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Fullerenes as Anti-Aging Antioxidants

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Abstract: Here we review fullerenes biological effects focusing on their antioxidant and anti-ageing action. A scope of various poisonous and healing properties reported in literature for fullerene and its derivatives is analyzed. The review begins with the history of fullerenes discovery and their main properties. Then we focus on the longevity and antioxidant action, including the confrontation of available experimental data and theoretical modeling of buckminsterfullerene C₆₀. Special attention is given to our hypothesis concerning the possibility of fullerenes to act as mitochondria protonophore and various simulations of the transport of C₆₀ and its hydroxylated and other derivatives through lipid bilayer membranes, which can account for scavenging capacity of fullerenes for reactive oxygen species and their acting as mild mitochondrial respiration uncouplers. Extension of the theoretical modeling to the mitochondria membranes and implications for the real biological systems is analyzed. Finally, we focus on the toxicity evaluation and current therapeutic usage of fullerenes. The review contains a comprehensive discussion of both papers published by 2016 and our own research results.

ARTICLE HISTORY

Received: March 15, 2016
Revised: April 20, 2016
Accepted: June 18, 2016

DOI:
10.2174/1874609809666160921120008

Keywords: Anti-aging, antioxidant protection, fullerenes, longevity, mitochondria, protonation, reactive oxygen species, safety, toxicity.

INTRODUCTION

Among numerous aging concepts, a viewpoint based on the idea proposed by D. Harman about free-radical oxidation participation in senescence is the most theoretically potent one [1]. Since 1956, Harman has formulated two important theses. The first one states that aging, as a whole, and many geriatric diseases, in particular, are just a slow poisoning by reactive oxygen species (ROS). The second thesis specifies that ROS are mainly generated as a result of “oxidative enzymes” functioning. As the development of oxidative stress biology was progressing, it became clear that main generators of ROS for the majority of eukaryotic cells concentrate in mitochondria. Therefore Harman augmented free-radical theory to transform it into mitochondrial aging theory in 1972 [2]. The latter has an advantage of not only explaining many known results but also exploring new fruitful lines of research. One of the latter is related to the search of antioxidants able to slow aging down. Countless studies over the last half-century showed that some synthetic and natural antioxidants exhibit a broad spectrum of oxyradical quenching activity based on reactions of single electron transfer, hydrogen atom transfer, sequential electron proton transfer, proton coupled electron transfer, radical adduct formation, and iron chelation [3-8].

Such kind of chemical activity correlates to the ability to increase average life expectancy of laboratory animals [9].

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Many antioxidants not only delay aging but also make it milder by reducing symptom severity of senile diseases. This aspect of anti-aging activity determines attractiveness of antioxidants for pharmacology [9].

Comparing maximal anti-aging effect peculiar to different classes of antioxidants, one should note a special behavior of spherical carbon nanoparticles – fullerenes [10].

FULLERENES DISCOVERY AND SOME PROPERTIES

Until 1985, molecular form of carbon did not seem conceivable, as pure carbon had already been well studied. That is perhaps why it took Harry Kroto from the University of Sussex, England, around one year to talk Richard Smalley (Rice University, USA) into using his equipment to study vaporized carbon in a mass spectrometer [10]. H. Kroto was interested in understanding the long linear carbon chain molecules found in interstellar space according to radio-astronomical spectra. R. Smalley had a powerful laser capable of heating a graphite target to as high as tens of thousands of degrees, which was used for an experiment. Cooling carbon vapor condensed into clusters of different sizes, which were then ionized by one more laser pulse in another vacuum chamber in order to finally analyze them in the mass spectrometer. In September 1985, an experiment revealed an unusually strong peak corresponding to the cluster consisting of sixty carbon atoms, and a less intense but still pronounced peak – corresponding to seventy carbon atoms, respectively [10].

Scientists had to admit that very stable C_{60} cluster formed and, furthermore, it did not react. The latter meant the mysterious cluster had no “dangling bonds” to be terminated with other elements. Considering plausible structures for C_{60} molecule, researchers came up with a soccer-ball sphere as the only feasible option. The novel compound was dubbed “buckyball” or “Buckminsterfullerene” after American architect Buckminster Fuller who designed a shape of geodesic domes in the form of C_{60} molecule before it was found in nature.

A concise letter to Nature [11] sent just a few days after discovery to report on C_{60} , was followed by a series of studies resulted in the eventual acceptance of fullerenes by scientific society by 1990, and synthesis of many similar compounds. There are spherical fullerenes with a number of carbon atoms ranging between 20 and hundreds [12] yet C_{60} and C_{70} forms are most common. Later, one more akin allotrope of carbon - nanotube or buckytube - was found by I. Sumio [13]. In 1996 Richard Smalley, Robert Curl, and Harry Kroto received the Nobel Prize for chemistry for the fullerene discovery.

Fullerene C_{60} is a hollow convex molecule made up of 12 pentagons separated by 20 hexagons (“isolated pentagon rule”), with 60 carbon atoms being located at vertices; every carbon atom valence is satisfied by two single and one double bonds. Buckminsterfullerene has 60 single C-C bonds of ~ 1.46 Å (between carbons comprising pentagons) and 30 double bonds of ~ 1.4 Å (between adjacent hexagons) leading to an average bond length 1.44 Å in accordance with nuclear magnetic resonance spectroscopy [14]. The diameter of C_{60} is ~ 7.1 Å and its van der Waals diameter is about 10 Å.

Discussing properties of fullerenes, we will further mostly refer to C_{60} as the most ubiquitous representative. It has carbon atoms with sp^2 and sp^3 hybridized valence shell yet it does not demonstrate super-aromaticity due to the lack of π -electron delocalization. C_{60} has considerable electron affinity to reversibly accept up to 6 electrons thanks to both triply-degenerate low-lying lowest unoccupied molecular orbital (LUMO), capable of acquiring e^- from donors, and geometric structure, allowing charge distribution over large volume thus minimizing electrostatic repulsion at the donor site [15]. On the other hand, oxidation is irreversible [16].

Compressed fullerenes compete with steel and diamond in hardness exceeding bulk modulus of both [17]. Besides, C_{60} additives strengthen metals and alloys, and fullerene is an excellent precursor to diamond. Fullerenes have non-linear optical response that can also be enhanced by atoms enclosed inside a cage, which can be used for optical limiting. Solid fullerene crystallizes within fcc (face-centered cubic) lattice at room temperature with a period of ~ 1.42 nm via van der Waals forces; below 255 K a first-order phase transition to the simple-cubic structure occurs [18, 19]. Such crystals have voids large enough to accommodate impurities, which may change conductivity from semiconductor to even superconductor in the case of some molecular solids with alkali or rare earth metals at the interstitial sites – fullerides [20-22]. Only solid state C_{60} demonstrates superconductivity and molecular ferromagnetism which may be used in future applications [19].

Promising applications of fullerenes include their usage as chemical sensors, molecular electric wires, electronic, and optical devices [19]. The latter is based on the ability of C_{60} to perform as electron transporter. Fullerenes can be used as catalysts [19].

Fullerenes are attractive as ideal electron acceptors in organic solar cells that would cost less and have flexibility in comparison with conventional silicon-based solar elements [19]. Unfortunately, current efficiency of the fullerene-based solar cells falls short of their inorganic counterparts [16, 23, 24]. Fullerenes can be reversibly hydrogenated to form polyhydrofullerenes (also called fullerenes) $C_{60}H_n$, where $n = 2, 4, 6, 18, 32,$ or 36 [24-34]. Besides, $C_{70}H_{38}$, $C_{70}H_{40}$, $C_{70}H_{36}$, and deuterated $C_{60}D_{18}$, $C_{60}D_{36}$, $C_{60}D_{38}$ have been synthesized [24, 25, 34, 35]. The nature of corresponding synthesis reactions is chemical, and can be envisioned considering fullerene being not completely aromatic, namely localized character of the double C=C bonds that add H atom upon breaking to form two single bonds C-C and C-H [24]. As C-H bonds are ~ 15 kcal/mole weaker according to the theoretical estimates [36], they break first when heated, and fullerene structure recovers. Therefore, among attractive fullerenes applications is hydrogen storage for e.g. vehicles, which would have superior storage capacity-to-weight ratio. Unfortunately, current implementations are far from being suitable because of the problems with loading/releasing hydrogen gas in an efficient and repeatable way [24, 37].

Of special interest are endohedral fullerenes – molecules containing impurity inside the carbon cage. For instance, metallofullerenes $M@C_{60}$ can serve as nano ball bearings (when $M=K$) and superconductors ($M=La$) [22]. Being incorporated inside a fullerene cage, spin carriers like N or P atoms can be applied for spintronics with possible use for quantum computing due to high spin relaxation times owing to shielding effect of C_{60} cage. Among reported fullerenes impurities are also mono- and di-metal rare-earth elements, refractory metals, rare-gases, He, CO, and CN [21, 38-40].

A problem of synthesis is of interest when it comes to the endohedral fullerenes. One can envision different ways of confining atoms inside a fullerene cage: accelerating ions to the right kinetic energy so that they could enter the cage but not escape after the collision, chemical reactions making carbon ring open, and synthesis of the endohedral fullerenes from the beginning [19]. Although only the latter way has been so far widely implemented by various synthesis techniques resulting in macroscopic fullerenes yield, empty fullerenes have been shown to be thermally penetrated by He and Ne noble gases that gives a hope for the future advance in this area. In such a process a 5-6 bond (between the pentagon and hexagon) is believed to break to form a nine-membered ring allowing noble gas atoms to pass through; then thermally-broken bond can be closed back resulting in an endohedral cage [19, 38, 41]. More recently, by chemical opening/closing of fullerenes (“molecular surgery” method), H_2 molecule was put inside C_{60} and C_{70} cages, respectively, with larger entities such as He, $2H_2@C_{70}$, $2H_2@C_{120}$, $N@C_{120}$ having been similarly synthesized [42-50].

Trapped elements are tightly bound with a negative charge transferred to the cage and are securely isolated from both mechanical and chemical interaction: endohedral

fullerenes prefer to lose carbon dimers C_2 and shrink but not lose an impregnated metal; it continues until a burst happens at the certain size conditioned by the impurity ionic radius [19, 21]. Depending on the manufacturing process, there may be formed a variety of endohedral fullerenes C_{2n} having even number of carbon atoms $60 \leq 2n \leq 84$, with divalent metals being prone to $M@C_{60}$ and trivalent ones to $M@C_{60}$, $M@C_{74}$, $M@C_{82}$ species [19, 21]. Interestingly, theoretical calculations favor sometimes off-center positions of the dopants resulting in lower-symmetry and additional electron transfer to the cage [19, 21].

