Review Article

Review on Fullerene: A Cutting Edge Trend in Drug Delivery

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ABSTRACT

Advanced therapeutic methods comprise a series of pioneer strategies to deliver substances to a specific target in the body. In this regard, fullerene derivatives due to their exceptional abilities are able to effectively deliver many molecules to targeted organs. Their unique carbon cage structure coupled with scope for derivatization make them feasible for therapeutic agent. Fullerene based nanoparticles interact well with biological environments and pass through cell membranes to deliver therapeutic molecules in particular target in controlled manner. In this review, we highlight the therapeutic application of Fullerene as anticancer, antiviral, antioxidant agent.

Keywords: Fullerene, derivatization, photodynamic therapy, gene delivery, antioxidant.

INTRODUCTION

In 1980, with the discovery of a new allotropic form of elemental carbon resulted in the birth of fullerene research.[1] Spherical fullerenes discovered by British scientist Harry Kroto in 1985 commonly referred to as buckyballs. American architect Richard Buckminster Fuller devised the arrangement of the atoms in buckyballs looked like the shape of the geodesic domes, so the name. Along with American researchers Richard Smalley and Robert Curl, Kroto synthesized buckyballs in the laboratory and were awarded a Nobel Prize in Chemistry in 1996.[2]

Fullerenes also referred to as Buckminsterfullerenes or Buckyballs have sp2 carbons that showed unique chemical and physical properties and a highly symmetrical cage with different sizes (C60, C76, etc.).[3,4] The most abundant fullerene in the synthesized composition is C60 [5]. C60 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.[6]

The fullerene cages, their sizes and shapes, have been exploited for bio-applications, such as plugging "holes" in viruses for inhibition purposes.[7-9] C60 is has a valuable prospect for photodynamic therapy (PDT) as it produces oxygen species upon exposure to visible light. Sometimes, it down regulates ROS, which can be used as a neuroprotective agent [3]. Functionalized Fullerenes have an immediate bioactivity such as antioxidant activity. Fullerenes also can function as drug vectors or drug delivery scaffolds with non-covalent linkages or with covalent linkages between the fullerene and a bioactive moiety.

CATEGORIES OF FULLERENE

The fullerenes can be categorized in different ways like

Alkali doped fullerenes

Fullerene readily forms compounds with electron donating species as it has high electronegetivity. E.g. alkali doped fullerides wherein alkali metal atoms fill in the space between buckyballs and donate valence electron to the neighboring C60 molecule. If the alkali atoms are potassium (K) or Rubidium(Rb), the compounds are superconductors and they conduct electric current without any resistance at temperatures below 20-40K.[4] e.g. K3C60, Rb3C60.

Endohedral fullerenes

When some other atoms can be enclosed inside the hollow shell of fullerenes, it results in the formation of inclusion compounds known as endohedral fullerenes. When the atom trapped inside is a metal, they are called metallofullerenes. Most of the endohedral materials are made out of C82, C84 or even higher fullerenes. As it is

extremely difficult to open up carbon cage molecules to surround a far off atom inside endohedral material must be synthesized while formation of cage itself.[6]

Exohedral fullerenes

Fullerenes have conjugated π system of electrons. So, two main types of primary chemical transformations are possible on fullerene surface: addition reactions which lead to covalent exohedral adducts and redox reactions yielding salts, respectively. Numerous derivatives of fullerenes have been synthesized with improved solubility profile.[10] Fullerene derivatives are also known as functionalized fullerenes.

DERIVATIZATION OF FULLERENE

Fullerenes are generally poorly soluble in commonly used organic solvents, let alone in aqueous solutions. For example, upon vigorous sonication of a C60 sample in water, large aggregates are found in the suspension [11]. The insolubility in aqueous media has been considered as a major obstacle. Many strategies have been demonstrated or applied to either solubilize or modify fullerenes for improving their utility in drug-delivery and medical applications. The following approaches: two-phase colloidal solutions preparation, synthesis of fullerene derivatives, fullerene polymers, encapsulation in special carriers (cyclodextrins, calixarenes, polyvinylpyrrolidone, micelles, liposomes, etc.), chemical modification [addition of hydrophilic substances such as amino acids, carboxylic acids, polyhydroxyl groups (fullerenols) and amphiphilic polymers], have all been explored by several entities throughout the world to overcome this problem with fullerenes.[12]

The solubility of Fullerenes in organic or aqueous media can be enhanced by derivatizations depending on the choice of attached functional group. Among the many water-soluble fullerene derivatives that can be synthesized, three synthetic approaches have shown to be particularly valuable.[13] They are shown in Fig. 1. These three are cyclopropanation to form methanofullerenes, polyhydroxylation (synthesis of fullerols, typically using biphasic organic/caustic media), and the cycloaddition of azomethine ylides (1,3 dipolar cycloaddition to form fulleropyrrolidines).

