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C_{60} Fullerene Derivatized Nanoparticles and their Application to Therapeutics

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Abstract: Fullerenes can be formed into many new materials and devices. They have a wide range of applications in medicine, electronics, biomaterials, and energy production. An overview of the nanostructure and the physical and chemical characteristics of fullerene-drug derivatives is given. The biological behavior of fullerene derivatives shows their potential to medical application fields because C_{60} is rapidly absorbed by tissues and is excreted through urinary tract and enterons, which reveals low toxicity *in vitro* and *in vivo* studies. Nanomedicine has become one of the most promising areas of nanotechnology, while many have claimed its therapeutic use against cancer, human immunodeficiency virus (HIV), and neurodegenerative disorders. Water-soluble C_{60} fullerene derivatives that come from chemical modification largely enhance the biological efficacy. The blood-brain barrier (BBB) is a physical barrier composed of endothelial tight junctions that restrict the paracellular permeability. A major challenge facing neuropharmacology is to find compounds that can be delivered into the brain through the bloodstream. Fullerene C_{60} was demonstratively able to cross the BBB by hybridizing a biologically active moiety dyad, which provides a promising clue as a pharmacological therapy of neural disorders.

Keywords: Antioxidant, apoptosis, autophagy, blood brain barrier (BBB), cytotoxicity, fullerene, fulleropyrrolidine-xanthine dyad, fullerene dyad, graphene, nanomedicine, nanoparticle, nanotechnology, neurodegenerative disease, neuroprotective, PEG-C_{60}-3, PTX-C_{60}-2.

INTRODUCTION

A fullerene is a molecule composed entirely of carbon in the form of a tube, a hollow sphere, or an ellipsoid [1]. The first fullerene was discovered in 1985 by Sir Harold W. Kroto, Richard E. Smalley, and Robert F. Curl Jr. In 1996, these three scientists were awarded the Nobel Prize for their pioneering efforts [2]. Prior to their discovery, only two well-defined allotropes of carbon were known: diamonds (composed of a three-dimensional crystalline array of carbon atoms) and graphite (composed of stacked sheets of two-dimensional hexagonal arrays of carbon atoms). The fullerenes constitute a third form, and their existence eluded discovery until nearly the end of the 20th century. The graphene also consists of sp^2-bonded carbon atoms having potential for various biomedical applications [3-5].

CHEMISTRY

Each molecule of the fullerene family (C_n) consists of 12 pentagons and *m* hexagons. C_{60} fullerene consists of rigid carbon spheres with a radius of 0.498 nm Fig. (**1**). It has two vacant tetrahedral sites and one octahedral site per C_{60} molecule. These sites are of sufficient size to accommodate sphere radii of 0.112 and 0.206 nm, respectively. The van der Waals diameter of a C_{60} molecule is approximately 1.1 nm [6]. Single-wall nanotubes and C_{60} fullerenes have diameters on the order of 1 nm, approximately half the diameter of an average DNA helix.

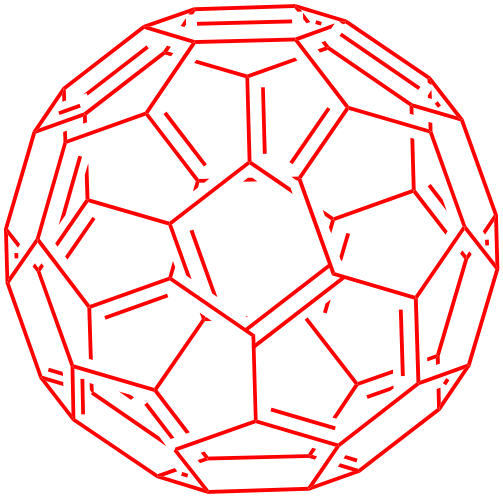

Fig. (1). Structure of C_{60} fullerene.