Limitless are possible biological applications of endohedral fullerenes ranging from drug carriers to magnetic resonance imaging agents. Unfortunately, larger cages of C_{2n} with $2n > 60$ are required to absorb drug molecules, while fullerenes' yield during synthesis falls drastically with increasing $2n$ [24]. Interesting point is that pure fullerenes are insoluble in water and only moderately soluble in certain organic solvents [19]. It appears to be another problem according to *in vitro* experiments (but not *in vivo*, surprisingly). Thus, there is a controversial point regarding whether biological utilization of fullerenes demands an attachment of polar functional groups to make them soluble or not [19]. Water-soluble fullerenes can be used in biology as a medication for different diseases, antibacterial agents, and enhancers in photodynamic cancer therapy. None of the listed applications is available to consumers to date. Perhaps the only exception overstepped research laboratories is a skin cream reportedly reducing free radical damage [51]. Fullerenes are found to have contradictory biological properties varying from toxic to medicinal effects discussed below.

FULLERENE INFLUENCE ON LONGEVITY

In the recent study, F. Moussa and H. Schwarz [52] showed that the oral administration of fullerene C_{60} dissolved in olive oil substantively retards senescence of rats. Herewith, median and maximum life span increases approximately twice. Although sample groups of animals in the experiments by Moussa and Schwarz were small-scale, relationship of mean values and parameters of longevity range allows one to conclude that obtained results have high statistical significance. Anti-aging effect of C_{60} and its derivatives manifests not only in mammals, but both *in vivo* and in model cell systems, as well as in members of other taxa [53]. Thus, it was found that water and oil emulsion of fullerenes can reduce skin premature aging, mediated by ROS-induced destruction of collagen [54].

As unmodified fullerenes are poorly soluble in water, many authors prefer to work with their modified derivatives and consider them of more use. Although it may be more convenient for *in vitro* experiments, it does not necessarily mean pristine fullerenes are less biologically active. In fact, chemically modified fullerenes may have dissimilar properties, and one should treat them separately. Hydroxylated fullerenes (fullerenols) and malonic acid substituted fullerenes (carboxyfullerenes) are two major groups of C_{60} derivatives [55, 56].

In 2010, a large-scale work of J. Gao *et al.* presented data on the influence of polyhydroxylated fullerenes (PHF) on the life expectancy [53]. It was studied in representatives of the

four kingdoms, the typical inhabitants of the aquatic environment and soil: green algae *Pseudokirchneriella subcapitata*, plant *Arabidopsis thaliana*, fungus *Aspergillus niger*, and invertebrate *Ceriodaphnia dubia*. The most impressive results were for *Ceriodaphnia dubia*: the life expectancy of individuals under the influence of PHF at the concentration of 20 mg/L increased by 38%. Applying of the 5 mg/L PHF solution stimulated growth of *Pseudokirchneriella subcapitata* culture. The concentration of 10 mg/L stimulated growth of *Aspergillus niger*. During germination of the *Arabidopsis* seed, treated with PHF at concentrations above 100 mg/L, a hypocotyl elongation compared to the control was observed, which the authors attributed to the likely effect on sensitivity of the receptors of phytohormones or their overproduction. Thus, PHF can act as a stimulator/regulator of growth and life expectancy, and, judging by the prevalence of impacts in different groups of living organisms, the mechanism of this action is to affect quite evolutionary ancient cellular structures.

In 2015, W. Cong *et al.* presented data that polyhydroxylated fullerene slowed down aging of nematode *Caenorhabditis elegans*, apparently reducing the level of oxidative stress, and also providing protection to *C. elegans* under stress conditions by up-regulating stress-related genes in the DAF-16 (forkhead transcription factor) dependent manner [57]. DAF-16 is known to be a key element in the regulation of longevity in insulin/IGF-1 pathway. It was observed that the motor activity of the adult PHF-treated nematodes decreases more slowly compared to the control. In addition, the survival rate in conditions of thermal and oxidative stress increased as compared to the control.

In 2013 O. I. Yablonskaya *et al.* found that the hydrated C_{60} fullerene at concentrations of 10^{-19} - 10^{-21} M affects the growth and aging of mammalian cells (in culture of transformed Chinese hamster cells) [58]. In these experiments, in contrast, the accelerating of cell aging was observed; however, the authors suggest that this effect may be specific to the particular transformed cells used.

As for the second group of derivatives (carboxyfullerenes), in 2008 Quick and colleagues have shown that chronic treatment with carboxyfullerenes reduced age-associated oxidative stress and mitochondrial radical production, and significantly extended lifespan (by 11%). Moreover, no age-related cognitive decline was observed in experimental mice compared to the control [59]. A derivative of fullerene C_{60} - C3, a fullerene connected with 3 carboxyl groups, was used. C3 is a mitochondrial active superoxide dismutase (SOD) mimetic, which manifested neuroprotective properties in the earlier studies of the same team [60]. Treatment of rats began at the age of 12 months, when the animals reached "maturity". As the main mechanisms of such effects, authors suggested reducing of superoxide anion radicals in the mitochondria, and indirect impact on systems controlled by mitochondria, in particular, insulin-dependent signaling or the sirtuins.

At the core of the observed effects may underlie the following properties of fullerene derivatives: radioprotective [61], antioxidant [62-66], anti-inflammatory [67], anticancer [68, 69], antibacterial [56], as well as reducing the level of apoptosis [70].

In the described above investigations both C_{60} and its hydrated forms $C_{60}\text{-OH}_n$ were used. Even though hydrated forms of fullerene significantly differ from C_{60} in water solubility, their adaptogenic and biophysics effects including those related to interaction with mitochondria are similar in general. In our opinion, it allows one to consider both molecules together also from the point of view of the action mechanism.

It follows also if we take into account that for our hypothesis on mitochondrial targeting and action of fullerene, discussed below in detail in the section "Mitochondrial targeting and uncoupling of respiration and phosphorylation", it is important the very possibility of the charge transport between membranes mediated by fullerene. Functionalization of fullerenes with -OH or other groups may alter their solubility (and hence aggregation chances) and the velocity of the intra-membrane translocation. However, fullerenes, regardless pure or with any additives that cannot be predicted for sure due to complicated cell medium, are shown to accumulate in mitochondria. Therefore, we may consider them together.

Experiments by F. Moussa and H. Schwarz also showed that rats treated with fullerene C_{60} demonstrated high resistance to carbon tetrachloride. Toxicity of this substance is mediated by ROS generation [71]. According to this fact and results of biochemical tests, fullerene C_{60} was proposed to possess high antioxidant activity *in vivo*. Taking into account free-radical theory of aging, highly active antioxidant activity can be the basis of unique anti-aging (geroprotective) properties.

ANTIOXIDANT ACTIVITY OF FULLERENES

Fullerene C_{60} and its modifications have been shown to demonstrate antioxidant properties in a range of chemical [64, 72, 73] and biological studies *in vitro* [65, 74] and *in vivo* [64, 75, 76], and thus were suggested for numerous applications [52, 77].

It is proposed that fullerenes act as anti-ROS factors by crossing the cell membrane and localizing preferentially in the mitochondria [78]; serving there and elsewhere as radical scavengers [78]; and inducing intrinsic cellular signaling response [74].

Molecules of C_{60} consist of 60 carbon atoms connected by sp²,5-bonds, which make it pseudo-aromatic thanks to (partially) delocalized π -electrons over its carbon core, or cage. Thanks to such a structure, C_{60} can readily react with oxygen free radicals. Since the first studies of C_{60} 's antioxidant abilities, it is supposed that an extended electron-conjugation system only determines the high reactivity of fullerene molecules towards ROS. Until recently, fullerene was considered to be a novel "structural" antioxidant and characterized as a "radical sponge" by Krusic *et al.* [73]. Nevertheless, based on the available data, it is obvious that C_{60} 's antiradical properties are not limited only by the direct reaction of the fullerene carbon cage with ROS [64].

Several research groups demonstrated that C_{60} and its hydroxylated derivative $C_{60}(\text{OH})_{24}$ are powerful antioxidants with radical scavenging activity for superoxide, hydroxyl, and lipid radicals [74].

Plenty of reports demonstrate antioxidant effects of fullerenes functionalized with various radicals.

Ali *et al.* synthesized a fullerene C_{60} derivative with superoxide dismutase mimicking properties (a tris-malonyl C_{60} derivate) [59, 63, 64]. Tris-malonyl C_{60} derivate is able to remove superoxide radical at a rate comparable to that shown for SOD [64]. Chen *et al.* found that fullerene derivatives protected RAW 264.7 macrophages from oxidative stress and were protective in ischemia-reperused lungs [79]. A glutathione linked fullerene derivative has been reported to protect pheochromocytoma cells from oxidative stress. A cysteine derivative of C_{60} fullerene and alanine [80, 81] and methionine [82] derivatives of C_{60} have also been shown to have antioxidant properties without overt toxicity [77]. Other amino acids, folacin/fullerene derivatives were synthesized and also demonstrated antioxidant effects [83], however these effects might be due to signaling induction of cellular intrinsic antioxidants by the derivatives.

In addition to chemical antioxidant activity, fullerenes demonstrate capability to induce antioxidant signaling pathways, and at least in part, this is due to their pro-oxidant effects.

Cai and co-authors report that $C_{60}(\text{OH})_{24}$ probably plays a significant indirect protective role through antioxidant pathways, especially nuclear factor erythroid 2-like 2 / activator protein 1 (NFE2L2/AP-1) pathway. The authors demonstrated that $C_{60}(\text{OH})_{24}$ fullerene elevated the total and nuclear NFE2L2 expression in an *in vitro* experimental model [74]. NFE2L2 controls several cyto-protective factors, starting with antioxidant enzymes, phase II and III detoxication proteins, and ending with ATP Binding Cassette (ABC) drug resistance pumps [84]. Thus, fullerenes may represent means to induce general cellular protection systems. However, it should be noted that whenever NFE2L2 is activated by a treatment - the treatment may not be considered entirely antioxidant, as NFE2L2 responses to pro-oxidants and electrophiles, as well as to stress signaling provoking stimuli, i.e. NFE2L2-activating treatments may actually be dangerous to the cell.

Ye and colleagues showed that at least in A549 cells, the $C_{60}(\text{OH})_{24}$ fulleranol induces the NFE2L2/AP-1 pathway via p38 mitogen-activated protein (MAPK) kinases [85]. They also demonstrated that NFE2L2/AP-1 pathway is essential for the fulleranol to exert its effects.

