress, and T2D

Although a causal link between iAs exposure and T2D has not been unequivocally established [88, 89], accumulating epidemiologic evidence, from studies carried out in Taiwan [90–94], Bangladesh [95–98], Sweden [99, 100], Mexico [101–103], Korea [104], the USA [105–108], and Denmark [109], has shown a strong correlation between iAs exposure and diabetes in humans [110]. In 2012, a systematic review by an expert panel coordinated by the National Toxicological Program of the USA demonstrated that existing human data at the end of 2011 provided limited to sufficient evidence to support an association between arsenic exposure and diabetes in populations with relatively high exposure levels (≥150 μg arsenic/L in drinking water) [110]. More recently, two systematic reviews [111, 112] revealed that exposure to moderate to high levels of iAs increased the risk of T2D and the same conclusion in Asia [113]. Given that accumulating evidence implicates iAs exposure as one factor in the current diabetes epidemic, the exact mechanism of action of the diabetogenic effects of iAs has become a new and urgent research focus in this field.

Two major pathophysiologic abnormalities, specifically insulin resistance and defects in pancreatic β-cell function, underlie most cases of T2D [114–116]. Normal β-cells can compensate for insulin resistance by increasing insulin production and secretion and/or increasing β-cell mass [117]. Insufficient compensation ultimately leads to the onset of glucose intolerance and T2D [118]. Among the various mechanisms proposed for insulin resistance and β-cell dysfunction in progression to overt diabetes, oxidative stress is a common denominator [118–124]. Interestingly, accumulating data [125–128] including our own *in vitro* [75, 77, 129–132], *in vivo* [133], and human studies [134] demonstrate that oxidative stress is associated with iAs exposure. It is generally accepted that iAs, including inorganic arsenite (iAs^{3+}) and inorganic arsenate (iAs^{5+}), are metabolized in human by enzymatic and nonenzy-

matic mechanisms [135, 136] into trivalent monomethyl arsenic (MMA^{3+}), pentavalent monomethyl arsenic (MMA^{5+}), trivalent dimethyl arsenic (DMA^{3+}) and pentavalent dimethyl arsenic (DMA^{5+}). MMA (MMA^{3+} + MMA^{5+}) and DMA (DMA^{3+} + DMA^{5+}) are the major metabolites of iAs in human blood and urine [134]. Although the detailed mechanisms of chronic arsenic poisoning (arseniasis) are still incompletely understood, it is generally considered that trivalent arsenicals, including inorganic and methylated forms, are potent oxidative stressors [75, 128, 133, 134, 137–140]. Acute and prolonged exposure to the arsenicals activates cellular antioxidant defense systems, including NRF2-mediated antioxidant response, in various cell types [75, 77, 141, 142]. Although the exact mechanisms for the diabetogenic effects of arsenic are still largely undefined, accumulating data [125–128] including our studies from cultured cells, animals, and humans [75, 129, 133, 134] indicate arsenic exposure will increase oxidative stress. Thus, oxidative stress is likely a key mechanism that links arsenic exposure to T2D.

6.2.2 Arsenic-Induced Pancreatic β-Cell Dysfunction

Evidence in β-cell lines, isolated pancreatic islets or rodents strongly suggests that exposure to arsenic or its metabolites can impair insulin synthesis or production and reduce insulin secretion in a concentration/dose-dependent manner [142–148]. These effects of arsenic are associated with oxidative stress and are followed by a typical antioxidant response [142–148]. High levels of iAs and its methylated metabolites are linked to severe oxidative stress, which can result in irreversible damage followed by necrosis or apoptosis [148–154]. However, low-level environmentally relevant arsenic exposure is potentially more pertinent to β-cell dysfunction, not direct β-cell damage, in which antioxidant response is involved [142, 145]. The dosage in the medium can have a mixed transitional effect between β-cell damage and glucose-stimulated insulin secretion (GSIS) dysfunction [144, 148].

6.2.2.1 Arsenic Induces β-Cell Death and Impairs Insulin Synthesis

Several *in vivo* studies, including prenatal arsenic exposure [155], show that arsenic exposure induces significant oxidative stress [151, 153, 156] and structural changes in pancreatic islets and surrounding regions [148, 157, 158] by directly binding to thiol or -SS- group [149] and inhibiting the activity of sensitive antioxidant enzymes [153] or mitochondrial enzymes [157]. This damage was largely reversed by supplementation with *N*-acetylcysteine (NAC) [152], folic acid or a combination of folic acid and vitamin B12 [151, 156]. In addition to inhibiting antioxidant enzymes, arsenic may disrupt demethylation of 5-methylcytosine (5mC) and/or 5-hydroxymethylcytosine (5hmC), an epigenetic modification, in the pancreas of rats subchronically exposed to the metalloid [159].

Consistent with *in vivo* investigations, arsenic is cytotoxic in pancreatic β-cell lines (e.g., MIN6, HIT-T15, INS-1, RIN-m5F) and isolated islets systems, as determined by cell viability or apoptosis measurement [132, 144, 148, 152, 154]. High concentrations of sodium arsenite decrease insulin transcription [144, 149], probably due to β-cell damage. Oxidative stress is consistently reported as the most important mechanism for arsenic-induced β-cell damage. ROS-dependent autophagy [154] and ROS-dependent ER stress [152] were often elucidated as an underlying mechanism. Furthermore, in a series of *in vitro* studies, isolated *Nrf2*-KO mouse islets and *Nrf2*-deficient MIN6 cells were more susceptible to iAs^{3+}- and MMA^{3+}-induced cell damage [132]. In contrast, pretreatment of cells with NRF2 activators, such as t-butylhydroquinone, protected the cells from arsenic- and direct oxidative stress-induced cell damage, indicating that the adaptive antioxidant response mediated by NRF2 protected β-cells against the high levels of arsenic-induced oxidative damage [132, 160].

6.2.2.2 Effects of Arsenic on Basal Insulin Release and GSIS in β-Cells

In vivo and *in vitro* studies demonstrate that low levels of arsenic impairs insulin secretion, and the underlying mechanisms have been variously attributed to impaired estrogen signaling [161], disrupted calcium-calpain pathway [143] or persistent activation of NRF2-mediated antioxidant response [142, 145]. Nontoxic or subtoxic concentrations of trivalent arsenicals inhibit GSIS in rat pancreatic β-cells [144, 145] and isolated islets [146]. However, the same exposures have little or no effect on insulin expression and insulin content, as well as the positive KCl-triggered insulin release, which suggests that arsenicals impairs GSIS by regulating glucose metabolism, and the translocation or exocytosis of insulin vesicles. Further studies find that the disturbance of calcium-calpain pathway impairs insulin exocytosis in RINm5F cells, by inhibiting the oscillations of free $[Ca^{2+}]$ and reducing calcium-dependent calpain-10 (CAPN-10) activity [143]. Calpains are calcium-dependent cysteine proteases, and calpain-10 is one of the calpains involved in the secretion and action of insulin. A subsequent population study shows that arsenic exposure and *CAPN-10* gene SNPs (SNP-43, SNP-44) both contribute to the outcome of β-cell dysfunction and T2D [98]. In addition to trivalent arsenicals, the relatively nontoxic arsenate (5 mM) inhibited mitochondrial activity and ATP production, thereby reducing the metabolic and secretory response to glucose in pancreatic islets [162].

6.2.3 Arsenic-Induced Insulin Resistance

It is well accepted that insulin resistance plays an important early pathogenic role in the development of T2D, and defects in insulin secretion and production by pancreatic β-cells are instrumental in the progression to overt hyperglycemia [116, 117].

Insulin resistance is a state in which the response to the hormone insulin is perturbed [163]. Insulin resistance has an impact at the cellular, organ or organismal level. Although insulin resistance may be initiated in white adipose tissues (WAT) [164], other tissues, including the skeletal muscle, liver and brain, are also critical in the pathogenesis of insulin resistance and T2D [150, 165–171]. Therefore, iAs-induced insulin resistance in mice could be derived from nonfat tissues. Nonfat tissues may play more important roles than WAT in the development of insulin resistance in the mice that are exposed to iAs.

6.2.3.1 Arsenic Induces Insulin Resistance in the Liver

iAs exposure impairs glucose homeostasis and hepatic metabolism of glycogen by inhibition of insulin signaling. After 4 h of exposure to iAs^{3+} or MMA^{3+} at non-toxic concentration, decreased glycogen content is observed in insulin-stimulated hepatocytes through inhibition of insulin-dependent activation of glycogen synthase (GS) and by induction of glycogen phosphorylase (GP) activity [172]. Further investigation reveal that both iAs^{3+} and MMA^{3+} inhibit insulin-dependent phosphorylation of protein kinase B/Akt, one of the mechanisms involved in the regulation of GS and GP by insulin. Another study shows that iAs^{3+} exposure produces a decrease in the intermediates of glycolysis and the TCA cycle while increasing ketones. Developmental iAs^{3+} exposure increases the expression of genes involved in fatty acid synthesis, lipogenesis, inflammation, and packaging of triglycerides [173].

In addition, arsenic-treated ovariectomized mice have higher blood glucose and insulin levels and increased glucose intolerance, insulin intolerance, and insulin resistance compared with arsenic-treated sham-operated mice [161]. Furthermore, liver phosphoenolpyruvate carboxykinase (PEPCK) mRNA expression is increased, and liver glycogen content is decreased in arsenic-treated ovariectomized mice compared with arsenic-treated sham mice [161].

6.2.3.2 Arsenic-Induced Insulin Resistance in the Skeletal Muscle

There are indications that arsenite may impair muscle function and myogenic differentiation [142–145, 174, 175]. In an *in vivo* study, mice received iAs in the drinking water (100 μg/L, 5 weeks) and suffered from increased perivascular ectopic fat deposition in skeletal muscle due to increased lipolysis and decreased lipid storage in adipose tissues through specific adipocyte G protein-coupled receptors (GPCRs) [176]. A *in vitro* study found that acute exposure to a toxic concentration of iAs (0.5 mM sodium arsenite, 30 min) impaired glucose uptake, insulin responsiveness, and mitochondrial function in L6 skeletal muscle cells, as determined by impairments of pyruvate kinase, glucokinase, ATP/ADP ratio, mitochondrial membrane potential, etc. [177]. These effects may be due to direct oxidative stress induced by this high concentration of arsenite.

Consistent with *in vivo* chronic study and environmental exposure, low concentrations (e.g., 20 nM) of arsenite reduced the expression of the transcription factors myogenin and Mef2c by changing the methylation patterns of the respective promoters, which resulted in the delay of myoblast differentiation in C2C12 cells [178]. Another study showed that 20 nM arsenite increased H3K9me2 and H3K9me3 near the transcription start site of myogenin and reduced H3K9 acetylation (H3K9Ac) [179]. This study also showed that the repression of myogenin expression in arsenic-exposed C2C12 cells was due to reduced expression of Igf-1 and enhanced nuclear accumulation and promoter recruitment of Ezh2 [179]. A sub-micromolar concentration (0.1–0.5 μM) arsenic trioxide (As_2O_3) markedly inhibited myogenic differentiation in an arsenic concentration-dependent fashion by inhibiting phosphorylation of Akt and p70s6k proteins [180]. Chronic arsenic exposure also reduced expression of insulin-regulated glucose transporter type 4 (Glut4) and decreased insulin-stimulated glucose uptake (ISGU) in mouse myotubes [181]. Consistent with this finding, arsenic exposure dramatically decreased the expression of Sirt3, a mitochondrial deacetylase, and its associated transcription factor, forkhead box O3 (FOXO3a). The reduction of FOXO3a activity caused by arsenic exhibited as decreased binding affinity to the promoters of its targets manganese superoxide dismutase (MnSOD) and peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1α, both of which are broad and powerful regulators of ROS regulation [181]. Another trivalent arsenical phenylarsine oxide (PAO) was shown to inhibit insulin-stimulated glucose transport in the skeletal muscle through interaction of vicinal sulfhydryls [182].

6.2.3.3 Arsenic Impairs Insulin Sensitivity in White Adipose Tissues

Various arsenicals, including iAs^{3+}, iAs^{5+}, MMA^{3+}, and DMA^{3+} and As_2O_3, are potent inhibitors of adipogenesis in the mouse pre-adipose embryo fibroblast (3T3-L1 cells) and human adipose-derived mesenchymal stem cells (ADSCs), probably by repressing the expression of Cebpα, Pparγ, and other genes which govern adipocyte differentiation [183]. In 3T3-L1 cells exposed to iAs^{3+}, iAs^{5+} or MMA^{3+}, CHOP10 was induced in expression and formed heterodimer with C/EBPβ. This heterodimer plays critical roles in the early stages of adipocyte differentiation, which delays the initiation of adipogenesis and finally impairs terminal differentiation [183]. As_2O_3 treatment suppresses the interaction between PPARγ and its ligand RXRα in 3T3-L1 preadipocytes [184]. This interaction normally drives the expression of a number of genes regulating differentiation of preadipocytes and maintaining the adipogenic phenotype. Endothelin-1 Gi protein-coupled receptor signaling is also activated by iAs^{3+} to inhibit differentiation of human mesenchymal stem cells into adipocytes [185].

Insulin-stimulated glucose uptake (ISGU) is inhibited by iAs^{3+} and MMA^{3+} in 3T3-L1 adipocytes through suppressing the insulin-dependent phosphorylation of protein kinase B (PKB/Akt) [170]. Further studies show that activity of PDK-1 catalyzing phosphorylation of PKB/Akt (Thr308), as well as a putative PKD-2

catalyzing phosphorylation PKB/Akt (Ser473), is inhibited by both iAs^{3+} (50 μM, 4 h) and MMA^{3+} (2 μM, 4 h) [171]. Prolonged iAs^{3+} exposure (up to 2 μM, 7 days) suppresses insulin-stimulated AKT S473 phosphorylation and glucose uptake in 3T3-L1 adipocytes. It has also been reported that arsenic induces insulin resistance in mouse adipocytes via oxidative stress-regulated mitochondrial Sirt3-FOXO3a signaling pathway [181].

6.2.4 Future Perspectives

Although emerging data suggest that early-life exposure to arsenic can result in long-term health consequences [39, 63, 186–188], the most sensitive exposure period, i.e., from conception through adulthood, the diabetogenic effect of arsenic is still unclear. Considering that millions of people might have been exposed to iAs in utero and in their early postnatal life, it is critically important to understand the effect of these exposures to iAs on human health, particularly the incidence of T2D. In contrast to the extensive epidemiological studies performed in various human populations with different levels of iAs exposure, there are limited mechanistic animal studies published on diabetes and on different arsenic levels. In particular, most of the animal studies were not designed specifically to study the diabetogenic effect of arsenic [110, 189]. Although these studies suggest pathways by which arsenic influences pancreatic β-cell function and insulin sensitivity, the findings in most cases are difficult to interpret, likely due to variations in experimental design, duration of exposure, routes of administration, arsenic species and doses, and animal models and strains. Thus, detailed mechanistic studies are required to systematically elucidate the diabetogenic mechanisms of iAs exposure. More systematic animal studies are required to correct the gap between human-based epidemiological investigations and *in vitro* mechanistic toxicity studies. Such studies will provide important novel insights into iAs-induced insulin resistance and β-cell dysfunction and advance innovative preventive and therapeutic concepts for iAs or other oxidative stressor-induced T2D.

6.3 Arsenic and Cardiovascular Disease

The incidence of cardiovascular disease (CVD) in general is rising. CVD is currently one of the leading causes of mortality worldwide [190]. Thus, the relationship between arsenic exposure and CVD has received intense attention in populations exposed to elevated arsenic in drinking water. The exposure levels of arsenic vary widely in different areas and vary with different endpoints within CVD, including overall CVD, coronary heart disease (CHD), peripheral arterial disease (PAD), and stroke [191]. In this section, we summarize the current epidemiology data concerning the association between chronic exposure to arsenic across populations exposed to low, moderate, and high levels of arsenic in drinking water and the risk of CVD.

6.3.1 *Epidemiology Studies on Arsenic and Cardiovascular Disease*

There are 40 studies on CVD outcomes in arsenic-exposed subjects. Of these studies, 27 were cross-sectional studies, ten were prospective cohort studies, and three were retrospective cohort studies. Eight of the studies were conducted in Taiwan, China, eight in Mainland, China, eight in the USA, ten in Bangladesh, one in Argentina, one in Mexico, and one in Turkey. Study outcomes included fetal CVD and CHD, atherosclerosis, hypertension, and PAD. Several epidemiology studies have reported that high arsenic levels in drinking water (>100 μg/L) increased the risk of CVD in several countries. In recent years, positive associations between arsenic exposure at low levels (<100 μg/L) and an increased risk for overall and subtypes of CVD in the USA, China, and Italy have also been found. We divided the studies above into two parts, low-moderate arsenic exposure and high arsenic exposure.

6.3.1.1 Arsenic and Fetal CVD

Some CVDs can be serious and even fatal. Thus, the mortality associated with CVD is a critical epidemiological data metric. There were ten studies focusing on the relationship of arsenic and CVD mortality, including seven prospective cohort studies, two retrospective cohort studies, and one cross-sectional study. Two of them were prospective cohort in a low arsenic-exposed area in the USA, which found that arsenic exposure was associated with increased CVD mortality [192–194]. The other prospective cohort study was conducted in Bangladesh. It is found that low levels of arsenic exposure were associated with increased risk of stroke mortality [195]. Several studies have also demonstrated an association between exposure to high levels of arsenic and CVD mortality, including studies conducted in Taiwan, China and Bangladesh [196–198]. However, a prospective cohort in the northeastern Taiwan reported there was no significant association between arsenic exposure and CVD mortality [199].

6.3.1.2 Arsenic, Cardiac Atherosclerosis, and Coronary Heart Disease

Chronic exposure to arsenic leads to increased cardiovascular lesions, such as carotid atherosclerosis, coronary heart disease (CHD), and a broad spectrum of heart diseases. One cross-sectional study in Taiwan, China reported the association between high levels of arsenic exposure and the incidence of cardiac atherosclerosis [200]. Two cross-sectional studies from Taiwan, China also reported that low arsenic exposure (10–50 μg/L) had a significant association with increased risk of cardiac atherosclerosis [201, 202]. In addition, CHD outcomes were assessed by the carotid artery intimal medial thickness (IMT) and QT prolongation—a risk factor for arrhythmia and sudden cardiac death. Two prospective cohort studies from Bangladesh, one cross-sectional study from Bangladesh, and one cross-sectional

study from Turkey reported an association between high levels of arsenic exposure and CHD lesions [203–206]. Based on the available epidemiologic evidence, the results remain inconsistent. There were significant associations between exposure to high levels of arsenic and CHD except in one prospective cohort study in Taiwan, China [200]. Three cross-sectional studies and one prospective cohort study in Mainland, China and the USA found that exposure to low levels of arsenic had an association with CHD [207–209]. However, there was one cross-sectional study with a negative result [210].

6.3.1.3 Arsenic and Hypertension

Hypertension is a high-risk factor for other cardiovascular diseases. Four cross-sectional studies from China, Iran, and Bangladesh were conducted to determine the association between exposure to high levels of arsenic and hypertension [148, 211–213]. All showed an association between arsenic exposure and hypertension except the study from Bangladesh [148]. Additionally, eight cross-sectional studies and one prospective cohort study from China, the USA, Iran, Bangladesh, and Argentina were conducted to detect a possible relationship between arsenic exposure at low levels and hypertension [203, 214–219]. All the studies indicated that low levels of arsenic exposure had an association with hypertension, with the single exception of one cross-sectional study from the USA which was negative.

6.3.1.4 Arsenic and Peripheral Artery Disease

Blackfoot disease is a unique vascular disease first reported from the southwestern area of Taiwan, China with endemic high arsenic exposure in well water. It is an end-stage peripheral artery disease (PAD) endemic in a limited area [220]. Three cross-sectional studies from Taiwan (China), Bangladesh, and the USA reported the association between arsenic exposure and PAD [221–223].

6.3.2 Arsenic and CVD in Animal Studies: Evidence and Mechanisms

Recently, animal models for arsenic-induced atherosclerosis, hypertension, vascular dysfunction, and cardiac disease have been developed. Initial studies showed that sodium arsenite exposure accelerated and exacerbated atherosclerosis in apolipoprotein E-knockout mice (ApoE) [224–230]. In a rat model, blood pressure was increased after long-term exposure to arsenic, and the relaxation of the vasculature was significantly declined as an impact of arsenic exposure [231–236]. Arsenic also impaired endothelial functions, such as angiogenesis [237], inflammation response [238], and the integrity of the endothelium [220]. Cardiac disease was correlated

with arsenic exposure in such models as well [239–241]. It can be concluded that the mechanism of arsenic-induced CVD is made up of four parts, oxidative stress, persistent inflammatory response, cytotoxicity, and direct effects on endothelial function.

6.3.2.1 Oxidative Stress

A number of risk factors for CVD are associated with increased vascular ROS generation when they exceed endogenous antioxidant capacity [242]. One of the major mechanisms is that arsenic induces ROS formation in most cell types. In the vascular system, and primarily in endothelial cells (ECs), exposure to arsenic results in overproduction of ROS [243], which modulate signaling not only in endothelial cells but also in vascular smooth muscle cells (VSMCs) and macrophages. ECs produce ROS via activation of various pathways, such as several NADPH oxidases (NOXs), endothelial nitric oxide synthase (NOS), the electron transport chain of mitochondria, the cytochrome P450 epoxygenases, xanthine oxidase, etc. [244]. In experimental studies, atherosclerosis has been frequently correlated with the ROS production induced by arsenic.

Animal studies indicate that oxidized lipids are present in all stages of atherosclerosis. Arsenic-exposed mice usually showed elevated lipid levels in the serum [227, 229, 230]. Early postnatal arsenic exposure increased atherosclerotic lesion by three to five fold in the aortic valve and the aorta, without altering plasma cholesterol [225]. Similar results were observed in adult mice exposed to iAs [225]. Another study showed that arsenic induced dyslipidemia in mice, including increased levels of cholesterol and triglycerides in the serum [226]. Malondialdehyde (MDA) and hydroxynonenal (HNE) are the most abundant saturated and unsaturated aldehydes generated from the oxidation of LDL. In the serum of ApoE mice, the markers of oxidative stress and lipid peroxide such as protein HNE, DHE, and MDA are markedly increased in lesions of arsenic-exposed mice [225, 228, 229], indicating that ROS play an important role in atherosclerosis progression resulting from arsenic exposure. Increased levels of MDA and HNE have been showed in both early and advanced lesions. Since lipid aldehydes are highly reactive and can increase monocyte adhesion, cytokine production, and lipid uptake by scavenger receptors, it is conceivable that excessive generation of these aldehydes or decreased detoxification after arsenic exposure exacerbates atherosclerotic lesion formation. NOX4 is the most abundant NOXs in endothelia. The possibility that arsenite induces EC oxidative stress via a mechanism involving a NOX-based oxidase was first alluded in studies of cultured porcine aortic ECs [245]. In arsenic-exposed rats, blood pressure was increased together with NOX4 expression in aorta [231]. The endothelium-dependent relaxation of rabbit iliac artery and aortic rings due to short-term arsenite exposure has been detected. It was found that the activity of NOXs but not NO was impacted by arsenite. Thus, simulated expression of endothelial NOX4 and formation of H_2O_2 from O^{2-} are likely to contribute to hypertension induced by long-term arsenite exposure.

Contraction and relaxation is the basic function for the vasculature. eNOS in endothelial cells is one of the most important genes regulating this function. In a study by Pi et al., it was found that both arsenite and arsenate exposures impaired eNOS function and decreased the NO generation in the vascular system. Long-term exposure to arsenic impaired vascular relaxation by regulating NO synthesis. In hypertensive rats, the expression of eNOS was decreased, and cGMP, which is related to NO synthesis, was also decreased [231, 235]. A similar pattern was seen in *in vitro* experiment, which observed that arsenite induced dysregulated mitochondrial respiration and ROS formation. All above studies indicated that arsenite, as an inducer of ROS, plays an important role in CVD.

6.3.2.2 Inflammation Response

A multitude of studies provide strong evidence that development of atherosclerosis is related to the inflammatory response. Endothelial cells typically express ICAM-1 and VCAM-1 on the membrane. ICAM-1 is a transmembrane protein and can bind to leukocytes. VCAM-1 mediates a similar function for adhesion of lymphocytes, monocytes, and eosinophils to the endothelium. Ninety days of continuous sodium arsenite in drinking water induced expression of VCAM-1 and ICAM-1 in rats [238]. Meanwhile, the pro-inflammatory cytokines, such as IL-6, IL-1β, and MCP-1 were increased [238]. These data suggest that arsenite induces endothelial dysfunction with inflammatory response, which contributes to the development of vascular disease.

Animal experiments have confirmed that arsenic exposure increases inflammatory response in ApoE mice. IL-6 is an interleukin that acts as both a pro-inflammatory cytokine and an anti-inflammatory cytokine. The expression of IL6 indicated by aortic valve of immunohistochemistry was increased after 24 weeks of exposure to sodium arsenite in ApoE mice. Similar alteration was seen with MCP-1 levels. MCP-1, also called CCL2, is a small cytokine that is able to recruit monocytes. When tissue injury occurs, CCL2 can be synthesized and released to recruit memory T cells and dendritic cells to the sites of inflammation. MCP-1 and IL-6 levels were increased in the serum of sodium arsenite-exposed ApoE mice [225]. Prostacyclin (PGI_2) is a prostaglandin member of the eicosanoid family of lipid molecules. It inhibits platelet activation and is an effective vasodilator. In a study conducted by Bunderson et al., the serum PGI_2 level was elevated along with increases in leukotriene E4 (LTE4), a reflection that the inflammatory response is more serious after arsenic exposure [224].

Results from *in vitro* assays are consistent with the *in vivo* studies. The mRNA levels of cytokines, such as TNF-α, IL-1β, and IL-6, were increased in a dose-response pattern in bone marrow-derived macrophages after being incubated with sodium arsenite for 6 h [225]. In many other cell lines, including HAECs, HUVEC, and PAECs, increased inflammatory responses were observed in response to arsenite exposure [227, 228, 246].

