Chapter 5
Fullerene-Derivatives as Therapeutic Agents in Respiratory System and Neurodegenerative Disorders

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Abstract Since the discovery of fullerenes in 1985, this class of spherical or ellipsoid molecules made entirely of carbon atoms has attracted great interest in the biological field because of its unique physical and chemical properties. However, due to the insolubility in polar solvents and the aggregation properties, the biological applications of these molecules have been restricted. To overcome this problem, water-soluble derivatives of fullerenes have been developed. Fullerenes and fullerene-derivatives react readily with electron rich species as radicals, and because of this property they have great potential to be used as antioxidants. Oxidative stress occurs when the cellular production of reactive oxygen species exceeds the capacity of antioxidant defenses within cells. In humans, oxidative stress is associated with the development and progression of several pathologies, including neurodegenerative and pulmonary diseases. This chapter brings together the current understanding of how fullerene-based materials may be used as therapeutic agents and contributes to reduce oxidative stress in respiratory system diseases and neurodegenerative disorders.

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5.1 Introduction

5.1.1 Oxidative Stress and Disease Development

Oxidative stress is a general term used to describe disturbances in the normal redox state of cells that occur when the cellular production of reactive oxygen species (ROS) exceeds the capacity of antioxidant defenses within cells [1]. The oxidative stress leads to damage of all components of the cell, including deoxyribonucleic acid (DNA), proteins, and lipids affecting the normal mechanism of cellular signaling. In humans, oxidative stress is associated with the development and progression of several pathologies, including cancer [2], Parkinson's and Alzheimer's diseases, amyotrophic lateral sclerosis [1], atherosclerosis, chronic congestive heart failure (CHF), myocardial infarction [3], asthma, acute lung injury/acute respiratory distress syndrome, sepsis-related lung injury, fibrosis and emphysema chronic obstructive pulmonary disease [4].

ROS can exert beneficial or deleterious effects that depend on the type and concentration of ROS produced in the organism [5]. Physiological levels of ROS can act as signaling molecules to drive several cellular functions activating redox-sensitive kinases, phosphatases, and transcriptional factors responsible for modulating gene transcription and affecting cell survival. On the other hand, high levels of ROS can cause significant damage to cellular proteins, membranes and nucleic acids, leading to cellular dysfunction and death [5–7].

This chapter summarizes fullerenes chemical properties (Sect. 5.1.2) and their antioxidant effects (Sect. 5.1.3). Sects. 5.2 and 5.3 describe fullerene-based materials effects in neurological and respiratory diseases, respectively. Sect. 5.4 gives a summary and discusses the perspectives for the use of fullerene and fullerene-based materials on both systems.

5.1.2 Fullerenes Chemical Properties

Fullerenes represent a class of spherical or ellipsoid molecules made entirely of carbon atoms in a cage-like structure composed of pentagonal and hexagonal faces. Fullerenes were discovery in 1985 by Harry Kroto, Richard Smalley and Robert Curl [8]. Further in 1996, this discovery led to the Nobel Prize of Chemistry.

The number of carbon atoms in each fullerene cage can vary, creating the possibility of numerous new structures, that are represented by the formula C_n (n = number of carbon atoms present in the cage). Since C_{60} discovery, several isomeric forms are confirmed to exist as stable clusters including C_{70}, C_{76}, C_{78}, C_{82}, C_{84}, C_{90} and C_{96}, however C_{60} is the most abundant and well characterized structure (Fig. 5.1). According to Euler's theorem, a closed structure can be construct with 12 pentagons, by this means all fullerenes consist of 12 pentagonal faces and a varying number of hexagonal faces, following the general formula C_{20+2n}.