It should also be mentioned that other fundamental chemical and physical mechanisms may explain the antioxidant effects of fullerenes. For example, Elswaifi and co-authors mention that cerium oxide nanoparticles (CeONP) have activity dictated by their quantum mechanical properties at the atomic scale, a poorly studied area. As such, it is known that CeONP have a high capacity to absorb and transfer electrons. Therefore, Elswaifi and colleagues conclude that nanoparticles may even serve as catalysts that absorb and transfer atomic entities. This catalytic activity indirectly restoring redox balance in a cell may be provided by CeONP's ability to facilitate channeling of electrons and may be explained at the quantum level [77]. At least some of these considerations may apply to fullerenes.

There are also numerous reports on pro-oxidative effects of fullerenes [86-91], a common observation with regards to antioxidants in general, including such classical antioxidants as vitamins C [80, 92, 93] and E [92, 94], carotenoids [92], and tert-butylhydroquinone (tBHQ) [95, 96], as well as other substances.

Usenko *et al.* reported on clear signs of oxidative stress induction by the pure fullerene C₆₀ and C₆₀(OH)₂₄ in zebrafish [97]. Pristine fullerene C₆₀ demonstrates these pro-oxidant, as well as pro-inflammatory effects in various organisms [98]. However, several studies demonstrated that pro-oxidant efficacy was only due to some impurities present in the used C₆₀ preparation, especially tetrahydrofuran (THF) [89, 99, 100].

Park and colleagues identified the post fullerene treatment decrease of intracellular glutathione (GSH) and the increase of nitric oxide production concurring with the decreased viability of RAW 264.7 cells [91]. Carbon fullerenes were also shown to induce the increase of serum cytokine levels of interleukins IL-1 and IL-6, and the expression of genes including HSP 8, iNOS, and COX-2 in the same study. The same group demonstrated *in vivo* pro-inflammatory effects of fullerene C₆₀ in mice [91]. The latter study utilized toluene solvent instead of THF in order to avoid THF-associated toxicity and obtain fullerenes in phosphate buffered saline without (hopefully) traces of solvent.

As seen from the above data, fullerenes and their derivatives cannot be definitely regarded as either pure antioxidants of pure pro-oxidants. One can speculate that their effects are always dependent on “biochemical environment” and traces of precursors and process-related impurities. On the other hand, many *in vivo* and *in vitro* studies indicate it is method of preparation that is responsible for fullerene toxicity [100]. Knowing how cellular context affects fullerene’s impacts on a cell is the relatively easy solution for the discouraging problem of complexity of biochemistry and ambiguity of bioeffects of this type of nanoparticles.

Fullerene C₆₀ is known to be able to inactivate hydroxyl radicals by attaching to double bonds [101]. However, this mechanism cannot fully explain the increase in lifespan of rats (near two times) mentioned above. Such kind of free radicals scavenging activity is also attributed to natural phenols and other classes of antioxidants; however, they do not exhibit high senescence retarding activity [102].

MITOCHONDRIAL TARGETING AND UNCOUPLING OF RESPIRATION AND PHOSPHORYLATION

Mitochondrial targeting could explain the mechanism of the anti-aging activity of C₆₀. Mitochondrial-targeted antioxidants such as lipophilic cations (Skulachev ions) with antioxidant load [103] are known to be the most effective antiaging agents among synthetic compounds. The ability of modified C₆₀ to accumulate in mitochondria is known since 2002 [104]. In the latter work, the authors used a C₁₄ labeled malonyl mono-adduct fullerene to study the intracellular distribution of this kind of C₆₀ derivative. After differential centrifugation, the data indicated that the radioactivity of the mitochondrial fraction exceeded that of cytosol and micro-

somes over 12 and 2 times, respectively. Surprisingly, bigger fullerenes, e.g. C₇₀, tend to uniformly distribute over endoplasmic reticulum and lysosomes, with less concentrations being found in mitochondria of human mast cells [105]. The difference in bio-distribution could be attributed to the difference of polarity/solubility as well as to the different type of cells used in these two experiments.

Taking into consideration that H₂@C₆₀ has been synthesized, it becomes obvious that there is enough space for at least a single hydrogen atom or a proton inside fullerene [24]. Despite the fact that such species have not yet been found, they should be quite stable once formed. The inter-mitochondria membranes charge transfer mediated by such fullerenes could in theory lead to remarkable biological implications in view of antioxidant and anti-aging effects of fullerenes described above.

Starting from the mentioned above evidence on fullerene accumulating predominantly in mitochondria, we proposed a hypothesis explaining its antioxidant action [106, 107] that may be achieved by only moderate decrease in mitochondrial potential Δψ. The inner mitochondrial membrane is negatively charged and may be a source of toxic ROS as it contains a respiratory chain. The membrane of mitochondria has a positive charge. If fullerenes could acquire the latter by e.g. absorbing an excess of proton(s) existent there, they would be able to transport positive charge through the mitochondrial membrane thus lowering Δψ. Described mechanism would elegantly account for mild uncoupling of respiration and phosphorylation promoted by fullerenes. Moreover, similar mechanism can be envisioned for fullerenes counterparts – carbon nanotubes [108] as they were also shown to aggregate in mitochondria and have antioxidant effect [109, 110].

In order to check this hypothesis, we have carried out Density Functional Theory (DFT) calculations of fullerenes in the presence of protons with ADF 2012 program suite [111, 112]. Briefly, calculations were done in a gas phase by minimizing the total energy of the “C₆₀ + hydrogen(s)” system with a positive charge equal to number of H atoms and probing different exchange-correlation potentials and Slater orbitals basis sets to achieve convergence [106, 107].

These calculations resulted in a stable structure consisting of fullerene and up to six protons forming C-H bonds from inside with relatively uniform electron charge transfer to the C₆₀ cage. Interestingly, as far as modeling of single-walled carbon nanotubes is concerned, a protonation occurred from external side of the tube but all other conclusions held true [108].

There are some reports on theoretical calculations of hydrogenated fullerenes performed on different levels of theory claiming various number of hydrogen, water, or even ammonia molecules that can be confined inside C₆₀ [24]. In contrast, Dodziuk carried out molecular mechanics simulation of C₆₀, C₇₀, C₇₆, C₈₀ isomers putting one to four hydrogen molecules inside the cage, and found only up to two H₂ can be stabilized inside a closed C₇₀ [113]. Certain calculations indicated stabilization of the HF and LiH polar moieties inside the cage, while H₂, N₂, and CO led to structure destabilization [114]. Nevertheless, one should take computational re-

sults with a pinch of salt as nevertheless all three latter molecules were later trapped inside fullerene [42, 115, 116]. As many as 24 or 29 hydrogen molecules were found to be able to get inside C_{60} [117-119]; however, such results seem disputable even from the geometrical point of view [120-122]. Some calculations showed impossibility of allocating even $2H_2@C_{60}$ [123]. Nevertheless, by using generalized gradient approximation potential for DFT modeling, single $H_2@C_{60}$ was found to be stable [124].

To sum up, simulations of the endohedral fullerenes with hydrogen and/or its ions inside are arguable and demand careful treatment. Fullerenes protonated from inside have not been synthesized or theoretically studied so far. However, the hypothesis on fullerenes being able to serve as protonophores in mitochondria membranes is corroborated by our calculations, which confirm the theoretical possibility of “proton charging” of fullerenes [106, 107]. Moreover, it is not very important how many – one or several – protons may be stabilized inside the cage. The very fact of bonding of even single proton to C_{60} allows one to envision charge transfer across mitochondria membrane, which eventually would lower $\Delta\psi$ and ROS production rate. As $H_2@C_{60}$ has been previously synthesized, and somewhat exotic way of chemical stabilizing of hydrogen by putting it into a partially opened C_{60} cage has been considered [24], protonated-inside fullerenes deserve further investigation. Theoretical studies suggest limitless possible interactions of fullerenes with biological systems. For example, X. Zhao *et al.* reported some results on DNA- C_{60} complexes formation in aqueous solution using molecular dynamics simulations that could explain some experimental results on C_{60} toxicity [125].

While Moussa *et al.* experimentally showed that C_{60} can cross membrane cells both *in vitro* and *in vivo* since 1995 [126, 127], several years later some interesting results on fullerenes behavior inside membrane cells have been researched computationally. Finally, there are interesting results on fullerenes behavior in membranes that was both studied experimentally and computationally. For instance, modeling of the C_{60} permeation through the lipid bilayer membranes in [128] with molecular dynamics showed that fullerene aggregates (formed in water, as fullerenes are hydrophobic) quickly disaggregate once inside the bilayer and easily move through it. More recent molecular dynamics study [129] suggested that pure fullerenes get inside a cell membrane modeled as 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), while their functionalization with polar groups leads to the controversial outcome: they can easily cross the membrane and escape it yet it is unlikely that they can enter the bilayer initially. Interestingly, molecular dynamics simulation using dipalmitoylphosphatidylcholine (DPPC) bilayers [130] showed that while C_{60} can easily penetrate such membranes, this is not the case for fullerenes functionalized with OH groups, which were “stuck” inside. Very recently, T. Rokitskaya and Y. Antonenko [131] measured the electric current across a cell divided by a diphytanoylphosphatidylcholine bilayer under various pH conditions where different concentrations of fullerenes were added to either one or both cell’s halves. The authors found that fullerenol $C_{60}(OH)_{20}$ can mediate the conductivity between the cell sides as a function of pH.

During the last 20 years, physicists, chemists, and biologists have published a series of theoretical and experimental findings, which almost confirm our theoretical model. In particular, one paper showed the possibility of reversible fullerene protonation yielding cations that are stable at room temperature both in solid state and in a solution [132]. Other calculations also indicated that fullerenes could be considered as efficient proton conductors [133]. It is obvious that proton conductivity of fullerenes is impossible without formation of proton-fullerene complexes. Such an assumption was recently proved by experimental data, which led Sony corporation to developing of a proton-conducting membrane based on the C_{60} -containing composite [134].

More recently, the interaction of pristine (C_{60}) and hydrated pristine ($C_{60}(OH)_{18-22}$) with liposomes and isolated mitochondria has been studied [135]. Obtained results validated previous models of fullerenes distribution in water and lipid bilayer [128]. Besides, it was demonstrated an ability of both C_{60} and $C_{60}(OH)_{18-22}$ to increase the inner mitochondrial membrane permeability to protons and decrease mitochondrial transmembrane potential ($\Delta\psi$). Surprisingly, the authors connect the ability of fullerenes to decrease $\Delta\psi$ with its potential toxicity. Interestingly, there are many examples of adaptogenic action of slight $\Delta\psi$ shift including the increase of longevity upon intake of low doses of dinitrophenol and other decouplers of respiration and oxidative phosphorylation [100, 136-138].