6.3.2.3 Cytotoxicity

There are many studies indicating that iAs exposure can be cytotoxic to cells, and typically there is a dose-response relationship with iAs exposure and cell viability [247].

6.3.2.4 Other Aspects

Arsenicals impair many functions of endothelial cells. Angiogenesis is one of the most important functions for vascular integrity and influences the transport of material and the union of endothelial cells. Dysfunction of angiogenesis is related to many disease states. For instance, when C57BL/6 mice were exposed to 50, 250, and 500 ppb sodium arsenite in drinking water, the blood vessel numbers and expression of genes related with angiogenesis were increased [237]. However, in an *in vitro* tube formation experiment, angiogenesis was increased in response to 5 μM of arsenic but decreased in response to 10 μM of arsenic. Long-term iAs exposure may impact a different pathological process within angiogenesis compared with acute treatment. As a nature container of blood, the endothelium is required to have a good integrity. As a vasoactive material, arsenic induces microvascular dysfunction with increased vascular leakage. When ICR mice were given iAs by gavage, they showed significantly increased PP2A activity and vascular leakage [220].

In conclusion, arsenic has toxic effects on the vascular system at the cell level, and cytotoxicity is a basic effect of arsenic in living organisms [248]. As an oxidizing agent, arsenic induces oxidative stress in many cell types such as ECs, VSMCs, and cardiac muscle cells. This is also the common initial stage in CVD (e.g., hypertension, cardiac disease, stroke, and atherosclerosis). The oxidative damage and cytotoxicity can cause endothelial dysfunction and cell death, all of which induce an inflammatory response [249]. Endothelial dysfunction is a systemic pathological state of the endothelium. It is thought to be a key event in the development of atherosclerosis. Inflammation is a part of complex biological response of the body and specific tissues to harmful stimuli. The function of inflammation is to eliminate the initial cause of cell injury. In the progress of atherosclerosis, the ensuring inflammation leads to formation of atheromatous plaques in the arterial tunica intima.

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Chapter 7
Metabolism and Toxicity of Organic Arsenic Compounds in Marine Organisms

Yang Cao, Ayako Takata, Toshiaki Hitomi, and Hiroshi Yamauchi

Abstract The ingestion of finfish has been recommended as a preventive measure for lifestyle-related diseases, and marine algae are gaining attention as a source of dietary fiber and essential nutrients, including minerals. However, given the recognition of a potential for high arsenic levels in marine organisms, these dietary recommendations may have neglected the necessity of verifying the absence of health risk from the ingestion of arsenic in marine-derived foods. Under such circumstances, it is clear that the toxicological effects of both arsenosugars and arsenolipids, common in marine-derived materials, are important among organic arsenic (As) compounds. This includes the recent identification of thio-dimethylarsinic acid (thio-DMA) as an arsenosugar metabolite and the demonstration that it is more cytotoxic than even inorganic arsenic (III) which is considered highly toxic. Moreover, multiple studies have found arsenic-containing hydrocarbons (AsHCs), a group of arsenolipids produced by marine organisms, are strong neurotoxins. Similar to thio-DMA, AsHCs are equally or more toxic than inorganic arsenic. Thus, future efforts need to elucidate the biological and toxic effects of organic As compounds by evaluating next-generation effects and brain dysfunction caused by genotoxicity. Although arsenobetaine (AB) is the organic As compound with the highest probability of ingestion, the conclusion that AB is a nontoxic arsenical seems probable.

Keywords Organic arsenic compounds · Arsenobetaine · Arsenosugars · Arsenolipids · Health effects · Toxicity

Y. Cao (✉) · A. Takata · T. Hitomi · H. Yamauchi
Department of Preventive Medicine, St. Marianna University School of Medicine, Kawasaki, Japan
e-mail: soyocao@marianna-u.ac.jp

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7.1 Viewpoints of Biological Effects of Organic Arsenic Compounds

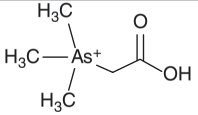
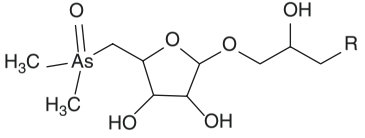
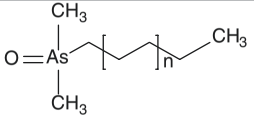
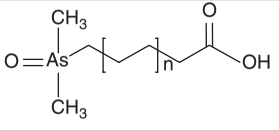
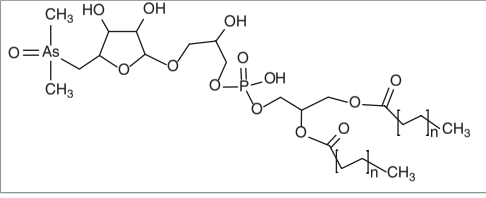
Chronic arsenic (As) poisoning caused by inorganic arsenic (iAs) compounds most frequently occurs because of the ingestion of contamination of well water and/or foods and occasionally due to occupational exposure. iAs is also considered an IARC Group 1 human carcinogen [1]. In terms of iAs exposure mitigation, the international community has made efforts regarding the safety management of the labor environment and for safe drinking water, considering the risk of toxicity and carcinogenicity of As. In contrast, international community may have neglected the necessity of verifying the risk of the ingestion of As derived from marine-derived foods on human health, despite the clear data that indicates there is often high As levels in marine organisms. Lunde reported initially that marine organisms contained high concentrations of water-soluble or fat-soluble organic arsenic (As) compounds contained and not iAs [2]. Subsequently, technologies for the isolation and purification of organic As compounds from marine organisms and the sensitivity and accuracy of As analysis dramatically improved. In 1977, Edmonds et al. identified arsenobetaine (AB) in rock lobster [3], and in 1981, they isolated arsenosugars from marine algae [4].

Recently, the ingestion of omega-3 fatty acid that is abundantly contained in finfish has been recommended as a preventive measure for various lifestyle-related diseases [5]. Moreover, marine algae are gaining attention as a source of dietary fiber and essential nutrients, including minerals. In fact, people in the East Asian countries customarily eat marine algae as a part of their dietary culture although this has not been the case in the West until recently. Opportunities to ingest marine algae in Western societies that were originally uncommon as this was a not traditionally utilized food materials have recently increased since sushi and other Japanese foods have gained popularity as healthy foods in terms of nutrition. However, the potential toxicological effects of organic As compounds in marine-derived foods are largely unknown and, relative to iAs, have only recently started to be studied. In this chapter, we attempt to provide a synopsis of the current knowledge of marine-derived organoarsenicals. We will introduce chemical structures of organic As compounds detected from both marine organisms and marine-derived foods available in the market, provide the latest information regarding their metabolism and toxicity, and provide potential biological effects of organic As compounds on humans.

7.2 Organic As Compounds Derived from Marine Organisms and Marine-Derived Foods

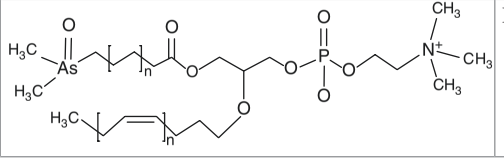
iAs contained in seawater is thought to be converted to organic As compounds via the marine ecosystem food chain [6]. Marine organisms contain water- or fat-soluble organic As compounds. Known water-soluble organic As compounds include AB, arsenosugars, and methylated As compounds. Known fat-soluble organic As compounds include arsenolipids. The frequency of detection and concentrations of these AB, arsenolipids, and arsenosugars in marine organisms are high (Table 7.1),

Table 7.1 Chemical structures of organic As compounds in marine organisms

No.	As compounds	Chemical structures[a]	Samples and As concentration		References
1	AB		Crustaceans (muscle) Finfish Shellfish (Bivalves)[b] Zooplankton	26 ppm[c] 13.6 μg/g wet wt.[c] 0.33–1.03 μg/g fresh tissue 0.21–17 mg/kg dry wt.	Edmonds et al. [3] Freeman et al. [7] Shibata et al. [17] Shibata et al. [22]
2	Arsenosugars[d]		Kombu Nori Shellfish (Bivalves)[b] Phytoplankton Zooplankton	10 mg/kg wet wt.[c] 8.7–21 mg/kg dry sample 0.2–1.63 μg/g fresh tissue Low concentration 0.16–2 mg/kg dry wt.	Edmonds and Francesconi [4, 26] Shibata et al. [31] Shibata and Morita [17] Shibata et al. [22] Shibata et al. [22]
3	Arsenolipids ①AsHCs		Fish oil (Capelin) Finfish (SASHIMI tuna) Cod liver Wakame Kombu	11.7 μg/g[c] (70% is AsHCs) 5.9 μg/g dry mass[c] (20% is AsHCs) 1.53 ± 0.02 mg/kg wet wt. 510 μg/kg (mean) 44.9–55.0 μg/g dry mass (mean)[c]	Taleshi et al. [33] Taleshi et al. [16] Arroyo-Abad et al. [34] Garcia-Salgado et al. [35] Yu et al. [36]
	②AsFAs		Cod-liver oil (crude) Wakame Cod-liver	5 μg/g[c] (20% is AsFAs) 5.7 ± 0.3 mg/kg dry mass[c] 1.53 ± 0.02 mg/kg wet wt.[c]	Rumpler et al. [37] Lischka et al. [38] Arroyo-Abad et al. [34]
	③AsPLs		Wakame Wakame Kombu	0.7 ppm[c] 1023 μg/kg (mean) 44.9–55.0 μg/g dry mass (mean)[c]	Morita and Shibata [39] Garcia-Salgado et al. [35] Yu et al. [36]

(continued)

Table 7.1 (continued)

No.	As compounds	Chemical structures[a]	Samples and As concentration		References
	④AsPCs		Herring caviar	0.8 μg/g dry mass[c]	Viczek et al. [40]

[a]Chemical structures of arsenolipids ($[\cdot]_n$) are reported by Taylor et al. [32]
[b]For the whole tissues of bivalves only
[c]Values refer to the total As concentration
[d]There are over 20 arsenosugars reported as natural products, of which four are the most detected compounds in marine organism which are as follows: arsenosugar-Gly (R=OH), arsenosugar-PO_4 (R=$OPO_3CH_2CHOHCH_2OH$), arsenosugar-SO_3 (R=SO_3), and arsenosugar-SO_4 (R=OSO_3)

whereas those of methylated As compounds, such as arsenocholine (AC; $(CH_3)_3As^+CH_2CH_2OH$), trimethylarsine oxide (TMAO; $(CH_3)_3AsO$), and tetramethylarsonium (TETRA; $(CH_3)_4As^+$), are generally low. However, the mechanisms of production, distribution, and other factors, like toxicity, associated with these organic As compounds remain relatively unexplored.

7.2.1 Arsenobetaine

The chemical structure of AB was first determined in the rock lobster [3] and has subsequently been identified in various marine-derived foods, including finfish [7–16], shellfish [10, 14, 15, 17, 18], and several crustaceans [10, 14, 19–21]. AB has also been detected in zooplankton [22], which are prey for finfish and shellfish.

Although the mechanism by which AB is produced has not yet been clearly elucidated, several inferences can be made. A potential production scheme is that phytoplankton convert iAs in seawater to arsenosugars in vivo, and zooplankton consume these phytoplankton to convert arsenosugars to AB [23]. Another potential production scheme is that the "N" forming the skeleton of betaine is converted to "As" as a part of the biosynthesis process of trimethylglycine ($(CH_3)_3N^+CH_2COOH$) in marine organisms. The salinity of the growth environment is also considered to be a potential factor influencing AB concentration in such marine organisms [24, 25]. An interesting and somewhat adventurous hypothesis is that AB exists in nature in order to actually mitigate iAs toxicity in marine ecosystems.

7.2.2 Arsenosugars

In 1981, the structures of arsenosugars were identified in water-soluble components of brown kelp (Ecklonia radiata) [4]. Subsequently, over 20 types of arsenosugars with different side chains in the skeletal structure have been identified, of which four types (arsenosugar-Gly, arsenosugar-PO_4, arsenosugar-SO_3, and arsenosugar-SO_4) are the most frequently detected types [4, 26, 27]. Regarding the origin of arsenosugars in marine algae, it is estimated that marine algae biosynthesize arsenosugars by directly absorbing iAs from seawater [28, 29]. Arsenosugars have also been detected in samples from phytoplankton [22, 30], in zooplankton that consume phytoplankton [22, 30], and in shellfish [17] that consume marine algae, in addition to marine algae (Table 7.1).

In a very recent study (unpublished results), we compared the As species found as arsenosugars in the edible algae, kombu and nori, which are produced and widely consumed in Japan (Fig. 7.1). Rishiri, Rausu, and Hidaka are three regions in Japan known for major kombu production. No significant difference was detected in the As species of arsenosugars in kombu among the three regions. The major As species was arsenosugar-Gly, followed by arsenosugar-PO_4 and arsenosugar-SO_3, whereas

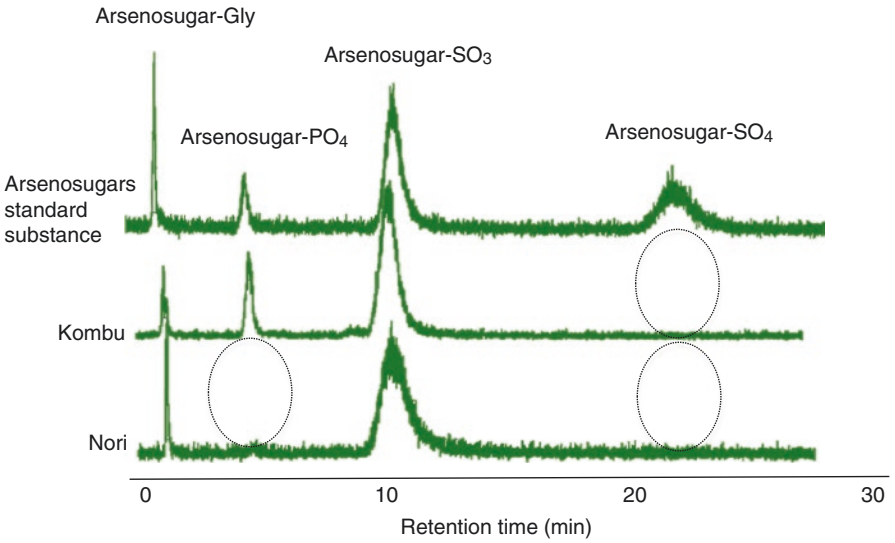


Fig. 7.1 Characteristics of the As species of arsenosugars detected from kombu and nori on the market. Experimental method: Kombu produced in three regions in Japan (Rishiri, Rausu, and Hidaka) and nori from two regions (Kisarazu and Ariake) was used. Kombu and nori weighing 50 and 10 g, respectively, were subjected to arsenosugar extraction for 8 h at room temperature in 1 L of ultrapure water. Measurement of arsenosugars used HPLC-ICP-MS (ELAN DRC-e, PeriknElmer) equipped with an anion-exchange column (PRP-X100, Hamilton). The standard solutions of arsenosugars (arsenosugar-Gly, arsenosugar-PO_4, arsenosugar-SO_3, and arsenosugar-SO_4) were provided by Dr. Francesconi

arsenosugar-SO_4 was not detected in any sample. The same analysis was conducted on nori (places of production: Kisarazu and Ariake). The results indicated that the major As species were arsenosugar-Gly and arsenosugar-SO_3, whereas arsenosugar-SO_4 and arsenosugar-PO_4 were not detected in any sample. Thus it appears that the arsenosugar is specific to the organism but not so much the variety in algae.

7.2.3 Arsenolipids

Arsenolipid is the general term for an organic As compound that exhibit lipophilic properties. From marine organisms, four main groups of arsenolipids have been identified based on chemical structures, namely, arsenic-containing hydrocarbons (AsHCs), arsenic-containing fatty acids (AsFAs), arsenic-containing phospholipids (AsPLs), and arsenic-containing phosphatidylcholines (AsPCs) (Table 7.1). In particular, AsHCs have been detected from finfish [16, 33, 34] and marine algae, such as wakame [35] and kombu [36]. AsFAs have been detected in finfish [34, 37] and wakame [38]. AsPLs have been detected from wakame [35, 39] and kombu [36]. AsPCs are rarely detected and have been found in herring caviar [40]. These reports

demonstrate a high arsenolipid concentration in finfish in lipid-rich sites, indicating that the behaviors of docosahexaenoic acid and eicosapentaenoic acid, which are omega-3 fatty acids recommended for the prevention of lifestyle-related diseases, may act in conjunction with those of arsenolipids. Thus, this possibility should be examined from a toxicological viewpoint in the future. According to recent studies, low concentrations of AsHCs and AsFAs have been detected in human breast milk [41]. This finding suggests that these compounds are biologically concentrated because of their lipophilic properties, and fetuses and/or infants may be preferentially exposed to As when compared to adults.

7.3 Metabolism and Toxicity of Organic As Compounds

Defining metabolism and assessing toxicity of organic As compounds in marine organisms can often have very substantial limitations when compared to iAs compounds. This is in part because with arsenosugars and arsenolipids, it is difficult to select the appropriate compound to research because there are multiple compounds with similar complex chemical structures, and it is difficult to synthesize test compound reagents for the various needs in complete toxicity assessment. In contrast, Japanese researchers have synthesized highly pure research reagents of methylated As compounds, including AB [42], AC [43], TMAO [44], and TETRA [45], to calculate toxic metrics like their 50% lethal doses and identify their basic metabolic pathways using experimental animals.

7.3.1 Arsenobetaine

Human experiments were performed earlier than animal experiments for the evaluation of the metabolism and excretion of AB, in which AB was prepared as a test reagent from the flesh of crab, prawns, and flatfish. The first study involving human subjects was conducted in 1977 by Crecelius and revealed a phenomenon in which an organic As compound (estimated to be AB) in crab meat was rapidly excreted into the urine without conversion [46]. Yamauchi and Yamamura examined the urinary excretion of AB (contained in deep-water shrimp) after a single ingestion by humans [19]. They found that AB was excreted nearly completely into urine without conversion within 72 h [19]. Moreover, in a metabolism experiment of flatfish AB, Tam et al. showed only trace amounts of the excretion of AB into feces [8]. In animal experiments, Vahter et al. revealed that when oral or intravenous ^{73}As-AB was administered to mice, rats, and rabbits, AB was excreted, without conversion, mainly into the urine [47]. Yamauchi et al. administered synthesized AB orally once to hamsters and found that the urinary excretion rate 12 h after administration was 70% of the dose, demonstrating rapid excretion [48]. Additionally, excretion into feces by 120 h after administration was as limited as approximately 1% of the dose

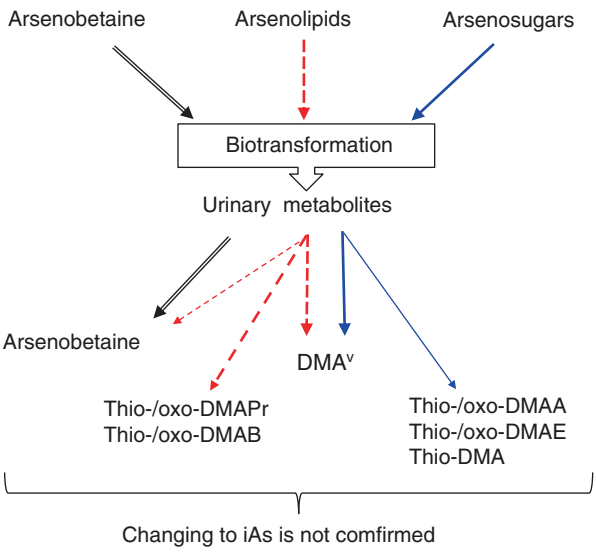


Fig. 7.2 Urinary metabolites of arsenobetaine (AB), arsenolipids, and arsenosugars in humans and mice* [69]. In rare cases, arsenolipids converted to AB in mice

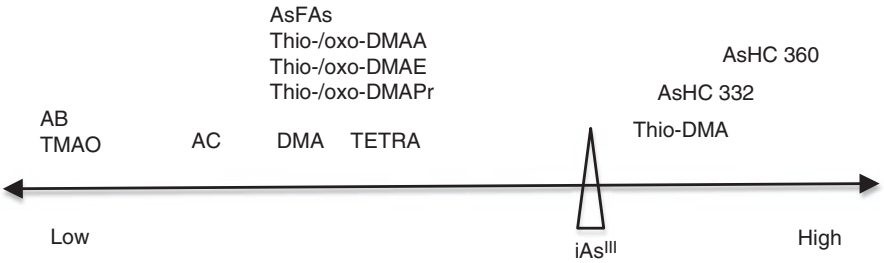


Fig. 7.3 Inferred coordinates of toxicity of organic As compounds in reference to that of iAs (50% lethal dose and cytotoxic concentration)

[48]. Regarding the toxicity of AB, Kaise et al. showed that the 50% lethal dose (LD_{50}) of orally administered AB for mice was 10 g/kg, without any finding of acute toxicity [42]. Thus, AB ingested by humans and animals is rapidly excreted mainly into the urine largely intact manner (Fig. 7.2), and it is thought that AB is the one of the least toxic (or the most non-toxic) among As compounds (Fig. 7.3).

7.3.2 Arsenosugars

Francesconi et al. administered an orally synthesized arsenosugar once to humans to investigate their urinary As levels and reported that 80% of the dose was excreted over 4 days, 67% of which was dimethylarsinate (DMA) [49]. Similarly, Raml et al. [50] administered an oxo-arsenosugar orally once to humans to investigate urinary As levels and found that DMA comprised 51% of urinary arsenicals and identified the following new metabolites: thio-dimethylarsenoacetic acid (thio-DMAA; 19%),

thio-dimethylarsenoethanol (thio-DMAE; 10%), oxo-dimethylarsenoacetic acid (oxo-DMAA; 2%), oxo-dimethylarsenoethanol (oxo-DMAE; <4%), and thio-dimethylarsinic acid (thio-DMA; trace) (Figs. 7.2 and 7.4). In several related works, organoarsenicals prepared from marine algae (kombu, nori, wakame) were used and administered to humans [21, 51–56]. The results of these studies demonstrated that DMA was the major metabolite excreted into the urine in all cases. Similar to the study described above, six types of arsenosugar metabolites containing DMA as the main constituent have been identified in humans according to the latest information. Among them, the presence of thio-DMA has been gaining attention.

The investigation of arsenosugars using an animal experiment revealed the major urinary metabolite of arsenosugars from marine algae ingested by sheep to be DMA [57, 58].

Regarding the systemic circulation of arsenosugars, a comparative analysis of intestinal bioavailability was performed among arsenosugars (arsenosugar-Gly, arsenosugar-SO_4, thio-arsenosugar-Gly), their metabolites (thio/oxo-DMAA, thio/oxo-DMAE, thio-DMA), and iAs^{III} using the Caco-2 intestinal barrier model. Among the examined As compounds, thio-DMA and thio-DMAE exhibited remarkably high intestinal bioavailability [59], and their activity levels were as high as those of iAs^{III}, which is highly toxic. Thus it appears that thio-DMA and thio-DMAE are easily absorbed by the intestinal tract and can be systemically circulated, leading to the development of the observed toxicity. Moreover, thio-DMAA, oxo-DMAA, and oxo-DMAE were confirmed to have a low intestinal bioavailability [59]. Regarding the absorption and digestion of marine algae in the human gastrointestinal tract, it has been shown that only ethnic Japanese individuals have the ability to

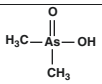
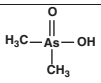
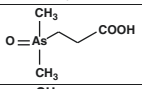
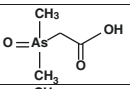
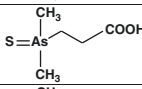
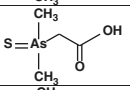
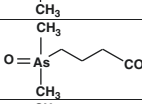
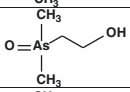
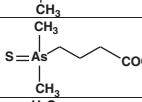
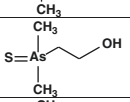
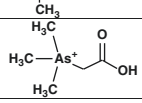
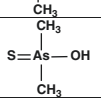
Metabolites of arsenolipids		Metabolites of arsenosugars	
Name	Chemical Structure	Name	Chemical Structure
Dimethylarsinate (DMA)		Dimethylarsinate (DMA)	
Oxo-dimethylarsenopropanoic acid (oxo-DMAPr)		Oxo-dimethylarsenoacetic acid (oxo-DMAA)	
Thio-dimethylarsenopropanoic acid (thio-DMAPr)		Thio-dimethylarsenoacetic acid (thio-DMAA)	
Oxo-dimethylarsenobutanoic acid (oxo-DMAB)		Oxo-dimethylarsenoethanol (oxo-DMAE)	
Thio-dimethylarsenobutanoic acid (thio-DMAB)		Thio-dimethylarsenoethanol (thio-DMAE)	
Arsenobetaine		Thio-dimethylarsinic acid (thio-DMA)	

Fig. 7.4 Chemical structures, names, and abbreviations of urinary metabolites of arsenolipids and arsenosugars

digest such algae because of the presence of catabolic enzymes [60]. Therefore, it may be necessary to take ethnic differences into consideration in verifications using cell model or human subjects in future studies.

Cytotoxicity tests have confirmed that arsenosugars [61–63] and their metabolites, namely, thio-DMAA, oxo-DMAA, thio-DMAE, and oxo-DMAE [50, 61], are significantly less toxic in all cases compared with DMA and iAs. In contrast, the findings that thio-DMA is significantly more cytotoxic than iAs^{III} has been deemed most noteworthy [64–66]. Indeed, in mice chronic oral administration of arsenosugar-Gly (20–50 mg/kg body weight; 40 days) caused an increase in brain DNA damage, oxidative stress, neurobehavioral impairment, and hyperkinesia [67].