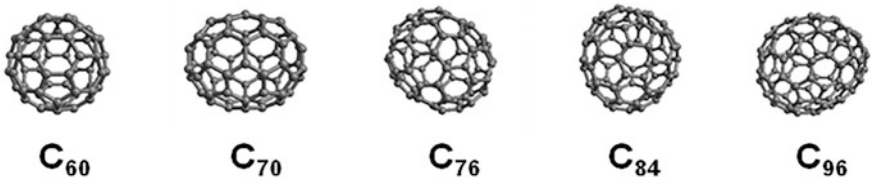

Fig. 5.1 Some fullerene isomeric isoforms [9]

The C_{60} plays significant features as all the 12 pentagons are isolated by hexagons and as it posses different types of bonds: the 6–6 junctions shared by two adjacent hexagons, also known as "short bonds" and the 5–6 junctions present in the juncture of a hexagon and a pentagon, the "long bonds" [10].

As a result of its bond-alternated structure, fullerene C_{60} should not be considered as a real aromatic compound but as a strained, electron-deficient polyolefin. The pyrimidalization of the sp^2-hybridized carbon atoms configure a non-fully planar surface without possibility of electronic ring current. The excess of strain on the surface of C_{60} is responsible for its high reactivity compared to other forms of carbon, because as they react, the functionalized fullerene carbon atoms change their hybridization from a trigonal sp^2 to a less strained tetrahedral sp^3. This particular characteristic of fullerenes makes them reacts readily with electron rich species as radicals, various nucleophiles, and carbenes and they also participate in many thermal cycloadditions reactions, including Diels-Alder [10]. Therefore, this rich chemistry allows a wide range of application for specific proposes [11].

In the biology field, the unique physical and chemical properties of these molecules led many scientists to predict several applications [12–17]. However, due to the insolubility in polar solvents and the aggregation properties, the biological applications have been restricted [18]. Indeed, fullerenes are hydrophobic molecules most common soluble in benzenes, naphtalenes and alkanes [19].

In order to overcome this disadvantage new approaches have been developed to provide its water solubility. Among these approaches we can cite: (i) chemical modifications of the fullerene cage by attachment of several functional groups. The most common chemical groups used are hydroxyl [20], carboxyl [21, 22] and amide [23]; (ii) covering the core of fullerenes with modifying agents such as surfactants, calixarenes [24] and cyclodextrins [25].

5.1.3 Antioxidative Properties of Fullerenes

Several lines of evidence support the fact that fullerenes are powerfull antioxidants, acting as a radical sponge [26]. The fullerene's antioxidant property is based on the fact that fullerenes possess an energetically low-lying, threefold-degenerate lowest unoccupied molecular orbital (LUMO) which can easily take up to six electrons, making an attack of radical species highly possible [27]. In addition, it is clear that nano-size

(diameter of ~1 nm), three-dimensionality and physical properties make the fullerenes and its derivatives, like fullerenol ($C_{60}(OH)_x$), very appealing subjects in medicinal chemistry [28, 29]. Due to these features fullerenes were considered to be the world's most efficient radical scavenger and were described as radical sponges [26].

The protective activity of fullerenes and derivatives to inhibit the chain reaction of lipid peroxidation is based on their capability to react with oxygen radical species such as superoxide, hydroxyl and peroxyl radicals, which attack lipids, proteins, DNA, and other macromolecules, which would lead to cell death [27, 29, 30].

5.2 Fullerene-Based Materials as Neuroprotective Agents

Oxidative stress is involved in the pathogenesis of a number of neurological disorders, including ischemia, and neurodegenerative diseases [31–33]. These states are associated with progressive impairment of mitochondrial function, increased oxidative damage and altered activities of antioxidant defense system. Toward the goal of reducing the oxidative stress in brain injuries, efforts have been focused on the development of new neuroprotective agents [34, 35]. Fullerene-based materials have been exploited for its purpose as excellent antioxidants, therefore possessing a broad spectrum of neuroprotective abilities [12, 36].

