Exposure Pathways and Toxicity of Fullerenes

Botao Liu *

School of Materials Science and Engineering, Central South University, Changsha, China

* Corresponding author: 8204190118@csu.edu.cn

Abstract. Fullerenes have become very popular nowadays in the field of cosmetic and medicine as "radical sponge" because of their high antioxidant activities. However, there are worries about the toxicity of fullerenes due to their special chemical properties and good ability to enter the human body. In this article, it is found that fullerenes have been proved to have the ability to enter animal body through skin penetration, peroral administration and pulmonary absorption beside initiative injection. The potential mechanism of fullerene toxicity is to interrupt the activation process of protein, cause mitochondrial dysfunction and generate ROS to damage organism. Nevertheless, the experiments result shows the negative effects of fullerenes only occur in a high dose and the assessments of fullerenes toxicity show a low result with the IC50 value and LD50 value to be 383.4 lg/mL to HEK293 and 721mg/kg to mice. It is suggested that fullerenes can rarely affect human health as long as people are exposed to the appropriate dose of fullerenes and the toxicity of fullerenes can further reduce via synthesis of fullerene derivates.

Keywords: fullerenes, toxicity, exposure pathway, mechanism.

1. Introduction

One of the most cutting-edge materials in use today is fullerene, which is the third allotrope of carbon after graphite and diamond. Fullerenes have only about four decades history since firstly being discovered by Kroto et al in 1985, but they have already had a significant impact on the world [1]. After "Krätschmer-Huffman Method" was introduced in 1990 [2], which made the massive production of fullerenes available, fullerenes have shown their great value in various fields like semiconductor, fuel cells, ultra-low pressure gas storage, superconductivities and so on.

With the new upsurge of nanomedicine in medical science and cosmetic industries, as a significant member of the carbon nanomaterials, fullerenes also have become attractive to chemical and material researchers very soon. People have subsequently discovered multitude potential applications of fullerenes and their derivatives, for instance, blocking the HIV-1 infection in vitro [3], photodynamic therapy [4], targeted drug delivery and so on. Fullerenes have also been regarded as a ''radical sponge" in cosmetic industries because of their incredible ability to neutralize radical oxygen species (ROS), and many products based on these high antioxidant activities of fullerenes like sun screams have already been put into practice. However, as fullerenes are now widely used in human daily lives, there are opinions supposed that fullerenes along with other nanomaterials would have negative impact on human and animal health. Fullerenes can easily enter human body because of their nano-scale size, and their special chemical properties have a great possibility to generate toxicities. In the following parts, this article is going to discuss fullerenes characteristics, fullerenes source and fullerenes toxicity.

2. Fullerenes Characteristics

The structure of fullerene is like a sphere or ellipse combined by carbon alloys. The most prevalent type of fullerenes is C60, which has a polygonal structure like a soccer ball with 60 vertices and 32 faces (Fig. 1). All the carbon alloys are localized on vertices and combine with each other via sp^2 hybridized electron orbitals and thus forming a unique pseudo-aromatic structure with a large delocalized π double bond system. The average diameter of C_{60} molecular is 0.7 nm. Fullerenes have many specific properties due to this structure.

For instance, in chemical properties, Carbon atoms are highly pyramidalized and very reactive due to the spherical form of fullerenes. The reactivity of fullerenes is like the reactivity of the electron deficient olefin which is prone to addition reaction. Besides, fullerenes have a high electron affinity, so they can easily attract oxides. The fullerenes can also react with metals, yielding complex productions in carbon cage or having bonding reactions out of the carbon cage. Under the light radiation, fullerenes show a trend to polymerize with each other to form giant fullerene molecule. In physical properties, fullerenes have extremely excellent high photostability, thermal stability and mechanical stability. These excellent properties give fullerenes great potency in various fields. However, there is a property having a negative impact in the usage of fullerenes in medicine that the fullerenes are extremely hydrophobic and only partially soluble in polar liquids. Nonetheless, this problem now seems to have been solved by adding of soluble fullerene derivates by adding solubilizing functional groups, forming suspensions with co-solvents, and encapsulating in specific carriers [5,6].