Several experiments have been conducted to check the ability of water-soluble fullerene derivatives to decrease mitochondrial transmembrane potential *in vivo* in the yeasts *Yarrowia lipolytica* experimental model [139].

Mitochondrial transmembrane potential of *Yarrowia lipolytica* cells was measured by the cytofluorimetric JC-1-based ratiometric assay. Applied fullerene derivatives significantly downregulated $\Delta\psi$, thereby decreasing the subset of cells with high mitochondrial potential as compared with intact control cells. These results can be considered as an *in vivo* experimental proof of our previous assumptions [106-108].

Hence, discovered by F. Moussa *et al.* fullerene’s ability to considerably slow down senescence rate for mammals naturally fits in a series of theoretical models and experimental evidence indicating high antioxidant activity of fullerenes and their derivatives.

FULLERENES SAFETY

Nowadays fullerenes are widely used in industry for new polymer synthesis. Besides, in the near future, fullerene applications are expected to grow due to the expanding range of potential applications in the fields of cosmetology and medicine [140, 141]. Therefore, it is natural to investigate fullerenes safety and environmental impact. Other carbon nanostructures – nanotubes – are known to have pronounced toxic effects [110, 142]; it makes an issue of health hazard of nanoparticles even more significant in the light of recent finding of carbon nanotubes in lungs of Parisian children [143]. Thus, it is necessary to assess safety of fullerenes’ usage, especially in medicine [144, 145].

Data on fullerene toxicity are controversial. A number of studies demonstrate that pristine C₆₀ has no acute or sub-acute toxicity in prokaryotic and eukaryotic organisms [127]. The majority of fullerene effects, which are said to be toxic, were found during *in vitro* studies, carried out with fullerene solutions and suspensions. Fullerenes and their derivatives can penetrate cells [146], incorporate into cellular membranes, and influence mitochondria function [135]. In addition, it was shown *in vitro* that fullerenes can induce ROS production and peroxidation of membrane lipids for human dermal fibroblasts, human liver carcinoma cells (HepG2), and neuronal human astrocytes [86, 87]. However, this was not confirmed *in vivo* [147]. Significant cytotoxicity is characteristic of some fullerene derivatives. For example, Bobylev *et al.* reported on pronounced toxicity of sodium salt of polycarboxylic derivative of fullerene C₆₀ in HEp-2 cells [148].

Interestingly, Injac and co-authors demonstrated that C₆₀(OH)₂₄ fullereneol is inherently cytotoxic to HepG2, but this property is masked by its antioxidant activity when these cells encounter a more dangerous condition than the fullereneol itself - oxidative stress [149].

Many researchers point out low genotoxicity of fullerenes. Absence of chromosome aberrations has been shown in models of mammals' cell cultures [150, 151]. Mutagenic activity of fullerenes was not found in studies carried out with DNA-comet assay [152] and Ames test [151, 153-155].

However, there are data contradicting to those described above. Dhawan *et al.* [156] investigated whether C₆₀ is able to inflict DNA damage within human lymphocytes, and such an effect was indeed detected using the comet assay. Using the same comet assay method, DNA breaks were also observed in marine mussels, *Mytilus* sp. [157].

In vivo studies demonstrated weak toxic effect of C₆₀. It was shown, that, once inhaled, fullerenes do not enter bloodstream from lungs but are withdrawn from an organism or absorbed by tissue macrophages after a while [150, 158-160]. On the other hand, there is an evidence that fullerenes' inhalation in lungs cells alters the expression of genes responsible for inflammation [161]. Long-term oral administration (92 days) of C₆₀ led to an increase of enzyme CYP2B1 activity, decrease of urea level in blood, and rise of uric acid yet all fluctuations were within the normal range. Similarly, at daily dose of 10 mg/kg there was observed an increase in absorption of antigenic proteins in gastrointestinal tract (morphology of intestine microvilli was unaffected), as well as changes in hepatic CD106(+) cells and granules accumulation in presumably Kupffer macrophages [162, 163]. Fullerenes are able to penetrate into deeper skin layers but also without causing any toxic effects and diffusion into bloodstream [164, 165].

Being intra-abdominal injected, fullerenes quickly distribute over an organism by blood, accumulating in liver and to a lesser extent in kidneys, lungs, spleen, and brain. Fullerenes were absorbed by tissue macrophages and caused antibodies production, while at the same time demonstrating antioxidant and anti-inflammatory properties [75, 166-168].

During lactation period, fullerenes can seep into breast milk [169].

However, one should note a research by Tsuchiya *et al.* [170] revealed death of all embryos within 18 hours after intraperitoneal administration of fullerenes to the pregnant mice. Authors suppose it occurred because of the constriction of the placenta blood vessels. On the other hand, studies exploiting much less C₆₀ concentration did not report on fetal death yet uterus and placenta vessels were found to be (vaso)hypertonic [169, 171].

Concentration plays an important role for manifestation of fullerenes toxic properties. Roursgaard *et al.* [172] showed fullereneols to have an anti-inflammatory effect within the mouse lung at low concentrations, which is related to their antioxidant action; however upon increase of concentration to 10 mg/kg described effect reversed to the pro-inflammatory one. A decrease in fullerenes aggregate size would also increase particle surface area, which would make this rather nonspecific mechanism even more potent [173, 174].

From the above said one may conclude that despite known data on fullerenes *in vitro* toxicity, they have low *in vivo* toxicity manifesting itself only at administration of rather high concentrations on the order of 10 mg/kg. Apparently, many effects arising from fullerenes interaction with cells are interpreted as toxic ones though in fact they are adaptive and reversible. Moreover, an increase in toxicity may be due to using of solubilizing agents or aggregation [175]. Overall, fullerenes at concentrations below 10 mg/kg are quite safe compounds and consequently have promising prospects in regards to their medical applications.

One should note there are several other factors that can affect an evaluation of fullerenes toxicity:

1. Surface Modification

Without any doubt, additional structures on the fullerene surface may considerably alter its chemical and physical properties such as solubility, tendency for clustering etc. Similarly, toxicity of fullerenes changes depending on surface structures. Cationic chains were shown to induce significant toxicity, while the presence of neutral or anionic moieties did not produce any hemolytic effects [176, 177]. Kamat *et al.* [178] demonstrated that C₆₀(OH)₁₈ was more toxic under the influence of light than underivatized C₆₀ *in vitro*.

2. Light Influence

Fullerene derivatives – fullerols – are toxic due to ROS generation, and there are studies demonstrating there is a photosensitive aspect of fullerol toxicity. Namely, there were shown cell damage of human HLE-B3 lens epithelial cells [179] and membrane damage under photosensitive conditions associated with lipid peroxidation [178]. Usenko *et al.* [97] explored the very same effect for C₆₀, when decrease of the illumination level considerably reduced malformation of zebrafish embryos.

On the other hand, Kato *et al.* [153] showed that fullerenes dissolved in squalene (as might be used in skin

creams) did not induce phototoxic effects under UVA-irradiation in fibroblasts. *In vivo* study by Huczko *et al.* [180], exploiting the Draize rabbit eye irritation test, did not show any activity of fullerenol. A mixture of C₆₀ and C₇₀ had no *in vivo* phototoxic effect on clipped free skin of guinea pigs [181].

3. Solvents and Metals Used During Synthesis

It is generally believed that majority of observed toxic effects of fullerenes are due to metal impurities introduced on the synthesis stage [77]. Fullerenes' toxic effects shown in some studies might depend not on fullerenes action itself but on solvents molecules remaining inside their clusters. For instance, Isakovic *et al.* [182] reported that C₆₀ solvated with tetrahydrofuran (THF) had considerable toxic effect on different types of cells related to ROS production, while irradiation in order to decompose residual THF (THFC₆₀) changed cytotoxic effect into cytoprotective and antioxidant ones. Zhang *et al.* [183] found the same tendency by comparing cleaned and initial THFC₆₀. Markovic *et al.* [184] noted the toxicity related to ROS-production is different for C₆₀ prepared within various solvents (THF or ethanol) or within an aqueous solution (aquC₆₀), with its being highest for THFC₆₀ and lowest for aquC₆₀.

FULLERENES IN THERAPY

Fullerenes applications in therapy are multitudinous. In most cases when fullerenes were proven to serve as antioxidants, the authors suggested the use of fullerenes as neuroprotective agents [82], future medications for the notoriously broad range of other oxidative stress-related pathologies [64], and even for HIV infection [185, 186]. Anti-cancer studies are an enormous field of application of fullerenes [52, 187].

I. Falynskova and colleagues revealed that fullerene-(tris-aminocaproic acid) hydrate is an effective medication for the model viral-bacterial pneumonia in mice [188]. Fullerenes exert microbicidal activity accounted for by different and even opposing mechanisms, e.g. by having either pro- or antioxidant properties [77].

Some fullerenes are applied in various fields of anti-viral studies, not limited to HIV, but including influenza [189, 190] and hepatitis C [191] viruses.

For therapeutic purposes, some advanced fullerene derivatives have also been synthesized, for example – a microbicidal photoexcited fullerene generating substantial amounts of ROS [192, 193]. Interestingly, C₇₀ fullerene can be modified to serve as an electron donor agent and produce hydroxyl-radicals thus fighting with infections [192].

Although much is unknown regarding the mechanisms of effects of fullerenes and their derivatives, there are already studies involving volunteers. Andrievsky and co-workers reported on the 3-year clinical observation of the beneficial effects of the hydroxylated fullerene administration to a volunteer with malignancy and detected a positive therapeutic effect of C₆₀HyFn during the course of radiotherapy, which was referred initially to its high antioxidant activity [64].

Importantly, even pure fullerene C₆₀ can cross the blood-brain barrier [194].

For a survey on fullerene C₆₀ derivatives applied in therapy, please refer to the review by Lin and Lu [194].

CONCLUSION

Thirty years after discovery, fullerenes are attracting more and more attention of synthetic chemists, physicists, engineers, physicians, and biologists by the vast prospective applications they offer. Without doubt, fullerenes are an extremely complex entity with unique and not fully understood properties to be studied with great deal of care. Although technical, health, and environmental risks should be taken into account, the practical value of fullerenes inspires and implies future studies that, we believe, will overcome the challenges. Current reports on toxicity and issues with synthesis of sufficient amounts of properly functionalized fullerenes seem to be obstacles one can cope with. Eventually, research may facilitate even such amazing fullerenes application as life prolongation.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by the Ministry of Education and Science of the Russian Federation (project No. 6.1202.2014/K); the publication was prepared as the part of the work "Providing scientific research No. 1032".

The authors would like to thank Dr. prof. Fathi Moussa for critical reading and extremely helpful comments.