One of the most promising and studied classes of water-soluble fullerene-derivatives is carboxyfullerenes, and its neuroprotective efficacy has been established in several models. Dugan et al. [12] using cortical cell cultures exposed to excitotoxic and apoptotic injuries have demonstrated that malonic acid derivatives of C_{60}, ($C_{63}((COOH)_2)_3$) decreased excitotoxic neuronal death in cortical neurons exposed to N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), or oxygen-glucose deprivation. Intriguing in vivo data by the same group showed that infusion of carboxyfullerenes in a transgenic mouse model for familial amyotrophic lateral sclerosis delayed onset of symptoms and death [12]. Remarkably, carboxyfullerene-treated mice showed improved functional performance when compared to saline treated mice. In addition, these authors found that carboxyfullerenes can also reduce apoptosis induced by serum deprivation or by the Alzheimer disease amyloid peptide (Aβ1–42) [12]. Further insight into the use of fullerene derivatives for the treatment of Alzheimer's disease came from the work of Makarova et al. [37]. In this study, the authors performed intrahippocampal microinjections of beta-amyloid peptide 25–35. Importantly, administration of aqueous colloidal solution of C_{60} prevented formation of beta-amyloid 25–35 deposits in hippocampal pyramidal neurons in vivo and neurodegeneration.

Focal cerebral ischemia is associated with a massive production of intravascular free radicals [38]. A variety of free radical removers and antioxidants are known to ameliorate the injury associated with ischemia reperfusion [39]. Huang et al. [40] investigating the effects of hexasulfobutylated C_{60} on the total volume infarct size elicited by focal cerebral ischemia in vivo reported beneficial effects in reducing

volume infarct size. Similar findings were reported by Lin et al. [41] in another model of focal ischemia-reperfusion injury in rat brain. In this study, carboxyfullerene was administered by intravenous injection or intracerebroventricular infusion to rats 30 min prior to the induction of transient ischemia-reperfusion. While no protection of cortical infarction was observed after intravenous injection of carboxyfullerene, intracerebroventricular injection significantly decreased the size of infarcted area and attenuated oxidative injury caused by transient ischemia-reperfusion. It is worth mentioning that these protective effects of carboxyfullerene were also associated with adverse behavioral changes, indicating that these fullerene-derivatives may present undesired side effects when administered in vivo [41]. This finding raises questions regarding toxicity issues related to C_{60} and its use in nanomedicine. In fact, in vitro experiments performed by Zha et al. [42] support the notion that fullerene derivatives present a dual effect, depending on the concentration used. In this study, it has been shown that, at low concentrations, water-soluble polyhydroxyfullerene significantly increased hippocampal neuronal viability and protected neurons from oxidative damage, while, at high concentrations, this fullerene-derivative decreased hippocampal neuron viability and induced apoptosis. Thus, these data highlight the importance of more fundamental research to be carried out, in order to better understand these concentration-dependent dual actions of fullerene derivatives and the underlying mechanisms.

The in vivo ability of carboxyfullerenes to exert neuroprotection was also evaluated in a study conducted by Lin et al. [43]. In this work, the authors used intrastriatal injection of iron to induce degeneration of the nigrostriatal dopaminergic system. Oxidative injury and decreased dopamine content in striatum were observed 7 days after iron infusion. The co-infusion of carboxyfullerene prevented the iron-induced oxidative injury. In the nigrostriatal dopaminergic system the authors did not observe toxic effects of carboxyfullerene.

Among the fullerene derivatives, the polyhydroxylated $C_{60}(OH)_{22}$ and malonic acid $C_{60}(C(COOH)_2)_2$ derivatives were show by Lao et al. [44] to protect against nitric oxide (NO)-induced apoptosis in sodium nitroprusside-treated rat cerebral microvessel endothelial cells (CMECs). This finding supports a potential application of fullerene derivatives in the treatment of NO related disorders.