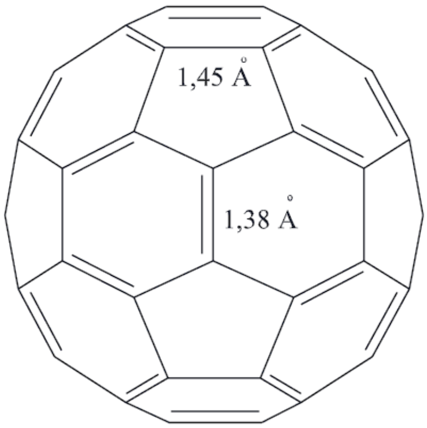

Figure 1. The structure of C_{60}. [7]

3. Exposure pathways

The size of fullerenes is extremely small, so they can easily enter the human body through several paths.

3.1. Skin penetration

The nanoparticles whose diameter is less than 10 nm seem to be able to penetrate across the stratum corneum barrier into the dermis and can accumulate in hair follicles, and there are some evidences showing C_{60} has the ability to penetrate deeply through the stratum corneum [8]. An experiment by Rouse et al. indicates that mechanical stimulation can have a significant impact on the penetration of fullerenes in skin [9]. The dermal penetration of fullerenes in skin flexed for 90 min can be palpably detected under 8 h exposure while in unflexed skin, most of fullerene nanoparticles merely localize in epidermis and little proportions of fullerenes can enter the dermis even after 24 h. What is more, they found this migration in the skin occurs between cells and penetrates the epidermis through passive diffusion via TEM. The fact that fullerenes can pass through these lipid lamellae suggests that nanomaterials may be absorbed by the capillaries of papillary layer and located somewhere else in the body.

3.2. Peroral administration

Fullerenes have shown strong stability so that they can hardly be degraded at natural environment [10], which means these nanomaterials are able to exist for a prolonged period and there is an enrichment effect that the particles can accumulate through food chain. Then these fullerenes may finally be orally taken by human, and it is not unusual for human gastrointestinal system to absorb particles within nanometers to micrometers size [11]. In fact, however, in the experiment given by Hendrickson et al., after intragastrical administration of fullerene C_{60} in rats, the majority of C_{60}

(>99%) had been excreted and only little of it could be absorbed into the gastrointestinal tract [12]. Therefore, oral intake seems not an effective path for fullerenes to enter the animal body.

3.3. Pulmonary absorption

The lung is permanently exposed to the external environment, and it has a surface area, so it is not surprising that lung is vulnerable to diverse pollutants. There is already proof that nanoparticles can cross the air-blood barrier (ABB) and enter the bloodstream from the lungs [13]. The study of Nance et al. reported there are two different mechanisms of the translocation of fullerenes through ABB, diffusion and caveolae-mediated pinocytosis [14]. And their regarded this diffusion effect as the most significant factor in this translocation. The C_{60} was detected mainly in the capillaries of pulmonary lymph nodes and the lumen of alveoli after inhalation exposure.

3.4. Pulmonary absorption

Injection of fullerenes is an effective way to deliver fullerenes into the body.

In intraperitoneal (i.p.) injections executed by Gharbi et al. of aqueous suspension of C_{60} at a dosage of 0.5 g/kg, C_{60} was detected entering the bloodstream and other organs and tissues [15]. In addition, they also found that C_{60} mainly accumulated in the livers of rats, reaching about 24% of the injected amount, and they were detected essentially inside Kupffer cells, some hepatocytes of the capsule as well as inside rare HSC. The experiment also showed the concentration of fullerene C_{60} in liver at day 14 and day 21 decreased to 5% and 1% of the value measured at day 1, which means C_{60} can be eliminated or transferred by livers.

