Fullerene-based delivery systems

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With the development of new drugs, there have been many attempts to explore innovative delivery routes. Targeted delivery systems are a desired solution designed to overcome the deficiency of routine methods. To transform this idea into reality, a wide range of nanoparticles has been proposed and studied. These nanoparticles should interact well with biological environments and pass through cell membranes to deliver therapeutic molecules. One of the pioneer classes of carbon-based nanoparticles for targeted delivery is the fullerenes. Fullerenes have a unique structure and possess suitable properties for interaction with the cellular environment. This short review concentrates on newly developed fullerene derivatives and their potential as advanced delivery systems for pharmaceutical applications.

Introduction

Routine methods for drug administration involve pulmonary, transdermal, transmucosal, ocular and other delivery systems among which injection and oral delivery are the most popular [1]. In these methods, one of the main problems is the poor pharmacokinetics and untargeted delivery of the drugs; therefore, in some applications such as chemotherapy there are increasing side-effects from the drug and the effectiveness of therapy could decrease. Beside the delivery of drugs, in some diseases there is also a need to deliver biological substances such as DNA and various small molecules through the cell membrane. In fact, the delivery of such substances to the nucleus and other organelles can engender new challenges owing to the specific barriers and features present in the cell.

To overcome these challenges, research in nanotechnology has offered a range of solutions to enhance the drug delivery effectiveness [2]. For instance, the pioneer nanoparticle: fullerene, with a specific geometry, size and surface characteristics, possesses a uniquely spherical structure with a strong apolar character [3]. These features enable fullerenes to be used in lipid-like systems, serving as a reservoir and even crossing cell membranes [4].

Structural features and activities

Fullerenes, also referred to as Buckminsterfullerenes or Buckyballs, are one of the allotropes of the carbon family of nanomaterials [5]. Fullerenes exhibit a structure consisting of sp^2 carbons that present unique chemical and physical properties and a highly symmetrical cage with different sizes (C60, C76, etc.) [6,7]. The most abundant fullerene in the synthesized composition is C60 [4]. It consists of 60 carbon atoms with C5–C5 single bonds (12 pentagons), and C5 = C6 double bonds (20 hexagons). Indeed, each fullerene with $2n + 20$ carbon atoms contains 'n' hexagons. C60 and C70 are produced at 1000 °C and the concentration increases as pulse duration increases [8]. Among different characteristics of this molecule, the dual behavior of C60 among reactive oxygen species (ROS) gave this nanoparticle the ability to act in different ways in specific situations. In some cases, C60 has the ability to produce oxygen species upon exposure to visible light, making it a suitable candidate for photodynamic therapy (PDT). In some other cases, it downregulates ROS, which can be used as a neuroprotective agent

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[9]. The mechanism of this action is still unknown and requires further investigation. An obstacle in exploiting this molecule in biological applications is its insolubility in water and low solubility in many organic solvents. Accordingly, in biological systems, the hydrophilicity of materials has greater importance in comparison with hydrophobicity and many methods have been used to increase its hydrophilicity and water solubility [4]. These include the preparation of two-phase colloidal solutions, synthesizing fullerene derivatives, fullerene polymers, encapsulation in special carriers (cyclodextrins, calixarenes, polyvinylpyrrolidone, micelles, liposomes, etc.), chemical modification [by adding hydrophilic substances such as amino acids, carboxylic acids, polyhydroxyl groups (fullerenols) and amphiphilic polymers], among others.

Applications in pharmaceutics

Nucleic acid delivery

Owing to their specific abilities, there have been several attempts to use fullerene and its functionalized derivatives for pharmaceutical applications (Fig. 1). Materials from this class can potentially act as targeted and controlled drug delivery systems. Nucleic acid delivery is a recently developed therapeutic tool for fullerene and its functionalized derivatives. The process is about targeted delivery in cells with nucleic acid deficiency. Most approaches use viral delivery for the transfer of DNA, RNA, siRNA, LNA and plasmid DNA to specific cellular locations [10]. In some studies, nanoparticles, such as fullerenes especially cationic ones, were used to deliver small molecules owing to their nonimmunological reactions, low cost and high efficacy [11]. A water-soluble cationic tetra-amino fullerene and siRNA form submicrometer complexes in a buffered solution [12]. It can be said that these complexes agglutinate further with plasma proteins in the bloodstream to form micrometer particles. The agglutinate rapidly clogs the lung capillaries, releases the siRNA into lung cells to silence the expression of targeted cancer genes and is then cleared rapidly from the lung after siRNA delivery [12]. In other research, C60-Dex-NH2 with a specific amphiphilic skeleton formed micelle-like aggregate structures in water, which could prevent siRNA from being destroyed by ROS. As it was exposed to visible light, C60-Dex-NH2 triggered controllable ROS generation causing lysosome membrane destruction, promoted lysosomal escape and enhanced gene silencing efficiency of siRNA *in vitro* and *in vivo*. The gene silencing efficiency was able to reach a maximum of 53% in the