Most studies attributed the protective effects of fullerene derivatives at least partly to its free radical scavenger properties [11]. Consistent with this idea, Ali et al. [45] proposed that C_3 malonic acid C_{60} derivative acts as a superoxide dismutase mimetic, and they demonstrated its presence in mitochondria, a major site of reactive oxygen species production in the cell. Based on the fact that fullerene derivatives are able to penetrate the blood-brain barrier and preferentially localize to mitochondria [46], Cai et al. [47] hypothesized that these compounds could be used as neuroprotective agents for treating Parkinson's disease. In this study, the authors treated human neuroblastoma cells with MPP1 (1-methyl-4-phenylpyridinium), a cellular model of Parkinson's disease, and evaluated the protective effects of $C_{60}(OH)_{24}$ on MPP1- induced mitochondrial dysfunction and oxidative stress. These results revealed that polyhydroxylated fullerene derivative is a powerful radical scavenger that protects the mitochondria. Thus, this finding is in agreement with previous

reports that support the role of fullerene-derivatives as potential neuroprotective agents capable of protecting against disorders associated with mitochondrial dysfunction, such as neurodegenerative diseases [48].

5.3 Fullerene-Based Therapeutics in Respiratory System

5.3.1 Reactive Oxygen Species in the Lungs

The reactive oxygen species (ROS), mainly derivate from oxygen and nitrogen, are continuously produced in several tissues and cells. At low concentration, ROS play an important physiological role, such as microbial defense system, vascular tonus control, cell signaling, and others [49, 50]. The excess is removed by enzymatic and non-enzymatic ways. However, when the production of ROS is high, the mechanisms of control fail, or there is imbalance between production and degradation, the organism suffers with oxidative stress that is a potential cause of damage [51].

Although oxygen is fundamental to life, high concentrations can exceed physiological limits and be dangerous to the cells [52]. Considering that the respiratory system is directly exposed to very high amounts of the oxygen [53–55], it is very important for the organ to possess defenses against oxidative attacks. Moreover, the lungs receive entire cardiac output, being the main target of systemic ROS. Thus, antioxidant defenses in lungs are primordial for the balance of ROS [52].

A wide variety of respiratory system cells can produce ROS, such as endothelial cells, Clara cells, alveolar type II cells (type II pneumocytes), neutrophils and macrophages. The process of production is the same of the other cells and systems [56]. The main enzymes involved in ROS synthesis are nicotinamide adenine dinucleotide phosphate—oxidase (NADPH oxidase), myeloperoxidase (MPO), xanthine oxidase and coupled and uncoupled nitric oxide synthases (NOS), and for ROS degradation, the most important enzymes are superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) [57].

Once produced, ROS can act in the respiratory system mainly in tracheal and bronchiolar smooth muscle and in pulmonary blood vessels. The primary effect of ROS in lungs is airways hyperresponsiviness to contractile agents [50, 51]. In pulmonary blood vessels the effect of ROS is variable. It is largely accepted that oxidative stress in lung can lead to vasoconstriction due to superoxide anion (O_2^-). However, in cases where there is the production of high amounts of nitric oxide (NO) it is observed pulmonary blood vessels hyporresponsiviness to contractile agents [58]. In both cases, pulmonary hypertension due to hypoxic vasoconstriction is observed [59, 60].

Notably, the lungs have particular mechanisms for its defense against massive production of ROS. Epithelial fluid is rich in glutathione (GSH), an important human pulmonary antioxidant. This substance is able to bind a free iron ion and other metals, and to inhibit synthesis of ROS through the Fenton reaction [61].

There are others antioxidants like water and fat-soluble vitamins on the mucosis secretion of airways that prevent changes on mitochondrial oxidant status. In addition, endogenous antioxidants enzymes such as SOD, catalase and others related with GSH metabolism—and exogenous antioxidants—like vitamins C and E, and N-acetylcistein may also help to prevent the imbalance between oxidant and antioxidant status [51].

In this context, new antioxidants can arise for the treatment of pulmonary diseases associated with the production of ROS. The polyhydroxylated fullerene (fullerenol) is a powerful antioxidant that can be useful because of its specific structure, electronegativity [28] and nanoscale [62], which drives its role like scavenger of ROS [55].