In intravenous injections executed by Rebecca et al. of ^{14}C-labled C_{60} (*C_{60}) into jugular cannulas of female Sprague-Dawley rats, results showed an extremely high speed of *C_{60} to be cleared from the circulation with less than 1% amount of *C_{60} remaining one minute after injection [16]. It was mainly because of their hydrophobic character. After 120 min of the intravenous administration, over 90% *C_{60} was found to be accumulated in the liver and 7% was detected in spleen, lung and muscle. *C_{60} showed a high resistance against the metabolism and can remain in liver for a long time (with 95% *C_{60} remaining in liver even after 5 days) which may lead to chronic toxicity to the liver. Meanwhile, the result of intravenous injection of some derivatives of fullerenes with higher hydrophilia (such as $C_{60}(OH)_x$) showed a more widely distribution in various tissues and can be easier excreted (56% of the injected dose was excreted via urine or feces with 72 h) [17].

4. Fullerenes toxicity mechanism

The toxicity of C_{60} is considered to occur via different approaches. Gieldon et al. investigated how C60 affected protein. The findings demonstrated that C60 aggregates could damage cell structure and were cytotoxic [18]. Their results presented that all the studied proteins readily interact with fullerenes, and that C_{60} tended to interact with the bonding sites of proteins (Fig. 2). The substantial number of fullerene particles bonding on the surface of the receptors of proteins showed an inevitable trend to disturb the normal biological function of proteins. Fullerenes cannot only block active sites of proteins but hamper their activation process, compete with initial substrates with the same surface charge as fullerene, impact the releasing of product as well. What is more, the fullerenes also showed a potentiality to interrupt the creation of protein-protein complex. Also, the phenomenon above may be one of the main factors of the biotoxicity of fullerenes on alive organisms.

Figure 2. Fullerene C_{60} binding at human serum albumin. [18]

In addition, the main mechanism of fullerene toxicity also involves mitochondrial dysfunction. Yang et al. have found that after free mitochondria were exposed to fullerenols ($C_{60}(OH)_{44}$), the permeability of mitochondrial inner membrane to H^+ and K^+ increased, mitochondria swelled, membrane potential collapsed, and membrane fluidity decreased [19]. It is worth noting that fullerenols have a significant effect on the mitochondrial inner membrane in the polar protein region, which may be a key step in its toxic effects. All results indicate that fullerenols not only affect the respiratory chain but destroy the inner membrane as well.

Additionally, the high photosensitivity of C_{60} makes it simple to create excited fullerenes under light excitation conditions, which is essential for C_{60}'s harmful effects on living things. Because C_{60} can produce singlet oxygen via energy transfer in the triplet state after being excited from the singlet state to the triplet state, The produced ROS can directly damage living things, causing a variety of alterations like DNA damage, protein oxidation, and membrane disintegration [20]. When the production of ROS outpaces the ability of the body's antioxidant defence system, the body will be experiencing oxidative stress, which will lead to cytotoxicity from protein oxidation, lipid peroxidation, and mitochondrial damage. It is important to note that the addition of antioxidants like L-ascorbic acid can completely inhibit the oxidative damage and subsequent toxicity of C_{60}.

5. Fullerenes toxicity

Recent research on the acute exposure of lungs to C_{60} fullerene indicated that the elasticity and viscosity of the lung tissues were enhanced in mice who had been intratracheally instilled with 1.0 mg/kg fullerenes C_{60} [21]. In addition, alveolar collapse, septal thickening, along with pulmonary edema were also detected in the mice. The researchers concluded that the exposure of C_{60} can cause harm to the function of lung and result in the impairment of lung parenchyma mechanical parameters and pulmonary tissue.