7.3.3 Arsenolipids

The arsenolipids are a relatively new field of study in specific As compounds. Schmeisser et al. studied the metabolites of cod-liver and liver oil arsenolipids in two subjects [68]. This showed that DMA is the main urinary metabolite of arsenolipids and that four types of metabolites [i.e., thio-dimethylarsenobutanoic acid (thio-DMAB), oxo-dimethylarsenobutanoic acid (oxo-DMAB), thio-dimethylarsenopropanoic acid (thio-DMAPr), and oxo-dimethylarsenopropanoic acid (oxo-DMAPr)] are produced. In contrast, Fukuda et al. synthesized phosphotidylarsenocholine, which has the same chemical structure as that of arsenolipids, and orally administered it to mice to investigate its metabolites [69]. The authors confirmed that AB was the main metabolite of the metabolites identified in the urine and also detected trace amounts of DMA and AC. The results obtained to date have indicated that metabolites of arsenolipids differ between humans and mice (Figs. 7.2 and 7.4). This is thought to be potentially because of the diversity in the chemical structure of arsenolipids, which is reflected in the difference in metabolites. Further investigation into this issue should be performed in the future.

Although limited number of studies has been conducted on the systemic circulation of arsenolipids, a study has been performed using the Caco-2 intestinal barrier model, which confirmed a significantly higher intestinal bioavailability of AsHCs, a group of arsenolipids [70]. Thus, we speculate that AsHCs are easily absorbed by the intestinal tract and would be available to the systemic circulation, leading to development of their toxic potential.

Pioneering studies were conducted on the biological effects of arsenolipids, in which both in vivo and in vitro studies were performed. As a result of the respective exposure of UROtsa and HepG2 cells to synthesized AsHC 332, AsHC 360, and AsHC 444, a substantial inhibition of the cell cycle was commonly observed [71]. Next, Meyer et al. separately exposed Drosophila to AsHC 332, AsHC 360, and AsHC 444 to identify a phenomenon in which the compounds influenced the late developmental stages [72]. In particular, it was confirmed the developmental toxicity of AsHC 332 and AsHC 360 occurred via the ability to inhibit hatching from pupae. Moreover, AsHC 332 accumulated in the Drosophila brain, indicating that the compound can permeate the blood-brain barrier [73]. Müller et al. examined the

influence of AsHCs on brain cells and tissues using porcine brain capillary endothelial cells in vitro and confirmed that AsHC 360 has the highest toxic potential, which is fivefold higher than that of iAs^{III}; moreover, AsHC 332 is 3.7-fold more cytotoxic than iAs^{III} [74] (Fig. 7.3). Using LUHMES cells, it was also shown that AsHCs (AsHC 332, AsHC 360, and AsHC 444) are remarkably neurotoxic [75–77], indicating that AsHCs may exhibit cranial nerve toxicity. A recent report has also shown that these AsHCs can be detected in human breast milk [41]. Because AsHCs have already been detected from marine-derived foods, the relationship between their ingestion and toxic effects should be examined in more detail in the future.

7.3.4 Methylated As Compounds

Water-soluble organic As compounds include AB and methylated As compounds. The methylated As compounds, such as AC [78], TMAO [78–80], and TETRA [78, 81, 82], have been identified in marine organisms. AB is detected in finfish, shellfish, and crustaceans at high concentrations, whereas AC, TMAO, and TETRA are all characterized by their detection in concentrations at typically low to trace levels.

To date, it has been a priority to clarify whether AC, TMAO, and TETRA are metabolites of iAs in humans and other mammals as well as define the associated metabolism that may occur with these compounds. The urinary metabolites of AC, TMAO, and TETRA following oral administrations have been identified. The metabolite of AC in mice [43, 83], rats, and rabbits [83] was found to be AB. In addition, it was confirmed that TMAO is not converted in mice [44] and hamsters [84]. However, DMA when orally administered to hamsters [85], or to mice, hamsters, and humans [86] in another experiment, is converted to TMAO (4–30% of the dose). It has been confirmed that TETRA is not altered in vivo [45].

The LD_{50} of orally administered AC, TMAO, and TETRA for mice was calculated using their artificially synthesized pure forms and equals: AC, 6.5 g/kg [43]; TMAO, 10.6 g/kg [44]; and TETRA, 0.9 g/kg [45]. Thus, AC and TMAO are nearly nontoxic arsenicals, similar to AB, whereas TETRA is considered a toxic arsenical (Fig. 7.3).

7.4 Changes in Cooking, Digestion, and Degradation

The physical and biological decomposition assuming cooking and digesting for organic As compounds from commercially available marine-derived foods has been studied. Shibata et al. used baked nori [31], and Wei et al. heat-treated nori at 100 °C [52] and found that the As species of arsenosugars remained unchanged. In our unpublished study, we immersed kombu in ultrapure water for 8 h at room temperature to extract arsenosugars and treated the extracted arsenosugars for 1 h by boiling with simulated gastric juice (pH 1.2) or digestive enzyme (pepsin) and found no

change in the As species of arsenosugars (Fig. 7.5). Similarly, Almela et al. found digestive enzyme exposure had no influence on the As species of arsenosugars derived from kelp [87]. Moreover, there was no influence of cooking conditions (i.e., frying, grilling, baking, or boiling) on organic As compounds contained in finfish (lean and fatty fish) [78, 88]. Although finfish AB was stable and maintained its skeletal structure in all cooking conditions, it changed to TETRA to a very slight extent. It is speculated that arsenosugars and AB are unlikely influenced by the physical and biological actions in food and the gastrointestinal tract. It has also been shown that these compounds are not degraded into iAs.

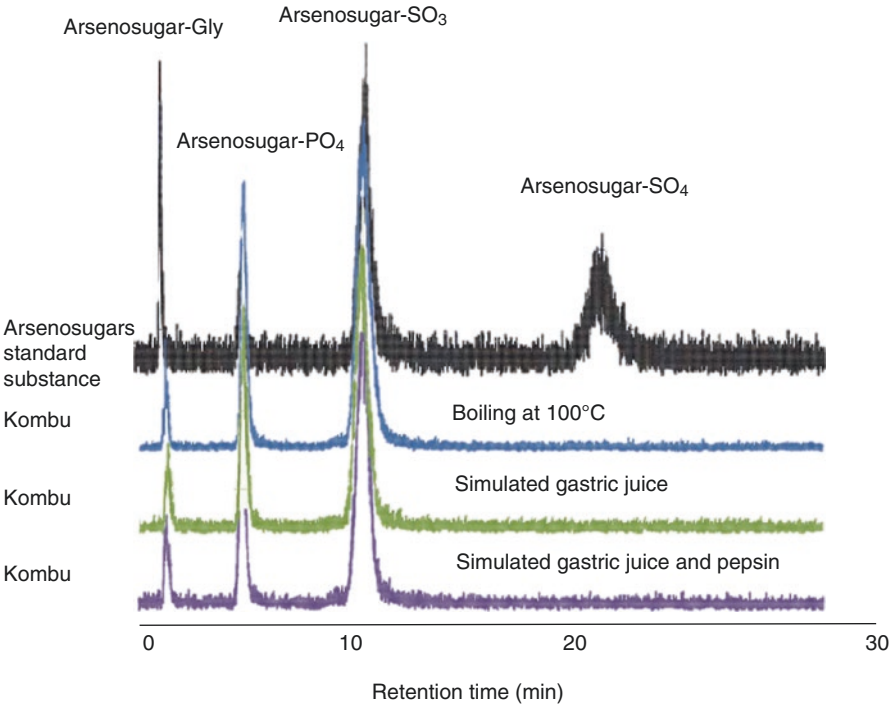


Fig. 7.5 Changes in arsenosugars in kombu following treatment with boiling, simulated gastric juice (pH 1.2), or a digestive enzyme (pepsin). Experimental method: Simulated gastric juice was prepared by mixing 2.0 g of sodium chloride with 7.0 mL of hydrochloric acid, followed by volume adjustment to 1 L with ultrapure water. The digestive enzyme solution was prepared by adding 2 g of porcine-derived pepsin to 100 mL of the prepared simulated gastric juice. Kombu produced in Rausu was used to prepare a test sample through the same treatment as in Fig. 7.1. Three aliquots of the test sample were separately placed in 10-mL Falcon tubes and subjected to 1-h treatment by boiling at 100 °C in a water bath, or in a water bath at 37 °C, after the addition of the simulated gastric juice or digestive enzyme solution. Arsenosugars were measured as described in Fig. 7.1

7.5 Conclusions

Information regarding the presence, metabolism, and toxicity of organic As compounds derived from marine organisms is important when assessing their potential toxicological effects in humans. Metabolic studies on organic As compounds from marine organisms using humans, animals, and cells have not shown a conversion to iAs under any conditions. Moreover, studies approximating their treatment in food or in the gastrointestinal tract did not detect degradation into iAs. Thus, it can be concluded that any toxicological effects of organic As compounds are not due to the derived iAs. Although AB is an organic As compound with the highest probability of ingestion due to its high content in finfish, shellfish, and crustaceans, the conclusion that AB is a nontoxic arsenical is appropriate. The ingestion of finfish is recommended as a preventive measure for lifestyle-related diseases, and dietary culture is becoming more diverse, as seen in the global spread of sushi and Japanese food throughout the international community. From this perspective, we can estimate that the opportunity and amount ingestion will increase for both arsenosugars and arsenolipids. However, a wide range of risk assessments into the toxicological effects of organic As compounds are needed, and many simply have not been sufficiently conducted particularly when compared with the vast amount of toxicological knowledge about iAs compound and their toxicity. The findings of recent studies organoarsenicals include the identification of thio-DMA as an arsenosugar metabolite and the alarming discovery that it is more cytotoxicity than even iAs^{III}. Moreover, multiple studies show AsHCs, a group of arsenolipids, can be potentially potent neurotoxins. This toxic potential could be exaggerated because these AsHCs are concentrated in vivo because of their lipophilic properties, and they have been detected high-fat biologic fluids like breast milk. The Joint FAO/WHO Expert Committee on Food Additives has developed comprehensive guidelines for the legal regulation of dioxins and methyl mercury incorporated through the ingestion of marine products and has conducted their own toxicological assessments [5]. Because the ingestion route of organic As compounds is typically the same in this case (i.e., through ingestion of marine organisms), we believe that new issues have been generated in food safety verification. Currently, individuals chronically exposed to iAs have serious problems, including cancer, developmental toxicity, brain dysfunction, etc. [89–92]. It is clear that the toxicity of thio-DMA and AsHCs is as high as or even higher than that of iAs. Thus, efforts should be made in the future to elucidate the biological effects of organic As compounds by evaluating next-generation effects and brain dysfunction caused by genotoxicity.

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Chapter 8
Arsenic Intake and Health Risk from Diet in Asia

Tomoko Oguri

Abstract Inorganic arsenic (InAs) is ubiquitous in the environment and has greater toxicity than do organic arsenic species. It is a proven fact based on epidemiologic studies in groundwater-contaminated regions that chronic ingestion of InAs can lead to adverse health effects. This chapter deals with InAs intake, as well as its health risk from diet in Asia. Dietary intake of InAs in contaminated regions is at least an order of magnitude higher than that in non-contaminated regions. In the contaminated region, daily intake of InAs increased not only by drinking contaminated water but also by the consumption of crops grown with contaminated water and/or by consumption of foods cooked with contaminated water. Consumption of rice is a dominant source of InAs intake in the non-contaminated regions. The mean dietary InAs intake ranges 36–1200 and 3.8–53 μg/day in contaminated and non-contaminated regions, respectively. On the basis of these data and the benchmark dose reported by the EFSA for InAs, the margin of exposure was estimated to be less than 200. Therefore, Asian people are exposed to the levels of InAs that could not be free from cancer and non-cancer risk in the contaminated and non-contaminated regions.

Keywords Diet · Inorganic arsenic · Drinking water · Rice · Health risk

T. Oguri
Research Institute of Science for Safety and Sustainability, National Institute of Advanced Industrial Science and Technology (AIST), Ibaraki, Japan
e-mail: oguri.tomoko@aist.go.jp

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8.1 Introduction

Chronic arsenicosis has been reported from all over the world and recognized as one of serious global public health issues. Millions of people in Asia, South America, and other regions suffer from symptoms of arsenicosis as a result of inorganic arsenic (InAs)-contaminated groundwater consumption. Of these, four major affected regions are located in Asia: South Taiwan, Bangladesh, West Bengal in India, and Inner Mongolia in China. The symptoms of chronic arsenicosis in humans include non-cancer diseases of dermal lesions, hypertension, cardiovascular diseases, and diabetes and skin, liver, lung, bladder, and kidney cancers. Epidemiological studies have demonstrated that cancer risk increased by consuming contaminated drinking water [1]. Although increased health risk of InAs through consumption of contaminated drinking water has been extensively studied in the past, its risk can also be present among people who consume InAs from other sources than drinking water in non-contaminated regions.

Diet is recognized as another possible major source of InAs intake [2, 3]. Dietary InAs intake has been attracting increasing attention of health authorities; therefore, health risk assessments of dietary InAs intake have been carried out by the European Union [2], by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) [3], and by health authorities in Asian countries, i.e., Hong Kong [4] and Japan [5]. These health risk assessments indicated that the general population is exposed to unacceptable levels of InAs.

Therefore, the health risk through daily InAs intake from diet is a major public health concern in Asian countries where major groundwater InAs contamination has not been recognized. This chapter deals with InAs intake and health risk from diet in Asian countries.

8.2 Arsenic Species in Water and Foods

Arsenic (As) is ubiquitous in the environment, which is present as various inorganic and organic species. Arsenic in groundwater is InAs, which can be present as arsenite [As(III)] and arsenate [As(V)].

Organic As and InAs are contained in most foodstuffs, such as crop, vegetable, meat, fish, shellfish, and fruits and pulses. Although InAs has greater toxicity than do organic As species, As in foods was reported as total-As without distinguishing the various As species in most of the previous literatures. This is because analytical methods of InAs in food were not standardized and not routine in most laboratories until recently [6]. Therefore, limited information is available to evaluate dietary InAs intake of subject populations from the literatures.

8.3 Dietary As Intake in Asian Countries

8.3.1 Taiwan

Occurrence of arsenicosis was first reported in southwestern Taiwan in the early 1960s [7]. The symptom, called as black foot disease (BFD), was a unique peripheral vascular disease identified in southwestern Taiwan. The cause of BFD was ascribed to drinking InAs-contaminated groundwater [8, 9]. In this region, total-As concentrations in the 155 well waters of 42 villages were found to be 10–1752 μg/L [10]. Another study reported that total-As concentrations in the 3901 well waters were <0.15–3590 μg/L [11]. Assuming that people in this region drink 3.5 and 2.0 L of water a day for male and female, respectively [12], the estimated maximum InAs intake would have been 13 mg/day. However, dietary intake of InAs of the people in this region during the period people utilized contaminated groundwater was not reported.

8.3.2 Bangladesh and Indian State of West Bengal

Tube wells were installed in Bangladesh and West Bengal to provide microbiologically safe drinking water since the 1970s [13]. In the 1990s, InAs contamination of groundwater in these regions was found in tube well waters in all of the 64 districts of Bangladesh and was analyzed for total-As (n = 52,202): the concentrations ranged <10–4730 μg/L. Forty-two percent of the sampled waters had total-As concentration above the drinking water quality standard of 50 μg/L in Bangladesh and India [14]. Additionally, in 19 districts of West Bengal, concentration of total-As in tube well water (n = 140,152) was <3–3700 μg/L; 24% of sample water also contained total-As above 50 μg/L [15]. Watanabe et al. [16] estimated maximum daily intake of total-As from drinking water to be 1.5 mg/day, by considering As content in tube well water and water intake of the people to be 3 L/day.

Daily InAs intakes from certain foods (i.e., rice, vegetables, and water) were estimated in the contaminated regions in Bangladesh and West Bengal. Smith et al. [17] estimated InAs intake based on total-As and InAs analyses of cooked rice (n = 38), vegetables (n = 9), and water (n = 46) collected in Bangladesh households. The daily amount of rice and water consumption was surveyed by face-to-face interview with study participants. Daily InAs intake was estimated by adding InAs intakes from cooked rice (mean InAs concentrations in rice multiplied by mean daily amount of rice consumption) and total-As intake from water (total-As concentration in tube well water multiplied by mean daily amount of water consumption). The amount of vegetables consumed were not clearly documented in the questionnaire, and therefore vegetables were not included in the daily InAs intake estimation. The mean daily InAs intake was estimated to be 1176 μg/day (range, 419–2053 μg/day) (Table 8.1).

Table 8.1 Comparison of dietary intakes of InAs in the InAs-contaminated region in the Asian countries

Country	Intake source	Intake (μg/day)		Intake (μg/kg/day)		Reference
		Mean	Min-max	Mean	Min-max	
Bangladesh	Rice, drinking water	1176[a]	419–2053[a]	19.6[b]	7.0–34[b]	[17]
West Bengal, India	Rice, vegetable, drinking water	151[a]	69–251[a]	2.5[b]	1.2–4.2[b]	[18]
Bangladesh	Total diet	43[a,c]		0.72[b,c]		[19]
Bangladesh	Total diet (except drinking water)	160 (male)[c] 120 (female)[c]		2.7[b,c] 2.0[b,c]		[20]
Cambodia	Rice, fish, vegetable	95.6 ± 126 (Kangal) 36.0 ± 29.1 (Kratie) 6.51 ± 5.50 (Kampong Chan)[d]	4.64–436 2.47–87.6 2.23–87.6	1.84 ± 2.42 (Kangal) 0.693 ± 0.560 (Kratie) 0.13 ± 0.35 (Kampong Chan)[d]	0.089–8.39 0.047–1.68 0.043–0.35	[28]
Cambodia	Rice, drinking water	265 ± 256	29.0–780	5.31 ± 5.02	1.16–19.5	[29]
Vietnam	Rice, drinking water	682	67–1520	11.4[b]	1.1–25.3[b]	[30]
Vietnam	Rice, water	1002 ± 727 (male) (before)[e] 652 ± 633 (female) (before)[e] 54 ± 17 (male) (after)[e] 47 ± 15 (female) (after)[e]	35–2941 35–2732 35–102 28–94	17 ± 12.5 (male) (before)[e] 13 ± 12.7 (female) (before)[e] 0.9 ± 0.3 (male) (after)[e] 0.9 ± 0.3 (female) (after)[e]	1–51 1–55 0.6–1.8 0.6–1.9	[31]

[a]In the drinking water, daily intake of InAs was estimated based on the total As concentration detected in the drinking water

[b]Intake (μg/kg/day) was calculated by body weight assumed 60 kg

[c]Daily intake of InAs was estimated based on the detection of total As and the use of conversion factors

[d]Control site

[e]Before and after technological countermeasure situation against As-contaminated groundwater was carried out

Signes-Pastor et al. [18] estimated dietary InAs intake of people in a rural village in West Bengal. The inhabitants used tube well water for drinking and irrigation: 70% of the wells were found to contain total-As at above 50 μg/L. The intake of InAs was estimated through a part of food items and water. Daily intake of InAs was calculated with quantity of water and food consumptions of inhabitants and measured InAs concentration of rice and vegetables collected from farms and local

markets. When total-As concentration in water was 50 μg/L, the mean daily intake of InAs from rice, vegetables, and water was 151 μg/day (range, 69–251) (n = 129) (Table 8.1).

Kile et al. [19] carried out a duplicate diet survey to quantify daily total-As intake of 47 women residing in Bangladesh. The median of total-As concentrations in drinking water of 47 tube wells was 1.6 μg/L (range, <1–450 μg/L). Median daily total-As intake (n = 47) was 48 μg/day (interquartile range (IQR), 33–67) from food and 4 μg/day (IQR, 2–152) from drinking water. The InAs accounted for 82.1 ± 13.9% of the total-As of the 35 diet samples. Daily InAs intake was estimated by adding InAs intake from food (median total-As intake from food multiplied by mean InAs/total-As ratio) and total-As intake from water. It was estimated that daily InAs intake is 43 μg/day (Table 8.1).

Joseph et al. [20] estimated dietary InAs intake in Bangladesh from literature data. The mean total-As concentrations in 13 food items and 6 composites (i.e., cereals, pulses, vegetables, spices, fruits, and meats and milk) were collected from literatures. Arsenic in all of the food items except for fish and chicken was assumed to be InAs. For fish and chicken, 5% and 1% InAs content was assumed, respectively. Amount of food consumption of Bangladeshi population was obtained from literature [21]. Estimated mean daily InAs intakes from diet were 160 and 120 μg/day for male and female, respectively (Table 8.1). Rice was found to be more important sources of InAs than other food items.

8.3.3 China

In Inner Mongolia (Northwest China) in the late 1970s, drinking water supply was shifted from shallow wells to tube wells, and drinking water quality of InAs deteriorated. Skin lesion caused by arsenicosis has been reported in this region since the 1990s [22]. Ning et al. [23] carried out a survey of InAs concentration in groundwater collected from 14,866 wells in Ba Men region, Inner Mongolia, from 1991 to 1997. The total-As concentration in the groundwater ranged from <20 to 1200 μg/L. Assuming that people in Inner Mongolia ingest 2.0 L of drinking water a day [12], it is estimated that daily intake of InAs by drinking water is approximately maximum 2.4 mg/day.

Liu et al. [24] estimated total-As intake in Inner Mongolia. Grain (wheat, n = 13; corn, n = 8), vegetables (bean, n = 6; cucumber, n = 8; kidney bean, n = 8; tomato, n = 8; pepper, n = 10; brinjaul, n = 6; scallion, n = 6; medlar, n = 7; rape, n = 6), and fruit (watermelon, n = 3; pear, n = 3) collected from the four typical villages in Inner Mongolia were measured for total-As concentration. Tap water samples were collected from 70 participants who resided in the study villages. The daily water consumption was surveyed by the questionnaire, and literature values were used for the amount of consumption of grains, fruits, and vegetables. Daily total-As intake was calculated by adding total-As intake from crop (total-As concentrations in crop multiplied by daily amount of crop consumption) and total-As intake from water (total-As concentration in water multiplied by daily amount of water consumption).

The mean daily total-As intake was estimated to be 18.6–22.8 μg/day in the four villages (range, 5.33–87.7 μg/day). The major sources of total-As intake were grain, fruits, and vegetables in the four villages with low As contents in drinking water. In Inner Mongolia, total dietary InAs intake (including all other foods than crops) of the people was not reported.

Wong et al. [4] reported the first comprehensive market basket survey in Hong Kong, China. Occurrence of arsenicosis from drinking InAs-contaminated water was not reported in Hong Kong. In this market basket survey, three samples of each food item were collected from supermarkets, groceries and restaurants, etc. Samples included drinking water. Six hundred food items were collected and InAs concentrations were measured. The amount of consumption of each food item was taken from the Hong Kong Population-based Food Consumption Survey conducted by the Centre for Food Safety of Hong Kong in 2005–2007. This market basket survey revealed that the mean and 95th percentile of InAs intake were 0.22 and 0.38 μg/kg body weight/day (Table 8.2), respectively, and cereals (53.5%), particularly rice, were found to be the major source of InAs intake.

Table 8.2 Comparison of dietary intakes of InAs in the non-contaminated regions

Country	Intake source	Intake (μg/day)		Intake (μg/kg/day)		Reference
		Mean	Min-max	Mean	Min-max	
Hong Kong	Total diet	13.2 [a]	22.8 (95th)[a]	0.22	0.38 (95th)	[4]
China	Total diet (except drinking water)	42.6 (National)[a] 42.6 (Urban)[a] 42.6 (Rural)[a] 28.2 (North)[a] 52.8 (South)[a] 46.2 (Coastal) [a] 42.0 (Inland)[a]		0.71 (National) 0.71 (Urban) 0.71 (Rural) 0.47 (North) 0.88 (South) 0.77 (Coastal) 0.70 (Inland)		[25]
Japan	Total diet	10.3 ± 5.5	1.8–22.6	0.17[a]	0.03–0.38[a]	[35]
Japan	Total diet	33.7 ± 25.1	8.34–101	0.56[a]	0.14–1.68[a]	[36]
Japan	Total diet	3.8[b] 27	2.0–57	0.063[a,b] 0.45[a]	0.033–0.95	[37]
Japan	Total diet	21		0.35[a]		[32]
France	Total diet	6.0[a]	16.2 (95th)[a]	0.1	0.27 (95th)	[3]
UK	Total diet	1.2–7.2[a]	3.0–9.6 (97.5th)[a]	0.02–0.12	0.05–0.16 (97.5th)	[3]
USA	Total diet	4.8–12[a]	9.6–20.4 (95th)[a]	0.08–0.20	0.16–0.34 (95th)	[3]

[a]Daily intake of InAs was calculated by body weight assumed 60 kg
[b]Median

On the other hand, Li et al. [25] estimated dietary InAs intake from literature data for different population groups in China. The amount of food consumption was adopted from the China National Nutrition and Health Survey. The reported concentrations of InAs in major food groups were used for the estimation. Dietary InAs intake was estimated by multiplying amount of daily food consumption with corresponding InAs concentrations. InAs intake through drinking water was not estimated in this study. The mean daily InAs intakes were 42.5, 42.8, 42.3, 30.6, 52.5, 46.0, and 42.3 μg/day for national, urban, rural, northern, southern, coastal, and inland, respectively (Table 8.2). This study concluded that rice was a dominant dietary source of InAs for Chinese populations.

8.3.4 Cambodia

Elevated water InAs concentrations have been found in tube wells in the Mekong River basin of Cambodia since the 2000s. Concentrations of total-As in well water exceeded 50 μg/L in 29% of all of the tube wells measured (n = 494) [26], and health risk of InAs intake through drinking groundwater of residents in the Mekong River basin indicated to be non-negligible [27] because As in tube well water could be assumed to be inorganic.

Recently, high total-As concentrations have been also reported in rice irrigated with InAs-contaminated groundwater in the Mekong River basin [28]. A couple of studies are available which estimated daily intake of InAs from selected foods in the Mekong River basin. Daily dietary InAs intakes were estimated based on the analyses of rice (n = 10), fish (n = 10), and vegetable (n = 15), but not from drinking water. The results were 95.6 ± 126, 36.0 ± 29.1, and 6.51 ± 5.50 μg/day for rice, fish, and vegetable in three villages near Mekong Delta, respectively [28]. In another village near Mekong Delta, the mean daily intake of InAs from rice and drinking water was estimated to be 69.0 ± 20.9 and 197 ± 243 μg/day, respectively, for 11 households, and total daily intake of InAs from rice and drinking water was 265 ± 256 μg/day (range, 29.0–780 μg/day) [29] (Table 8.1).