5.3.2 Fullerene-Based Materials and the Respiratory System

Several respiratory diseases, such as asthma, acute lung injury/acute respiratory distress syndrome, sepsis-related lung injury, fibrosis, emphysema chronic obstructive pulmonary disease and hypovolemic shock are associated with an increased production of ROS.

Hypovolemic shock after exsanguination induces increase of ROS production [63]. Subsequently, afferent C-fibers are activated [64] producing tachykinins release and noncholinergic airway constriction. Lai and Chiang [28] evaluated whether fullerenol changed the pulmonary function and whether fullerenol attenuated the exsanguination-induced bronchoconstriction in guinea-pigs. Firstly, they observed that fullerenol does not usually cause alteration in pulmonary function unless it is administered via an intratracheal route in a high dosage (2 mg/kg). The reason for this slight alteration in respiratory function with a high dosage of intratracheal fullerenol was not clear. A possible explanation is that at this dose fullerenol can generate more ROS and cause a mild airway constriction [28]. In a second time they showed that fullerenol ameliorated the exsanguination-induced bronchoconstriction. The antioxidant action of fullerenol probably antagonized the exsanguination-induced increase in ROS production, thereby decreasing the activation of afferent C-fibers, tachykinin release, and airway constriction following exsanguinations in those animals [28].

Lung transplantation is an important way to treat a variety of end-stage lung diseases. Despite this fact, a central problem following this procedure is ischemia-reperfusion injury. This damage happens when blood supply returns to the lung after the completion of the implantation procedure [65]. The alveolar oxygen represents a risk for generation of ROS and continuous oxygen supply can increase ischemia-reperfusion injury [66]. Antioxidant therapy arises like a potential approach to attenuate this injury. Chen et al. [67] carried out experiments in ischemia-reperfused lungs to examine whether fullerene derivatives [$C_{60}(OH)_{7\pm2}$] can protects against injury induced by ROS. When applied in lungs during

ischemia-reperfusion procedure, sodium nitroprusside (SNP) increased pulmonary artery pressure and capillary filtration pressure. The fullerene derivative [$C_{60}(OH)_{7\pm2}$] (10 mg/Kg) attenuated SNP-induced lung injury during ischemia-reperfusion. Chen et al. [67] also investigated whether fullerene derivatives [$C_{60}(OH)_{7\pm2}$] protects against oxidative stress in a murine macrophage RAW 264.7 cell line. They used SNP and hydrogen peroxide (H_2O_2) to enhance NO and oxidants, respectively. They showed that $C_{60}(OH)_{7\pm2}$, in a concentration-dependent manner (10–50 μM), inhibited the increase in ROS production, and the decrease in mitochondrial membrane potential and cell viability induced by SNP and H_2O_2. Based on the above results, Chen et al. [67] suggested that due to their antioxidant characteristics, fullerene derivatives [$C_{60}(OH)_{7\pm2}$] can be used to prevent ischemia-reperfusion injury that follows lung transplantation. They additionally, hypothesized that fullerene derivatives could be potentially useful for the treatment of ROS-induced pulmonary diseases such as sepsis-related lung injury, acute respiratory distress syndrome, fibrosis, emphysema, and asthma.