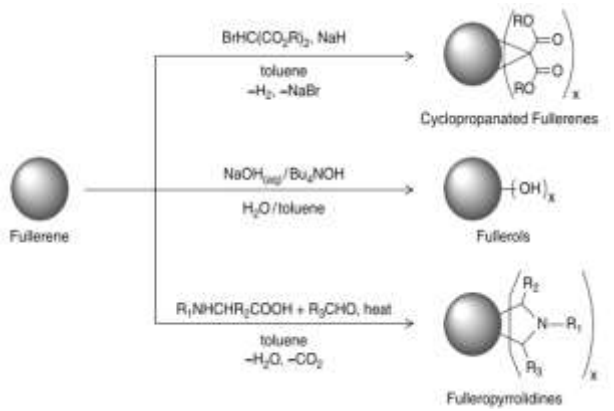

Figure 1: Three main synthetic approaches of Fullerene for drug delivery

APPLICATION OF FULLERENE

Fullerenes as carrier of drug

The fullerene cage is structurally stable, but readily functionalized, thus serving as versatile three-dimensional scaffolds for carrying various drugs. There are three running modalities available by which derivatized fullerene can be employed in drug delivery nanomaterials. First, the derivatized fullerene solely can serve as a biomedically active agent. Secondly, the fullerene derivative can assist as a non-covalent carrier of bioactive drug species. Thirdly, the initial fullerene derivative can be secondarily derivatized via covalent attachment of drug molecule.[14]

Components need in fullerene based drug delivery the fullerene cage, the surface derivative groups/linker unit, and the attached drug or otherwise biomedically active molecule(s).[15] Functionalized fullerenes with covalently attached drug agents are highly amenable to bringing in biofunctions such as controlled and targeted drug release.

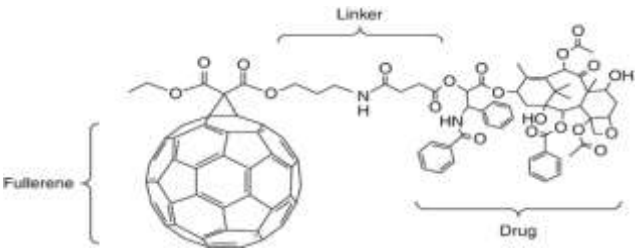

Figure 2: Fullerene conjugation with active moiety[15]

Many drug molecules are often attached to a fullerene. The medicine loaded fullerene can then be attached to an antibody which is Y-shaped proteins that can recognize and attach to antigens. Viruses, bacteria and diseases within the body have unique antigens. The antibody finds the disease in the body, and then the attached fullerene delivers the appropriate medicine. Just like with magnetic nanoparticles, medicine can be sent only to place where it is needed, leaving healthy cells alone.[16]

Figure 3: Drug loaded Fullerenes (green) attached to an antibody (pink) which will look for specific disease antigen[16].

Doxorubicin (DOX), a first line anticancer drug. However, doxorubicin has several side effects; and it is considered most of the toxicity is developed by oxidative stress and the formation of free radicals. DOX, conjugated to fullerenols through a carbamate linker and produces fullerenol−doxorubicin conjugate. This conjugate block the G2-M cell cycle *in vitro* in cancer cell line. Finally results in apoptosis by inhibiting the proliferation of cancer cell.[17]

Covalently attached DOX and polyethylene glycol onto fullerenol not only provided the aqueous solubility but also enabled a high loading of drugs. In the cell viability MTS assay, the cytotoxicity of the fullerenol-DOX conjugate against mouse melanoma cell line B16-F10, mouse lung carcinoma (LLC1), and metastatic human breast cancer cell line MDA-MB-231 was found to be in a time dependent fashion, coherent with a lessen segmentation of DOX from conjugate. Following the treatment with the fullerenol-DOX conjugate, there was an elevated level of DOX in the tumor as compared with that post-treatment with free DOX. This effect was happened may be deposition of fullerene based nanoparticle through enhanced permeability and retention (EPR) effect.[18]