8.3.5 Vietnam

Groundwater InAs contamination was found in the Red River and the Mekong River delta basin in Vietnam. In Red River delta, Agusa et al. [30] estimated daily InAs intake from water and rice. The mean concentration of total-As in 28 tube well water samples was 1.8–486 μg/L, and average daily intake of InAs from drinking water (n = 28) was estimated to be 624 μg/day. Rice was collected from ten households, and average intake of InAs from rice was estimated to be 58 μg/day. Thus, average total daily intake of InAs was 682 μg/day (range, 67–1520 μg/day) (Table 8.1), and drinking water contributed 91% of total (water and rice) intake indicating that drinking water was a major source in this region.

In Mekong River delta, daily intake of InAs was estimated before and after transition of water source [31]. The residents stopped drinking of InAs-contaminated tube well water and started using alternate sources of drinking water by 2008. The mean daily InAs intakes from drinking water and rice were 1002 ± 727 and 652 ± 633 μg/day for male and female, respectively, before 2008. After 2008, they were 54 ± 17 and 47 ± 15 μg/day for male and female, respectively (Table 8.1). When tube well water was used for drinking, contribution of rice consumption was 14.4 ± 25.0% and 20.2 ± 27.6% of total InAs intake for male and female, respectively. After the transition to safer water source, contributions of rice increased to 97.5 ± 4.1% and 96.5 ± 9.7% for male and female, respectively. These results showed that daily intake of InAs is substantially decreased by transition to uncontaminated water source, whereas contribution of rice to total InAs intake relatively increased.

8.3.6 Japan

In Japan, occurrence of arsenicosis from InAs-contaminated water has not been reported. Oguri et al. [32] carried out a market basket survey in Japan. Collected 159 food items were divided into 19 food categories, and corresponding 19 composites were measured for InAs concentrations after extraction with synthetic gastric juice. The amount of food consumption was based on the National Health and Nutrition Survey in Japan. Total daily InAs intake was 21 μg/day on a bioaccessible-fraction basis and 24 μg/day on a content basis. It was found that approximately 60% of daily InAs intake of the Japanese was from consumption of rice and 28% was from hijiki (*Hizikia fusiforme*). The great contribution of rice was partly due to large amount of daily consumption (312.5 g/day), because rice is a staple food of Japanese, and relatively higher InAs concentration in rice than in other foods. The contribution of hijiki to daily InAs intake was due to its high InAs concentration, although the amount of daily consumption was small (3.18 g/day) [32]. Hijiki is one of seaweeds consumed as daily food in Japan and is known for its high level InAs [33, 34].

There are three literatures on duplicated diet studies of small-sized population in Japan. Mohri et al. [35] reported daily InAs intakes of four volunteers for 7 consecutive days. The mean InAs intake of 7 days for the four volunteers was 10.3 μg/day and ranged from 1.8–22.6 μg/day. Yamauchi et al. [36] measured daily InAs intakes of 35 subjects to find average intake being 33.7 μg/day with a range of 8.34–101 μg/day. Oguri et al. [37] estimated InAs intake by measuring the InAs concentration in two different sets of total diet sample: duplicated diet samples collected from 25 subjects and a certified reference material prepared from duplicated diet samples (NIES CRM No. 27 Typical Japanese Diet, TJD). The median InAs intake for the 25 subjects was 3.8 μg/day with a range of 2.0–57 μg/day. Daily InAs intake estimated from TJD was 27 μg/day (Table 8.2).

These studies showed moderately large variation in InAs intake levels between and within study populations in Japan. This large variation may be explained by variation in frequency and/or amount of InAs-rich food items, such as hijiki. Nakamura et al. [38] reported that the consumption of hijiki was only 2.3 times a month but InAs intake from one serving of cooked hijiki was up to 202 μg/serving. Thus, whether or not hijiki was consumed on the day of duplicated diet sampling greatly affects the daily intake estimation of the subject, which eventually results in large inter- and intraindividual variation.

8.4 Overview of Dietary InAs Intake in Asian Countries

Table 8.1 compares daily InAs intake from diet in InAs-contaminated regions of Asian countries reported in the previous studies. In Table 8.2, dietary intakes of InAs in uncontaminated regions are summarized. This table contains InAs intakes of Western countries (France, USA) for comparison purpose.

The reported mean dietary InAs intake ranged 36–1200 and 3.8–53 μg/day in the InAs-contaminated and non-contaminated regions of Asian countries, respectively (Tables 8.1 and 8.2). Dietary intakes of InAs in contaminated regions were at least an order of magnitude higher than that in non-contaminated regions. In the contaminated regions, daily intake of InAs increased not only by drinking contaminated water but also by the consumption of crops grown in contaminated area. This is partly because rice is a plant that accumulates As more than other grain crops [39]. In addition, rice cultivation using InAs-contaminated water can further increase the As content in rice grains [40].

Moreover, cooking rice by using InAs-contaminated water can also increase As content of cooked rice. Roychowdhury [41] found higher As content of cooked rice than that of uncooked rice. They reported that the mean total-As concentration in uncooked rice (n = 52) collected in West Bengal was 222 μg/kg-dry with the range of 43–662 μg/kg-dry. In comparison, the mean total-As concentration in cooked rice (n = 22) was 518 μg/kg-dry (range, 105–1030 μg/kg-dry) when water containing 110 μg/L of InAs was used for cooking.

Although InAs intake was generally much higher in contaminated regions than in uncontaminated regions, intakes can be lowered by the control of contaminated water use as shown in the studies of Kile et al. [19] and Hanh et al. [31] in Table 8.1. Kile et al. [19] pointed out that relative contribution of InAs from foods becomes significant in total InAs intake when As concentration in drinking water decreases to 50 μg/L [19].

In the non-contaminated regions, the mean of dietary InAs intake in the non-contaminated regions of Asia ranged 3.8–53 μg/day (Table 8.2). On the other hand, it was estimated to be 1.2–12 μg/day in other non-Asian countries (Table 8.2). Abundance of rice in the diet should be the reason of this difference, because rice was identified as a dominant source of InAs intake in the non-contaminated regions [4, 32]. Additionally, hijiki consumers may ingest additional InAs [32].

It has to be pointed out that dietary InAs intake has been estimated based on the analyses of limited food items, e.g., rice and vegetables, but not based on comprehensive total diet sampling, in most of the studies (Tables 8.1 and 8.2). Moreover, diet/water has been analyzed for total-As but not InAs in many studies. Comprehensive diet study for specifically InAs is requisite for better characterization of InAs intakes in different regions of the world with different dietary habits.

8.5 Health Risk of InAs Intake in Asian Countries

Health risks of InAs are assessed by margin of exposure (MOE) approach. MOE is a ratio of the dose at which a small and measureable adverse effect is first observed to daily InAs intake. The smaller MOE means higher potential risk posed by the InAs intake. Confidence interval of the benchmark dose was estimated by the EFSA to be 0.3–8 μg/kg/day that can cause 1% increased risk of lung, skin, and bladder cancer, based on the past epidemiologic studies [2]. For non-cancer risk, confidence interval of benchmark dose (0.9–5.7 μg/kg/day) was estimated to cause 1% increased risk of dermal lesions [2]. The current mean and maximum dietary intake of InAs estimates in Asian countries, both contaminated and non-contaminated regions, are compared with benchmark doses of extra cancer risk (Fig. 8.1) and non-cancer risk (Fig. 8.2) estimated by EFSA [2]. It was evident that many reported InAs intakes in contaminated and non-contaminated regions exceeded the minimum estimated 1% benchmark dose ($BMDL_{01}$) for both cancer and non-cancer endpoint. The MOE calculated for cancer in contaminated and non-contaminated regions were 0.015–12

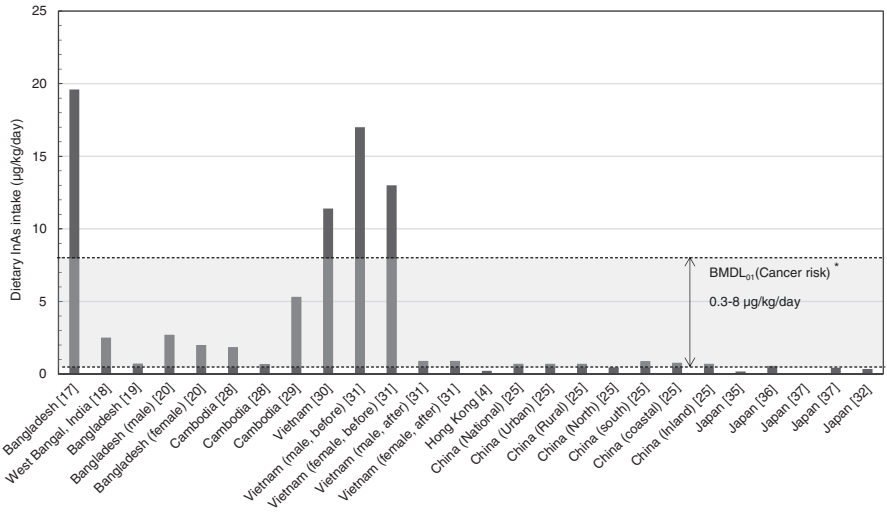

Fig. 8.1 Comparison of dietary InAs intake and BMDL of cancer risk. (*Asterisk*) $BMDL_{01}$: lower 95% confidence limit for the benchmark dose for 1% increased incidence of cancer over background [2]

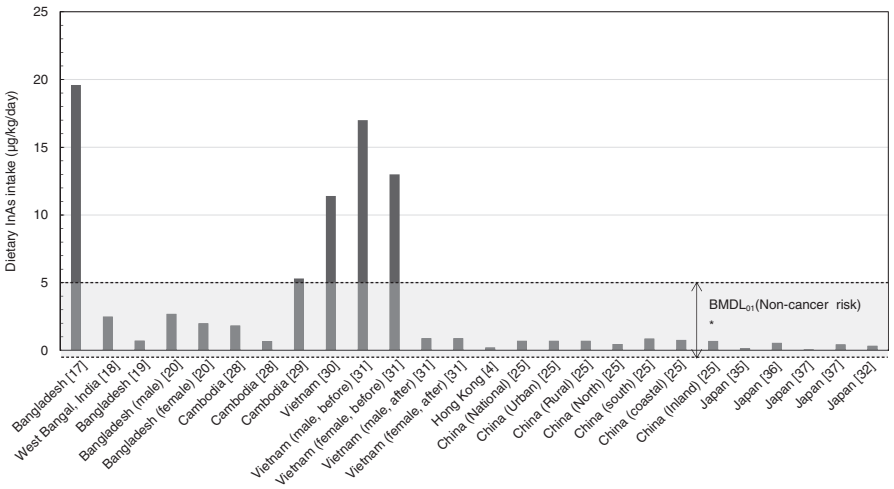

Fig. 8.2 Comparison of dietary InAs intake and BMDL of non-cancer effect. (*Asterisk*) $BMDL_{01}$: lower 95% confidence limit for the benchmark dose for 1% increased incidence of non-cancer effect over background [2]

and 0.34–130, respectively, and for non-cancer endpoint (skin lesion) were 0.046–8.7 and 1.0–95, respectively.

The MOE results indicated that both cancer and non-cancer risks were evident in contaminated regions as expected. However, more importantly, the MOE results indicated that cancer risk posed by InAs intake is present (MOE < 1) even in general populations of non-contaminated regions of Asia. In fact, most of the reported intakes in non-contaminated regions exceeded the estimated minimum $BMDL_{01}$ value (0.3 μg/kg/day) (Table 8.2; Fig. 8.1). This would be one of the most serious public health problems in many Asian countries. Effective and reasonable countermeasures to reduce InAs intake levels of general population would be warranted.

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Chapter 9
Preventive Agents and Phytochemicals for Reducing the Adverse Health Effects of Arsenic

Yumi Abiko and Yoshito Kumagai

Abstract Arsenic, a metalloid abundant in the earth's crust, is ingested mainly through contaminated drinking water. Arsenite [As(III)], which is known to be toxic to humans, is detoxified through conjugation with glutathione (GSH), followed by excretion of the As(III)-GSH adducts via phase III transporters such as the multidrug resistance-associated protein. The Keap1-Nrf2 system regulates phase II xenobiotic-metabolizing enzymes such as glutamate-cysteine ligase and glutathione *S*-transferase, which are required to produce GSH and to conjugate chemicals and phase III transporters. Activation of the Keap1-Nrf2 system to upregulate downstream targets protects cells from As(III) toxicity. Multiple phytochemicals have been identified as Nrf2 activators and may reduce the adverse health effects of arsenic. These phytochemicals are briefly introduced in this review.

Keywords Arsenic · Electrophile · Alkenal · Keap1 · Nrf2 · Coriander

Abbreviations

ARE	Antioxidant response element
BAL	British anti-Lewisite
Cyt19	Arsenic methyltransferase
DMPS	2,3-Dimercapto-l-propanesulfonic acid
DMSA	Meso-dimercaptosuccinic acid
EpRE	Electrophile response element
GCL	Glutamate-cysteine ligase

Y. Abiko (✉) · Y. Kumagai (✉)
Faculty of Medicine, University of Tsukuba, Ibaraki, Japan
e-mail: yumi.abiko@md.tsukuba.ac.jp; yk-em-tu@md.tsukuba.ac.jp

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GST Glutathione *S*-transferase
Keap1 Kelch-like ECH-associated protein 1
MEF Mouse embryo fibroblast
MRP Multidrug resistance-associated protein
NQO1 NAD(P)H quinone oxidoreductase 1
Nrf2 NF-E2-related factor-2
ROS Reactive oxygen species
SFN Sulforaphane

9.1 Introduction

While arsenic has long been used in industry and as a medicine and pesticide, this metalloid is toxic to humans, and thus its intake represents a health risk (see Chaps. 6 and 8). Arsenic is ubiquitous, and drinking arsenic-polluted well water is a major source of chronic exposure worldwide and especially in East Asia (see Chaps. 3 and 4). Replacing the source of drinking water may reduce the risk of exposure and reverse chronic arsenicosis [1]. A 13-month study in residents of Inner Mongolia found that switching to a source of low-arsenic drinking water decreased arsenic levels in blood and urine and restored systemic production of nitric oxide, a reliable marker of vasorelaxation, indicating that arsenicosis can be reversed by exposure cessation [1]. Nonetheless, switching sources of drinking water is almost impossible because of the expense involved. In this chapter, we introduce strategies for decreasing the risk of arsenic poisoning by ingesting preventive agents and phytochemicals.

9.2 Detoxification of Arsenic

9.2.1 Chelation

Inactivating ingested arsenic is essential to reducing its adverse health effects. Generally, chelation therapy is the primary treatment of metal poisoning because chelating agents can sequester metal ions by covalent and/or coordinate binding. 2,3-Dimercaptopropanol (British anti-Lewisite, BAL) was developed as an antidote to 2-chlorovinyldichloroarsine (Lewisite) poisoning during World War II [2, 3]. BAL chelates Lewisite using its two thiol groups to form {2-[(E)-2-chloroethenyl]-1,3,2-dithiarsolan-4-yl}methanol [4] but has limited water solubility, low efficiency, high toxicity, and several side effects [5]. For this reason, meso-dimercaptosuccinic acid (DMSA) and 2,3-dimercapto-l-propanesulfonic acid (DMPS), water-soluble analogs of BAL, have been studied extensively, and DMSA has been used as a major chelation agent [5, 6]. DMSA was found to be effective against arsenic poisoning in humans, mice, and rats [6], and its therapeutic index and half-maximal

lethal dose are higher than those of DMPS [7]. Few epidemiological studies comparing chelation agents for chronic arsenic poisoning have been published. Guha Mazumder et al. compared DMPS and DMSA in a randomized placebo-controlled trial for patients with chronic arsenicosis [8, 9] and found that treatment with DMPS, but not DMSA, significantly reduced symptoms such as physical weakness, skin pigmentation, and symptoms of lung diseases [8, 9].

9.2.2 A Cellular Defense System Against Arsenite

The aquaglyceroporins AQP7 and AQP9 are known to take up arsenite into cells [10] in mammalian systems [11–13]. Following cellular uptake, arsenite will metabolize to monomethylarsenite [MMeAs(III)] catalyzed by *S*-adenosylmethionine-dependent arsenic methyltransferase (Cyt19) or arsenic-glutathione (GSH) adduct [As(III)-SG] catalyzed by glutathione *S*-transferases (GSTs) [14] in the presence of GSH produced by glutamate-cysteine ligases (GCLs) (Fig. 9.1). MMeAs(III) also undergoes GSH conjugation by enzymatic and nonenzymatic reactions of GSTs to form MMeAs(III)-SG adducts that are further converted to MMeAs(III) and dimethylarsenic-GSH adducts [DMeAs(III)-SG] in the presence of Cyt19 [15]. The GSH adducts, but not DMeAs(III)-SG, are excreted from hepatic cells into bile by phase III transporters such as multidrug resistance-associated proteins (MRPs) [16, 17]. Thus, detoxification pathway cascades, GSH conjugation, and excretion into the extracellular space are crucial steps in preventing arsenic toxicity (Fig. 9.1). Indeed, pretreatment with GCL, GST, and MRP inhibitors has been shown to significantly enhance inorganic As(III) [iAs(III)]-mediated toxicity in primary mouse hepatocytes [18].

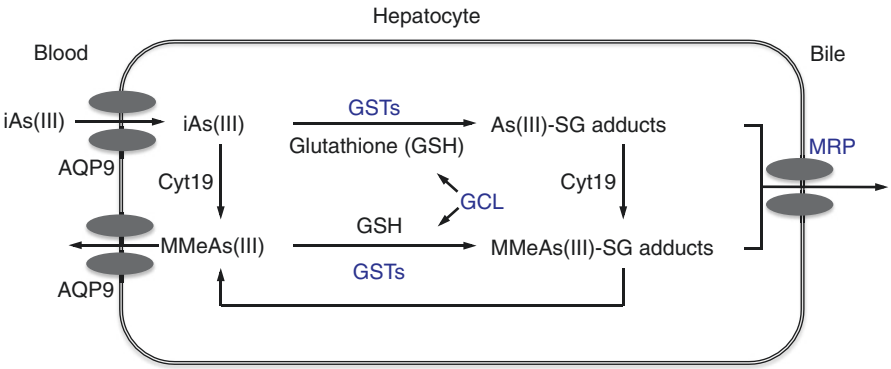

Fig. 9.1 Proposed metabolic pathway of inorganic arsenite [iAs(III)] in hepatocytes. *AQP* aquaglyceroporin, *Cyt19* arsenic methyltransferase, *GCL* glutamate-cysteine ligase, *GSH* glutathione, *GST* GSH *S*-transferase, *MMeAs(III)* monomethylarsenite, *MRP* multidrug resistance-associated protein

9.3 Keap1-Nrf2 System

Transcriptional factor NF-E2-related factor-2 (Nrf2), which is negatively regulated by E3 ligase Kelch-like ECH-associated protein 1 (Keap1), undergoes degradation via the ubiquitin proteasome pathway, resulting in minimal cellular levels of Nrf2 under normal conditions (Fig. 9.2) [19]. Once cysteine (Cys) residues of Keap1 are modified by electrophiles or reactive oxygen species (ROS), Nrf2 newly synthesized is able to avoid such a degradation and thus translocates into the nucleus, where it is activated by binding to the antioxidant response element (ARE) or electrophile response element (EpRE) on DNA following interaction with small Maf proteins (Fig. 9.2) [20]. The transcriptional factor regulates antioxidative proteins such as heme oxygenase 1 (HO-1) and GCLs, phase II xenobiotic-metabolizing enzymes such as GSTs and UDP-glucuronosyltransferases, and phase III transporters such as MRPs (Fig. 9.2). As mentioned earlier, iAs(III) and MMeAs(III) undergo GSH conjugation and subsequent excretion of the polar metabolite into the extracellular matrix, indicating that Nrf2 plays a key role in As(III) detoxification [10]. Pancreatic β-cells with stable knockdown of Nrf2 and pancreatic islets from Nrf2 knockouts ($Nrf2^{-/-}$) have been shown to be more susceptible to acute cell damage caused by iAs(III) and MMeAs(III) [21]. Wang et al. demonstrated that exposure of siNrf2-treated human bladder cells and mouse embryo fibroblast (MEF) cells from a Nrf2 knockout to iAs(III) or MMeAs(III) increased arsenic

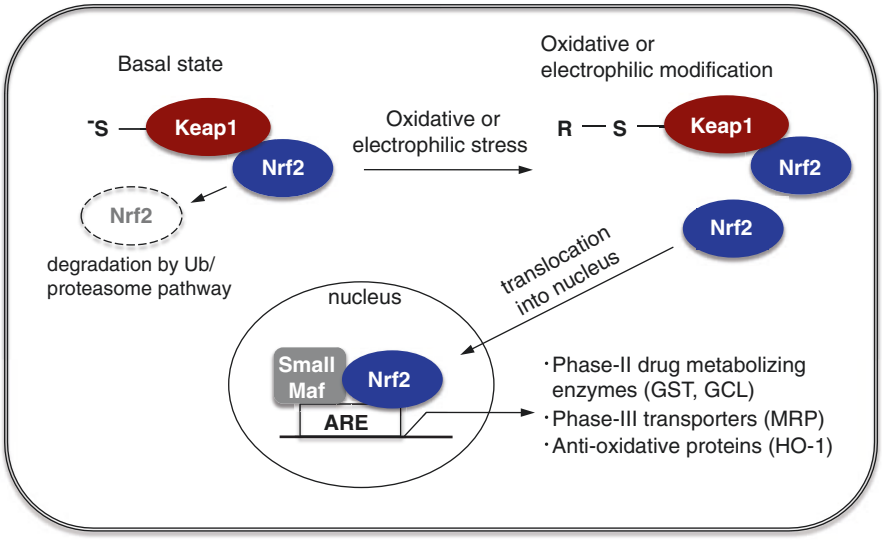

Fig. 9.2 Activation of Keap1-Nrf2 system by oxidative and electrophilic stresses. *ARE* antioxidant response element, *GCL* glutamate-cysteine ligase, *GST* GSH *S*-transferase, *HO-1* heme oxygenase 1, *Keap1* Kelch-like ECH-associated protein 1, *MMeAs(III)* monomethylarsenite, *MRP* multidrug resistance-associated protein, *Nrf2* NF-E2-related factor-2

toxicity [22]. The same group later showed that adding 10 or 100 ppm iAs(III) to the drinking water of Nrf2$^{-/-}$ and wild-type mice caused interstitial edema, congestion, and apoptosis in bladder tissues in the Nrf2$^{-/-}$ mice [23]. Nrf2 deficiency in mice did not alter the iAs(III) methylation profile during treatment with 5 mg/kg for 12 h, indicating that methylation of iAs(III) may not be important for Nrf2-related protection against arsenic toxicity [24]. We previously reported that iAs(III) activates Nrf2, at least in part, by generating ROS that oxidize Cys and upregulate Keap1's downstream targets in human keratinocytes [25].

Knockdown of HO-1 by its siRNA or preincubation with an HO-1 inhibitor prior to iAs(III) exposure enhanced iAs(III) toxicity in HepG2 cells [26], suggesting that antioxidant profile in cells is corresponding to iAs(III)-mediated cell damage. It has also been shown that arsenic-induced inhibition of the ubiquitin proteasome pathway can activate Nrf2 [27]. Taken together, the data suggest that iAs(III)-mediated Nrf2 activation is a cytoprotective response to reduce acute toxicity, although the actual activation mechanisms are still unclear.

9.4 Nrf2 Activation in Plants

9.4.1 Brassica oleracea italica *(Broccoli)*

Prochaska et al. reported a rapid method to detect NAD(P)H:quinone oxidoreductase (NQO1, a phase II enzyme) inducers in vegetable extracts; they found that compounds in broccoli and Brussels sprouts were potent inducers of NQO1 [28]. A separate study found that sulforaphane [(−)-1-isothiocyanato-(4R)-(methylsulfinyl)butane, SFN] in broccoli extract could induce NQO1 [29]. Although NQO1 gene expression is regulated by the xenobiotic response element regulating phase I reactions and ARE/EpRE [30], SFN-induced NQO1 expression is predominantly regulated by ARE through Nrf2 activation [31]. The SFN molecule is highly reactive against nucleophiles such as the thiolate anion because of its isothiocyanate (–N=C=S) moiety that contains electrophilic carbon. SFN easily reacts with Cys residues in the Keap1 protein, such as Cys151, Cys278, and Cys288, which are known to be highly reactive and involved in Nrf2 activation [32, 33]. In accordance, SFN failed to activate Nrf2 in MEFs and macrophage cells from mice expressing a Keap1-C151S mutation [34]. Treatment of primary mouse hepatocytes with 10 μM SFN activated Nrf2 in a time-dependent manner and upregulated Nrf2 downstream proteins such as HO-1, GCLM, GCLC, GSTs, and MRP1 in a concentration-dependent manner [18]. SFN (1, 2.5, 5 μM) also significantly enhanced intracellular GSH levels, presumably by upregulating GCLs. Pretreatment with 5 μM SFN for 24 h prior to iAs(III) (5 μM) exposure significantly reduced arsenic accumulation in the cells and iAs(III)-induced cytotoxicity [18]. While pretreatment with 40 μM SFN for 12 h suppressed acute toxicity of 2 mM iAs(III) in zebrafish, the effect of SFN in a zebrafish mutant (*nrf2*^{fh318}) lacking defenses against oxidative stress was fairly weak [35],

indicating that SFN can protect cells from iAs(III)-induced damage through Nrf2 activation to induce its downstream targets. It should be noted that SFN is stored as an inert precursor glucosinolate, which is converted to SFN by enzymatic reaction of myrosinase in plants [36]. In general, glucoside cannot be absorbed in the body through the gastrointestinal tract. After glucoside is decomposed by intestinal bacterial flora, the aglycone corresponding to SFN would be absorbed. Glucosinolates of isothiocyanates such as SFN must also be converted to the free isothiocyanate by intestinal flora [37].