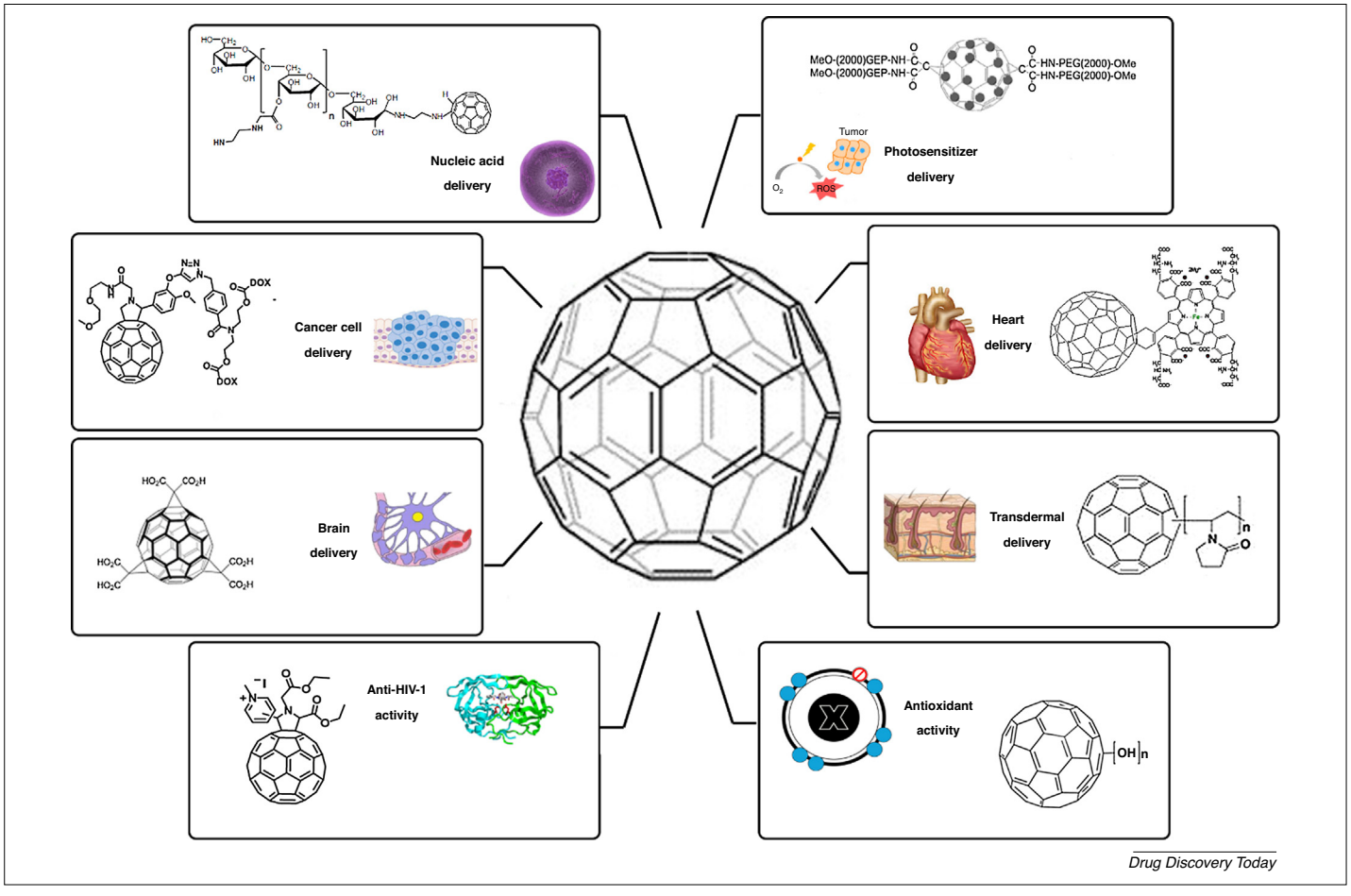

FIGURE 1

Fullerene-based delivery systems. Nucleic acid delivery: penetration through cell membrane and non-immunological reaction; photosensitizer delivery: targeted delivery to cancer cells and activation of ROS system owing to the activation of photosensitizer by UV light; cancer cell delivery: targeted delivery of chemotherapeutic agents and decreasing side effects and increasing efficacy; heart delivery: targeted delivery of highly potent cardiovascular medicines and decreasing side-effects and increasing efficacy; brain delivery: unique nanostructure and appropriate interaction with tight assembled cellular structure of blood–brain barrier; topical delivery: unique and flexible structure and good interaction with keratinocytes; anti-HIV-1 activity: blocking the hydrophobic pocket of protease and blocking lifecycle of HIV-1; anti-oxidant activity: downregulating the ROS system and inactivating the oxygen species as neuroprotective and antiaging agents.