REFERENCES

- [1] Harman D. Aging: a theory based on free radical and radiation chemistry. *J Gerontol* 1955; 11(3):298-300.
- [2] Harman D. The Biologic Clock: The Mitochondria? *J Am Geriatr Soc* 1972; 20(4):145-7.
- [3] Small DM, Coombes JS, Bennett N, Johnson DW, Gobe GC. Oxidative stress, anti-oxidant therapies and chronic kidney disease. *Nephrology* 2012; 17:311–21.
- [4] Leopoldini M, Russo N, Chiodo S, Toscano M. Iron chelation by the powerful antioxidant flavonoid quercetin. *J Agric Food Chem* 2006; 54:6343–51.
- [5] Leopoldini M, Russo N, Toscano M. Gas and liquid phase acidity of natural antioxidants. *J Agric Food Chem* 2006; 54:3078–85.
- [6] León-Carmona JR, Galano A. Is caffeine a good scavenger of oxygenated free radicals? *J Phys Chem B* 2011; 115:4538–46.
- [7] Galano A, Martínez A. Capsaicin, a tasty free radical scavenger: mechanism of action and kinetics. *J Phys Chem B* 2012; 116:1200–8.
- [8] Martínez A, Galano A, Vargas R. Free radical scavenger properties of α -mangostin: thermodynamics and kinetics of HAT and RAF mechanisms. *J Phys Chem B* 2011; 115:12591–8.
- [9] Sadowska-Bartosz I, Bartosz G. Effect of antioxidants supplementation on aging and longevity. *BioMed Res Int* 2014; 2014.
- [10] Ginsberg J. American Chemical Society National Historic Chemical Landmarks. Discovery of Fullerenes. : American Chemical Society; 2010 [updated October 11, 2010; cited 2016 March, 20]. Available from: <http://www.acs.org/content/acs/en/education/whatschemistry/landmarks/fullerenes.html>.
- [11] Kroto HW, Heath JR, O'Brien SC, Curl RF, Smalley RE. C₆₀: Buckminsterfullerene. *Nature* 1985; 318(6042):162-3.
- [12] Frederick N, Yoshida M, Tomanek D. Cn Fullerenes: Michigan State University Computational Nanotechnology Lab; 2015

- [updated April 29, 2015; cited 2016 March, 20]. Available from: <http://www.nanotube.msu.edu/fullerene/fullerene-isomers.html>.
- [13] Iijima S. Helical microtubules of graphitic carbon. *Nature* 1991; 354(6348):56-8.
- [14] Jensen AW, Wilson SR, Schuster DI. Biological applications of fullerenes. *Biorg Med Chem* 1996; 4(6):767-79.
- [15] Byszewski P, Klusek Z, editors. Some properties of fullerenes and carbon nanotubes. SPIE proceedings series; 2001: Society of Photo-Optical Instrumentation Engineers.
- [16] Dresselhaus MS, Dresselhaus G, Eklund PC. Science of fullerenes and carbon nanotubes: their properties and applications: Academic press; 1996.
- [17] Ruoff RS, Ruoff AL. The bulk modulus of C60 molecules and crystals: a molecular mechanics approach. *Appl Phys Lett* 1991; 59(13):1553-5.
- [18] Prassides K, Kroto HW, Taylor R, *et al.* Fullerenes and fullerides in the solid state: Neutron scattering studies. *Carbon* 1992; 30(8):1277-86.
- [19] Hirsch A. The chemistry of the fullerenes: Wiley Online Library; 1994.
- [20] Prassides K, Takabayashi Y, Nakagawa T. Mixed valency in rare-earth fullerides. *Phil Trans R Soc A* 2008; 366(1862):151-61.
- [21] Bethune D, Johnson R, Salem J, De Vries M, Yannoni C. Atoms in carbon cages: the structure and properties of endohedral fullerenes. *Nature* 1993; 366(6451):123-8.
- [22] Hebard A, Rosseinsky M, Haddon R, *et al.* Potassium-doped C60. *Nature* 1991; 350:600-1.
- [23] Soga T. Nanostructured materials for solar energy conversion: Elsevier; 2006.
- [24] Cataldo F, Graovac A, Ori O. The mathematics and topology of fullerenes: Springer Science & Business Media; 2011.
- [25] Henderson CC, Cahill PA. C60H2: synthesis of the simplest C60 hydrocarbon derivative. *Science* 1993; 259(5103):1885-7.
- [26] Henderson CC, Rohlfing CM, Assink RA, Cahill PA. C60H4: kinetics and thermodynamics of multiple addition to C60. *Angew Chem Int Ed Engl* 1994; 33(7):786-8.
- [27] Bergosh RG, Meier MS, Laske Cooke JA, Spielmann HP, Weedon BR. Dissolving Metal Reductions of Fullerenes. *J Org Chem* 1997; 62(22):7667-72.
- [28] Meier MS, Spielmann HP, Haddon RC, *et al.* Reactivity, spectroscopy, and structure of reduced fullerenes. *Carbon* 2000; 38(11):1535-8.
- [29] Athans AJ, Briggs JB, Jia W, Miller GP. Hydrogen-protected acenes. *J Mater Chem* 2007; 17(25):2636-41.
- [30] Briggs JB, Montgomery M, Silva LL, Miller GP. Facile, Scalable, Regioselective Synthesis of C 3 v C60H18 Using Organic Polyamines. *Org Lett* 2005; 7(25):5553-5.
- [31] Wägberg T, Johnels D, Peera A, *et al.* Selective Synthesis of the C 3 v Isomer of C60H18. *Org Lett* 2005; 7(25):5557-60.
- [32] Darwish AD, Avent AG, Taylor R, Walton DR. Structural characterisation of C 60 H 18; a C 3v symmetry crown. *J Chem Soc, Perkin Trans 2* 1996; (10):2051-4.
- [33] Nossal J, Saini R, Sadana A, *et al.* Saunders: M.; Khong, A.; Weisemann, R. *J Am Chem Soc* 2001; 123:8482-95.
- [34] Taylor R. Addition reactions of fullerenes. *C R Chim* 2006; 9(7-8):982-1000.
- [35] Darwish A, Abdul-Sada A, Langley G, Kroto H, Taylor R, Walton D. Polyhydrogenation of [60]- and [70] fullerenes with Zn/HCl and Zn/DCl. *Synth Met* 1996; 77(1):303-7.
- [36] Withers JC, Loutfy RO, Lowe TP. Fullerene commercial vision. *Fuller Nanotub Car N* 1997; 5(1):1-31.
- [37] Zhang Z, Han P, Liu X, *et al.* First Principle Calculations of the Electronic Properties of the Fullerene Derivative as an Electron Acceptor in Organic Solar Cells. *J Phys Chem C* 2008; 112(48):19158-61.
- [38] Saunders M, Jiménez-Vázquez HA, Cross RJ, Poreda RJ. Stable compounds of helium and neon: He@ C60 and Ne@ C60. *Science* 1993; 259(5100):1428-30.
- [39] Christian JF, Wan Z, Anderson SL. Nitrogen ion (N+) + C60 fullerene reactive scattering: substitution, charge transfer, and fragmentation. *J Phys Chem* 1992; 96(26):10597-600.
- [40] Christian JF, Wan Z, Anderson SL. O++C60- C60O+ production and decomposition, charge transfer, and formation of C59O+. Dopeyball or [CO@C58+]. *Chem Phys Lett* 1992; 199(3):373-8.
- [41] Andreoni W. The physics of fullerene-based and fullerene-related materials: Springer Science & Business Media; 2000.
- [42] Komatsu K, Murata M, Murata Y. Encapsulation of molecular hydrogen in fullerene C60 by organic synthesis. *Science* 2005; 307(5707):238-40.
- [43] Murata M, Maeda S, Morinaka Y, Murata Y, Komatsu K. Synthesis and Reaction of Fullerene C70 Encapsulating Two Molecules of H2. *J Am Chem Soc* 2008; 130(47):15800-1.
- [44] Murata Y, Maeda S, Murata M, Komatsu K. Encapsulation and Dynamic Behavior of Two H2 Molecules in an Open-Cage C70. *J Am Chem Soc* 2008; 130(21):6702-3.
- [45] Rubin Y, Jarrosson T, Wang G, *et al.* Insertion of helium and molecular hydrogen through the orifice of an open fullerene. *Angew Chem Int Ed* 2001; 40(8):1543-6.
- [46] Murata Y, Murata M, Komatsu K. 100% encapsulation of a hydrogen molecule into an open-cage fullerene derivative and gas-phase generation of H2@ C60. *J Am Chem Soc* 2003; 125(24):7152-3.
- [47] Sawa H, Wakabayashi Y, Murata Y, Murata M, Komatsu K. Floating Single Hydrogen Molecule in an Open-Cage Fullerene. *Angew Chem Int Ed* 2005; 44(13):1981-3.
- [48] Dodziuk H, Hara T, Konno T, *et al.* Fullerenes. Strained Hydrocarbons: Wiley-VCH Verlag GmbH & Co. KGaA; 2009. p. 205-333.
- [49] Murata M, Murata Y, Komatsu K. Synthesis and properties of endohedral C60 encapsulating molecular hydrogen. *J Am Chem Soc* 2006; 128(24):8024-33.
- [50] Goedde B, Waiblinger M, Jakes P, Weiden N, Dinse K-P, Weidinger A. Nitrogen doped C 60 dimers (N@ C 60-C 60). *Chem Phys Lett* 2001; 334(1):12-7.
- [51] Lalwani G, Sitharaman B. Multifunctional fullerene-and metallofullerene-based nanobiomaterials. *Nano Life* 2013; 3(03):1342003.
- [52] Baati T, Bourasset F, Gharbi N, *et al.* The prolongation of the lifespan of rats by repeated oral administration of [60] fullerene. *Biomaterials* 2012; 33(19):4936-46.
- [53] Gao J, Wang Y, Folta KM, *et al.* Polyhydroxy fullerenes (fullerols or fullerlenols): beneficial effects on growth and lifespan in diverse biological models. *PLoS One* 2011; 6:e19976.
- [54] Ngan CL, Basri M, Tripathy M, Karjiban RA, Abdul-Malek E. Skin intervention of fullerene-integrated nanoemulsion in structural and collagen regeneration against skin aging. *Eur J Pharm Sci* 2015; 70:22-8.
- [55] Grebowski J, Kazmierska P, Krokosz A. Fullerenols as a new therapeutic approach in nanomedicine. *BioMed Res Int* 2013; 2013.
- [56] Tsao N, Luh T-Y, Chou C-K, *et al.* *In vitro* action of carboxyfullerene. *J Antimicrob Chemother* 2002; 49:641-9.
- [57] Cong W, Wang P, Qu Y, *et al.* Evaluation of the influence of fullereneol on aging and stress resistance using *Caenorhabditis elegans*. *Biomaterials* 2015; 42:78-86.
- [58] Yablonskaya OI, Ryndina TS, Voeikov VL, Khokhlov AN. A paradoxical effect of hydrated C60-fullerene at an ultralow concentration on the viability and aging of cultured Chinese hamster cells. *Moscow Univ Biol Sci Bull* 2013; 68:63-8.
- [59] Quick KL, Ali SS, Arch R, Xiong C, Wozniak D, Dugan LL. A carboxyfullerene SOD mimetic improves cognition and extends the lifespan of mice. *Neurobiol Aging* 2008; 29:117-28.
- [60] Dugan LL, Lovett EG, Quick KL, Lotharius J, Lin TT, O'Malley KL. Fullerene-based antioxidants and neurodegenerative disorders. *Parkinsonism Relat Disord* 2001; 7:243-6.
- [61] Theriot CA, Casey RC, Moore VC, *et al.* Dendro [C60] fullerene DF-1 provides radioprotection to radiosensitive mammalian cells. *Radiat Environ Biophys* 2010; 49:437-45.
- [62] Ma H, Liang X-J. Fullerenes as unique nanopharmaceuticals for disease treatment. *Sci China Chem* 2010; 53(11):2233-40.
- [63] Ali SS, Hardt JI, Quick KL, *et al.* A biologically effective fullerene (C 60) derivative with superoxide dismutase mimetic properties. *Free Radical Biol Med* 2004; 37:1191-202.
- [64] Andrievsky GV, Bruskov VI, Tykhomyrov AA, Gudkov SV. Peculiarities of the antioxidant and radioprotective effects of hydrated C 60 fullerene nanostructures *in vitro* and *in vivo*. *Free Radical Biol Med* 2009; 47:786-93.
- [65] Chen Y-W, Hwang KC, Yen C-C, Lai Y-L. Fullerene derivatives protect against oxidative stress in RAW 264.7 cells and ischemia-reperfusion lungs. *Am J Physiol Regul Integr Comp Physiol* 2004; 287:R21-R6.
- [66] Injac R, Perse M, Cerne M, *et al.* Protective effects of fullerene C 60 (OH) 24 against doxorubicin-induced cardiotoxicity and