According to studies on the toxicity of C60 in vitro, increasing the concentration of fullerenes to 36 mg/mL will result in a 20% reduction in the viability of HEK293 cells [22], and that fullerene C60 has a low toxicity to HEK293 cells with an estimated IC50 value of 383.4 lg/mL.

Though the photosensitivity of fullerenes was a worrying problem of the usage of fullerenes in cosmetic, several studies have shown that under the radiation of ultraviolet light, fullerenes did not present any indication of phototoxicity, while in contrast, the value of ROS and the amount of apoptosis were declined in the skin [23].

In vitro and in vivo tests on C_{60} aqueous colloid solution's toxicity were conducted. On the 14th day following an intraperitoneal injection, researchers looked into the acute toxicity of C_{60} at doses ranging from 75 to 1800 mg/kg in mice. No harmful effect on C_{60} was found in the dose range from 75 to 150 mg/kg. But when mice were exposed to C_{60} at doses of 300 mg/kg and higher, the blood toxicity, behavioural abnormalities, and pathological alterations in the spleen, liver, and kidney tissues were all clearly felt. Leukocytes and platelets were suppressed by fullerene C_{60} at a dose of 1200 mg/kg, and signs of inflammation were seen in mice treated with it at a level of 600 mg/kg. The value of LD50 of C_{60} was estimated to be 721 mg/kg And The study findings indicated that C_{60} aqueous solution is safe for use in biomedicine at doses between 75 and 150 mg/kg.

6. Conclusions

There have already been many studies on the toxicity of fullerenes. It has been proved that fullerenes can enter animal body through skin penetration, peroral administration and pulmonary absorption beside initiative injection. Various probable mechanisms of fullerenes toxicity are discussed in this article. Fullerenes has the potence to interrupt the activation process of protein, cause mitochondrial dysfunction and generate ROS to damage organism. Recent researches have detected the negative effect of fullerenes to pulmonary tissue and fullerenes induction of inflammation in mice. However, all the toxicities of fullerenes that have been observed in experiments are based on high dose. In fact, the volume of fullerenes in actual exposures are far less than that in experimental conditions. The assessment of the toxicity of fullerenes shows a low result with the IC50 value of fullerene C_{60} is calculated to be 383.4 lg/mL to HEK293 and the LD50 value is calculated to be 721mg/kg to mice.

It is noteworthy that the toxicity and excretion of a fullerene is highly depended on its size and its functional group, so synthesis of fullerene derivates which remain the function of fullerenes but have lower toxicity to human body seems to be a promising approach to solve the problem of fullerenes toxicity. Consequently, though there are some worries about the toxicity of fullerenes, their toxicity can rarely affect human body in an appropriate dose.

References

[1] Kroto, H. W., Heath, J. R., O'Brien, S. C., et al. (1985). C60: Buckminsterfullerene. nature, 318(6042), 162 - 163.

[2] Krätschmer, W., Lamb, L. D., Fostiropoulos, K. H. D. R., et al. (1990). Solid C60: a new form of carbon. Nature, 347 (6291), 354 - 358.

[3] Khakina, E. A., Ol'ga, A. K., Popova, M. L., et al. (2017). Synthesis of different types of alkoxy fullerene derivatives from chlorofullerene C60Cl6. Organic & Biomolecular Chemistry, 15 (4), 773 - 777.

[4] Franskevych, D., Palyvoda, K., Petukhov, D., et al. (2017). Fullerene C60 penetration into leukemic cells and its photoinduced cytotoxic effects. Nanoscale Research Letters, 12 (1), 1 - 9.

[5] Krusic, P. J., Wasserman, E., Keizer, P. N., et al. (1991). Radical reactions of C60. Science, 254(5035), 1183 - 1185.

[6] Akçan, R., Aydogan, H. C., Yildirim, M. Ş., et al. (2020). Nanotoxicity: A challenge for future medicine. Turkish journal of medical sciences, 50 (4), 1180 - 1196.