9.4.2 Coriandrum sativum *L. (Coriander)*

Coriander leaves, often termed cilantro, paxi, or Chinese parsley, are used to flavor dishes and as a remedy for gastrointestinal disorders, pain, inflammation, and oxidative stress [4, 38]. The key phytochemicals driving these effects are not well-characterized, although multiple compounds have been identified in coriander leaves. The n-pentane extract of coriander leaves contains fatty acids and (*E*)-2-alkenals that account for approximately 70% of the oil [39] and its characteristic odor. (*E*)-2-Alkenals are electrophilic because of their α,β-unsaturated aldehyde moiety and easily bind to thiol groups, resulting in formation of the protein adduct [40, 41] and suggesting a possible interaction with Keap1. The Keap1 protein can be modified by 4-hydroxynonenal, which has an α,β-unsaturated aldehyde group, resulting in Nrf2 activation [42]. It has been reported that a 70% ethanol extract of coriander could activate Nrf2 in the HaCaT human keratinocyte cell line and protect the cells from hydrogen peroxide-induced toxicity [43]. To our knowledge, there is no published study identifying coriander phytochemicals that activate Nrf2. We therefore presume that (*E*)-2-alkenals in plants would activate Nrf2 by modifying Keap1, resulting in detoxification of iAs(III) (Fig. 9.3). We identified a series of (*E*)-2-alkenals in coriander hexane extract, including (*E*)-2-hexenal (C_6), (*E*)-2-decenal (C_{10}), (*E*)-2-undecenal (C_{11}), (*E*)-2-dodecenal (C_{12}), (*E*)-2-tridecenal (C_{13}), (*E*)-2-tetradecenal (C_{14}), (*E*)-2-pentadecenal (C_{15}), (*E*)-2-hexadecenal (C_{16}), and (*E*)-2-heptadecenal (C_{17}). These (*E*)-2-alkenals were detected by 2-diphenylacetyl-1,3-indandione-1-hydrazone, which can label aldehyde groups using UPLC-MS and activated Nrf2 (Fig. 9.3) [44]. We found that the (*E*)-2-alkenal group in coriander is essential for activating Nrf2 through *S*-modification of Keap1 and inducing its downstream targets [44]. Of interest, pretreatment of HepG2 cells with (*E*)-2-butenal, but not butanal, prior to iAs(III) exposure decreased intracellular arsenic levels and repressed iAs(III)-mediated cytotoxicity (Abiko et al., in preparation). Overall, we speculate that coriander leaf extract would reduce iAs(III) toxicity via Nrf2 activation by (*E*)-2-alkenals. Since fatty acids such as linoleic acid in plants are biotransformed to (*E*)-2-hexenal, a compound responsible for the odor of green leaves [45, 46], plant matter containing (*E*)-2-hexenal may decrease the adverse health effects of environmental pollutants such as iAs(III).

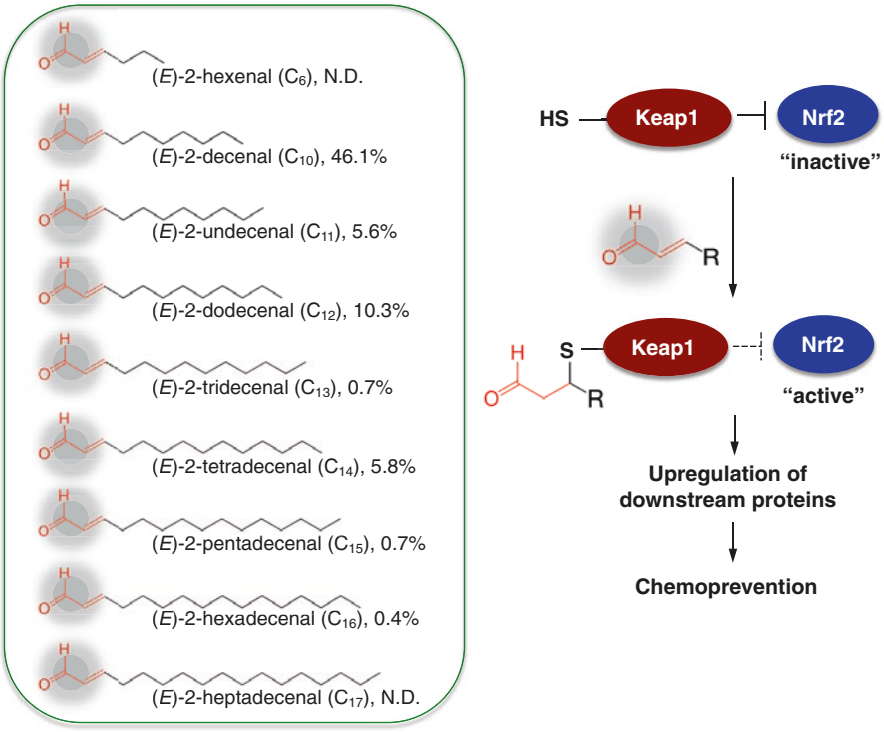

Fig. 9.3 (*E*)-2-alkenals in coriander hexane extract and mechanism of Nrf2 activation by phytochemicals. (*E*)-2-Alkenals detected in coriander hexane extract are shown. The percentages of each (*E*)-2-alkenal by total ion current detected by GC/MS were reported in Potter and Fagerson [61]. Once Keap1 is modified by (*E*)-2-alkenal, Nrf2 is activated and induces its downstream targets to detoxify xenobiotics such as arsenic. *N.D.* not detected

9.4.3 Curcuma longa *L. (Turmeric)*

Curcumin, a major component of turmeric, can exert anti-inflammatory, antitumor, and antioxidative effects in vivo [47, 48]. A field study in India found that curcumin supplementation for 3 months reduced DNA damage and suppressed ROS generation in individuals drinking arsenic-polluted water, presumably by enhancing GSH levels and the activity of antioxidative enzymes such as catalase, superoxide dismutase, GST, and GSH peroxidase [49]. The underlying molecular mechanism that enhances antioxidant activity and suppresses arsenic-induced toxicity, however, is unknown. Catalase and superoxide dismutase are also partially regulated by Nrf2 [50]. Curcumin with two α,β-unsaturated aldehyde carbonyl moieties acts as a Michael acceptor, similarly to SFN and (*E*)-2-alkenal, and has been reported to activate Nrf2 [51–53]. In addition, curcumin is also recognized as a potent antioxidant that scavenges radical species [54]. These findings indicate that curcumin may suppress arsenic toxicity by increasing the expression of Nrf2-regulated enzymes

and scavenging ROS. Apart from turmeric, other *Curcuma* species also contain curcumin and curcuminoids such as demethoxycurcumin, bis-methoxycurcumin, and tetrohydrocurcumin [54]; these phytochemicals may protect cells from arsenic toxicity.

9.4.4 Other Phytochemicals

Simultaneous treatment of rats with rutin (2 g/L in drinking water), a glucoside of quercetin, and iAs(III) (10 mg/kg by gavage) for 6 weeks decreased arsenic levels in the cortex and liver and suppressed arsenic-mediated functional neurotoxic effects [55], suggesting that rutin reduces iAs(III) toxicity by mediating its excretion. As rutin can scavenge ROS and be oxidized into a quinone derivative that can activate Nrf2 [56], the flavonoid may also block iAs(III) toxicity. Curcumin, capsaicin, ellagic acid, fisetin, quercetin, and resveratrol, compounds that activate Nrf2 [53, 57, 58], have been shown to inhibit iAs(III)-induced DNA damage in V79 Chinese hamster lung fibroblasts [59]. From the observations described here, along with reviews from other groups [53, 60], it appears likely that electrophilic phytochemicals in foods may be candidate agents for reducing the adverse health effects of arsenic.

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Chapter 10
Development of Arsenic Removal Technology from Drinking Water in Developing Countries

Yong Fang Li, Da Wang, Bing Li, Liangjie Dong, and Guifan Sun

Abstract At the global scale, drinking arsenic-contaminated groundwater is the most common way for people exposed to arsenic. A number of developing countries have serious arsenic contamination. And thus, developing technologies that could remove arsenic from drinking water has become a major focus of researchers. For developing countries, the technologies applied for arsenic removal are most given consideration of not only effectiveness but also the cost-effectiveness. In this chapter, we reviewed the methods that could be used for arsenic removal from drinking water. It includes coagulation–flocculation, adsorption, membrane technology, oxidation, ion exchange, phytoremediation, and electrokinetics. Of them, the coagulation–flocculation and adsorption were relatively cost-effective and used more often in developing countries. Additionally, we introduced the methods of arsenic removal in drinking water in China and the experience from our group, including a series of research and development of adsorbent material development that could be effective in removing arsenic from drinking water. We hoped that the chapter could provide basic information for researchers in this field and be helpful for them to develop much more effective and cost-effective arsenic removal technologies.

Keywords Arsenic · Arsenic removal · Developing countries · Coagulation–flocculation · Adsorption

Y. F. Li · D. Wang · B. Li · G. Sun (✉)
Research Center of Chronic Diseases and Environment, School of Public Health, China Medical University, Shenyang, Liaoning, People's Republic of China
e-mail: gfsun@cmu.edu.cn

L. Dong
College of Tropical Agriculture and Human Resources, University of Hawaii, Honolulu, HI, USA

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10.1 Introduction

Arsenic contamination in drinking water is a global concern, particularly in developing countries [1]. It has been estimated that more than 30 developing countries around the world were influenced by the arsenic contamination, including Bangladesh, India, China, Vietnam, Thailand, Argentina, Chile, Bolivia, Mexico, Nepal, Pakistan, Afghanistan, Mali, Nigeria, and so on [2–5]. Since water deficiency is also a problem in these countries, developing methods or technologies that could remove arsenic is very necessary [6–8]. Importantly, due to the limitation of economic development and because most of arsenic contamination occurred in rural areas, the arsenic remediation methods applied in these developing countries needed to be efficient, low cost, easy to use, and safe [2]. Therefore, in this chapter, we summarized the development of arsenic removal technology from drinking water in developing countries and aimed to provide some insights from researchers in this field.

10.2 Coagulation–Flocculation

Coagulation–flocculation is the most common method used to remove arsenic from drinking water. In the coagulation processes, chemicals are firstly added to destabilize and convert the dissolved arsenic compounds into insoluble precipitate, and then the solids can be removed through sedimentation and/or filtration [9–14]. Various chemicals can be used as a coagulant in this process, including alum coagulation, iron coagulation, enhanced coagulation by ferric ions with coarse calcite, lime softening, coprecipitation with manganese and iron, and electrocoagulation. Of them, aluminum salts such as aluminum sulfate [$Al_2(SO_4)_3 \bullet 18H_2O$] and ferric salts such as ferric chloride [$FeCl_3$] or ferric sulfate [$Fe_2(SO_4)_3 \bullet 7H_2O$] are commonly used in developing countries because of their low cost and relative ease of handling [15]. At pH 7.6 or lower, iron and aluminum coagulants are of equal effectiveness in removing arsenic (V). The effectiveness of iron coagulants in removing arsenic (III) diminishes at pH 6.0. However, iron coagulants could also show significant higher advantages than aluminum coagulants under pH values above 7.6, or soluble coagulant metal residuals are problematic, or arsenic (III) is present in the water [16]. The results suggested that iron coagulants have much wider scope of application in contrast with aluminum coagulants. And therefore, correctly selected coagulants on the basis of water characteristic can be helpful for achieving satisfactory arsenic removal results. In addition, studies have also shown that the dosage of coagulant usage is associated with arsenic removal, which characterized that the more dosage of coagulant, the higher arsenic removal efficiency achieved. The pH values are another influencing factor for arsenic removal efficacy, and the optimal pH values differed for different coagulants, which implied that adjusting pH values

could be one of the methods to achieve satisfactory results in arsenic removal. Instead of conventional aluminum and iron salts, titanium tetrachloride ($TiCl_4$) was used to remove the particulate and dissolved organic matter from wastewater in sewage treatment plants. The most significant advantage of using $TiCl_4$ as a coagulant is that sludge recovery produces a valuable by-product, namely, titanium dioxide, which is the most widely used metal oxide. Compared with the aluminum and iron coagulants, the cost of titanium tetrachloride is higher which made it limited to be widely used in developing countries.

Coagulation–flocculation is one of the cost-effective arsenic removal technologies and is suitable for treatment of large volume of water with high concentrations of arsenic [2]. A previous report showed that a water treatment system built based on coagulation using ferric chloride was used in 15 hand tube well-sourced drinking water systems in a village in Comilla, Bangladesh, which reduced arsenic from initial maximum concentration of 400 μg/L to less than 20 μg/L in most of the tube wells [17]. The overall cost for this technology was estimated to be in the range of \$0.73–1.70 per m^3 of water [18]. In addition, this technology is also frequently used in combination with other technologies to deal with complex arsenic-contaminated water samples. However, the weakness for the technology is that it required regularly putting chemicals into the water and will produce a large amount of sludge in the process of flocculation. Up to now, how to reasonably dispose sludge to avoid secondary arsenic pollution has become the biggest challenge for popularization and application of this technology.

10.3 Adsorption

Adsorption is a process that uses solids for removing substances from liquid solutions. The technology has been used most widely because of its high removal efficiency, easy operation and handling, and low cost, and it is sludge-free [1]. Several adsorbents have been developed, including metal-based adsorbents (e.g., iron oxide-coated sand, ferrihydrite, red mud, activated alumina, TiO_2, $FePO_4$, MnO_2, MnO_2-loaded resin), carbon-based adsorbents, iron oxide, and activated alumina, to adsorb arsenic from water [19–25]. In contrast to other technologies, adsorption attracted much attention because (1) it usually does not need large volume and additional chemical, (2) it does not need large corollary equipment to establish arsenic removal process [26], (3) it does not produce sludge or other by-products that arouse secondary arsenic pollution risk [27, 28], and (4) various materials could be used as adsorbents which are usually cost-effective [29]. In general, the rate of arsenic removal by adsorption techniques is related with pH values and the chemical forms of arsenic (III or IV). Most studies indicated that the arsenic (V) removal efficacy is higher than arsenic (III) and the removal efficiency is better shown at pH values lower than 7 [30]. In addition, phosphate, silicate, HCO_3^-, and Ca^{2+} in water were also confirmed to have an influence on arsenic removal efficacy

through competing for the adsorption sites [31]. Among the entire spectrum of adsorbents, the activated alumina, iron-based sorbents (IBS), and zerovalent iron (ZVI) were most widely used for arsenic removal from drinking water. However, the major issue for this technology was the secondary arsenic release after the materials that adsorbed arsenic. And then, developing the new materials with high removal efficacy and without secondary arsenic release has become the key point in this field.

10.3.1 Activated Alumina Sorbents

Activated alumina is the first adsorptive medium to be successfully applied for the removal of arsenic from water supplies [32]. It is produced from the thermal dehydration of aluminum hydroxide $Al(OH)_3$ at high temperature. The activated alumina presented a high surface area for good sorption properties. In the adsorption process, water is continuously passed through a bed packed with activated alumina under pressure, and then arsenic was adsorbed on the surface [31, 33, 34]. When adsorption sites on the activated alumina surface become filled, activated aluminum must be replaced. The arsenic removal capacity of activated alumina is related with pH values, arsenic oxidation state, the presence of competing ions, and contact time. Researchers have shown that the relatively high removal capacity of activated alumina for arsenic (V) was observed at pH values 5–6. In contrast, the optimal pH values for high arsenic (III) adsorption capacity of activated alumina were observed at 7–8 [34]. The conventional activated alumina for arsenic removal is relatively low, with a maximum arsenic (V) adsorption capacity of 15.9 mg/g [33]. Along with the development of mesoporous materials, mesoporous alumina gradually attracted much attention in the field of arsenic removal. Patra et al. reported a highly efficient synthetic strategy for self-assembled mesoporous alumina, which has high surface area and high adsorption efficiency for the dissolved arsenic from contaminated aqueous solutions [35]. Another study from Yu et al. reported another method to synthesize mesoporous alumina [36]. The synthesized mesoporous alumina material contains a wormhole-like mesoporous structure and showed 483 m^2/g surface area and 0.82 cm^3/g pore volume. The comparisons of adsorption behavior of mesoporous alumina and conventional activated alumina revealed that the adsorption capacity of mesoporous alumina for arsenate was 5.1 times (61.3 mg/g) higher and the adsorption rate was 3.8 times (1.5 mg/g min) faster than that obtained with an activated alumina. The good performance of mesoporous alumina on adsorption behavior is majorly associated with the larger surface area and pore volume of the mesoporous alumina.

The cost of activated alumina adsorbents in arsenic removal depends on the synthesized methods [37]. Sen and Pal et al. developed a low-cost ($2.39–3.20 per m^3 of water) activated alumina with specific surface area of 335–340 m^2/g from a gibbsite precursor through partial thermal dehydration method [38]. The producing

method was reported to be $1152 per ton, which was approximately 39% lower than the gel precipitation method. But, the cost to synthesize mesoporous alumina is relatively higher than conventional activated alumina [37, 39].

10.3.2 Iron-Based Sorbents (IBS)

In recent years, the enthusiasm for developing and using iron-based sorbents to treatment of arsenic in drinking water is increased [40]. The underlying mechanism for iron-based sorbents to remove arsenic is thought to be ion exchange, specific adsorption to surface hydroxyl groups, or coprecipitation. There are two important iron-based materials, which are hydrous ferric oxide (HFO) and goethite [39]. The two materials are used as sorbents, but goethite is less reactive than HFO due to the lack of sufficient surface area. Similar with activated aluminum, the removal efficiency of iron-based sorbents was associated with arsenic chemical form, pH values, water quality, contact time, and dosage of iron-based adsorbent used [41].

10.3.3 Zerovalent Iron

Zerovalent iron (ZVI) consisted of an elemental iron core surrounded by a shell of corrosion products, especially magnetite [42, 43]. Since ZVI is nontoxic, abundant, cheap, and easy to produce, a great number of studies had been carried out to explore its efficiency in removal or immobilization of a variety of contaminants, including arsenic in drinking water [42]. These studies also provide evidence on the influence factors of arsenic removal of ZVI. Studies conducted field column and laboratory batch experiments to assess the performance of ZVI in removing arsenic from geothermal waters, in which phosphates and nitrates were present [44–46]. The field study demonstrated that ZVI could effectively remove not only arsenic but also phosphate and nitrate from water. In addition, batch study results showed that arsenic (V) exhibited greater removal rates than arsenic (III) and the removal efficacy decreased when phosphate and nitrate were present in water. The temperature of the water was found to play a dominant role on the kinetics of arsenic removal [47]. Another study from Biterna et al. showed that ZVI could effectively remove arsenate from tap water under dynamic conditions. The concentrations of arsenic in treated water were under the limitation of arsenic proposed by the WHO [48]. However, the efficiency of ZVI to remove arsenate decreased at the presence of borate and organic matter, particularly at higher concentrations [49]. Bang et al. also conducted batch and column experiments to investigate the effects of dissolved oxygen (DO) and pH on arsenic removal with ZVI [50]. Their findings suggested that arsenic removal was significantly affected by DO content and the pH values of the

solution. Under toxic conditions, arsenic (V) removal efficiency by ZVI was faster than arsenic (III). At the pH value of 6, more arsenic (V) was also removed compared to arsenic (III) [99.8% vs. 82.6%]. The authors also proposed that the removal of arsenic by ZVI was attributed to adsorption by iron hydroxides generated from the toxic corrosion of ZVI, which might be an underlying removal mechanism of arsenic [51]. Findings showed that the removal of arsenic is a two-step reaction with fast initial disappearance of arsenite followed by a slow subsequent removal process. Kinetic analysis indicated that arsenic removal behaves as a zero-order reaction at high arsenic concentrations. The arsenic removal capacity of ZVI in this study was determined to be approximately 7.5 mg arsenic/g Fe [52]. According to the above researches from the laboratory, ZVI could effectively remove different forms of arsenic from drinking water, and the capacity was influenced by pH, DO, ion in water, and temperature. Apart from these laboratory experiments, up to now, there are also lots of filters constructed on the basis of ZVI and had been applied in some developing countries, such as India, Bangladesh, and Pakistan. A study from Bangladesh reported that the arsenic concentrations in groundwater treated by a filter developed based on ZVI were lower than 10 μg/L. And the filter could work without any chemical treatment, without regeneration, and without producing toxic wastes. The costs of this filter were about $40/5 years, and it could treat 20–30 L/h for daily drinking and cooking that is needed by 1–2 families. Given the highly effective arsenic removal efficacy and safety, this filter was approved by the Bangladesh government thereafter, and about 30,000 SONO filters were deployed all over the country and continue to provide more than a billion liters of safe drinking water.

In recent decades, nano-ZVI, a nanoparticle, is effective and extensively used for the removal of arsenic from contaminated water. As listed in the study from Zhu et al., nanoscale ZVI was supported onto activated carbon (NZVI/AC) by impregnating carbon with ferrous sulfate followed by chemical reduction with NaBH4. The adsorption capacity of arsenite and arsenate by NZVI/AC at pH 6.5 calculated was 18.2 and 12.0 mg/g, respectively. And the study revealed that phosphate and silicate in water markedly decreased the removal efficacy of both arsenite and arsenate, while the influence of other anions and humic acid (HA) on the removal efficacy was not significant. In addition, the metal cations (Ca^{2+}, Mg^{2+}) could enhance arsenate adsorption, but ferrous iron was demonstrated to suppress arsenite adsorption [53]. Tanboonchuy et al. investigated the removal efficacy of air and/or CO_2 bubbling NZVI for the removal of high concentration of arsenate (3000 μg/L) [54]. In this study, CO_2 bubbling at 300 mL/min for 5 min was taken to adjust the solution pH to an acidic environment, followed by air bubbling at 300 mL/min for 10 min to increase DO in the solution. In the treatment period, NZVI was applied to remove arsenate which resulted in outstanding performance in arsenate removal. Later, another report from the same group investigated the influence of background species on the removal of arsenic (III) and arsenic (V) in groundwater by NZVI process [55]. They found that Ca^{2+} plays a promoting role, while PO_4^{3-} and HA play an inhibiting role on removal of arsenic.

In arsenic removal with Cl^- and HCO_3^-, the former enhances arsenic (III) removal, whereas the later inhibits arsenic (III) removal; arsenic (V) removal was affected slightly in the presence of Cl^- and HCO_3^-. Although NZVI is effective for the removal of arsenic from contaminated water, the underlying mechanism is not clearly understood.

10.3.4 Other Adsorbents

Apart from adsorbents developed based on active alumina and ZVI, a number of other adsorbents were also synthesized for the removal of arsenic. Activated carbon [42], aluminum-loaded shirasu zeolite, iron oxide-coated polymeric materials, polymeric hybrid sorbent, zirconium-loaded activated carbon, natural hematite, fly ash [56], modified fly ash [57], and nanoparticles of hydrous iron oxide [58] have been reported to be used as adsorbents for the removal of arsenic from water.

Activated carbon was produced from coconut shell, peat, and coal. Rajakovic et al. observed a high arsenic adsorption capacity of activated carbon, and they found that carbon pretreated with Ag+ or Cu^{2+} ions improved the arsenic (III) adsorption but reduced the arsenic (V) adsorption [59]. In addition, carbon was also used as a medium to impregnate different metal ions to improve their adsorption capacity. For example, Gu et al. prepared iron-containing adsorbents for granular activated carbon (GAC) for effective removal of arsenic from drinking water [60]. They found that GAC could remove arsenic most efficiently on the condition that the iron content was approximately 6%. The removal of arsenate occurred in a wide range of pH (4.4–11), but the efficiency was decreased when pH was higher than 9.0. In addition, the presence of phosphate and silicate could significantly decrease arsenic (V) removal at pH > 8.5, while the effects of sulfate, chloride, and fluoride were found to be minimal. Groundwater containing 50 μg/L of arsenic treated by GAC could result in both arsenic (V) and arsenic (III) concentration reduction to less than 10 μg/L within 6000 empty bed volume. The removal efficiency of activated carbon is found to be associated with arsenic chemical form, pH values, ions' presence in water, and contact time and also depends on carbon type and carbon pretreatment.

10.4 Membrane Technology

Typically, membranes are synthetic materials with billions of pores acting as selective barriers, which allow some constituents of water to pass through while others are excluded or rejected. A driving force, such as pressure difference between the two sides of the membrane, is needed to transport the water through the membrane. Generally, the membrane filtrations could be divided into two types, including low-pressure membrane processes and high-pressure membrane processes [61, 62].

10.4.1 The Low-Pressure-Driven Membrane Process

Microfiltration (MF) and ultrafiltration (UF) belong to low-pressure membrane process. MF was used for separating colloidal and suspended particles in the range of 0.1–10 μm. And thus, the MF alone cannot remove the dissolved arsenic from water. The particle size of arsenic-bearing species must be increased prior to MF process, and the most popular methods to increase the particle size of arsenic species are coagulation and flocculation. Han et al. [63] previously invested the removal capacity of MF on arsenic. The authors combined MF with flocculation wherein ferric chloride ($FeCl_3$) and ferric sulfate [$Fe_2(SO_4)_3$] were used as flocculants. Results showed that the arsenic removal efficiency obtained through the combination of flocculation and MF is higher than MF alone. However, the pH of the water and the presence of other ions are major factors affecting the efficiency of this arsenic immobilization. Another study from Ghosh et al. [64] explored the arsenic removal efficacy of a combination of electrocoagulation (EC) and MF by using a ceramic membrane. The findings showed that arsenic concentration decreases from 200 to 8.7 μg/L, which suggested a good performance.

Similar with MF, UF alone is also not an effective technique for the treatment of arsenic-contaminated water due to large membrane pores. Surfactant-based separation, such as micellar-enhanced ultrafiltration (MEUF), was used as major combination method to remove arsenic from water. Several studies have already focused on arsenic removal using MEUF. In a previous study, the authors compared the difference of arsenate removal efficiency among different cationic surfactants, including benzalkonium chloride (BC), hexadecylpyridinium chloride (CPC), hexadecyltrimethylammonium bromide (CTAB), and octadecylamine acetate (ODA) [65, 66]. Among these four cationic surfactants, the findings showed that CPC demonstrated to be the most effective material with regard to arsenic removal efficiency. Despite the effective removal of arsenic, the concentration of the surfactant in the effluent is relatively high, and thus it needs to be further treated with powdered activated carbon (PAC) before being discharged to the environment.