- hepatotoxicity in rats with colorectal cancer. *Biomaterials* 2009; 30:1184–96.
- [67] Shershakova N, Baraboshkina E, Andreev S, *et al.* Anti-inflammatory effect of fullerene C 60 in a mice model of atopic dermatitis. *J Nanobiotechnology* 2016; 14:1.
- [68] Zhu J, Ji Z, Wang J, *et al.* Tumor-Inhibitory Effect and Immunomodulatory Activity of Fullerol C60 (OH) x. *Small* 2008; 4:1168–75.
- [69] Yin J-J, Lao F, Meng J, *et al.* Inhibition of tumor growth by endohedral metallofullerenol nanoparticles optimized as reactive oxygen species scavenger. *Mol Pharmacol* 2008; 74:1132–40.
- [70] Lai H-S, Chen W-J, Chiang L-Y. Free radical scavenging activity of fullereneol on the ischemia-reperfusion intestine in dogs. *World J Surg* 2000; 24:450–4.
- [71] Manibusan MK, Odin M, Eastmond DA. Postulated carbon tetrachloride mode of action: a review. *J Environ Sci Health C* 2007; 25:185–209.
- [72] Chiang LY, Lu F-J, Lin J-T. Free radical scavenging activity of water-soluble fullereneols. *J Chem Soc, Chem Commun* 1995:1283–4.
- [73] Krusic PJ, Wasserman E, Keizer PN, Morton JR, Preston KF. Radical Reactions of C60. *Science* 1991; 254(5035):1183–5.
- [74] Cai X, Jia H, Liu Z, *et al.* Polyhydroxylated fullerene derivative C(60)(OH)(24) prevents mitochondrial dysfunction and oxidative damage in an MPP(+)-induced cellular model of Parkinson's disease. *J Neurosci Res* 2008; 86(16):3622–34.
- [75] Gharbi N, Pressac M, Hadchouel M, Szwarc H, Wilson SR, Moussa F. [60] fullerene is a powerful antioxidant *in vivo* with no acute or subacute toxicity. *Nano Lett* 2005; 5:2578–85.
- [76] Tykhomyrov AA, Nedzvetsky VS, Klochkov VK, Andrievsky GV. Nanostructures of hydrated C 60 fullerene (C 60 HyFn) protect rat brain against alcohol impact and attenuate behavioral impairments of alcoholized animals. *Toxicology* 2008; 246:158–65.
- [77] Elswaifi SF, Palmieri JR, Hockey KS, Rzigalinski BA. Antioxidant nanoparticles for control of infectious disease. *Infect Disord Drug Targets* 2009; 9:445–52.
- [78] Gruber J, Fong S, Chen C-B, *et al.* Mitochondria-targeted antioxidants and metabolic modulators as pharmacological interventions to slow ageing. *Biotechnol Adv* 2013; 31:563–92.
- [79] Chen B, Liu Y, Song WM, Hayashi Y, Ding XC, Li WH. *In vitro* evaluation of cytotoxicity and oxidative stress induced by multiwalled carbon nanotubes in murine RAW 264.7 macrophages and human A549 lung cells. *Biomed Environ Sci* 2011; 24(6):593–601.
- [80] Wei Y, Song J, Chen Q, Xing D. Enhancement of photodynamic antitumor effect with pro-oxidant ascorbate. *Lasers Surg Med* 2012; 44(1):69–75.
- [81] Hu Z, Guan W, Wang W, Huang L, Xing H, Zhu Z. Synthesis of beta-alanine C60 derivative and its protective effect on hydrogen peroxide-induced apoptosis in rat pheochromocytoma cells. *Cell Biol Int* 2007; 31(8):798–804.
- [82] Chen T, Li YY, Zhang JL, *et al.* Protective effect of C(60) -methionine derivate on lead-exposed human SH-SY5Y neuroblastoma cells. *J Appl Toxicol* 2011; 31(3):255–61.
- [83] Hu Z, Huang Y, Guan W, Zhang J, Wang F, Zhao L. The protective activities of water-soluble C 60 derivatives against nitric oxide-induced cytotoxicity in rat pheochromocytoma cells. *Biomaterials* 2010; 31(34):8872–81.
- [84] Zolotukhin P, Kozlova Y, Dovzhik A, *et al.* Oxidative status interactome map: towards novel approaches in experiment planning, data analysis, diagnostics and therapy. *Mol Biosyst* 2013; 9(8):2085–96.
- [85] Ye S, Chen M, Jiang Y, *et al.* Polyhydroxylated fullerene attenuates oxidative stress-induced apoptosis via a fortifying Nrf2-regulated cellular antioxidant defence system. *Int J Nanomedicine* 2014; 9:2073.
- [86] Sayes CM, Gobin AM, Ausman KD, Mendez J, West JL, Colvin VL. Nano-C 60 cytotoxicity is due to lipid peroxidation. *Biomaterials* 2005; 26:7587–95.
- [87] Sayes CM, Fortner JD, Guo W, *et al.* The differential cytotoxicity of water-soluble fullerenes. *Nano Lett* 2004; 4:1881–7.
- [88] Isakovic A, Markovic Z, Todorovic-Markovic B, *et al.* Distinct cytotoxic mechanisms of pristine versus hydroxylated fullerene. *Toxicol Sci* 2006; 91(1):173–83.
- [89] Oberdörster E, Zhu S, Blickley TM, McClellan-Green P, Haasch ML. Ecotoxicology of carbon-based engineered nanoparticles: effects of fullerene (C 60) on aquatic organisms. *Carbon* 2006; 44(6):1112–20.
- [90] Lovorn SB, Klaper R. Daphnia magna mortality when exposed to titanium dioxide and fullerene (C60) nanoparticles. *Environ Toxicol Chem* 2006; 25(4):1132–7.
- [91] Park E-J, Roh J, Kim Y, Park K. Induction of inflammatory responses by carbon fullerene (C60) in cultured RAW264. 7 cells and in intraperitoneally injected mice. *Toxicol Res* 2010; 26:267.
- [92] Rietjens IM, Boersma MG, de Haan L, *et al.* The pro-oxidant chemistry of the natural antioxidants vitamin C, vitamin E, carotenoids and flavonoids. *Environ Toxicol Pharmacol* 2002; 11:321–33.
- [93] Chakraborty A, Ramani P, Sherlin HJ, Premkumar P, Natesan A. Antioxidant and pro-oxidant activity of Vitamin C in oral environment. *Indian J Dent Res* 2014; 25(4):499–504.
- [94] Pearson P, Lewis SA, Britton J, Young IS, Fogarty A. The pro-oxidant activity of high-dose vitamin E supplements *in vivo*. *Biodrugs* 2006; 20:271–3.
- [95] Taguchi K, Fujikawa N, Komatsu M, *et al.* Keap1 degradation by autophagy for the maintenance of redox homeostasis. *Proc Natl Acad Sci U S A* 2012; 109:13561–6.
- [96] Jung K-A, Choi B-h, Nam C-W, *et al.* Identification of aldo-keto reductases as NRF2-target marker genes in human cells. *Toxicol Lett* 2013; 218:39–49.
- [97] Usenko CY, Harper SL, Tanguay RL. Fullerene C 60 exposure elicits an oxidative stress response in embryonic zebrafish. *Toxicol Appl Pharmacol* 2008; 229:44–55.
- [98] Zhu S, Oberdörster E, Haasch ML. Toxicity of an engineered nanoparticle (fullerene, C 60) in two aquatic species, Daphnia and fathead minnow. *Mar Environ Res* 2006; 62:S5–S9.
- [99] Brant JA, Labille J, Bottero J-Y, Wiesner MR. Characterizing the impact of preparation method on fullerene cluster structure and chemistry. *Langmuir* 2006; 22(8):3878–85.
- [100] Kolosnjaj J, Szwarc H, Moussa F. Toxicity Studies of Fullerenes and Derivatives. In: Chan WW, editor. *Bio-Applications of Nanoparticles. Advances in Experimental Medicine and Biology.* 620: Springer New York; 2007. p. 168–80.
- [101] Wang IC, Tai LA, Lee DD, *et al.* C(60) and water-soluble fullerene derivatives as antioxidants against radical-initiated lipid peroxidation. *J Med Chem* 1999; 42(22):4614–20.
- [102] Banks R, Speakman JR, Selman C. Vitamin E supplementation and mammalian lifespan. *Mol Nutr Food Res* 2010; 54:719–25.
- [103] Skulachev MV, Antonenko YN, Anisimov VN, *et al.* Mitochondrial-targeted plastoquinone derivatives. Effect on senescence and acute age-related pathologies. *Curr Drug Targets* 2011; 12(6):800–26.
- [104] Foley S, Crowley C, Smahli M, *et al.* Cellular localisation of a water-soluble fullerene derivative. *Biochem Biophys Res Commun* 2002; 294(1):116–9.
- [105] Dellinger A, Zhou Z, Norton SK, Lenk R, Conrad D, Kepley CL. Uptake and distribution of fullerenes in human mast cells. *Nanomed Nanotechnol Biol Med* 2010; 6:575–82.
- [106] Chistyakov VA, Smirnova YO, Prazdnova EV, Soldatov AV. Possible mechanisms of fullerene C₆₀ antioxidant action. *BioMed Res Int* 2013; 2013:821498.
- [107] Chistyakov V, Prazdnova E, Soldatov A, Smirnova YO, Alperovich I. Can C60 fullerene demonstrate properties of mitochondria-targeted antioxidant from the computational point of view? *BIBIEN* 2014; 8:59–62.
- [108] Chistyakov VA, Zolotukhin PV, Prazdnova EV, Alperovich I, Soldatov AV. Physical consequences of the mitochondrial targeting of single-walled carbon nanotubes probed computationally. *Physica E Low Dimens Syst Nanostruct* 2015; 70:198–202.
- [109] Zhou F, Xing D, Wu B, Wu S, Ou Z, Chen WR. New insights of transmembranal mechanism and subcellular localization of noncovalently modified single-walled carbon nanotubes. *Nano Lett* 2010; 10(5):1677–81.
- [110] Ma X, Zhang LH, Wang LR, *et al.* Single-walled carbon nanotubes alter cytochrome c electron transfer and modulate mitochondrial function. *ACS Nano* 2012; 6(12):10486–96.
- [111] Velde GT, Bickelhaupt FM, Baerends EJ, *et al.* Chemistry with ADF. *J Comput Chem* 2001; 22(9):931–67.
- [112] Guerra CF, Snijders JG, te Velde G, Baerends EJ. Towards an order-N DFT method. *Theor Chem Acc* 1998; 99(6):391–403.
- [113] Dodziuk H. Modeling complexes of H 2 molecules in fullerenes. *Chem Phys Lett* 2005; 410(1):39–41.