The development of versatile functionalization chemistry allowed for a large variety of organic reactions to be carried out with fullerene, such as arylation, halogenation, hydroxylation, alkoxylation, and osmylation [7, 8]. CNTs have also gained increasing interest among polymer chemists as building blocks for constructing novel materials with interesting properties [8]. A number of metal-intercalated complexes are

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known, and fullerenes show a pronounced tendency to crystallize with solvent molecules trapped in the lattice to form host-guest complexes, such as [C_{60}(g-cyclodextrin) $_2$] and [C_{60}(1,4-hydroquinone)$_3$]. They are also able to form charge-transfer complexes with, for example, bis(ethylenedithio) tetra-thiafulvene. Fullerenes pass through addition reactions with various types of reagents forming oxygen, carbon, nitrogen, and metallic bridges. Addition may take place across either the 6,6-ring junction or the 5,6-ring junction, with the rings being either open fulleroid or closed fullerene structures [1].

Macromolecular fullerene derivatives combine the unusual properties of fullerenes with the specific properties of many polymers. The first attempts to produce 'side-chain' polymers of C_{60} included the use of diphenyl fulleroid as a building block [9]. Liu *et al.,* [10] prepared C_{60}-polystyrene polymers using Friedel-Crafts reactions, which include alkylation and acylation reactions. Geckeler and Hirsch [11] prepared C_{60}-on-chain polymers by titrating toluene solutions of C_{60} with the aminopolymers, poly(ethylenimine), and poly(4-[[(2-aminoethyl)imino] methyl]styrene). Fullerene-based polymers, which were soluble in hexane or tetrahydrofuran (THF), were prepared by covalently attaching fullerenes to amine-containing hydrocarbon polymers. Fullerene alone is essentially insoluble in these solvents [12] (Table **1**). Fullerenes are also insoluble in water but sparingly soluble in many solvents. Common solvents for fullerenes include aromatics, such as toluene, bromoform, benzene, and others solvents such as carbon disulfide, carbon tetrachloride and chloroform. Fullerenes are the only known allotrope of carbon that disolve in solvents at room temperature [13-14]. Water-soluble C_{60} fullerene derivatives were carefully studied because of the broad range of biological activities these compounds possess. The size, geometry, and surface characteristics of these structures make them appealing for use as drug carriers. Sun *et al.,* [15] synthesized and characterized a water-soluble C_{60} fullerene-poly (propionyl-ethylenimine-co-ethylene) polymer.

TOXICITY

Mice tolerated a dose of 5000 mg/kg of body weight, and no evidence of toxicity was found *in vivo* for C_{60} after intraperitoneal administration of large doses (Moussa *et al.,* 1996, 1997) [16, 17]. Mori *et al.,* (2006) [18] did not find toxicity in rodents with C_{60} and C_{70} mixtures after oral administration of a dose of 2000 mg/kg body weight. Moreover, no evidence of genotoxic or mutagenic potential was observed *in vitro*. Gharbi *et al.,* (2005) [19] http://en.wikipedia.org/wiki/Fullerene - cite_note-46 suggested that aqueous C_{60} suspensions, which failed to produce acute or subacute toxicity in rodents, might protect their livers against free-radical damage in a dose-dependent manner. In 2007, Kolosnjaj *et al.,* [20-21] produced a comprehensive review of fullerene toxicity. These authors reviewed work on fullerene toxicity since the 1990s and concluded that little evidence gathered since the discovery of fullerenes indicated that C_{60} is toxic. However, the extreme hydrophobicity and potential toxicity of fullerene limits its application as a therapeutic antioxidant [22].