Inflammation plays a central role in eliminating pathogens and promoting repair of injured tissue. The resolution of this process is fundamental, since excessive or persistent inflammation can lead to tissue damage and exacerbation of diseases, including inflammatory lung diseases, such as acute lung injury [49], chronic obstructive pulmonary disease [68] and silicosis [69]. Exposure to quartz particles leads to ROS production, which leads to lung inflammation. Additional injuries can occur in response to the arrival of inflammatory cells [69]. The quartz-induced inflammation is characterized by neutrophilic inflammation in rodents [70]. Roursgaard et al. [71] studied neutrophilic lung inflammation induced by quartz and evaluated whether pre-treatment with fullerenol [$C_{60}(OH)_{20\pm2}$] at low doses can attenuate this pulmonary disease. They hypothesized that fullerenol would be able to neutralize ROS and thereby attenuate inflammation induced by ROS. Quartz induced a marked inflammation characterized by increase in the number of neutrophil in bronchoalveolar lavage (BAL). Interestingly, fullerenol (0.2, 2.0 and 20 μg) given to mice before quartz instillation decreased neutrophilic lung inflammation in a dose-dependent manner. As a control, mice were exposed to increasing doses of fullerenol alone given intratracheally. The BAL data indicated that only at the highest dose (200 μg), fullerenol induced lung inflammation primarily caused by neutrophils infiltration. This increased number of neutrophils was associated with an increased level of macrophage inflammatory protein 2 (MIP-2), which is a very important neutrophil attractant chemokine. At the doses of 0.02, 0.2, 2.0 and 20.0 μg, fullerenol alone was without inflammatory effect. The authors suggested that this compound has an anti-inflammatory effect at low doses but a pro-inflammatory effect at higher doses and concluded that quartz-induced neutrophilic inflammation in the lungs can be attenuated by administration of the water-soluble polyhydroxylate fullerene derivative (fullerenol) at low doses [71].

Similar results were found by Sayes et al. [72] that showed that intratracheal instillation of low doses (0.2 mg/Kg) of fullerenol [$C_{60}(OH)_{24}$] did not induce neutrophilic inflammation, whereas high doses (1.5 mg/Kg) of the same compound produced marked inflammation in rats.

Doxorubicin, an anthracycline antibiotic, is considered as a broad spectrum antineoplastic agent, which represents one of the most effective chemotherapy agent currently in use for cancer treatment [55, 73]. Doxorubicin antitumor effects include mechanisms related to alterations of DNA and production of free radicals [74]. The most frequent investigated effect of this agent at a molecular level is its tendency to generate large amounts of ROS [54]. In addition, doxorubicin inhibits ROS neutralizing enzymes [54, 55, 75]. A variety of tissues like heart, liver [76], lung [54], testes and kidneys [55], are also affected by this agent. Due to doxorubicin's toxicities, its use in chemotherapy has been largely limited [55, 73, 74]. It is believed that oxidative stress and formation of free radicals play a crucial role in doxorubicin's toxic mechanism. Thus, the use of antioxidants as protective agents could be a potential solution for doxorubicin-induced toxicity [77] in a variety of tissues.

Injac et al. [54] investigated the potential protective effect of 100 mg/kg of fullerenol [$C_{60}(OH)_{24}$] on the lungs of Sprague-Dawley rats with mammary neoplasm after doxorubicin (8 mg/kg). This study indicated that doxorubicin treatment markedly impairs pulmonary function and that pre-treatment with fullerenol (100 mg/kg) prevented this toxicity in rats via inhibition of oxidative stress. In that paper, the authors showed that fullerenol normalized the activity of antioxidants enzymes (catalase, glutathione reductase, GPx, SOD) and lipid peroxidation to the control levels.

The above results are in accordance with Srdjenovic et al. [78], which showed that fullerenol [$C_{60}(OH)_{24}$] inhibited doxorubicin-induced toxicity in lungs, kidney and testes of Wistar rats. They analyzed lipid peroxidation and antioxidant enzymes activity (catalase, glutathione reductase, GPx, glutathione S transferase and SOD) and found that fullerenol in a dose of 100 mg/kg inhibited the oxidative stress in kidney, testes and lungs 2 days after doxorubicin treatment. Moreover, the results with the dose of 50 mg/kg also indicated that, at the period of 14 days after doxorubicin, fullerenol [$C_{60}(OH)_{24}$] induced an important tissue-protection in the analyzed organs.