- [114] Cioslowski J. Endohedral chemistry: Electronic structures of molecules trapped inside the C60 cage. *J Am Chem Soc* 1991; 113(11):4139-41.
- [115] Peres T, Cao B, Cui W, *et al.* Some new diatomic molecule containing endohedral fullerenes. *Int J Mass spectrom* 2001; 210:241-7.
- [116] Suetsuna T, Dragoe N, Harneit W, *et al.* Separation of N2@ C60 and N@ C60. *Chem Eur J* 2002; 8(22):5079-83.
- [117] Türker L, Erkoç Ş. AM1 treatment of endohedrally hydrogen doped fullerene, nH 2@ C 60. *Comp Theor Chem* 2003; 638(1):37-40.
- [118] Yang C-K. Density functional calculation of hydrogen-filled C 60 molecules. *Carbon* 2007; 45(12):2451-3.
- [119] Pupyshva OV, Farajian AA, Yakobson BI. Fullerene nanocage capacity for hydrogen storage. *Nano Lett* 2007; 8(3):767-74.
- [120] Dolgonos G. How many hydrogen molecules can be inserted into C 60? Comments on the paper ‘AM1 treatment of endohedrally hydrogen doped fullerene, nH 2@ C 60’ by L. Türker and Ş. Erkoç [J. Mol. Struct.(Theochem) 638 (2003) 37-40]. *Comp Theor Chem* 2005; 732(1):239-41.
- [121] Dolgonos G. A commentary on ‘Density functional calculation of hydrogen-filled C 60 molecules’ by CK. Yang [Carbon 2007; 45 (12): 2451-53]. *Carbon* 2008; 46(4):704-5.
- [122] Dodziuk H. Reply to the ‘Comment on ‘Modelling complexes of H 2 molecules in fullerenes’ by H. Dodziuk [Chem. Phys. Lett. 410 (2005) 39]’ by L. Turker and S. Erkoç. *Chem Phys Lett* 2006; 426(1):224-5.
- [123] Kruse H, Grimme S. Accurate quantum chemical description of non-covalent interactions in hydrogen filled endohedral fullerene complexes. *J Phys Chem C* 2009; 113(39):17006-10.
- [124] Bai Yu-Jie FS-Y, Deng Kai-Ming, Tang Chun-Mei, Chen Xuan, Tan Wei-Shi, Liu Yu-Zhen, Huang De-Cai. Density functional calculations on the geometric and electronic structures of the endohedral fullerene H₂@C₆₀ and its dimmer. *Acta Phys Sin* 2008; 57(6):3684-9.
- [125] Zhao X, Striolo A, Cummings PT. C 60 binds to and deforms nucleotides. *Biophys J* 2005; 89(6):3856-62.
- [126] Moussa F, Chretien P, Dubois P, *et al.* The Influence of C60 Powders On Cultured Human Leukocytes. *Fullerene Sci Technol* 1995; 3(3):333-42.
- [127] Moussa F, Trivin F, Céolin R, *et al.* Early effects of C60 Administration in Swiss Mice: A Preliminary Account for *In vivo* C60 Toxicity. *Fullerene Sci Technol* 1996; 4(1):21-9.
- [128] Wong-Ekkabut J, Baoukina S, Triampo W, Tang I-M, Tieleman DP, Monticelli L. Computer simulation study of fullerene translocation through lipid membranes. *Nat Nanotechnol* 2008; 3(6):363-8.
- [129] Sridhar A, Srikanth B, Kumar A, Dasmahapatra AK. Coarse-grain molecular dynamics study of fullerene transport across a cell membrane. *J Chem Phys* 2015; 143(2):024907.
- [130] Qiao R, Roberts AP, Mount AS, Klaine SJ, Ke PC. Translocation of C60 and its derivatives across a lipid bilayer. *Nano Lett* 2007; 7(3):614-9.
- [131] Rokitskaya TI, Antonenko YN. Fullerene C 60 (OH) 24 increases ion permeability of lipid membranes in a pH-dependent manner. *Biochim Biophys Acta* 2016; 1858(6):1165-74.
- [132] Reed CA, Kim K-C, Bolskar RD, Mueller LJ. Taming Superacids: Stabilization of the Fullerene Cations HC60+ and C60+. *Science* 2000; 289(5476):101-4.
- [133] Tasaki K. Carbonaceous Materials as Proton Conductors: I. Fullerenes and Polyaromatic Hydrocarbons. *J Electrochem Soc* 2006; 153(5):A941-A9.
- [134] Nuber B, Pietzak B, inventors; Sony Corporation, assignee. Fullerene based proton conductive materials. patent US8377997 B2. Feb 19, 2013.
- [135] Santos SM, Dinis AM, Peixoto F, Ferreira L, Jurado AS, Videira RA. Interaction of Fullerene Nanoparticles With Biomembranes: From the Partition in Lipid Membranes to Effects on Mitochondrial Bioenergetics. *Toxicol Sci* 2014; 138(1):117-29.
- [136] Brand MD. Uncoupling to survive? The role of mitochondrial inefficiency in ageing. *Exp Gerontol* 2000; 35(6-7):811-20.
- [137] Severin FF, Severina II, Antonenko YN, *et al.* Penetrating cation/fatty acid anion pair as a mitochondria-targeted protonophore. *Proc Natl Acad Sci U S A* 2010; 107(2):663-8.
- [138] Kolosnjaj J, Szwarc H, Moussa F. Toxicity Studies of Carbon Nanotubes. In: Chan WW, editor. *Bio-Applications of Nanoparticles*. Advances in Experimental Medicine and Biology. 620: Springer New York; 2007. p. 181-204.
- [139] Kalacheva NV, Gubskaya VP, Fazleeva GM, *et al.* Novel water-soluble methanofullerenes C60 [C13 H18 O4 (OH)4]6 and C60 [C9 H10 O4 (OH)4]6: Promising uncouplers of respiration and phosphorylation. *Biorg Med Chem Lett* 2015; 25:5250-3.
- [140] Aitken R, Chaudhry MQ, Boxall ABA, Hull M. Manufacture and use of nanomaterials: current status in the UK and global trends. *Occup Med* 2006; 56:300-6.
- [141] Halford B. Fullerene for the face. *Chem Eng News* 2006; 84:47.
- [142] Cui D, Tian F, Ozkan CS, Wang M, Gao H. Effect of single wall carbon nanotubes on human HEK293 cells. *Toxicol Lett* 2005; 155(1):73-85.
- [143] Kolosnjaj-Tabi J, Just J, Hartman KB, *et al.* Anthropogenic Carbon Nanotubes Found in the Airways of Parisian Children. *EBioMedicine* 2015; 2(11):1697-704.
- [144] Magrez A, Kasas S, Salicio V, *et al.* Cellular toxicity of carbon-based nanomaterials. *Nano Lett* 2006; 6(6):1121-5.
- [145] Chan WCW. *Bio-Applications of Nanoparticles*. New York, NY: Springer New York; 2007.
- [146] Wang F, Jin C, Liang H, Tang Y, Zhang H, Yang Y. Effects of fullerene C 60 nanoparticles on A549 cells. *Environ Toxicol Pharmacol* 2014; 37:656-61.
- [147] Sayes CM, Marchione AA, Reed KL, Warheit DB. Comparative pulmonary toxicity assessments of C60 water suspensions in rats: few differences in fullerene toxicity *in vivo* in contrast to *in vitro* profiles. *Nano Lett* 2007; 7:2399-406.
- [148] Bobylev AG, Okuneva AD, Bobyleva LG, *et al.* Study of cytotoxicity of fullerene C60 derivatives. *Biophysics* 2012; 57:572-6.
- [149] Injac R, Perse M, Obermajer N, *et al.* Potential hepatoprotective effects of fullerene C60(OH)24 in doxorubicin-induced hepatotoxicity in rats with mammary carcinomas. *Biomaterials* 2008; 29(24-25):3451-60.
- [150] Shinohara N, Gamo M, Nakanishi J. Risk assessment of manufactured nanomaterials—fullerene (C60). Interim Report, Executive Summary, NEDO Project - Research and Development of Nanoparticle Characterization Methods, 2009 P06041.
- [151] Mori T, Takada H, Ito S, Matsubayashi K, Miwa N, Sawaguchi T. Preclinical studies on safety of fullerene upon acute oral administration and evaluation for no mutagenesis. *Toxicology* 2006; 225:48-54.
- [152] Jacobsen NR, Pojana G, White P, *et al.* Genotoxicity, cytotoxicity, and reactive oxygen species induced by single-walled carbon nanotubes and C60 fullerenes in the FE1-Muta™ Mouse lung epithelial cells. *Environ Mol Mutagen* 2008; 49:476-87.
- [153] Kato S, Aoshima H, Saitoh Y, Miwa N. Biological Safety of LipoFullerene composed of Squalane and Fullerene-C60 upon Mutagenesis, Photocytotoxicity, and Permeability into the Human Skin Tissue. *Basic Clin Pharmacol Toxicol* 2009; 104:483-7.
- [154] Çavaş T, Çinkılıç N, Vatan Ö, Yılmaz D. Effects of fullerene C60 nanoparticles on acetamidiprid induced cytotoxicity and genotoxicity in cultured human lung fibroblasts. *Pestic Biochem Physiol* 2014; 114:1-7.
- [155] Ema M, Tanaka J, Kobayashi N, *et al.* Genotoxicity evaluation of fullerene C 60 nanoparticles in a comet assay using lung cells of intratracheally instilled rats. *Regul Toxicol Pharm* 2012; 62:419-24.
- [156] Dhawan A, Taurozzi JS, Pandey AK, *et al.* Stable colloidal dispersions of C60 fullerenes in water: evidence for genotoxicity. *Environ Sci Technol* 2006; 40:7394-401.
- [157] Al-Subiai SN, Arlt VM, Frickers PE, *et al.* Merging nanogenotoxicology with eco-genotoxicology: An integrated approach to determine interactive genotoxic and sub-lethal toxic effects of C 60 fullerenes and fluoranthene in marine mussels, *Mytilus* sp. *Mutat Res Genet Toxicol Environ Mutagen* 2012; 745:92-103.
- [158] Baker GL, Gupta A, Clark ML, *et al.* Inhalation toxicity and lung toxicokinetics of C60 fullerene nanoparticles and microparticles. *Toxicol Sci* 2008; 101:122-31.
- [159] Morimoto Y, Hirohashi M, Ogami A, *et al.* Inflammogenic effect of well-characterized fullerenes in inhalation and intratracheal instillation studies. *Part Fibre Toxicol* 2010; 7(1):1-18.
- [160] Walker NJ, editor *The toxicology of fullerenes: current efforts at the national toxicology program*. Society For Risk Analysis Annual Meeting 2009; 2009 December 7, 2009; Baltimore, Maryland,