MDA-MB-231-EGFP cells and 69% in the 4T1-GFP-Luc2 tumor-bearing mice [11]. The reported data in the literature support the use of conjugated nucleic acids with fullerene-based delivery systems to significantly enhance the efficacy in a target-specific manner. There is growing interest toward this strategy because the healthy cells are somewhat less vulnerable to adverse reactions.

Topical drug delivery

Antioxidant activity of fullerenes and interactions with epidermal keratinocytes enabled this spectacular nanomaterial to have enormous abilities to be used in transdermal delivery and cosmetic applications [13]. Owing to their antioxidant activity and the ease of topical delivery, this class of materials has been frequently used in different forms, such as moisturizers, anti-inflammatory substances, cytoprotective, UVB inhibitory and barrier-repair agents, hair growth stimulators and antimelanogenesis agents [14,15].

In the area of drug delivery, fullerene-based peptides were examined and the results demonstrated adequate penetration, as related to mechanical flexion [16]. Basic cosmetic formulations with transcutol/isopropyl myristate without harsh organic solvents showed a high potential for applications in biopharmaceuticals and cosmetics [17]. Furthermore, there are a few reports on the use of noncovalently adsorbed drugs and fullerenes (showing reasonable stability at room temperature); therefore, at an optimum concentration they could present acceptable and efficient transdermal drug delivery [18,19].

Cancer therapy

Cancer is the uncontrolled growth of abnormal cells in the body and is caused by many factors such as chemical or toxic compounds, ionizing radiation, pathogens and human genetics [20]. Many drugs with different effects have been used to treat cancer. The main problem in cancer therapy is the significant side-effects of the drugs, and they require targeted delivery [21]. Another drawback in cancer treatment is drug resistance; therefore, there is a need for the use of multiple drugs, which further increases their negative side-effects.

One promised nanoparticle in drug delivery is fullerene with its strong structural features. Fullerene, with the ability of carrying a multiple drug payload and with targeted drug delivery, could solve many of the side-effects of chemotherapy [22,23]. For instance, the cardiomyopathy-related side-effects of doxorubicin made this compound a candidate for conjugation with fullerene [24]. This conjugation was examined at different pH values and the results demonstrated 100% drug release at pH 5.25. The results enable us to use this conjugation for targeted drug delivery and to decrease side effects. The challenge in this approach was that doxorubicin was water-soluble whereas fullerene was hydrophobic, so ethylene glycol spacers were used for conjugating methano-C60 with doxorubicin, improving the water solubility of this conjugation [4]. In another study, Buckysomes, spherical nanostructures that are made of amphiphilic fullerenes with hydrophobic regions, were used to mask the hydrophilic surface of paclitaxel. This study indicated that the proposed complex could effectively enhance drug absorption [25].

A new, novel drug delivery system was based on an 'on–off' drug delivery strategy developed by the conjugation of doxorubicin and fullerene after attachment of a hydrophilic shell to the outer surface of the conjugation. This drug delivery system is very stable in physiological solutions even with a pH ~5.5 in the 'off' state; by contrast, in the 'on' state the generation of ROS by fullerene results in two treatment types. First, generation of oxygen species and causing cell death (PDT) and, second, the breaking of the ROS-sensitive linkers, enabling the exploding release of doxorubicin (chemotherapy) [26]. It should be mentioned that, in the development of innovative strategies for cancer treatment, especially in chemotherapy and PDT, many nanomaterials have been tested so far but fullerene and fullerene-based systems are among the most promising candidates owing to their unique structures and properties.