10.4.2 The High-Pressure-Driven Membrane Process

Nanofiltrations (NFs) and reverse osmosis (RO) belong to high-pressure membrane process. NFs can significantly remove arsenic in water with less suspended solids. Findings from Uddin et al. [66] have shown that the arsenic (V) removal efficiency of NFs was better than arsenic (III). Even when the feed solutions contain 50 μg/L arsenic, arsenic (III) could not be reduced to the maximum contaminant level (MCL), and thus oxidation of arsenic (III) to arsenic (V) might be an essential pretreatment method. Findings from Sato et al. also reported similar results [67].

The history of RO use to remove arsenic is long. In the 1980s, researchers have developed cellulose acetate RO membrane and evaluated the arsenic removal efficacy [68]. The researchers found that arsenic (V) removal efficiency could achieve higher than 90% with the RO system, while arsenic (III) removal efficiency was less than 70%. The difference of removal efficacy with regard to arsenic (V) and arsenic (III) was similar with NF. And the findings further demonstrated the significance of pre-oxidation stage in arsenic removal when using both NF and RO.

10.5 Other Technologies and Methods

In addition to the abovementioned methods, oxidation, ion exchange, phytoremediation, and electrokinetics were also used to remove arsenic from water. The main purpose of oxidation is to convert the soluble arsenic (III) to arsenic (V), which is then followed by precipitation of arsenic (V). The technology is necessary and essential for arsenic removal because arsenic (III) is the predominant form of arsenic at neutral pH conditions. Various traditional chemicals including chlorine, chlorine dioxide, ozone, hydrogen peroxide, chloramine, permanganate, and ferrate have been used to convert arsenic (III) into arsenic (V) in many studies. Among these oxidants, chlorine, ozone, and permanganate are faster compared to hydrogen peroxide and chloramine for the oxidation reaction of arsenic (III) to arsenic (V). Apart from these chemical oxidants, UV light is also widely used as a method to induce oxidation reaction [69]. Studies have shown that the oxidation rate of arsenic (III) in water can be increased by UV irradiation in the presence of oxygen. However, the economic benefit of UV light in reducing arsenic from drinking water needs to be evaluated [70, 71].

Ion exchange has been used for removal of arsenic in drinking water for a long time [72]. The efficacy of ion exchange process was influenced by several factors, such as arsenic form, pH values, the concentration of competing ions (e.g., sulfates, nitrates, and phosphate), and total dissolved solids (TDS). In general, the ion exchange for arsenic removal is only applicable for source water with low TDS and low sulfate.

Phytoremediation is an environment-friendly technology to remove arsenic. A number of plants (e.g., *Pteris vittata*), bacteria, and other species (e.g., *Paenibacillus*, *Pseudomonas*, *Haemophilus*) have been demonstrated to have the capability of hyperaccumulating large amounts of arsenic [73–75].

Apart from these technologies, the storage of rainwater is also an important way to avoid arsenic exposure in Southeast Asian countries where rainwater is rich. In the high-arsenic-exposure region, the storage cistern was built by family and used for collecting rainwater for cooking and drinking (Fig. 10.1).

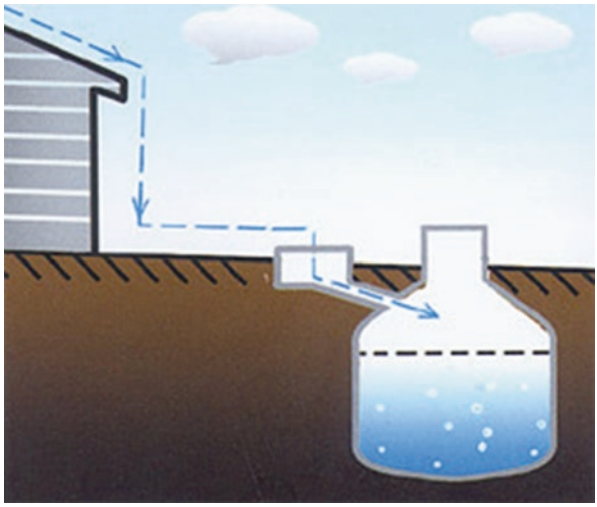

Fig. 10.1 Rainwater storage tank used for drinking and cooking installed in the house

10.6 China's Methods and Researches on Arsenic Removal from Our Group

10.6.1 Experiences from China

China is one of the most serious arsenic-contaminated countries around the world. Apart from drinking-water-type endemic arsenism, coal-burning-type endemic arsenism also occurred in China [76]. During the past decades, a series of methods have been applied to reduce the health hazards caused by arsenic. The most effective and directive method is water improvement program throughout the whole country. Since 2006, almost all arsenic-contaminated villages (containing wells with arsenic concentration higher than 50 μg/L) started to use tap water instead of arsenic-contaminated well water [76]. The water sources used in the "water improvement program" included river, lake, rainwater, as well as groundwater without arsenic contamination. The Water Conservancy Department is responsible for finding available water resources, and the Ministry of Health is responsible for detecting arsenic concentration. Until 2015, almost all high-arsenic-exposed villages have conducted water improvement of tap water in family. With respect to those rural regions and sporadic arsenic-contaminated wells, the abovementioned arsenic removal technologies could be used based on family.

10.6.2 Researches on Arsenic Removal from Drinking Water by Our Group

Since the occurrence of arsenic poisoning in China, our research group has focused on investigating the biological role of arsenic and the epidemiological characteristics and the mechanism of arsenic poisoning [77–86]. Under the support of the United Nations Children's Fund (UNICEF), our group compiled an atlas of clinical diagnosis of endemic arsenism and extended it to other arsenic-contaminated countries [87]. Given that the wells with high levels of arsenic showed a scatter-distributed pattern, our group firstly developed a "10% sampling method" to increase the efficiency of field survey [88]. In 2013, our group in cooperation with the Swiss Federal Institute of Aquatic Science and Technology (Eawag) developed the first predictive risk model for geogenic groundwater arsenic contamination in China. The model provides another convenience method to screen arsenic-contaminated areas in China [40].

As in other countries, drinking-water-type endemic arsenism in China is majorly occurring in rural areas. Along with the large-scale water improvement which has been finished by the government, the main issue for arsenic removal is focused on the remote mountainous areas and residents who live in scattered rural areas, where the government-centralized water supply is very difficult. Therefore, the removal of arsenic from water in the family unit is the main problem to eliminate arsenic pollution in drinking water.

For these regions, developing arsenic removal materials or technologies that could be used in the unit of family is an imperative requirement. Although the water purification devices developed on the basis of the abovementioned technology are everywhere in the market, the limited arsenic removal efficacy, secondary arsenic release from the used materials, high price, and inconvenient use have gradually become the main concerns. Given the background, our group together with researchers from the University of Hawaii developed a ceramic-based adsorbent material, which is characterized by high arsenic removal efficacy through adsorption, without secondary arsenic contamination, cheap price, and convenient use [89]. After numerous laboratory experiments and field verifications, the arsenic removal materials named as "MesoNose ceramic" were successfully developed.

Through nanotechnology using natural ingredients, the materials were manufactured into mesoporous (about 40–50 nm which is called meso) materials. There are two generation products based on this technology: (1) MesoNose water pitcher, which uses "MesoNose ceramic" particles to be the pitcher filter element, and (2) Mesopaper (Company No. 93335/Est No. 93335-CA-1) which sandwiched the

"MesoNose ceramic" powder between two layers of bamboo fibers to create a special paper for water and wastewater filtration removing arsenic. The MesoNose water pitcher with ceramic particles as the carrier of adsorption is the first product granted with the certification of safely reducing arsenic in drinking water by NSF International [90]. And the Mesopaper is the first paper water filter in the world that removes arsenic and lead and inactivates bacteria and viruses which was just granted with the certification of safely reducing arsenic in drinking water by NSF International in March of 2018. Currently, the water purification devices and equipment based on this material have been sold in the market.

For the products, our group has made a large fieldwork to evaluate the efficacy in removing arsenic in China's arsenic-contaminated regions (Fig. 10.2). All findings have shown that more than 90% arsenic could be filtered using the products. The products are of simple usage, highly efficient, cost-effective, and safe. There are no hazardous filtration by-products. We called the meso-ceramics approach to be achieving sustainability via five Cs: clean water, clean soil, clean air, clean energy, and cost-effective.

Clean water: the solution of arsenic filtration does not generate wastewater.
Clean soil: arsenic is immobilized in the material, and used materials nor hazard waste has no pollution to the soil.
Clean air: the circle of material has no arsenic emission to air.
Clean energy: the micro- or mesoporous ceramic material makes the filtration achieve gravity flow or low pressure flow that does not require energy consumption.
Cost-effectiveness: the clay and iron are used as key ingredients, and low energy is used; therefore, it will make the material affordable for end users in the developing countries.

It can be recycled or landfilled without any secondary environmental negative impacts. It can be used for household, industrial, and municipal wastewater treatment sites because the self-standing paper can be molded into many shapes such as pour-through filters, straw filters, and industrial filtration cartridges.

Fig. 10.2 The field test conducted in Gansu (left) and Hunan (right)

Additionally, based on this technology, our research team currently is trying to find out a more effective way which can remove not only arsenic but also other heavy metal elements from water simultaneously and diversify its practical application.

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Chapter 11
Agronomic Strategies for Reducing Arsenic Risk in Rice

Satoru Ishikawa, Tomohito Arao, and Tomoyuki Makino

Abstract Dietary exposure to arsenic (As) has become a serious issue because it may pose a health risk. In particular, rice is a major source of inorganic As (the more toxic form) for a large part of the world's population. The greater assimilation of As by rice than by other crops is mainly attributed to two reasons: the high arsenite bioavailability in reductive paddy soil and the high ability to transport arsenite through silicon transporters. The fundamental mechanisms relating to As uptake and transport from soil to rice grains have been increasingly explored. Along with an advanced understanding of As mechanisms, techniques have been developed to minimize As levels in rice. Here, we propose three strategies that can be applied to paddy fields as countermeasures for As reduction. First, intermittent irrigation is effective at reducing As levels in rice grains, but this approach may increase cadmium (Cd) levels. Consequently, growing low-Cd cultivars aerobically is the practical way to simultaneously reduce the Cd and As levels in the rice grains. Second, the application of iron-bearing materials is effective at reducing As and Cd in rice grown under flooded conditions. Third, the selection of low-As cultivars and the modification of the genes responsible for grain As levels are promising possibilities. We confirmed that the As levels in the grains were significantly lower in rice lines overexpressing *OsPCS1*, which encodes an enzyme for synthesizing the phytochelatin necessary to conjugate arsenite. These strategies would help greatly reduce the As levels in paddy rice.

Keywords Arsenic species · Cadmium · Iron-bearing materials · Irrigation Low-cadmium cultivar · Phytochelatin · Paddy field · Plant breeding · Rice Transporters

S. Ishikawa (✉) · T. Makino
Institute for Agro-Environmental Sciences, National Agriculture and Food Research Organization (NARO), Ibaraki, Japan
e-mail: isatoru@affrc.go.jp

T. Arao
Institute Central Region Agricultural Research Center, National Agriculture and Food Research Organization (NARO), Ibaraki, Japan

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11.1 Introduction

Arsenic (As) is a metalloid that is widely dispersed in the environment, and it is a primary concern in the public health field because it is a carcinogen. Arable soils inevitably contain naturally derived As, and crops grown on these fields assimilate As from the soil. The As levels in crops vary depending on the species, growing conditions, and As availability in soils. Paddy rice is a plant of particular interest because it accumulates As at higher concentrations in the grains than other crops [1]. Arsenite, which is inorganic As (iAs) and has high toxicity in living organisms, is present as a major As species in paddy soils under aerobic conditions [2], and rice can efficiently absorb this species via silicon transporters [3]. Rice is a major contributor to iAs exposure for Asian populations that consume rice as a staple food. For example, in Japan, rice and rice cakes contributed 97% of the estimated daily iAs intake from cereals, occupying the greatest proportion (62%) of the total daily iAs intake [4]. Therefore, we urgently need to establish practicable techniques for reducing iAs in rice to diminish the risk it poses to human health. In this chapter, we provide an overview of the standard regulatory law and current status relating to As levels of rice in Japan and several other countries. We also present the latest and most promising techniques to mitigate As accumulation in rice as developed by our research group at the National Agriculture and Food Research Organization (NARO). We believe that these techniques are applicable to Japanese paddy fields and would also help prevent risk of As exposure from rice throughout Asia.

11.2 Current Status and Regulations of As Contamination in Rice

In several areas of Japan, agricultural lands were heavily contaminated with As by irrigation using river flow from the area near the mine, and agricultural damage was observed [5]. To prevent metal pollution in the agricultural lands of Japan, including from As, the *Agricultural Land Soil Pollution Prevention Law* was enforced in 1971; the criterion for As was set at 15 mg kg^{-1} of 1 M HCl-extractable As from soils to prevent rice yield losses of more than 10% [6]. An area of 391 ha was designated as As-contaminated fields with soil As concentrations of over 15 mg kg^{-1}; by 2016, an area of 326 ha was already cleaned up by soil dressing that replaced the polluted soil with unpolluted soil. For the regulation of residual agrochemicals in food, the As concentrations in some fruits and vegetables have been limited to 1 mg kg^{-1} (3.5 mg kg^{-1} for the exocarp of some fruits) by the *Food Sanitation Law*, although the registrations of pesticides containing As have already expired in Japan. The Codex Alimentarius Commission of the Joint FAO/WHO has adopted maximum levels (MLs) for iAs in rice of 0.2 mg kg^{-1} for polished rice [7] and 0.35 mg kg^{-1} for husked rice [8]. The EU and China also set a maximum allowable level of iAs in rice at 0.2 mg kg^{-1}. Furthermore, the EU has set a 0.1 mg kg^{-1} level for rice-based

foods destined for infants and young children because these populations have a higher consumption rate per body weight unit than adults [9]. Japan does not have a regulation for iAs in rice.

Geographical variations in the iAs concentrations of rice grains from within and across countries have been well investigated. For example, Li et al. [10] analyzed the iAs concentrations of 446 rice samples collected from 15 primary rice-growing provinces and autonomous regions in China. The iAs concentrations varied widely, ranging from 0.071 to 0.567 mg kg^{-1} for husked rice and from 0.028 to 0.217 mg kg^{-1} for polished rice, and regional differences in the mean iAs concentrations of both types of rice were found. A market basket survey by Meharg et al. [11], which collected 901 polished rice samples from ten countries, showed that the median total As concentrations of rice varied sevenfold depending on the country of origin, with Egypt (0.04 mg kg^{-1}) and India (0.07 mg kg^{-1}) being the lowest and France (0.28 mg kg^{-1}) and the USA (0.25 mg kg^{-1}) being the highest. The median iAs concentrations ranged from 0.03 to 0.12 mg kg^{-1} for a subset of 63 samples from five countries.

According to the data cited from a document by the Codex Committee on Contaminants in Foods, the mean iAs level of husked rice was highest in Japan (0.21 mg/kg, $n = 600$) among the nine countries included in the comparison [12]. The iAs concentrations varied widely, ranging from 0.03 to 0.59 mg kg^{-1}, indicating that there must be geographical variation because there is little genotypic difference in grain iAs among Japanese cultivars [13]. China showed the second highest mean iAs level in husked rice (0.17 mg kg^{-1}, $n = 942$), and the Republic of Korea had the lowest (0.10 mg kg^{-1}, $n = 250$). Specific water management choices for paddy rice might lead to higher iAs levels in rice grains produced in Japan than in other countries. To reduce the cadmium (Cd) level in rice grains, maintaining flooded conditions for 3 weeks before and after heading is recommended and practiced in some areas of Japan. In addition, this submerged condition is an effective way to prevent the reduction of grain quality caused by global warming because high temperatures cause poor starch synthesis [14]. Although flooded conditions are profitable for reducing grain Cd levels and improving grain quality, these conditions increase the As concentrations in rice because of the high solubility of arsenite in paddy soil (see Sect. 11.3.1). Further analyses on the high iAs level of rice grains produced in Japan are necessary.

Apart from iAs, aromatic arsenical contamination in rice has been reported as a special case. Chemical warfare agents containing aromatic arsenicals (AAs), such as Clark I (diphenylchloroarsine), are well documented. They were primarily produced as emetic and vesicant agents during World Wars I and II; after World War II, these agents were dumped into the sea or buried underground in Europe, China, Japan, and other countries. In 2002, residents of the Kizaki area in Kamisu, Ibaraki, Japan, exhibited uncommon clinical symptoms of the central nervous system. Ishizaki et al. [15] analyzed the well water in the area and detected AAs, such as diphenylarsinic acid (DPAA). The origin of the DPAA in the groundwater was not determined. The groundwater was also used for the irrigation of paddy fields in the area, and the rice grains harvested from the contaminated paddy fields in 2004

contained DPAA and methylphenylarsinic acid (MPAA) [16]. Concrete block containing DPAA at a high concentration was discovered around the well, and it was suspected that this is an origin of contamination [17]. The concrete block was removed from the surrounding polluted soil, and the contaminated groundwater was cleaned up from 2009 to 2011. As a result, the arsenic concentration in the groundwater has decreased greatly [18].

The uptake of AAs from agricultural soils was investigated through pot experiments [19, 20]. MPAA was detected in brown rice grown on contaminated soil. Dimethylphenylarsine oxide (DMPAO) and methyldiphenylarsineoxide (MDPAO) were detected in the straw but not in the grains grown in the contaminated soil. In the contaminated soil, the phenylarsonic acid (PAA) and MPAA concentrations decreased, and DMPAO concentration increased under the flooded conditions; however, their concentrations remained unchanged under upland conditions [19]. Activated charcoal amendments were effective at reducing the AAs in rice and soybeans [21]. In autoclaved soil under anaerobic conditions, DPAA underwent little degradation during the 24-week incubation. In unautoclaved soils, the DPAA concentration clearly decreased after 24 weeks of incubation, indicating that the DPAA degradation was driven by microbial activity [22]. AAs were adsorbed more strongly onto the Andosol than the Fluvisol. The mobility of MDPAO and DMPAO in acidic soil with low clay and oxide contents (Fluvisol) was higher than that of PAA and MPAA [23].

11.3 Strategies for Reducing As in Rice

11.3.1 Water Management

When rice plants are cultivated under flooded conditions, the iAs in paddy soil is more easily solubilized and accumulated in rice grains than under aerobic conditions. By contrast, more Cd accumulates in rice under aerobic conditions [24]. The relationships between the dissolved As and Cd concentrations and the air-filled porosity suggest that there is an optimum soil wetness that simultaneously minimizes the solubilization of both As and Cd [25, 26].

The effects of water management on the Cd and As levels in rice grains were investigated in pot experiments [24]. Flooding for 3 weeks before and after heading was most effective at reducing the grain Cd concentration, but this treatment increased the As concentration considerably. Conversely, the aerobic treatment during the same period was effective at reducing the As concentrations but markedly increased the Cd concentrations.

Field experiments were conducted to investigate the optimal Eh and pH conditions of the soil that simultaneously decreased iAs and Cd accumulation in rice [27]. The water management techniques during the experiments included continuous flooding and intermittent irrigation at different intervals after midseason drainage. Intermittent irrigation with 3 days of flooding and 5 days of drainage was effective simultaneously decreasing the accumulation of iAs and Cd in the grains (Fig. 11.1).

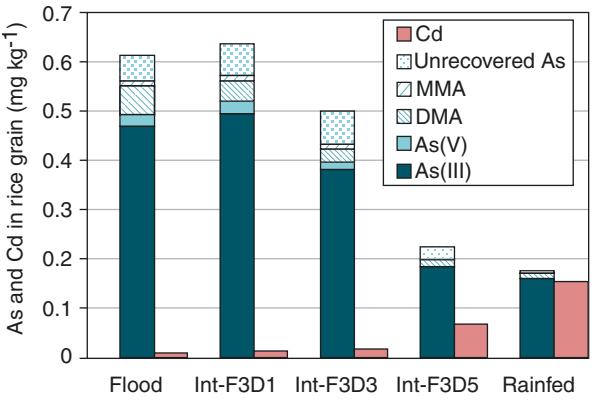

Fig. 11.1 Effects of different water management choices on the As and Cd concentrations and As speciation in rice grains. *DMA* dimethylarsinic acid, *MMA* monomethylarsonic acid [27]

The Eh, pH, dissolved As, Cd, and Fe(II) concentrations in soils were strongly affected by the water management practices. Neither the grain yield nor the growth of the rice plants was significantly different among the plots. The water management choice did not affect the grain quality. In practical situations, the efficiency of water management practices may be affected by the size of the paddy fields, owing to a possible delay in drainage. Further research is needed to confirm whether the optimal irrigation interval is widely applicable across different soil types, weather conditions, and As and Cd concentrations in soils.

A *japonica* rice mutant with nearly undetectable levels of Cd in the grain was produced [28]. The name "Koshihikari Kan No. 1" was chosen, and in 2015, the cultivar was registered in Japan. This cultivar and "Koshihikari," its parent and the most popular cultivar in Japan, were grown in paddy fields with different soil properties under three water regimens, namely, flooded conditions (FLD), with flooding for 2 or 3 weeks before and after heading; alternate wetting and drying conditions (AWD), with the re-flooding of the soil just after the disappearance of the ponding water; and water-saving conditions (WAS), with irrigation water being reapplied after drying of the soil surface. FLD is normally implemented in areas in which rice could potentially exceed the maximum allowable Cd level (0.4 mg kg^{-1}) in rice grains. AWD is the conventional regimen for cultivating paddy rice in Japan. For WAS, the duration of the dry soil period during one cycle ranged from 2 to 7 days, depending on the water-holding capacity of the soil at each site. To prevent water stress at heading under WAS, AWD was applied for 10 days after the emergence of several panicles, and WAS was then repeated. For all fields, the irrigation water was drained 7 days before harvesting. All water regimens were designed such that they could be easily applied by farmers.

We analyzed the As species in rice samples from Ishikawa et al. [29], and effects of water regimens on the concentration of iAs and Cd in brown rice were evaluated (Fig. 11.2). Inorganic As was the dominant species in the rice grains, irrespective of the site, cultivar, and water regimen. The mean grain iAs concentrations of "Koshihikari" and "Koshihikari Kan No. 1," except at sites D and F, were 23.5% and 20.6% less under the AWD regimen and 43.3% and 45.9% less under the WAS

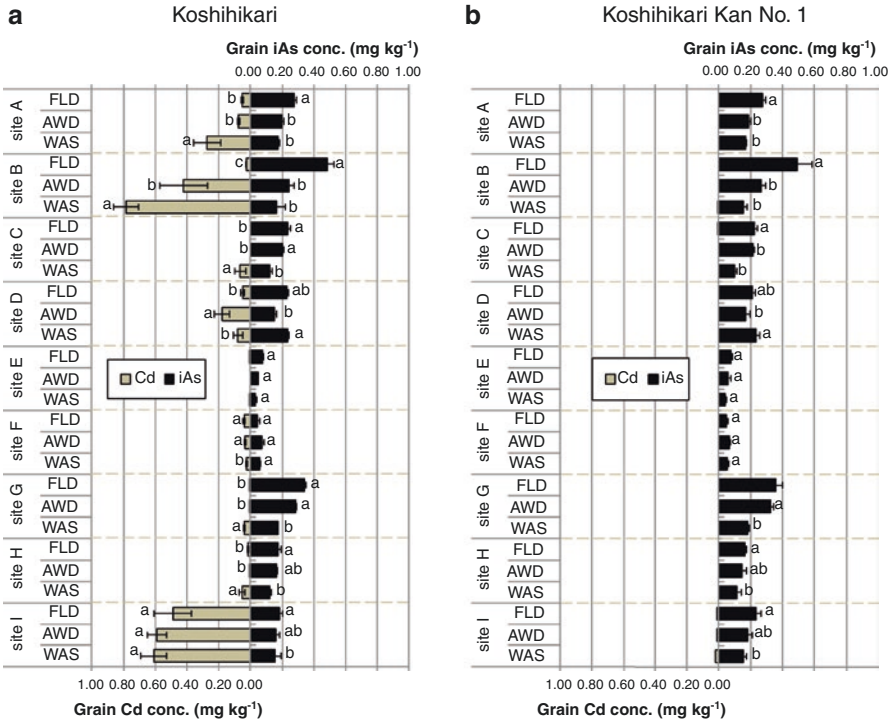

Fig. 11.2 Effects of the three water regimens (*FLD* flooding, *AWD* alternate wetting and drying, *WAS* water-saving) on the concentrations of inorganic As and Cd in brown rice of (**a**) Koshihikari and (**b**) Koshihikari Kan No.1 grown at the nine experimental sites (the analyzed inorganic As in rice samples from [29]). Bar at a site labeled with different letters differs significantly (Tukey-Kramer test, $p < 0.05$)

regimen than under FLD. Similar results were observed in total As concentrations in the same rice samples [29].

Because of the high rainfall at site D, the paddy soil remained wet from midseason drainage to heading, and the soil Eh remained negative even under WAS, resulting in no difference in the grain As concentrations between WAS and FLD for both cultivars. Arsenic was not detected in the soil solution at sites E and F (both Andosols), and the grain As concentrations were also markedly lower at these sites than at other sites, even under the FLD regimen. In addition to the high amounts of noncrystalline Fe, Andosols generally have high organic matter contents, leading to decreased As bioavailability for plants. Thus, rice grown in Andosols is likely to have lower grain As concentrations than rice grown in other soil types.

Although the AWD and WAS treatments markedly increased the grain Cd concentrations in "Koshihikari," "Koshihikari Kan No. 1" had nearly undetectable levels of grain Cd in all treatments. Compared with that of "Koshihikari" under FLD, the grain yield of "Koshihikari Kan No. 1" and "Koshihikari" decreased by an average of 2% for AWD and 4–6% for WAS. In addition, the WAS treatment tended to

decrease the grain quality slightly for both cultivars. Although aerobic conditions such as WAS have somewhat adverse effects on grain yield and quality, growing the low-Cd cultivar aerobically is the most practical way to simultaneously reduce Cd and As contents in the rice grains [29].