- USA: National Toxicology Program, National Institute of Environmental Health Sciences, NIH.
- [161] Fujita K, Morimoto Y, Endoh S, *et al.* Identification of potential biomarkers from gene expression profiles in rat lungs intratracheally instilled with C60 fullerenes. *Toxicology* 2010; 274(1-3):34-41.
- [162] Shipelin VA, Smirnova TA, Gmshinskii IV, Tutelyan VA. Analysis of Toxicity Biomarkers of Fullerene C60 Nanoparticles by Confocal Fluorescent Microscopy. *Bull Exp Biol Med* 2015; 158:443-9.
- [163] Shipelin V, Avren'eva L, Guseva G, *et al.* [Characterization of fullerene C60 peroral toxicity to rats in the 92-day experiment] (article in Russian language). *Vopr Pitan* 2012; 81(5):20-7.
- [164] Xia XR, Monteiro-Riviere NA, Riviere JE. Skin penetration and kinetics of pristine fullerenes (C 60) topically exposed in industrial organic solvents. *Toxicol Appl Pharmacol* 2010; 242:29-37.
- [165] Kato S, Aoshima H, Saitoh Y, Miwa N. Fullerene-C60/liposome complex: Defensive effects against UVA-induced damages in skin structure, nucleus and collagen type I/IV fibrils, and the permeability into human skin tissue. *J Photochem Photobiol B: Biol* 2010; 98:99-105.
- [166] Chen HH, Yu C, Ueng TH, *et al.* Acute and subacute toxicity study of water-soluble polyalkylsulfonated C60 in rats. *Toxicol Pathol* 1998; 26:143-51.
- [167] Chen B-X, Wilson S, Das M, Coughlin D, Erlanger B. Antigenicity of fullerenes: antibodies specific for fullerenes and their characteristics. *Proc Natl Acad Sci U S A* 1998; 95(18):10809-13.
- [168] Yamago S, Tokuyama H, Nakamura E, *et al.* *In vivo* biological behavior of a water-miscible fullerene: ¹⁴C labeling, absorption, distribution, excretion and acute toxicity. *Chem Biol* 1995; 2:385-9.
- [169] Snyder RW, Fennell TR, Wingard CJ, *et al.* Distribution and biomarker of carbon-14 labeled fullerene C60 ([¹⁴C (U)] C60) in pregnant and lactating rats and their offspring after maternal intravenous exposure. *J Appl Toxicol* 2015; 35:1438-51.
- [170] Tsuchiya T, Oguri I, Yamakoshi YN, Miyata N. Novel harmful effects of [60] fullerene on mouse embryos *in vitro* and *in vivo*. *FEBS Lett* 1996; 393:139-45.
- [171] Vidanapathirana AK, Thompson LC, Mann EE, *et al.* PVP formulated fullerene (C60) increases Rho-kinase dependent vascular tissue contractility in pregnant Sprague Dawley rats. *Reprod Toxicol* 2014; 49:86-100.
- [172] Roursgaard M, Poulsen SS, Kepley CL, Hammer M, Nielsen GD, Larsen ST. Polyhydroxylated C60 fullerene (fullerenol) attenuates neutrophilic lung inflammation in mice. *Basic Clin Pharmacol Toxicol* 2008; 103:386-8.
- [173] Johnston HJ, Hutchison GR, Christensen FM, Aschberger K, Stone V. The biological mechanisms and physicochemical characteristics responsible for driving fullerene toxicity. *Toxicol Sci* 2010; 114:162-82.
- [174] Piotrovskiy LB, Eropkin MY, Eropkina EM, Dumpis MA, Kisekev OI. Mechanisms of biologic action of fullerenes - dependence on aggregate state (article in Russian language). *Psikhofarmakologia i biologicheskaya narkologia* 2007; 7:1548-54.
- [175] Szwarc H, Moussa F. Toxicity of [60]fullerene: Confusion in the scientific literature. *Journal of Nanoscience Letters* 2011; 1(1):61-2.
- [176] Bosi S, Feruglio L, Da Ros T, *et al.* Hemolytic Effects of Water-Soluble Fullerene Derivatives. *J Med Chem* 2004; 47(27):6711-5.
- [177] Lucafò M, Gerdol M, Pallavicini A, *et al.* Profiling the molecular mechanism of fullerene cytotoxicity on tumor cells by RNA-seq. *Toxicology* 2013; 314:183-92.
- [178] Kamat JP, Devasagayam TPA, Priyadarsini KI, Mohan H. Reactive oxygen species mediated membrane damage induced by fullerene derivatives and its possible biological implications. *Toxicology* 2000; 155:55-61.
- [179] Roberts JE, Wielgus AR, Boyes WK, Andley U, Chignell CF. Phototoxicity and cytotoxicity of fullerol in human lens epithelial cells. *Toxicol Appl Pharmacol* 2008; 228:49-58.
- [180] Huczko A, Lange H, Calko E. Short communication: fullerenes: experimental evidence for a null risk of skin irritation and allergy. *Fullerene Sci Technol* 1999; 7:935-9.
- [181] Aoshima H, Saitoh Y, Ito S, Yamana S, Miwa N. Safety evaluation of highly purified fullerenes (HPFs): based on screening of eye and skin damage. *J Toxicol Sci* 2009; 34:555-62.
- [182] Isakovic A, Markovic Z, Nikolic N, *et al.* Inactivation of nanocrystalline C 60 cytotoxicity by γ -irradiation. *Biomaterials* 2006; 27:5049-58.
- [183] Zhang B, Cho M, Fortner JD, *et al.* Delineating oxidative processes of aqueous C60 preparations: role of THF peroxide. *Environ Sci Technol* 2008; 43(1):108-13.
- [184] Markovic Z, Todorovic-Markovic B, Kleut D, *et al.* The mechanism of cell-damaging reactive oxygen generation by colloidal fullerenes. *Biomaterials* 2007; 28:5437-48.
- [185] Gharbi N, Burghardt S, Brettreich M, *et al.* Chromatographic separation and identification of a water-soluble dendritic methano [60] fullerene octadecaacid. *Anal Chem* 2003; 75:4217-22.
- [186] Fakhraee S, Souri M. Deformation density components analysis of fullerene-based anti-HIV drugs. *J Mol Model* 2014; 20:1-10.
- [187] Prylutskyy YI, Evstigneev MP, Pashkova IS, *et al.* Characterization of C 60 fullerene complexation with antibiotic doxorubicin. *Phys Chem Chem Phys* 2014; 16:23164-72.
- [188] Falynskova IN, Leonova EI, Fedyakina IT, *et al.* Effectiveness of fullerene-(tris-aminocaproic acid) hydrate in the model of experimental viral-bacterial pneumonia of mice. *Zh Mikrobiol Epidemiol Immunobiol* 2014:32-9.
- [189] Shoji M, Takahashi E, Hatakeyama D, *et al.* Anti-influenza Activity of C 60 Fullerene Derivatives. *PLoS One* 2013; 8:e66337.
- [190] Tollas S, Berezcki I, Borbas A, *et al.* Synthesis of a cluster-forming sialylthio-D-galactose fullerene conjugate and evaluation of its interaction with influenza virus hemagglutinin and neuraminidase. *Biorg Med Chem Lett* 2014; 24(11):2420-3.
- [191] Xiao S-I, Wang Q, Yu F, *et al.* Conjugation of cyclodextrin with fullerene as a new class of HCV entry inhibitors. *Biorg Med Chem* 2012; 20:5616-22.
- [192] Huang L, Wang M, Dai T, *et al.* Antimicrobial photodynamic therapy with decacationic monoadducts and bisadducts of [70]fullerene: *in vitro* and *in vivo* studies. *Nanomedicine* 2014; 9(2): 253-66.
- [193] Zhang Y, Dai T, Wang M, Vecchio D, Chiang LY, Hamblin MR. Potentiation of antimicrobial photodynamic inactivation mediated by a cationic fullerene by added iodide: *in vitro* and *in vivo* studies. *Nanomedicine* 2015; 10:603-14.
- [194] Lin C-M, Lu T-Y. C60 fullerene derivatized nanoparticles and their application to therapeutics. *Recent Pat Nanotechnol* 2012; 6:105-13.