Photodynamic therapy

Over the past decades, PDT has proved its potential in the treatment of a series of diseases ranging from bacterial infection to cancer [27]. This method is based on the absorption of photosensitizers in targeted tissue, activation by a specific wavelength, activation of the ROS system and oxygen species and, finally, subsequent cell death [28]. A key limitation in PDT is the poor water solubility of many photosensitizers [29,30]. Some of the great advantages of fullerenes include their potential in PDT – the absorption of visible light combined with an efficient intersystem crossing to a long-lived triplet state that makes fullerenes generate ROS upon illumination and allows fullerenes to act as photosensitizers [4]. Many substances conjugated with fullerenes have been synthesized in past years. In innovative research, iron oxide nanoparticles (IONPs) were first decorated on the surface of fullerenes and then PEGylated. C60-IONP-PEG nanocomposite demonstrated a strong superparamagnetism and powerful PDT capacity [31]. In another study, C60-lysozyme showed effective endogenous ROS activity after exogenous H_2O_2 generation owing to its photodynamic activity in HeLa cells [32].

With the aim of developing smart photosensitizers with tumor-microenvironment-activatable fluorescence turn-on and singlet oxygen generation for tumor bioimaging and PDT, Tang *et al.* [33] proposed that a pH-sensitive photosensitizer, C60-rhodamine B, having a spirolactam structure, was activated in acidic pH by its ring-opening structure. In this system, enhancement of visible light absorption and singlet oxygen generation occurred. Advances in this field suggest that PDT can be considered as a strong treatment method owing to the massive terminating effect on cells such as cancer cells. But the main problem was that the effect on healthy cells was equal to cancer cells. Fullerene-based delivery systems could begin to solve this problem. This class of materials, with the ability of targeted delivery, decreased the absorption in healthy cells and increased it in cancer cells. This supreme ability of fullerene-based delivery systems should be noted and more experiments should be conducted to be used in clinical applications.

Crossing the blood–brain barrier

The blood–brain barrier is a physical barrier composed of endothelial tight junctions that inhibit the paracellular permeability and therefore create a major challenge for delivering drugs to the central nervous system (CNS) [34]. Fullerene and its water-soluble derivatives are among the nanomaterials with a high potential for delivering drugs into the CNS. In a study, a complex of fullerene and hexamethonium was developed and compared with the

hexamethonium delivery system alone. The research demonstrated a 40-times boosted potency in the complex drug delivery system [35]. In another study, water-soluble C60 fullerene derivatives with four types of linkages between the fullerene cage and the solubilizing added atoms were synthesized [36]. Fullerene derivatives 1–6 (compounds 1–3: C–C bonds; compounds 4–5: C–S bonds; compound 6: C–P bonds) were observed to induce neural stem cell (NSC) proliferation *in vitro* and rescued the function of the injured CNS in zebrafish. Surprisingly, compound 3 bearing residues of phenylbutyric acid significantly promoted NSC proliferation and neural repair with a change in the metabolism of cells by reducing ROS activity and Inducing ATP activity. Fullerene derivatives 7–9 (compounds 7–9: C–N bonds) were found to act as glioblastoma cell proliferation inhibitors in zebrafish. Compound 7 with phenylalanine appendages significantly inhibited glioblastoma growth; therefore, acting as an antitumor agent. This effect was also correlated to the cell metabolic changes in the tail of the induced ROS activity and reduced ATP activity [36]. In addition, carboxyfullerenes have been exploited in the treatment of neurodegeneration caused by amyotrophic lateral sclerosis (ALS) [37]. The tight complex structure of the brain does not naturally let any chemical pass the barrier. As stated, fullerene-based delivery systems do not usually obey this rule, being able to deliver therapeutics through their twisted structures.

Antioxidant activity and radical scavenging

Balanced oxidation and antioxidant activity of the body is indeed an important function for the safety of our biological system. With our incomplete endogenous antioxidant defense system and massive free-radical production by our normal cellular metabolism as well as abnormal reactions, there is always a need for exogenous antioxidants to prevent toxicities and diseases caused by excess free radicals such as cancer and atherosclerosis [38]. The presence of several double bonds in the fullerene cage makes this class of nanoparticles capable of reacting with free radicals such as superoxide, hydroxyl radicals and hydrogen peroxide [4]. In a recent study, two different fullerenes, C60 and C82, conjugated with three transition metals (i.e., copper, silver and gold) were used as an antioxidant. The results demonstrated that the antiradical capacity of fullerenes was boosted as a result of the presence of the metals [39]. In other research, the antioxidant ability of fullerene was used in cosmetics and skincare products as an anti-ageing agent [40]. With the ability of crossing the blood–brain barrier, a suspension of fullerene with a maximum size of 450 nm was prepared and injected into the hippocampi of Wistar rats. Although the results demonstrated impaired spatial memory with a significant decrease in brain-derived neurotrophic factor (BDNF) protein levels, a decrease in ROS was also observed [41]. Antioxidant activity of fullerenols in oxidizer solutions with luminous bacteria and their enzymes was tested. The results demonstrated that the effect on bacterial cells was attributed to the hormesis phenomenon, whereas the intensification of biochemical reactions was related to its catalytic activity [42]. One of the most important problems for citizens today is related to oxidative stress. It is expected that fullerene-based systems could balance the oxidative stress system. Many investigations should be conducted to fully understand the vital role of fullerene-based delivery systems in controlling oxidative stress.