In vivo gene therapy

Delivery of gene through non viral routes has become a powerful and popular research tool which helps in the elucidation of gene structure, regulation, and function [19]. Fullerene based nanoparticles have nonimmunological reaction, minimum cost and relatively higher efficacy. Due to these characteristics, fullerene particularly cationic ions are applied to deliver small molecules such as segment of gene, protein.[20]. Cationic fullerene molecules such as tetraaminofullerene have the capacity to make globules of DNA smaller than 100 nm which helps in penetrate into the cell.[21–23]

A study was conducted on *in vivo* delivery of enhanced green fluorescent protein gene (EGFP) by TPFE tetra (piperazino) fullerene epoxide on pregnant female ICR mice. The result showed distinguishable organ selectivity and higher gene expression in liver and spleen, but not in the lung. TPFE showed no acute toxicity in the liver and kidney. TPFE-based gene delivery also helps in insulin 2 gene delivery. In this study, female C57/BL6 mice showed elevated plasma insulin levels and reduced blood sugar concentrations, pointing the possibility of therapeutic application of TPFE-based gene delivery.[24]

In Photodynamic therapy and cancer treatment

In some sorts of cancer and premalignant conditions, photodynamic therapy represents an alternate treatment with great potential. Singlet oxygen ($^1O_2$) is toxic for cells. Using this principle, photodynamic therapy combines a nontoxic photosensitizer (PS) that upon visible light activation generates singlet oxygen ($^1O_2$) which is reactive oxygen species (ROS). The PS causes localized oxidative damage after accumulation in tumor cells. Fullerene's has expanded p-conjugated system of molecular orbitals in which 60-100 carbons that are inert physiologically are aligned in a soccer-ball structure. The presence of p-conjugated system helps fullerene in the absorption visible light at a particular wave length ($\lambda \geq 600$ nm).[25] Cationic quaternary ammonium groups in a soccer-ball structure of fullerene provide water solubility and biological targeting ability. To aid the photodynamic therapy, different functional groups like pyrrolidinium salts, more complex decacationic chains, and light-harvesting antennae are attached to C60, C70 and C84 cages.

The first report of phototoxicity in malignant cells was found in 1993 which is mediated by fullerenes. In this research functionalized fullerenes with carboxylic acid at 6.0 M concentration and white light were used to inhibit cancer cells growth.[26] Pristine C60 at 10 M with visible light from a mercury lamp were used to produce some phototoxicity in Ehrlich carcinoma cells or in rat thymocytes.[27] TA dendritic C60 monoadduct and malonic acid C60 trisadduct, two water soluble functionalized fullerene were tested for cytotoxic and photocytotoxic effects. UVA or UVB light was used to irradiate Jurkat cells and the cell death was mainly caused by membrane damage which was UV dose-dependent.[28]

Another study was conducted using monopyrrolidinium fullerene on three mouse cancer cell lines: (J774, LLC, and CT26) incubated for 24 h and illuminated with white light. The results showed that phototoxicity may be mediated both by superoxide and by singlet oxygen.[29]

HIV Inhibitor

HIV is a lentivirus responsible for AIDS with the help of reverse transcriptase enzyme. The patient suffering from HIV dies by cancer or opportunistic infections. A large number of antiviral drugs are available in the prevention or delaying the attack of acquired immunodeficiency syndrome (AIDS). But most of them are not as efficacious as thought. So fullerene derivatives are studied.

Funclionalized fullerene has the nature of complexation with HIV protease (HIV-P) enzyme and suppresses the growth of HIV. The active site of the enzyme 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. The fullerene can enter into the pocket and bind

to the active site via van der Waals interactions. Hence it showed antiviral activity.[30]

Specifically synthesized water-soluble fullerene derivative was found to be active against HIV-1 and HIV- 2 *in vitro.*[31] The compound was otherwise non-cytotoxic (up to 100M) in uninfected peripheral blood mononuclear cells, H9, Vero, and CEM cells.