Table 1. Chemical Modification Methods

Methods	Fullerene Compounds
Metal-intercalate [1]	Ba_2CsC_{60}
Crystallizing with solvent molecules	C_{60}(g-cyclodextrin) $_2$ [1], C_{60}(1,4-hydroquinone)$_3$ [1]
Charge-transfer complexes	With bis(ethylenedithio) tetra-thiafulvene [1]
Hydroxylation [7, 8]	
Alkoxylation [7, 8]	
Osmylation [7, 8]	
Forming polymers	
Friedel-Crafts reactions	C_{60}-polystyrene polymers [10]
Titrate toluene solutions	With aminopolymers [11], poly(ethylenimine) [11] and poly(4-[[(2-aminoethyl)imino]methyl]styrene) [11]
Covalent attaching	Amine-containing hydrocarbon polymers [12]
Water-soluble	Fullerene-poly(propionyl-ethylenimine-co-ethylene) [15]

GENERAL APPLICATIONS

Time-of-flight secondary ion mass spectrometry (TOF-SIMS) is a powerful tool in surface science and analysis. Fletcher *et al.,* [23] found C_{60} increased the sputter yield and secondary ions when used as a primary particle in SIMS analysis. They also investigated depth profiling of organic samples when using C_{60}. No increase in damage was evident in the mass spectra when the impact energy was increased. A solid-phase extraction of the stationary phase with silica particles of different porosities was modified with an aminopropyl linker and then covalently bound to C_{60}-fullerenoacetic acid or C_{60}-epoxyfullerenes. The materials were successfully applied as an alternative to commercially available reversed-phase materials for solid-phase extraction. These novel materials were applied for the desalting and preconcentration of proteins, peptides, and, especially, phosphopeptides. In addition, C_{60}-fullerene silica, with a recovery rate of 99%, was applied for the solid-phase extraction of selected flavonoids [24]. Chiral porphyrin-fullerene dyads were produced for their potential use in photovoltaics [25]. A novel oligo(p-phenylenevinylene) (OPV)-fullerene dyad, with strong intermolecular pi-pi interactions between the donor groups led to improvements in the fill factor and power conversion efficiency of dyad-based solar cells of up to 0.44% and 1.28%, respectively. These are the highest values reported for dyad-based solar cells so far [26]. Furthermore, in a 2010 report, the Norwegian Scientific Committee for Food Safety evaluated the application for the use of fullerene C_{60} as a food additive.

MEDICAL APPLICATIONS

C_{60} Derivatives

Nanomedicine has become one of the most promising areas of nanotechnology. Current problems in nanomedicine involve understanding the toxicity and environmental impacts of nanoscale materials. Researchers in the conjectural field of molecular nanotechnology believe that cell repair machines could revolutionize medicine [27]. The biological behavior of fullerene derivatives shows their potential applications to various medical fields. Fullerenes are promising carriers of bioactive molecules and demonstrate increased circulation time and acceptable functionality [28]. Kirpatrick *et al.,* (2011) [29] complexed fullerene compositions with multiple bioactive agents. C_{60} is rapidly absorbed by tissues, especially by the coronal bone, breastbone, backbone, extremity honeycomb, liver, and spleen. Clearance was slow from all tissues except the brain. The compound is possibly excreted through urinary tract and enterons [30]. A method of treating free radical-related medical condition by using water-soluble fullerene derivatives had been developed. The method included the step of administering to a subject in need of such treatment an effective amount of a compound of the formula F(-X)m wherein F is a fullerene core; each X is independently OH, (CH_2)n-SO_3H, or a metal salt of (CH_2)n-SO_3- [31]. Some studies were carried out on the biological efficacy of water-soluble fullerenes *in vitro* [32-33] and *in vivo* [34-35]. The results revealed low toxicity. The administration of an aqueous solution of hydrated C_{60} fullerenes ($C_{60}H_yF_n$), with a 30 nM concentration of C_{60} as drinking water during chronic alcoholization of rats, protected tissues of the central nervous system from damage caused by oxidative stress and prevented the pathological loss of both astrocytes and astrocytic markers and glial fibrillary acidic proteins. Due to its adaptogenic effects, $C_{60}H_yF_n$ improved behavioral responses and eliminated emotional problems induced by chronic alcohol uptake. It also treated alcohol-induced encephalopathy and alcoholism prophylaxis (Table **2**) [36]. A water-soluble nanoformulation of fullerene (C_{60}) was produced with a poly(N-vinyl pyrrolidine) (PVP) or poly(2-alkyl-2-oxazoline)s (POx) homopolymer and a random copolymer. Chung (2011) [37] developed a method for preparing a water-soluble fullerene derivative with excellent fluorescence by mixing a fullerene and a ligand containing a hydroxyl group which possesses bioaffinity, and thus can be useful in the biomedicine. Tong *et al.,* [38] evaluated the cytotoxicity of these C_{60}-polymer complexes, and the results demonstrated that C_{60}-Pox complexes are non-toxic, neuronal cell-permeable, superoxide-scavenging antioxidants that might be candidates for treating brain-related diseases associated with increased levels of reactive oxygen species (ROS). A water-soluble compound of fullerene polycarboxylic anions was produced for inhibiting membrane viral infection [39]. The advancement of fullerene-specific antibodies using C_{60} broadens the application of fullerenes as medicinal agents, diagnostic agents, imaging vectors, and drug delivery vehicles. The derivatives in various tissues and/or cells of experimental animal models were examined to identify any therapeutic activities [40].