Anti-HIV activity of fullerenes

HIV is a lentivirus (a retrovirus subgroup) that ultimately causes AIDS [43]. With the lack of immune activity, the patient dies either by cancer or opportunistic infections [44]. In acknowledging the mechanism-of-action of HIV, many drugs such as nucleic and non-nucleic reverse transcriptase inhibitors, protease inhibitors, and so on, have been synthesized [45]. There are several reports on fullerene and its ability to inhibit HIV-1 protease activity. The active site is a semi-open hydrophobic ellipsoid, with Asp-25 and Asp-125 standing out on the surface of the cavity and catalyzing the protease function. So the antiviral activity was caused by the entrance of fullerene into the pocket, attaching to the active site via van der Waals interactions [46]. Some derivative fullerenes inhibit HIV-1 viruses that are multidrug-resistant [47]. The mechanism-of-action was suggested to be via a strong interaction with the immature capsid in pull-down experiments. No toxicity occurred during the treatment with these compounds; therefore, they could be among the next-generation candidates for HIV treatment [47]. Furthermore, HIV-1 reverse transcriptase inhibitory activities of cationic, anionic and zwitterion fullerene derivatives were tested, in which cationic derivatives showed lower activity [47]. Pyridine/pyridinium-type fullerene derivatives without any carboxylic acid inhibit the HIV-1 reverse transcriptase in a cell-free environment. The results showed the important role of the fullerene cage without any sign of cytotoxicity [48]. Many approaches for HIV treatment consist of vaccines and/or drugs, in which vaccines are of great interest. HIV is a special virus with an amazing lifecycle that has a supreme defense against drugs. Fullerene-based delivery systems could be the answer to control this virus and its lifecycle, but we shall see what this system can do through further investigation.

Antibacterial activity of fullerenes

Along with many antibacterial agents such as beta lactams, several functionalized fullerene derivatives showed acceptable inhibitory effects on bacteria. Carboxyfullerene antibacterial activity was examined against *Streptococcus pyogenes* infection. *In vitro* experiments showed that this derivative could suppress growth of *S. pyogenes* and its administration could protect 33% of mice from death [4]. Furthermore, this compound exhibited more activity against Gram-positive bacteria than Gram-negative bacteria (almost none).

The biological responses against alkaline-synthesized fullerenol were examined on *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The results demonstrated that toxicity thresholds for commercially prepared fullerenol were lower for all species, which was attributed to the presence of impurities. A mechanistic analysis of membrane damage on bacteria by laboratory-prepared fullerenol indicated necrotic and apoptotic responses with and without photoactivation [49].

It has been frequently reported that fullerenes could directly interact with biomolecules, such as aromatic mutagens or anticancer drugs. In a recent study, aqueous solutions containing C60 and an aromatic mutagen called ICR-191 were prepared and further evaluated against *Salmonella typhimurium*. The results demonstrated a massive affinity to the bacterial cell wall that altered cellular morphology [50].

C60/C3N4 and C70/C3N4 hybrids synthesized by a hydrothermal method exhibited stronger bacterial inactivation than C3N4 against

Escherichia coli O157:H7. The enhancement of photocatalytic activity of composites could be attributed to the effective transfer of the photoinduced electrons under visible light irradiation [51]. Bacteria are the most familiar microorganisms and many types of them are completely understood. The main problem is the development of their mechanisms and increasing antibiotic resistance. It is expected that proposing innovative delivery systems could provide us with alternative solutions for bacterial resistance to control and terminate bacteria in a different mechanism to that of antibiotics.