[7] Martins, M., Azoia, N. G., Melle-Franco, M., et al. (2017). Permeation of skin with (C60) fullerene dispersions. Engineering in Life Sciences, 17 (7), 732-738.

[8] Ruoff, R. S., Tse, D. S., Malhotra, R., et al. (1993). Solubility of fullerene (C60) in a variety of solvents. The Journal of Physical Chemistry, 97 (13), 3379 - 3383.

[9] Da Ros, T., & Prato, M. (1999). Medicinal chemistry with fullerenes and fullerene derivatives. Chemical Communications, (8), 663 - 669.

[10] Torres, V. M., Posa, M., Srdjenovic, B., et al. (2011). Solubilization of fullerene C60 in micellar solutions of different solubilizers. Colloids and Surfaces B: Bio interfaces, 82 (1), 46 - 53.

[11] Florence, A. T. (1997). The oral absorption of micro-and nanoparticulates: neither exceptional nor unusual. Pharmaceutical research, 14 (3), 259 - 266.

[12] Hendrickson, O. D., Morozova, O. V., Zherdev, A. V., et al. (2015). Study of distribution and biological effects of fullerene C60 after single and multiple intragastrical administrations to rats. Fullerenes, Nanotubes and Carbon Nanostructures, 23 (7), 658 - 668.

[13] Geiser, M., Rothen-Rutishauser, B., Kapp, N., et al. (2005). Ultrafine particles cross cellular membranes by nonphagocytic mechanisms in lungs and in cultured cells. Environmental health perspectives, 113 (11), 1555 - 1560.

[14] Naota, M., Shimada, A., Morita, T., et al. (2009). Translocation pathway of the intratracheally instilled C60 fullerene from the lung into the blood circulation in the mouse: possible association of diffusion and caveolae-mediated pinocytosis. Toxicologic pathology, 37 (4), 456 - 462.

[15] Gharbi, N., Pressac, M., Hadchouel, M., et al. (2005). [60] Fullerene is a powerful antioxidant in vivo with no acute or subacute toxicity. Nano Letters, 5 (12), 2578 - 2585.

[16] Bullard-Dillard, R., Creek, K. E., Scrivens, W. A., et al. (1996). Tissue sites of uptake of14C-labeled C60. Bioorganic Chemistry, 24 (4), 376 - 385.

[17] Ji, Z. Q., Sun, H., Wang, H., et al. (2006). Biodistribution and tumor uptake of C60 (OH) x in mice. Journal of Nanoparticle Research, 8 (1), 53 - 63.

[18] Giełdoń, A., Witt, M. M., Gajewicz, A., et al. (2017). Rapid insight into C60 influence on biological functions of proteins. Structural Chemistry, 28(6), 1775 - 1788.

[19] Yang, L. Y., Gao, J. L., Gao, T., et al. (2016). Toxicity of polyhydroxylated fullerene to mitochondria. Journal of hazardous materials, 301, 119 - 126.

[20] Sayes, C. M., Gobin, A. M., Ausman, K. D., et al. (2005). Nano-C60 cytotoxicity is due to lipid peroxidation. Biomaterials, 26 (36), 7587 - 7595.

[21] Caldeira, D. D. A. F., Mesquita, F. M., Pinheiro, F. G., et al. (2021). Acute exposure to C60 fullerene damage's pulmonary mitochondrial function and mechanics. Nanotoxicology, 15 (3), 352 - 365.

[22] Kartel, N. T., Ivanov, L. V., Lyapunov, A. N., et al. (2020). Study of cytotoxicity performance of carbon nanohorns by method of spin probes. Fullerenes, Nanotubes and Carbon Nanostructures, 28 (9), 737 - 744.

[23] Aschberger, K., Johnston, H. J., Stone, V., et al. (2010). Review of fullerene toxicity and exposure–appraisal of a human health risk assessment, based on open literature. Regulatory Toxicology and Pharmacology, 58 (3), 455 - 473.