Traditional oral and injection drug delivery methods are not efficient for certain therapies. The rate of drug diffusion and transport is highly dependent on the size of the medicine. Typically, the skin cell membrane, made of phospholipids, cholesterol and lipoproteins plays an active role in controlling the ion transport into, out of and across the channels or ion pores using active energy against the concentration gradient. The small size of C_{60} nanoparticles, can keep the bound drug molecules safe while passing across the membrane and can later release them to a suitable pH to act at the tissue target site [28]. The mechanisms used to achieve alternative routes for drug delivery typically unite one or more biologic, polymer, silicon-based, and carbon-based materials as well as metals. The materials used for biologics are lipids, peptides, nucleic acids, polysaccharides, and viruses in the form of vesicles, nanotubes, rings, and nanoparticles. The materials for polymerics are poly(glycolic acid), poly(alkylcyanoacrylate), and poly(3-hydroxybutanoic acid) are in the form of vesicles, spheres, nanoparticles, and dendrimers. For silicon- (like silicones), carbon- (like carbon), or metallic-based (like silver, palladium, platinum and gold) materials, they are in the form of porous particles, nanoparticles, and nanoshells [41]. The advantages of nanostructure-mediated drug delivery include the ability to directly deliver drug molecules into cells [42], and the capacity to target tumors in healthy tissues [43]. Fullerenes have been studied for potential medicinal use. They bind specific antibiotics to their structures to target resistant bacteria and can even target certain cancer cells like melanomas. The October 2005 issue of *Chemistry & Biology* [44] contains an article explaining the use of fullerenes as light-activated antimicrobial agents. Fullerene derivatives were used as a carrier for serum protein profiling, which is a powerful tool used to distinguish protein signatures for pathologies and biomarker findings. This tool uses a material-enhanced laser desorption/ionization (MELDI) technique. MELDI is a new form of laser desorption/ionization that was introduced in 2005 by Bakry' laboratory [45-47].

RADICAL SCAVENGING AND BLOOD BRAIN BARRIER (BBB) PENETRATION CHARACTERISTICS

The exceptional radical-scavenging property of fullerene makes it a favorable pharmacophore that can be used to target and counterbalance cellular harm caused by the overexpression of radicals [33, 48-50]. This radical-scavenging characteristic was shown to protect cell growth from various toxins that can cause apoptotic injuries *in vitro* [51-53] to neuronal [33,54], hepatoma [55], and epithelial cells [56].