11.3.2 Amendments

Under flooded conditions, the concentration of Cd in soil solutions decreases, while the concentration of As increases, as mentioned under Sect. 11.3.1. Therefore, a promising strategy to simultaneously mitigate the concentration of Cd and As in rice grains is a combination of flooded cultivation and soil amendments, respectively. Various materials have been used to treat As-contaminated water, including various oxides, treated slags, carbons derived from agricultural waste (char and coconut husk carbons), biosorbents (immobilized biomass and orange juice residue), goethite, and some commercial adsorbents, including resins, gels, silica, and treated silica [30]. Ferrihydrite, a short-range ordered iron hydroxide common in soil, has a high specific surface area and adsorption capacity [31]. Furthermore, zerovalent iron (ZVI) also efficiently removes As(III) and As(V) from water [32].

Due to the high affinity of As for Fe materials, amendments with iron can be useful to remediate As-contaminated soils. For instance, the precipitate of polysilicate-iron solution can be a novel and promising As adsorbent for soil under flooded anaerobic conditions [33]. Furthermore, ferrihydrite and Al-substituted ferrihydrite have been reported to be effective in reducing the concentration of dissolved As in the tested soils.

In our studies, we verified the effect of three commercially available iron materials—ZVI, iron oxide material (FH), and iron and steel slag (SCS)—on arsenic absorption by rice crops [34–36]. By X-ray diffraction, we determined that the structure of FH was similar to that of ferrihydrite.

The results, in general, showed a significant correlation between the concentration of As and Fe in soil solution or As-sol and Fe-sol (Fig. 11.3). The mean As-sol values decreased in the order REF > SCS > FH ≫ ZVI with no significant difference between REF and SCS. However, the mean Fe-sol values did not differ significantly among the treatments, except for SCS, which was significantly low. The mean ratio of As-sol to Fe-sol ($\Delta As/\Delta Fe$) tended to decrease in the following order: REF > SCS > FH ≫ ZVI. These data indicate that the iron-bearing materials suppress the dissolution of soil iron minerals, followed by a decrease in the dissolution of As. This decrease in the dissolution of iron minerals requires an electron donor from some soil constituents; easily reducible organic matter might play a primary role. The total amount of easily reducible organic matter in soils is the same before and after the application of iron-bearing materials. Therefore, the total amount of electron donors would also remain the same. The decrease in electron transfer to soil iron hydroxide ratio due to the application of iron-bearing materials might probably suppress the dissolution of soil iron minerals on which As is adsorbed.

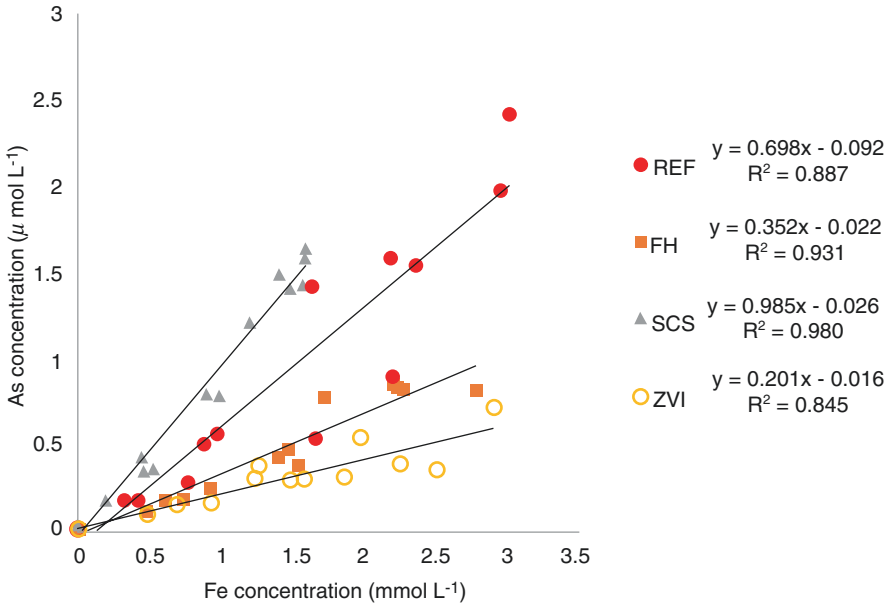

Fig. 11.3 Relationship between the Fe and As concentrations in soil solution (soil depth, 5 cm). The applications per square meter were iron oxide material 1 kg (FH), iron and steel slag 2 kg (SCS), and zerovalent iron 1 kg (ZVI) [35]

This might explain the mechanism underlying the inhibitory effect of iron-bearing materials on the dissolution of As in soil.

Another possible explanation for As-sol mitigation by iron-bearing materials is that they can readsorb the released As, probably through the coordination exchange bond between their surface hydroxyl groups and As. With ZVI, the surface became highly reductive. Thus, it is possible that low-soluble arsenic sulfide is produced during this process, suppressing the elution of soil arsenic.

The application of iron materials significantly decreased the mean As concentration in brown rice in the order REF > SCS ≈ FH > ZVI (Fig. 11.4) with significant differences between REF and SCS and between FH and ZVI. The concentration of Cd in the rice grains was significantly low due to the submerged water management treatment (Fig. 11.3). These data indicate that iron-bearing materials along with the water management choice can simultaneously decrease the concentration of As and Cd in rice grains. As there was a significant correlation between the average As-sol and As in brown rice, the decrease in As-sol might be primarily responsible for mitigating the accumulation of As in rice plants. The total iron inputs were 0.4, 0.64, and 0.98 kg m^{-2} for SCS, FH, and ZVI, respectively. This order was consistent with that of decreased As ratio in rice grains. These results suggest that the iron content can be an important factor to suppress As dissolution from soils irrespective of the chemical form of iron in these materials.

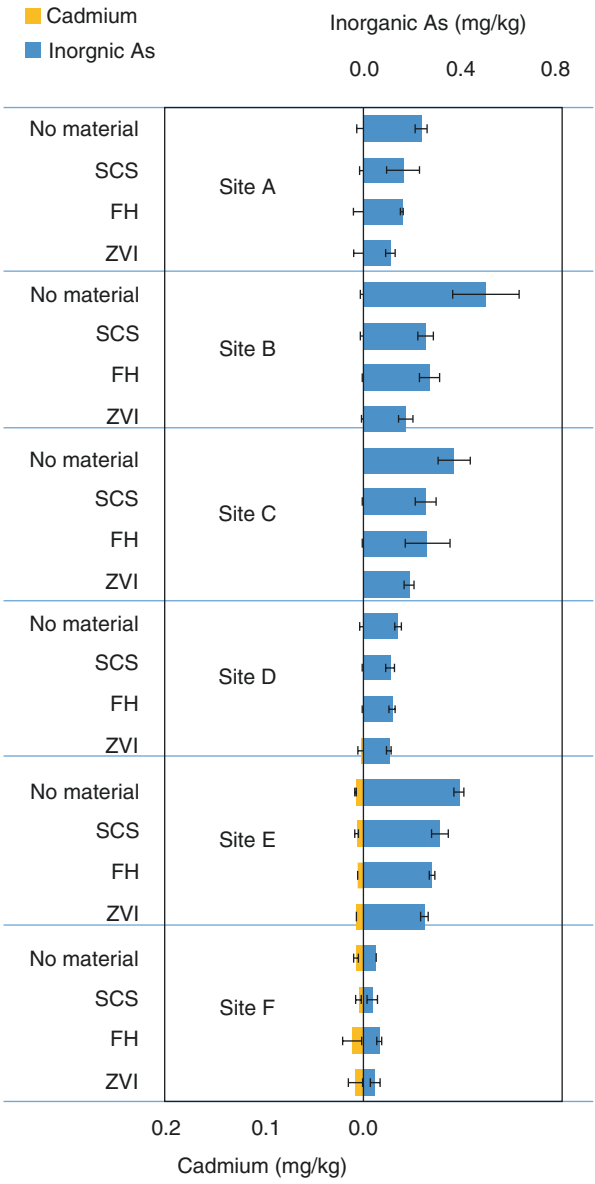

Fig. 11.4 Concentrations of inorganic As and Cd in rice grains during the field experiment. The applications per square meter were iron oxide material 1 kg (FH), iron and steel slag 2 kg (SCS), and zerovalent iron 1 kg (ZVI) [35]

However, the concentration of As-sol in SCS tended to be higher than that in FH. Furthermore, the concentration of As was similar in SCS and FH (Figs. 11.3 and 11.4). This difference might be partially attributed to the difference in the concentration of silicic acid (Si) in soil solution, which was significantly higher in SCS than in the other treatments. Silicic acid is known to compete with As in terms of plant uptake due to similarity in the chemical forms of silicate and arsenate. Arsenite transport in rice

roots shares a highly efficient pathway similar to that of Si transport; the transporters are Lsi1 and Lsi2 [3]. Silicic acid fertilization decreased the concentration of total As in straw and grain, even though Si addition increased the concentration of As in the soil solution [37]. The study also reported that Si decreased the concentration of inorganic As (iAs) in rice grain. A similar trend was observed in the present study. The percentages of iAs in the total concentration of As in the rice of REF, SCS, FH, and ZVI treatments were 79.0%, 73.7%, 78.5%, and 86.5%, respectively. Furthermore, SCS contained a high amount of silicates, which can dissolve in soil solution and suppress As uptake by rice plants. Although the stem yield decreased significantly in ZVI, there was no negative effect of iron-bearing materials on the grain yield and percent of perfect grain. The significantly higher percent of perfect grain in FH and ZVI than in REF indicates a positive effect of iron-bearing materials on the quality of rice. It is well documented that slag-based silicon fertilizers have beneficial effects on the growth and disease resistance of rice plants [38]. Iron and steel slag, a steel converter furnace slag, is expected to have positive effects on the growth and yield of rice with successive applications.

11.3.3 Breeding Rice Cultivars with Low As Accumulation

The selection and breeding of low As-accumulating cultivars are also practical options for reducing the risk of contamination from As in food. By using the diverse germplasm in rice, genotypic variations in grain As concentrations have been widely studied. We found approximately threefold differences in the grain As concentrations of the World Rice Core Collection (WRC), consisting of 69 accessions, which cover the genetic diversity of almost 32,000 accessions of cultivated rice [39]. This difference was quite smaller than the difference in grain Cd concentrations, in which an approximately 67-fold difference was found in the same WRC [40]. A similar result was reported by Duan et al. [41], who found that the Cd and As concentrations in the grains varied by 10- to 32- and 2.5- to 4-fold, respectively, among 471 local cultivars grown in China. These reports suggest low genetic diversity in the grain As concentration among rice cultivars. In addition, previous studies have shown larger environmental effects than genetic effects for the variation in grain As concentrations in rice cultivars [42]. The difference in the heading time may also influence the genotypic variation in the grain As concentration, and the quantitative trait loci (QTLs) for both traits were co-localized in chromosomes 8 and 10 [43]. However, it should be noted that differences in heading times might interfere with the detection of QTLs specific to the grain As concentration because of the different growth periods resulting within the mapping population, as suggested by our previous research on detecting a specific QTL for Cd concentrations in rice grains [44]. Thus, the low genetic diversity and high environmental effects on grain As concentration and the difference in heading times among cultivars or in the mapping population lead to difficulty in identifying low-As genes that can be useful for breeding. To our knowledge, this specific gene has not been identified yet, although a number of QTLs are reportedly associated with the As concentration of the rice grain [39, 45, 46].

Several reports have shown that basmati rice sold in the USA and Europe markets had a significantly lower iAs content than other types of rice [47, 48]. Because the iAs level in rice varies greatly depending on where the rice is grown, a low-iAs level in basmati rice might be attributable to the geographical region, and India is a major country for basmati rice production. According to data from the Codex Committee on Contaminants in Foods report, the iAs level of rice grown in India tends to be lower than that in other countries [12]. In addition, the genetic effect may also contribute to the low-iAs level in basmati rice because we showed the lowest grain As concentration of basmati rice (variety name: Local basmati) in the WRC accessions grown in the same field over 3 years [39]. These findings indicate that basmati rice could be a good genetic resource to confer a low-iAs trait to other types of rice, and we now seek to understand the genetic mechanisms related to low grain iAs in basmati rice.

In addition to iAs, methylated As, primarily in the form of dimethylarsenate (DMA), is present in rice grains. The DMA in the rice grain originates from rhizosphere soil via As methylation by specific microbes and not from methylation within rice plants [49]. The proportion of DMA to total As in rice grains varies greatly depending on where the rice is grown, and it becomes increasingly dominant as the total As concentration rises above 0.6 mg kg^{-1} [50]. In addition to regional variations, genotypic differences in the proportion of DMA were observed in the WRC accessions grown in the same field with low As [39]. Padi Perak, a tropical *japonica* variety, presented approximately 45% DMA within its total iAs and methylated As in the grain, and Nipponbare, a temperate *japonica* variety, displayed approximately 15%, although the total As concentrations of the grains in both cultivars were almost similar at approximately 0.2 mg kg^{-1}. The physiological mechanism responsible for the genotypic difference in the DMA proportion is still unclear, and three QTLs were identified as genetic factors related to the high proportion of DMA in Padi Perak. One strategy for developing rice cultivars with a low level of toxic iAs would be to change the proportion of methylated As on the basis of a low level of total As content, and Padi Perak could be used as a starting material to develop this type of rice cultivar through DNA marker-assisted selection.

The molecular mechanisms of As uptake and transport in rice are increasingly elucidated in accordance with the identification of the relevant transporter genes, and the recent progress is well summarized by several review papers [51–53]. Rice plants are likely to discriminate among As species in terms of uptake and translocation, and the arsenite pathway in rice is more notable than other species because arsenite is the predominant form of As in paddy soil. Studies have shown that the two silicic acid (Si) transporters Lsi1 (OsNIP2;1) and Lsi2 mediate the influx of arsenite into the roots and the efflux of arsenite toward the xylem, respectively [3]. An *lsi1* knockout line showed lower concentrations of As in straw than the wild type, but the grain As was not decreased. By contrast, two *lsi2* mutants showed significantly decreased As levels in the straw and grain, at 13–19% and 51–63% of the corresponding wild-type rice, indicating that it is possible to reduce the As concentration in rice grains by mutating *Lsi2*. However, the grain yield of the *lsi2*

mutant was only 40% that of wild-type rice due to the lower Si in the shoot. Therefore, Ma et al. [3] proposed the identification of allelic variations in *Lsi2* that could favor the uptake of silicon over arsenite for developing low-As cultivars without growth inhibition.

Recent studies have revealed that rice nodes are key tissues for reducing As accumulation in the rice grain. The nodes have a firewall system to sequester phytochelatin (PC)-arsenite complexes in the vacuoles of the phloem companion cells of diffuse vascular bundles via OsABCC1 (gene locus: Os04g0620000), an ATP-binding cassette transporter [54]. In addition, the PCs biosynthesized by OsPCS1 (gene locus: Os05g0415200), a phytochelatin synthase, could be a rate-limiting step for As transport into the vacuoles [55]. The wild type (cv. Koshihikari) and two mutants (*osabcc1* and *ospcs1*) were cultivated in a paddy field under continuously flooded conditions, and the As concentrations in each shoot tissue were compared (Fig. 11.5). A loss of function of either OsABCC1 or OsPCS1 greatly affected the As distribution in the shoot parts. In the wild type, the As concentration in the nodes was highest, and it was approximately 40-fold higher than that in brown rice. By contrast, the As concentrations in the nodes of *osabcc1* and *ospcs1* were approximately one-tenth of those in the wild type, whereas the As concentrations of the grains were five times higher in the *osabcc1* and *ospcs1* than in wild type. These results suggest that these mutants have defects in As trapping in the nodes, and both OsABCC1 and OsPCS1 are essential for reducing As levels in the grains.

Strengthening the As firewall system in the nodes may lead to dramatically reduced concentrations of As in the grain. To verify this possibility, we produced transgenic lines that overexpressed *OsPCS1* under the control of the strong constitutive promoter *CaMV 35S*, and these transgenic lines were cultured in paddy soil or in hydroponic systems. The result showed that the total As levels in the grains were significantly lower in *OsPCS1* overexpressing lines than in the wild type [55]. Furthermore, we analyzed the As species in rice samples from Hayashi et al. [55]. Although the DMA levels did not change in the transgenic plants, the inorganic As levels were significantly lower in *OsPCS1* overexpressing lines than in the wild type (Fig. 11.6). The nodes of the overexpressing line showed higher As levels than those of the wild type, indicating that OsPCS1 overexpression strengthens the As-sequestering ability in nodes. Rice has another PCS, which is designated as OsPCS2 (gene locus: Os06g0102300) in the Rice Annotation Project Database (RAP-DB), and the overexpression of *OsPCS2* also decreased the grain As levels, although its effect was much smaller than that of *OsPCS1* [55]. Rather, OsPCS2 may contribute to Cd detoxification because OsPCS2 was more strongly activated by Cd than by As according to an in vitro PC synthesis assay. We confirmed that there were no adverse effects on plant growth in the *OsPCS1* overexpressing lines. Therefore, modifying the *OsPCS1* expression would be an approach to breeding rice cultivars with low inorganic As in the grains.

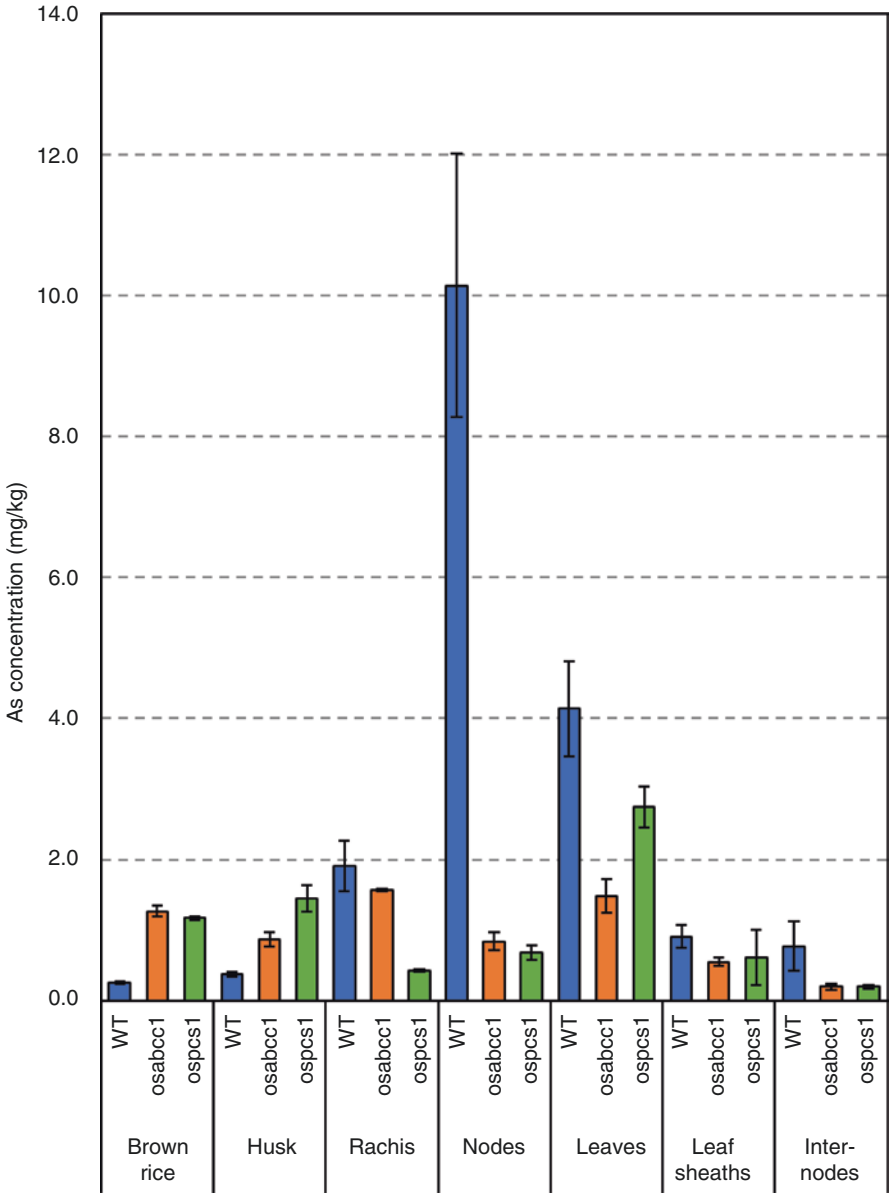

Fig. 11.5 As concentrations in each shoot tissue of wild type (cv. Koshihikari) and two mutants (*osabcc1* and *ospcs1*) grown in a paddy field (Ishikawa et al., unpublished data)

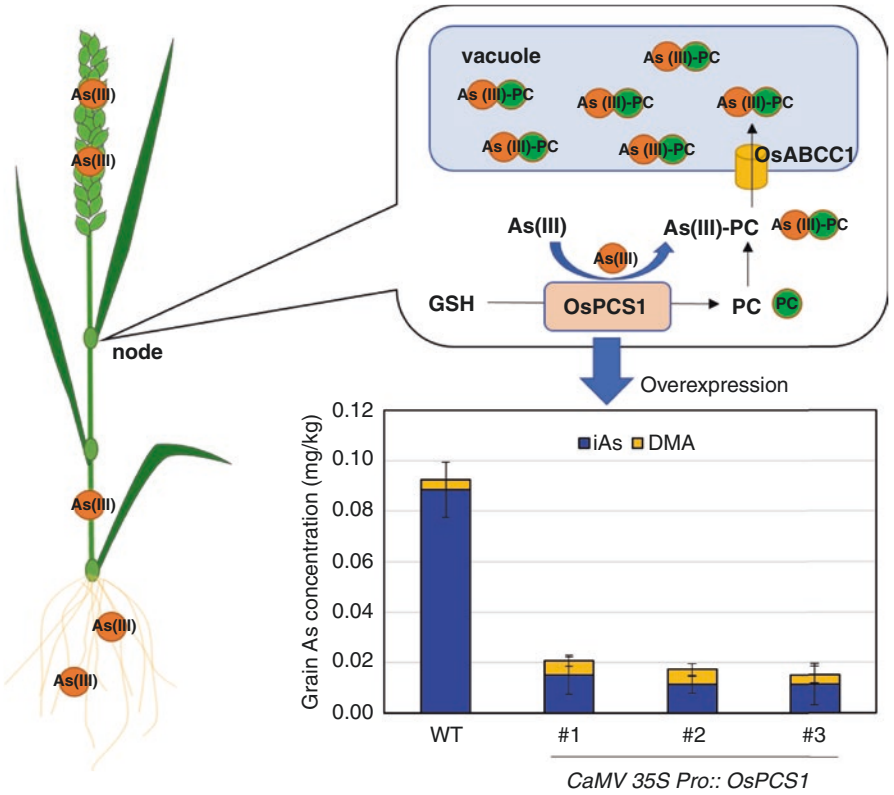

Fig. 11.6 Schematic mechanism of As trapping in the nodes and grain As concentrations of *OsPCS1* overexpressing lines of rice (the analyzed As species in rice samples from [55])

11.4 Perspective

To reduce the As levels in rice, we propose three strategies: (I) intermittent irrigation, (II) the application of Fe materials, and (III) the breeding of low-As rice cultivars. For strategy I, the daily management of the water supply and drainage of rice fields for intermittent irrigation is a heavy burden, particularly for farmers managing large and dispersed paddy fields. A new water control system in paddy fields (the farm-oriented enhanced aquatic system) has been developed [56]. This system automatically supplies water during droughts and drains excess water during heavy rainfall, thereby maintaining suitable soil moisture conditions. Another system that allows for the remote control and automatic monitoring of the water supply and drainage in rice paddy fields using a smartphone or PC has been developed [57]. With these systems, it could be possible to save labor in relation to water management.

For strategy II, the application of materials to mitigate the concentration of As in rice must be cost-effective and acceptable by farmers. In this milieu, it is important to evaluate

soil amendments to verify if they can simultaneously improve soil quality (plant growth) and decrease the concentration of As in rice grain at a low concentration and for long period. Iron and silicate amendments are promising. Furthermore, materials to decrease the concentration of As and Cd in rice and maintain soil oxidative condition need to be investigated in the future. Recently developed technologies to evaluate the properties of materials, such as nuclear magnetic resonance (NMR) and X-ray absorption near-edge structure (XANES), are expected to clarify the relationship between the property of amendment and their effectiveness for rice growth and As/Cd mitigation.

Regarding strategy III, gene-editing technology must be a powerful tool to develop low-As rice because low-Cd *indica* rice was produced by knocking out the metal transporter gene *OsNramp5* using a CRISPR/Cas9 system [58]. Low-As rice could be developed without decreasing the Si level by modifying the ion selectivity of *Lsi1/Lsi2* through gene editing. Moreover, we might be able to create low-As rice without the control of the strong constitutive promoter *CaMV 35S* if gene editing can modify endogenous *OsABCC1* and *OsPCS1* expression, although many countries are still discussing whether plants developed by gene-editing technology should be treated as genetically modified organisms (GMO).

Another important finding is the effect of high air temperatures after rice heading on the iAs concentration of grain [59]. Global warming will increase the temperatures in rice-growing areas, which could result in higher concentrations of iAs in rice grains. Late or early transplanting to avoid high air temperatures around rice heading could be a countermeasure.