Fullerenes as neuroprotective agents

Excess production of oxygen species and hyperactivation of *N*-methyl-D-aspartic acid or *N*-methyl-D-aspartate (NMDA) and glutamic receptors can lead to neurodegenerative diseases such as Alzheimer's and Parkinson's [52]. Radical scavenging ability, ROS activation and ability to interact with peptides were the main reasons for the usage of fullerene derivatives in neurodegenerative diseases [4]. Their ability to interact with peptides was also used to prevent formation and progression of Alzheimer's disease. In a study, the effect of hydrophobic fullerene C60 on the oligomeric structures of the hydrophilic GNNQQNY peptide, the key amyloid-forming fragment of yeast prion protein Sup35, was tested [53]. The results demonstrated that fullerenes completely prevent fibril-like bilayer β-sheets, formed by GNNQQNY peptides, and also blocked the inter-peptide interactions that are crucial for oligomerization and β-sheet formation [53]. In some innovative research, UCNP@C60–pep under near-infrared light produced ROS species, in which the oxygen species hindered A-beta and mitigated attendant cytotoxicity [9]. Fullerene and fullerenols have shown spectacular antioxidant activity. Their abilities to reduce apoptosis in cortical neurons and to block glutamic acid receptors have been reported [54]. Hexa(sulfobutyl) fullerenes and trimesic acid (TMA) fullerenes also demonstrated their ability to trap free radicals and play an efficient part in the treatment of neurodegenerative diseases [55,56]. Based upon various studies, water-soluble derivatives, such as fullerenols and malonic acid fullerenes, can react with hydroxyl and superoxide free-radicals and reduce neuronal degeneration related to ROS [57]. Neurodegenerative diseases are developing rapidly, reducing quality-of-life whether physical or social. This could be related to life expectancy increasing and systemic deficiency occurring in the body over time. With the use of fullerene-based delivery systems we can overcome, control or prevent this major problem.

From basic findings toward clinical applications

One of the most important approval tests in drug manufacturing processes after evaluation in animal models is promising clinical trials. Owing to their superior advantages over conventional delivery systems, there have been several attempts to take advantage of nanocarriers for the delivery of drugs. For the case of fullerene-based delivery systems, despite the incredible abilities mentioned in the previous sections, no clinical trials have been announced yet [58]. One of the most important obstacles restricting this approach from human usage is the inherent toxicity of fullerene nanomaterials [58]. Nowadays, a series of tests conducted by several independent teams, such as C60 nanoaggregates prepared with tetrahydrofuran (THF), confirmed the safety of pure C60 in various experimental models in the laboratory [59]. Although there have been some scattered reports in the literature, there is still a long way to go before we fully understand the actual performance and function of this class of nanomaterials. Several successful preclinical trials have been conducted in different animal models, indicating that there is a need for clinical evaluation.

In recent innovative research, hydroxylated fullerenes have been used to investigate the analgesic effect in clinically relevant lumbar radiculopathy [60]. Remarkably, single and local use was sufficient to decrease ipsilateral paw pain sensation in mice up to 2 weeks post-surgery. Furthermore, micro-CT data suggested that hydroxylated fullerenes potentially promoted disc height recovery following injury-induced disc herniation. To understand the mechanism of these effects, an *in vitro* study on mouse dorsal root ganglia (DRG) culture demonstrated that fullerol attenuated tumor necrosis factor (TNF)-α-elicited expression of transient receptor potential cation channel subfamily V (TRPV)-1 and neuropeptide release (substance P and calcitonin gene-related peptide). This action was mentioned to be through protein kinase B (AKT) and extracellular protein regulated kinase (ERK) pathways. Furthermore, Alcian blue and picrosirius red staining also suggested that fullerol promoted regeneration of extracellular matrix proteins visualized by the presence of abundant, newly formed collagen and proteoglycan in herniated discs (Fig. 2).

In other research, a lethal pulmonary disease called pulmonary fibrosis was tested via inhalation of gadofullerenol (GF-OH) and fullerenol nanoparticles [61]. The results demonstrated that gadofullerenols and fullerenols substantially decreased the collagen deposition induced by acute lung injury. The mechanism-of-action was observed to be via antioxidant and anti-inflammatory functions for the modulation of ROS-mediated inflammation and indirectly modulating transforming growth factor (TGF)-β1 expression (Fig. 3). Furthermore, gadofullerenol exhibited a higher performance than fullerenol owing to the larger quantity of the residual conjugated double bonds.

In a study by Chen *et al.* [62], carboxyfullerenes were used in rat models in acute hepatic injury caused by severe hemorrhagic shock. In hemorrhagic shock there is a great deal of activity of the ROS system, which could further lead to oxidative stress, inflammation and subsequent tissue injury. The results demonstrated a decrease in alanine aminotransferase (ALT) activity, methemoglobin content, malondialdehyde content and myeloperoxidase activity. Carboxyfullerenes also decreased levels of TNF-α and interleukin (IL)-6 with an increase in superoxide dismutase activity in the liver. It seems that these protective effects are related to the inhibition of the nuclear factor (NF)-κB pathway via reducing phosphorylation of the NF-κB p65 subunit in the liver.