Vapa et al. [55] also showed that fullerenol [$C_{60}(OH)_{24}$] (50 and 100 mg/kg) had protective effect against lipid peroxidation in testes, kidneys and lungs after the application of doxorubicin (10 mg/kg) in rats.

The toxicity of fullerene derivates in vitro has been investigated in cell lines. Cytotoxicity effects have been related, mainly to high concentrations, but a correlation between in vitro and in vivo toxicity measurements was not observed [72]. In addition, the underivatized fullerene (C_{60}) is 3–4 orders of magnitude more toxic to human dermal fibroblasts, lung epithelial cells and normal human astrocytes when compared to a fully derivatized, highly water-soluble derivate, fullerenol [$C_{60}(OH)_{24}$] [79].

Some pulmonary diseases, such as asthma and chronic obstructive pulmonary disease, require treatment by inhalation. This administration route provides rapid delivery of drugs to the lungs for local treatment of respiratory diseases, avoiding systemic side effects and evading liver first-pass effects [80, 81]. At this regard, the effect of fullerene derivatives like fullerenol given by inhalatory route deserves further investigation. Sayes et al. [72] carried out a study, where they assessed the

lung toxicity of intratracheal instilled fullerene (C_{60}) and fullerenol [$C_{60}(OH)_{24}$] in rats. They applied these compounds at the doses of 0.2, 0.4, 1.5 and 3.0 mg/kg and analyzed the effects 24 h, 1 week, 1 month and 3 months after the instillation and compared with α-quarts-induced lung disease. Exposures to fullerene (C_{60}) and fullerenol [$C_{60}(OH)_{24}$] suspensions did not produce any significant adverse pulmonary effects. Results from the BAL fluid and cell proliferation evaluations demonstrated that pulmonary exposures to α-quartz particles, specially at the higher doses (1.5 and 3 mg/kg), produced significant adverse effects as compared to the controls in pulmonary inflammation (neutrophils number and histology), cytotoxicity (dehydrogenase latic, alkaline phosphatase and total protein content) and lung parenchymal cell proliferation indices. In contrast, both compounds produced transient and reversible inflammation, similar to exposures to Milli-Q water (vehicle), showing that inflammatory response was likely related to the effects of the instillation procedure.

Xu et al. [62] also measured fullerenol [$C_{60}(OH)_{22–24}$] effects after intratracheal instillation in Sprague-Dawley rats at the doses of 1, 5 or 10 mg/rat. Exposures to 1 mg per rat did not induced adverse pulmonary toxicity, while 5 and 10 mg per rat induced cell injury effects (increased pulmonary vascular permeability and increased activity of dehydrogenase lactic and, alkaline and acid phosphatases), oxidative/nitrosative stress (increased lipid peroxidation and NOS activity and, reduced glutathione content and SOD activity) and inflammation (increased TNF-α, IL-1β, IL-6 and neutrophils number on BAL and blood). Their results showed that only the highest dosages (5 and 10 mg per rat) of fullerenol [$C_{60}(OH)_{22–24}$] produced pulmonary inflammatory response. The aggregation of the fullerenol particles in these high-doses may lead to inflammation and be responsible for the pulmonary toxicity. Thus, they concluded that fullerenol particles would have a potential risk for producing pulmonary toxicity and suggested the use of fullerenol as an inhaled drug in short-term and low doses.

The literature about fullerenol value on respiratory system is poor. Therefore, more investigations are essential because the potential use of this compound to improve and treat lung diseases is considerable.

5.4 Summary and Perspectives

The value and applicability of fullerene and fullerene derivatives (fullerenol) for the treatment of neurological and respiratory disorders that are associated with oxidative stress is undoubted. However, caution must be taken with dose and via of administration. Moreover, the difficulty of interpretation and extrapolation of in vitro toxicity measurements to in vivo effects, highlight the complexity associated with probing the relevant toxicological response of fullerene nanoparticle systems. Therefore, more studies are necessary to elucidate the most suitable dosage and via of administration of fullerenol that induce the best results.

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