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Chapter 12
The Development and Purposes of Arsenic Detoxification Technology

Hiroshi Yamauchi, Ayako Takata, Yang Cao, and Koichiro Nakamura

Abstract Currently, factors that adversely impact human health all too frequently exist within various industrial settings and in the natural environment, and the continued diversification of industrial activities further exacerbates these health risks. As there is no definitive cure for chronic arsenic poisoning, it is paramount to take prophylactic measures to minimize the chance of accidental inorganic arsenic (iAs) exposure. To that end, we have been attempting to develop detoxification techniques for neutralizing iAs at its sources by focusing on a mechanism for the stimulation of the biosynthesis of methylated arsenic compounds from iAs, a process that naturally occurs in some marine ecosystems. Arsenobetaine (AB) was chosen as a candidate for a detoxified iAs derivative based on its toxicological profile as it is much less toxic than iAs. The detoxification of iAs was verified with arsenical chemical weapons (abandoned stockpiled chemical weapons containing arsenic) and arsenic-contaminated well water. The photocatalytic titanium dioxide system was shown to convert other non-iAs compounds to AB. However, further development of efficiency and economy is considered needed to reach a level of the practical use for any system, and the disposal of arsenic waste generated is still an issue. For the future of humankind, we believe that it is a critical endeavor to further refine the concept and practice of iAs detoxification to minimize iAs-related health impact.

Keywords Arsenic · Detoxication · Arsenical chemical weapons · Arsenic-contaminated water · Chronic arsenic poisoning

H. Yamauchi (✉) · A. Takata · Y. Cao
Department of Preventive Medicine, St. Marianna University School of Medicine, Kawasaki, Japan
e-mail: hyama@marianna-u.ac.jp

K. Nakamura
R&D Department, Nippon Sheet Glass Co. Ltd., Tokyo, Japan

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12.1 Background on the Need for Detoxification of Arsenic

In the twentieth century, numerous cases of acute and chronic arsenic poisoning from inorganic arsenic (iAs) exposure emerged in several advanced economies, due to various sources including occupational exposure and food contamination [1, 2]. These exposures resulted in an associated increase in lung and skin cancer [3]. Meanwhile, in various developing countries, particularly in East Asia and Central or South America, large-scale areas of endemic chronic arsenic poisoning were detected among people who consumed well water contaminated with iAs from the 1980s onward [4, 5]. The consumption of iAs-tainted well water in these countries actually increased due to an effort change sources to well water in order to prevent waterborne diseases contracted from consumption of surface waters. Based on the World Health Organization (WHO) and United Nations Children's Fund estimates, the number of individuals at risk to chronic arsenic poisoning now is in the tens of millions [4, 5]. Currently, factors that adversely affect human health relentlessly exist within various industrial settings and in the natural environment (Table 12.1), and the diversification of industrial activities may continue to further exacerbate these risks.

To prevent the health hazard caused by arsenic, we believe that the most effective countermeasure is to ensure that the toxic iAs is not left in the living environment. To this end, we have been attempting to develop detoxication techniques for neutralizing highly toxic iAs sources by focusing on a mechanism for the biosynthesis of methylated arsenic compounds from iAs, a process that naturally occurs in some marine biosystems. Our recent studies have been aimed at reproducing this same process under artificial conditions.

Table 12.1 Potential sources of arsenic contamination in industrial settings and natural environments

Copper smelting and non-iron metal smelting	*Soil contamination*
• Residues of copper smelting • Residues of non-iron metal smelting • Recycling of III–V semiconductors (GaAs, InAs)	• Termite repellent (Cu-Cr-As; CCA) for various wooden materials • Use of herbicides (cacodylic acid) in cotton plants and orange and grapefruit plantations • Plant site for arsenic compound handling • Coal burning ashes
Oil fields, coal mines, and geothermal power plants	*Chronic arsenic poisoning*
• Mining of shell oil or gas • Mining of sand oil • Development of copper mines and coal mines • Geothermal power generation as clean energy development • Hot spring water	• iAs in well water • iAs removed from environmental sources • Smoke from burning coal

This chapter summarizes some of our latest findings regarding this biodetoxification of iAs, an environmental toxicant that has been causing socially contentious health issues pertaining to various industrial activities and from contamination of the natural environment. We also briefly elaborate on methods for achieving the optimum detoxication of iAs, on problems inherent in detoxification techniques, and on the social significance of and future prospects for arsenic detoxification.

12.2 Arsenic-Related Health Impact Within the Industrial and Natural Environment

Table 12.1 presents some sources of iAs exposure from the environment and industrial activities that can affect the human health. The demand for and use of iAs in industrial applications has greatly varied. The most commonly used raw material inorganic arsenic is arsenic trioxide, which is purified from by-products generated during copper and non-iron metal smelting processes. In the past, arsenic trioxide was often used to produce arsenical agrochemicals, termite repellents, and herbicides, as well as fining agents used during the manufacture of high-quality liquid crystal and crystallized glass panels. However, the restrictions on the use of certain hazardous substances in electrical and electronic equipment directive, which was enforced by the European Union in 2011 [6], effectively banned the use of arsenic trioxide in industrial applications, which has drastically reduced the demand for this compound. As a result, a new concern in recent years has been the possible accumulation of by-products containing arsenic at copper smelters worldwide, which may be a new source of arsenic contamination. Nowadays, industries are shifting their focus to the manufacture of semiconductors based on arsenic compounds, such as gallium arsenide (GaAs) and indium arsenide (InAs). Of these, GaAs semiconductors are particularly in high demand as electronic components for communications equipment. Both Ga and In are precious metal resources and, therefore, are highly recycled. During this recycling, iAs-containing substances are often converted to arsenic trihydride (arsine). There have been sporadic reports of health effects among workers exposed to this highly toxic arsenic compound [7]. Studies have shown that GaAs and InAs of III–V semiconductors are both toxic and, at least with GaAs, carcinogenic [8–11].

Environmental problems posed by iAs-containing natural substances present serious social challenges. The primary source of environment-related chronic arsenic poisoning is well water contaminated with iAs [12, 13]. In regions where chronic arsenic poisoning is prevalent, iAs concentration in well water is commonly 10–100 times that of the standard limit concentration recommended by the WHO (10 μg/L) [14]. International organizations, including the WHO, World Bank, Red Cross, and Japan International Cooperation Agency, have been continuously striving to alleviate the problem of chronic arsenic poisoning, but the results have been less than satisfactory. From the standpoint of international assistance, countries have been cooperat-

ing to develop and share diverse technologies for removing iAs compounds from contaminated well water. Despite such efforts, the failure to properly store or discard iAs-containing substances that have been removed from well water often results in repeated incidents of human exposure to these substances, thus forming a vicious cycle. In addition, floods caused by heavy rainfall, often during the monsoon season, return previously removed contaminated iAs compounds to the living environment.

Soil contamination due to chromated arsenicals (Cu-Cr-As, CCA) and cacodylic acid (dimethylarsinic acid, DMA) is a serious environmental problem related to arsenic compounds. In the past, CCA was often used as a termite repellent for various wooden materials used in housing. In the United States, people used recycled wood chips to prepare compost for horticultural and gardening purposes. However, the US Environmental Protection Agency has since then banned the recycling of wooden construction materials containing CCA to prevent arsenic contamination of the soil [15, 16]. In contrast, DMA is still widely used in the United States to remove weeds in orange and grapefruit orchards and to wither stalks and leaves of cotton plants for the cotton harvest. These practices have caused soil arsenic contamination in vast areas. According to the criteria proposed by the International Agency for Research on Cancer, DMA is classified as a Group 2B possible human carcinogen [17, 18]. Furthermore, some argue that iAs contamination has occurred in soil and the local environmental water due to the development of oil fields [19, 20], coal mines [21, 22], geothermal power plants [23], and other facilities and that exposure in this manner should be considered as a potential risk factor. Nevertheless, there has been no scientific evidence to suggest any correlation between the exposure to iAs and occurrence of health problems in these particular industrial settings to date [19–23].

As a more unusual example, operations are being conducted to dismantle long-abandoned chemical weapons containing arsenic [24]. Several international conventions prohibit the manufacture and use of chemical weapons. During the Second World War, the former Imperial Japanese Army manufactured approximately 7000 tons of chemical weapons, such as the blistering agent, lewisite ($C_2H_2AsCl_3$), and sneezing gases, diphenylcyanoarsine [$(C_6H_5)_2AsCN$] and diphenylchlorarsine [$(C_6H_5)_2AsCl$]. These chemical weapons were abandoned in several locations in China and Japan by the Imperial Japanese Army when it was defeated in the war and since have been left untouched to the present day. Currently, efforts are underway to properly dismantle and discard these abandoned chemical weapons, pursuant to provisions stipulated in international treaties. To properly dispose of these weapons, contractors perform controlled detonations using the Detonation of Ammunition in a Vacuum-Integrated Chamber (DAVINCH™) technology [25], followed by high-temperature combustion. During both processes, iAs compounds are generated. Methods to implement the final disposal of these iAs compounds are currently being deliberated.

Recently, numerous studies have revealed that iAs contamination and accumulation to unsafe levels may cause health complications in humans engaged in relevant industrial operations or exposed to certain contaminated natural environments. These reports signify the need for the development of measures to purify and preserve the environment in as safe manner as possible, rather than by simply attempting to isolate and store iAs compounds, to avoid eventual health hazards associated with iAs exposure.

12.3 Arsenic Detoxification and the Detoxificated Derivatives

Established conventional detoxication methods involve the conversion of water-soluble iAs compounds into insoluble sulfides or solidifying iAs compounds within molten glass [26]. These methods could be reversible or could generate large quantities of glass with a vast need for storage/disposal. However, to achieve a more complete and irreversible detoxification of iAs, an entirely different approach is needed.

Recent studies have yielded key findings regarding widely varying degrees of toxicity within arsenic compounds depending on their chemical structure, conformation, and species. Arsenic compounds are roughly divided into inorganic and organic compounds, with iAs being generally much more toxic than organic arsenic [27, 28]. Organic arsenic compounds include methylated organic arsenic compounds, arsenosugars, and arsenolipids. Of these, arsenobetaine [$(CH_3)_3CH_2COOH^+$; AB] is a trimethylated organic arsenic compound that has been most commonly isolated from marine organisms. In 1977, Edmonds et al. identified the chemical structure of AB extracted from rock lobsters [29]. Later studies detected AB in various marine organisms such as finfish, shellfish, and crustaceans [30–32]. In relation to these findings, trimethylarsine oxide [$(CH_3)_3AsO$; TMAO] was also shown to be present in marine organisms, although to a lesser extent than AB (for more information on this field of research, refer to other chapters).

During the same time period, human and animal experiments on the metabolism and toxicity of AB revealed that AB is not metabolized in vivo but is swiftly excreted unchanged from the body via the urine [30, 33, 34]. Furthermore, separate animal experiments demonstrated that TMAO remains unaltered in the body [35, 36]. The median lethal doses (LD_{50}) of AB and TMAO are 10 g/kg [37] and 10.6 g/kg [35], respectively, when orally administered to mice, showing the toxicity of both substances to be very low or negligible. Compared with the iAs compound arsenic trioxide, the most toxic of all arsenic compounds (LD_{50}, 0.026 g/kg) [35], AB and TMAO are approximately 1/300 as toxic.

Thus, from a toxicological perspective, AB and TMAO are excellent potential candidates as detoxificated iAs derivatives.

12.4 Detoxication Methods

12.4.1 Efforts to Synthesize AB

To synthesize AB from iAs, we conceived a biomimetic system, then developed it into a bioinspired catalytic system to solve the problems of the biomimetic system, and finally established a photocatalytic titanium dioxide system for practical use. Our first attempts involved the trimethylation of iAs compounds using a natural vitamin B_{12} derivative, methylcobalamin ($C_{63}H_{91}CoN_{13}O_{14}P$,), and a reducing agent, glutathione (GSH), as well as the synthesis of AB via carboxymethylation using

iodoacetic acid and GSH [38]. Subsequent testing confirmed that less expensive, simple amino acids possessing sulfhydryl (SH) groups, such as cysteine and homocysteine, could be used as alternative reducing agents to GSH. These amino acids successfully catalyzed the trimethylation and betaine-synthesizing reactions to produce an intermediate metabolite, TMAO, which was then converted to AB [39]. However, there were several inherent problems in the synthesis of AB using this biomimetic system in terms of both efficiency and cost-effectiveness.

To improve the efficiency of the synthesis of AB from iAs, we then developed a "bioinspired catalytic system" which involved the use of vitamin B_{12}, oxidized titanium, and a methyl-group donor (XCH_3), a system characterized by an ability to frequently activate the catalytic cycle responsible for transmethylation reactions of arsenic compounds [turnover (B_{12} equivalent), >150 times] [40]. Using this system, we increased the efficiency of conversion to AB. Additionally, to examine the feasibility of future practical applications, we tested a "photocatalytic titanium dioxide system" (Fig. 12.1), which is a simplified reaction system for converting iAs to AB and is characterized by its ability to synthesize AB from iAs via a one-step reaction under ultraviolet light in the presence of oxidized titanium and acetic acid. Oxidized titanium is highly adsorptive of exceedingly toxic iAs compounds and exhibits very low adsorptive activity to the less toxic AB and TMAO. Thus, AB can easily desorb into a liquid solution from the surface of the oxidized titanium substrate, thereby considerably improving the recycling efficiency of oxidized titanium. A separate experiment ascertained that this photocatalytic titanium dioxide system can also be applied to other iAs and methylated compounds (data not published).

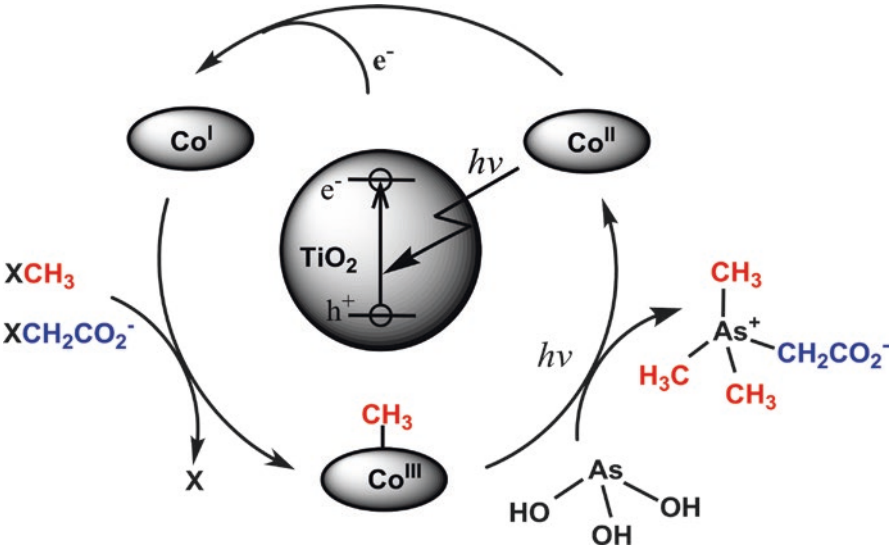

Fig. 12.1 Conversion of iAs to AB using the photocatalytic titanium dioxide system

12.4.2 Problems Presented by Methylated Mercury, Lead, and Tin

iAs compounds often are intermixed with other metallic elements, such as mercury, lead, and tin, in natural environments or in industrial wastes. Therefore, measures need to be taken to prevent coexisting or intermixed toxic elements from being converted to highly toxic substances via the methylation of iAs in an attempt to detoxicate the metalloid. Particularly, the inadvertent synthesis of methylmercury, which has caused a major pollution-related disease (Minamata disease) [41] in Japan in the 1950s, must be avoided at all costs. Likewise, measures should be taken to ensure that lead is not methylated to generate alkyl-lead compounds [42, 43], which are another group of highly toxic substances. We unequivocally confirmed in a separate study that our photocatalytic titanium dioxide system does not methylate mercury, lead, or tin under the conditions used for the methylation of iAs (data not published). Verifying that no new toxic substances are generated during iAs detoxication is essential to further advance this technology as useful, relevant, and, perhaps most importantly, safe.

12.5 Practical Example of Arsenic Detoxication

12.5.1 Arsenical Chemical Weapons

As a practical test to handle abandoned chemical weapons, we attempted to detoxify artillery shells containing a sneezing agent manufactured by the former Imperial Japanese Army. These shells were salvaged from Japanese domestic waters after being abandoned several decades earlier. The shells were first pulverized into minute particles in a controlled detonation chamber (Fig. 12.2) [25], and then iAs contained within these pulverized particles was converted to AB using the biomimetic system [39, 40]. Subsequently, cytotoxicity and apoptosis assays were performed using a commercial kit (Cell Counting Kit-8; Dojindo Laboratories, Japan) to examine the toxicity of the synthesized AB in the treated sample and iAs in the nontreated pulverized particle sample on human promyelocytic leukemia cells (HL-60) and human cervical cancer cells (HeLa) (data not published). iAs in the pulverized particle sample consistently exhibited cytotoxicity in a concentration-dependent manner. This cytotoxicity resembled that of arsenic trioxide, which is the most highly toxic inorganic arsenic compound. In contrast, the synthesized AB in the treated sample did not exhibit cytotoxicity. Similarly, the apoptosis assay confirmed a high rate of apoptosis due to iAs in the untreated pulverized particle sample but no apoptosis from the treated sample where it was treated to synthesize AB (Fig. 12.3). These results demonstrate that our system can convert and detoxify iAs contained in chemical weapons into nontoxic AB.

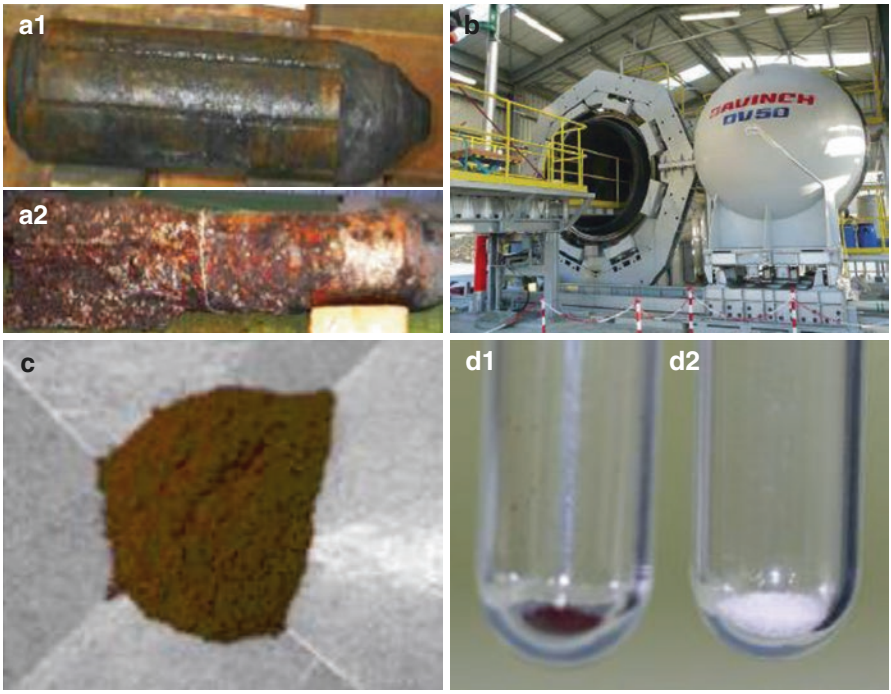

Fig. 12.2 Dismantling and detoxication of abandoned arsenical chemical weapons (containing diphenylcyanoarsine (DA) and diphenylchlorarsine (DC)) (converted to AB). The Abandoned Chemical Weapons Office operated by the Cabinet Office of Japan [24] is undertaking a project to dismantle and dispose of abandoned chemical weapons. The controlled detonation process was outsourced to Kobe Steel, Ltd. [25]. The biomimetic system was employed to convert iAs in the sample to AB [39, 40]. (**a**1) Abandoned chemical weapons (containing DA and DC), (**a**2) abandoned chemical weapons (containing lewisite + mustard gas), (**b**) Detonation of Ammunition in a Vacuum-Integrated Chamber (DAVINCH™), (**c**) fine particles produced by DAVINCH™, (**d**1) raw AB, (**d**2) purified AB

12.5.2 iAs in Well Water Causing Chronic Arsenic Poisoning

To prevent chronic arsenic poisoning, various measures have been taken to remove iAs compounds from iAs-contaminated well water to make it potable. However, these efforts are currently facing challenges in terms of cost, waste disposal, management, and technology. To alleviate such problems, we developed a new technique to directly convert and detoxicate highly toxic iAs contained in well water to nontoxic AB. We tested this technique using water samples from a well in China that produced the hallmark symptoms of chronic arsenic poisoning by the local people that consumed its water [12]. Our photocatalytic titanium dioxide system enabled us to successfully convert the iAs contained in the samples to AB (Fig. 12.4) (data not published). These results demonstrate that our system can detoxicate iAs-contaminated water without involving numerous intermediate detoxication steps and can, therefore, serve as a foundation for future environmental purification

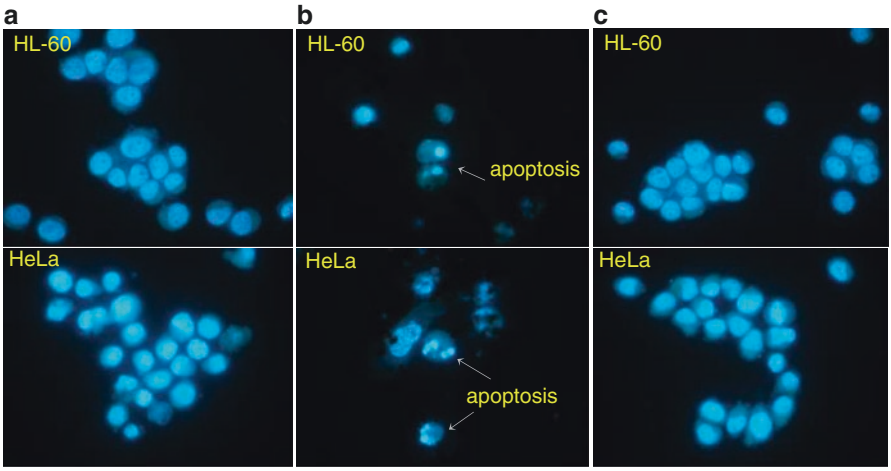

Fig. 12.3 Apoptosis assay on the detoxificated product (AB) in the treated abandoned chemical weapons. The iAs and AB present in the treated sample shown in Fig. 12.2 were used as test specimens. Human promyelocytic leukemia cells (HL-60) and human cervical cancer cells (HeLa) were used for the assay. Chromatin condensation was observed in apoptotic cells stained with Hoechst 33342. Apoptosis was not observed in synthetic AB. (**a**) Controls, (**b**) synthesized AB, (**c**) AB synthesized from iAs in fine particles

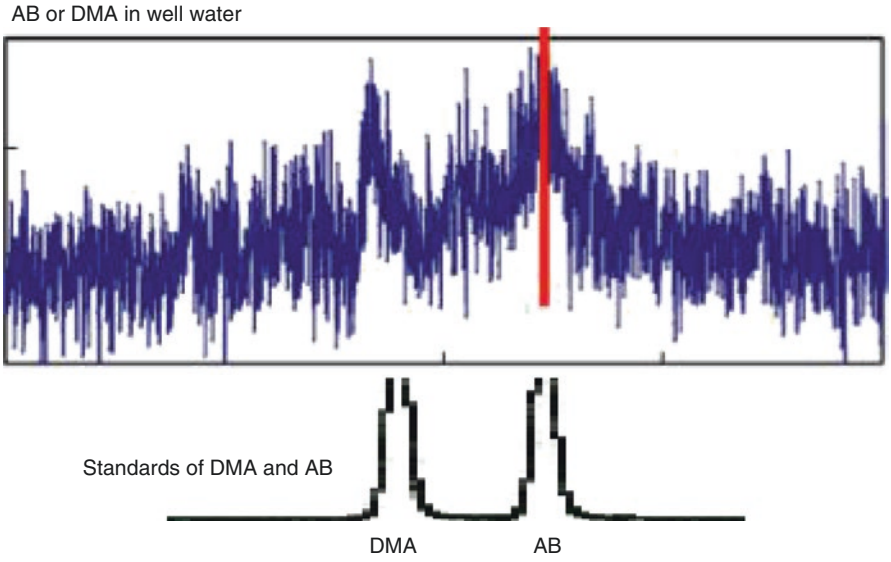

Fig. 12.4 Direct conversion of iAs in contaminated well water consumed by patients who experienced chronic arsenic poisoning to AB. Well water samples (as iAs 200 μg/L) were collected from a well that was used by patients who experienced chronic arsenic poisoning in China [12]. The photocatalytic titanium dioxide system was employed to convert iAs contained in well water to AB. The ratio of AB in total arsenic was 65% or 35%, respectively. Measurement of dimethylarsinic acid (DMA) and AB used HPLC-ICP-MS (7500ce, Agilent)

technologies. The relatively simple technology combined with the lack of the need for storage of waste would be major positives for this system as well.

12.6 Conclusion

Currently, exposure to iAs is causing widespread health problems among millions of people drinking water naturally contaminated with iAs, and there is also concern for workers engaged in certain emerging industrial activities. While the international community has been most concerned about the risk of iAs exposure and cancer in adulthood [3], recent data indicate that early life exposure carries comparable degrees of risk for various disorders [44] including brain dysfunction [45] and can impact both pregnant women and infants. Because there is no definitive cure for chronic arsenic poisoning, we feel it is paramount to take active measures to minimize the chance of undue exposure to iAs. To this end, we sought a system to convert iAs compounds to other nontoxic substances, at the highest extent possible. AB was chosen as a candidate for a detoxificated iAs derivative based on toxicological data. To convert iAs to nontoxic AB, we used the methylation mechanism of iAs demonstrated in several marine organisms. To artificially synthesize AB from iAs, we experimented with a biomimetic system using a vitamin B_{12} derivative. However, given the inherent problems with a lack of efficiency and cost-effectiveness that might preclude this system from being useful in society, we developed and tested improved systems (i.e., the bioinspired catalytic system and photocatalytic titanium dioxide system). The photocatalytic titanium dioxide system, in particular, was shown to be capable of converting other non-iAs compounds to AB. Although advances have been made in technologies for removing iAs from drinking water, no concrete methods have been established to properly dispose of the removed iAs compounds. This waste disposal issue might be why we have yet to achieve a system for prevention of chronic environmental arsenic poisoning. For both the present and future of humankind, we believe that it is a critical endeavor to further refine the concept and technique of iAs detoxication to prevent iAs-related human diseases.

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