Fulleropyrrolidines have activity against HIV-1 and HIV-2.[32] The relative positions two ammonium groups in the side chains give a robust influence on antiviral effect. Derivatized fullerenes C60 activity is studied in lymphocyte CEM cell cultures infected with HIV-1 and HIV-2.[33] Fullerene C60 derivatized with amino acid also effective against HIV and human cytomegalovirus replication.[34] Some water-insoluble fullerene (C60) derivatives have antiviral activity against enveloped viruses. The virulence capacity of semliki forest virus (SFV, *Togaviridae*) or vesicular stomatitis virus (VSV,*Rhabdoviridae*) in the presence of C60 is lost after visible light illumination for 5 h.[35] HIV-reverse transcriptase and hepatitis C virus replication can be inhibited by different types of functionalized fullerene with Cationic, anionic and amino acid group .[36] HIV has a complicated lifecycle which give it supreme protection against drugs. Fullerene-based delivery systems can be a potential option to control this virus as well its lifecycle. Perhaps further investigation may be needed to make it more feasible.

Antioxidant and Radical Scavenging

The presence of free radical species in human body is natural, but their overproduction may be harmful and cause serious diseases. Therefore, there has been extensive research on the development of free radical scavengers for biomedical applications.[37, 38]

Fullerenes possess great deal of conjugated double bonds and low lying lowest unoccupied molecular orbital (LUMO). This property helps in taking up an electron, making an attack of radical species highly possible. All these make fullerene capable for antioxidant property.

It has been described that one C60 molecule can take up to 34 methyl radicals. This quenching action involves in catalysis.[39] Dugan, *et al.* synthesized two regioisomers (*C*3 and *D*3) of trismalonyl C60 with carboxylic acids to serve as radical scavengers. Both regioisomers inhibited excitotoxic death of cultured cortical neurons induced by the exposure to *N*-methyl-Daspartate (NMDA), -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), or oxygen-glucose deprivation, though the regioisomer *C*3 was more effective than the regioisomer *D*3.[40]

Experiments on CCl_4 treated rats done by using C60 suspensions prepared in aqueous media without any polar organic solvent. This intoxication cause extreme damage to liver due to formation trichloromethyl radical (CCl3•) after reaction with oxygen. The result showed this suspension have protective effect on liver from free-radical damage produced by in dose dependant manner.[41]

It also has no acute or sub acute toxicity in rats. C60 and C82 fullerene conjugated with different transition metals like copper, silver and gold showed antioxidant effect. The presence of metals is supercharged the antiradical capacity of fullerenes.[42] Water soluble fullerenol and C60 tris(malonic)acid can cross the plasma membrane of mitochondria and produces free radicals within the cell. Fullerene also scavenges various toxins that can induce apoptotic injuries *in vitro* in different cell types such as neuronal cells or epithelial cells.[40] It is found that carboxyfullerenes, also known as C60 tris(malonic)acid, is able to protect quiescent human peripheral blood mononuclear cells (PBMCs) against apoptosis by preserving the mitochondrial membrane potential integrity, which is the early stage of apoptosis.[43]

Several fullerenols derivatized from C60 and the gadolinium endohedral fullerene, C60 (C(COOH)2)2, C60(OH)22, and Gd@C82(OH)22, were studied for their scavenging superoxide radical anion (O2), singlet oxygen, hydroxyl radical (HO), and the nitrogen-centered 2,2-diphenyl-1-picryhydrazyl free radical (DPPH).[44,45]

Oxidative stress is one of the common problems for today's world. Fullerene-based drug systems could counterweight the oxidative stress system. More and more experiments should be carried on to have a clear idea on fullerene-based drug delivery systems in the management free radical produced by oxidation.

DISCUSSION

Fullerene cages apparently offer great opportunities for the exploration of their biological and biomedical applications due to their unique carbon nanostructures. There have already been many successes in the exploration, which should inspire more fundamental studies and technological development. Functionalized or derivatized fullerene has better aqueous solubility and bioavailability and makes it suitable in biomedicine. C60 fullerene is mostly studied in biomedical applications, but the other fullerenes such as C80, C82 and metallofullerenes also have prospects to be an important vector to deliver drugs. At the same time, research should be extended on design of new derivative and synthesis of bioactive fullerene conjugates.

CONCLUSION

Fullerenes as a unique class of carbon allotropes have been studied extensively for their distinctive material properties and potential technological applications, including those in biology and medicine. It has application in distinct biological field like neuroprotection, can cross the blood brain barrier, radical scavenging and antioxidant activity, anti viral activity etc. Fullerene based drug delivery can be a solution for different incurable disease, and targeted drug delivery.

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