It is worth mentioning that all of the stated preclinical trials could have some similarities to the mechanism in humans but the main obstacle is that the human body is a special case and sometimes acts differently from data collected in animal tests. Despite many criticisms, the use of fullerenes for delivery purposes remains a very attractive concept and should be contemplated very carefully. Addressing this, this article offers another proof-of-concept toward better understanding the essential factors governing different aspects of fullerene delivery systems. Although some researchers have previously confirmed preclinical success, recent debates failed to comprehensively discuss and acknowledge the effectiveness of fullerene delivery systems in the clinic.

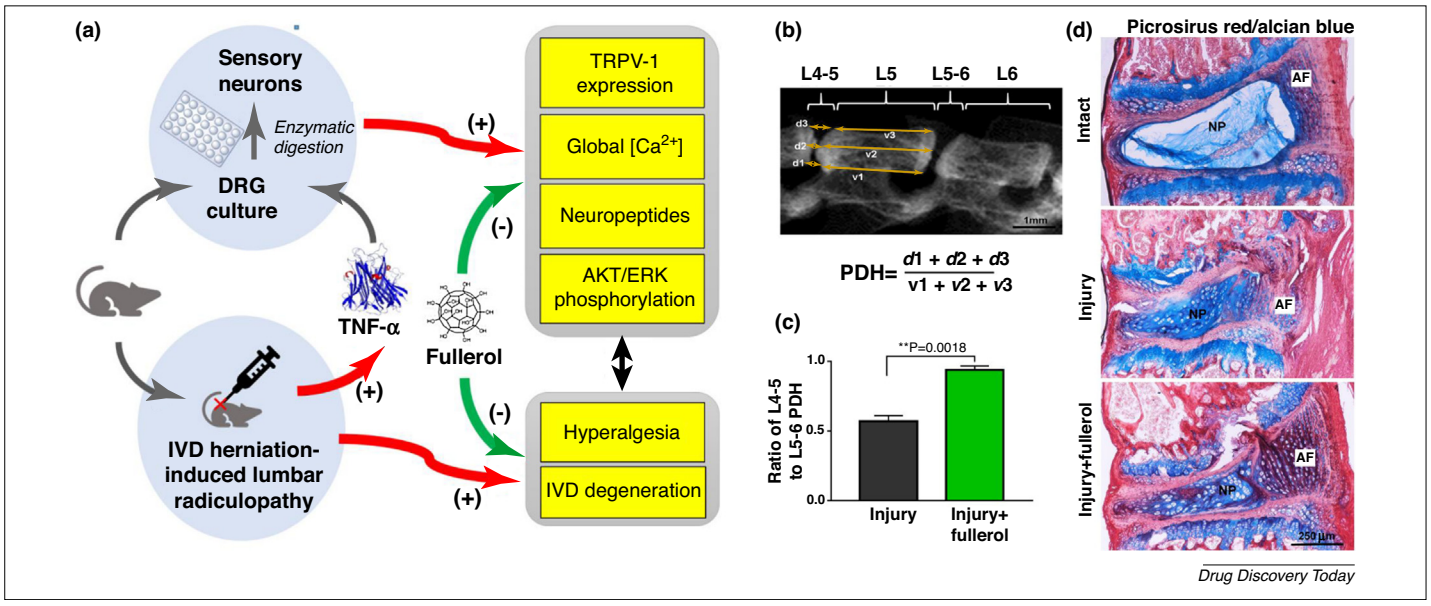

FIGURE 2

Hydroxylated fullerene to treat lumbar radiculopathy. **(a)** Schematic hypothesis of therapeutic mechanism of fullerol in lumbar radiculopathy secondary to disc herniation. (Upper row) *in vitro* mechanism via antagonizing TNF-α-induced neuroinflammation and alternation in nociceptive factors, ion channel, intracellular [Ca^{2+}] and signaling pathways in DRG. (Lower row) *in vivo* regenerative effect in clinical model of lumbar radiculopathy secondary to disc herniation. **(b)** Micro-CT image of mouse spine 2 weeks after surgery indicated restored disc height via fullerol restoring effect. **(c)** The injury + fullerol group showed an increase in the ratio of L4–5:L5–6 PDH. **(d)** Alcian blue/picrosirius red staining models demonstrating ongoing regenerative process of injured IVD post-fullerol treatment. After 2 weeks post-surgery, compared with intact disc (top row), injury + saline (middle row) discs demonstrated classical manifestations of disc degeneration such as ruptured lamina, enlarged and proliferated chondrocytes. The fullerol-treated group (bottom row) demonstrated much better purple staining in the inner and red staining in the outer regions, suggesting an active regenerative process. Reproduced, with permission, from Ref. [60].

The state-of-the-art and future perspectives

With the growing interest in the use of fullerene-based delivery systems, many innovative systems are being proposed. Although there are many promising examples, there is still room for more-advanced systems against challenging diseases. In the first case ever reported on the fullerene-based low toxic nanocationite particles designed for targeted delivery, porphyrin adducts of cyclohexyl-fullerene were prepared for the delivery of Mg^{2+} to the myocardium [63]. In a single injection, release of Mg^{2+} due to the acidic metabolic shift by nanoparticles was used to stimulate ATP overproduction in oxygen-depleted cells and, therefore, 80% of the tissue that was suffering from hypoxia was recovered after 24 h. The positive changes in heart cell energy metabolism could assist in the treatment and prevention of local myocardial hypoxic disorders and protect heart muscles in different hypoxia-caused clinical situations.

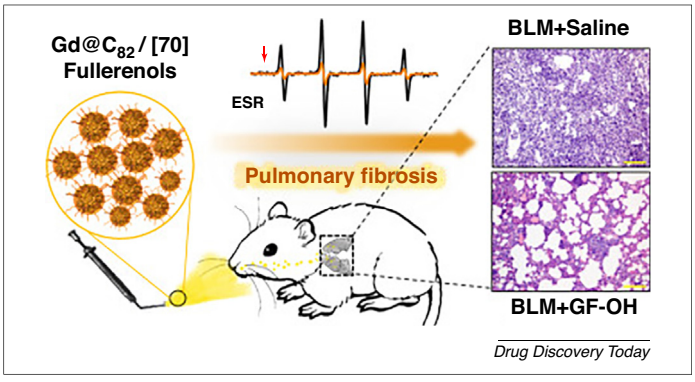

FIGURE 3

Inhalable gadofullerenol/[70] fullerenol for pulmonary fibrosis therapy. Inhalants containing gadofullerenol and fullerenol nanoparticles with excellent free-radical scavenging ability were used as an essential therapy for pulmonary fibrosis. Both of them effectively attenuated bleomycin (BLM)-induced acute lung injury. The mechanism-of-action was due to improvement of collagen accumulation and reduction in oxidative stress damage. These events are related to direct ROS modulating ability and indirect inducing TGF-β1 expression accomplished by gadofullerenol and fullerenol. Furthermore, gadofullerenols displayed better antioxidant activity owing to the larger quantity of the residual conjugated double bonds. Reproduced, with permission, from Ref. [61].

Fullerenes also have been used in delivery of highly bioactive molecules like warfarin – a coumarin anticoagulant drug [64]. This drug has shown a very low therapeutic index and therefore is indeed a candidate for drug delivery in order to adjust its dosage. Conjugation of warfarin to fullerene can alter its biological profile and prevent variation of its concentration in the blood. Furthermore, this conjugate modulates warfarin dosage and makes this drug controllable. This is an important concept that should be further developed.

Erythropoietin (EPO), a hormone mainly produced by the kidneys, which increases red blood cell production, can also be linked to fullerenes and can be used in diseases such as bone marrow suppression [65]. Upon i.v. injection of EPO, its biological activity is reduced abruptly. To overcome this problem, nanoparticles of EPO were synthesized for effective administration of EPO. For instance, EPO was absorbed on porous materials containing fullerene and its bioavailability studied via pharmacokinetics and the results demonstrated three-times higher bioavailability than intraperitoneal administration.

Among the mentioned conjugated nanoparticles, some poly-conjugated fullerenes were used to treat cancer. Conjugation of malonodiserinolamide plus fluorophore and fullerene exhibited promising outcomes in in vivo liver cancer therapy and breast adenocarcinoma in murine cells [66]. In other research, conjugated PEI-C60-folic-acid-(via an amide linker)-docetaxel exhibited reasonable cell membrane permeation, increasing the apoptosis and decreasing the possible side-effects of the docetaxel. Furthermore, conjugation of hyaluronic acid with transferrin showed high efficacy in PDT [67].

Concluding remarks

In this concise review, many fullerene derivatives have been mentioned and their uses in different pharmaceutical applications have been discussed. With the specific structure and methods used to increase water solubility, there is no doubt that fullerene-based delivery systems offer many opportunities in disease treatment. For this to be realized, more in vivo tests should be conducted before clinical examinations. Systemic pharmacokinetics, toxicological studies, therapeutic index and side-effects must be understood. With the significant input that will be gained from in vivo tests, we can rapidly improve this class of delivery system.

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