The blood-brain barrier (BBB) is a physical barrier composed of endothelial tight junctions that restrict the paracellular permeability. The barrier is formed by capillary endothelial cells surrounded by basal lamina and astrocytic perivascular endfeet Fig. (**2**). The functional intricacy of these membranes is attributed mainly to brain microvessel endothelial cells and the manifestation of complex tight junctions between the endothelial cells of the BBB [57-59]. Efflux transport systems consisting of energy-dependent membrane protein may target the drugs and export them from the brain [57, 60]. The BBB, therefore, acts as an obstacle for the systemic delivery of neurotherapeutics. To cross the BBB, a central nervous system (CNS) drug should be relatively small and hydrophobic to mix well with membrane layers and un-charged at blood pH. One nano-enabled drug delivery

Table 2. Medicinal Application of Fullerene Derivatives

Fullerene Derivatives	Medicinal Applications
$C_{60}H_yF_n$ [36]	Improve behavioral response eliminate emotional problems induced by chronic alcohol uptake
	Protect tissues of the central nervous system
C_{60}-polymer complexes	Treat brain-related diseases [38]
Fullerene polycarboxylic anions	Inhibit membrane viral infection [39]
Fullerene-specific antibodies [40]	Apply as medicinal agents, diagnostic agents, imaging vectors, drug delivery vehicles
Nanostructure-mediated drug	Deliver drug molecules directly into cells [42] Target tumors [43]
Binding specific antibiotics [44]	Target resistant bacteria Target certain cancer cells like melanomas
Fullerene derivatives	Act as a carrier for serum protein profiling [44] Identify pathology and biomarkers [45-47] Treat HIV infection [47]
Monocationic fullerene	Kill cancer cells [66]
Compositions comprising C_{60} fullerene	Ameliorate, treat and reverse CNS disorder [70]
Fullerene dyad	
PEGylated (PHDCA) [71]	Allow interactions with BBB endothelial cells
Polysorbate 80-coated	Produce endocytosis followed by possible transcytosis [72] Deliver drugs to the brain [73]
Doxorubic bound	Treat glioblastoma [74]
Fullerene core and glutamate receptor ligand residues	Neuroprotective agents [79]
Fulleropyrrolidine-thalidomide dyad [80]	Suppress the release of nitric oxide (NO) and tumor necrosis factor (TNF)-α
Fulleropyrrolidine-xanthine dyad [80, 81]	Suppress LPS-induced NO production Suppress inflammation
PEG-C_{60}-3 [82]	Increase cell viability Reduce β-amyloid $(A\beta)_{25\text{-}35}$-induced cytotoxicity
PTX-C_{60}-2 [83]	Induce cell autophagy
Synthetically modified fullerene	Treat inflammatory disorders or inhibit the build-up of arterial plaque [84]

system used for the *in vivo* administration of drugs targeting the brain is a rapidly biodegradable poly(butylcyanoacrylate) (PBCA) nanoparticle. PBCA nanoparticles coated with polysorbate 80 are thought to cross the BBB via plasma adsorption of apolipoproteins, which results in a receptor-mediated endocytosis by brain capillary endothelial cells as apolipoproteins cross the BBB [61]. Fullerene molecules rapidly accumulate in water but disaggregate after entering the membrane interior. The compound was also able to cross the BBB [34, 49]. The apoptosis of cerebral microvessel endothelial cells (CMECs) induced by oxidative stress injury plays a key role in the dysfunction of the BBB. Using CMECs as an *in vitro* BBB model, Lao *et al.*, (2009) [62] demonstrated that $C_{60}(C(COOH)_2)_2$ nanoparticles can selectively enter oxidized CMECs rather than normal cells, and then protect them from apoptosis [63].

PHOTODYNAMIC THERAPY (PDT)

Another potential medical application of C_{60} is associated with the photoexcitation of fullerenes. An excited fullerene can be reduced under biological conditions in the presence of biological reducing agents, such as guanosine. In addition, singlet oxygen and superoxide radical anions are renowned reactive species towards DNA [64]. This property of fullerenes makes them potential photosensitizers for use in PDT. The cytotoxicities of a dendritic C_{60} monoadduct and malonic acid C_{60} trisadduct were investigated on Jurkat cells, and upon exposure to UV light, the cell number was found to descend by approximately 19% within 2 weeks [65]. The photodynamic activities of fullerenes derivatized with hydrophilic and cationic groups against a range of mouse cancer cell lines were studied. Monocationic fullerene was

found to be a highly effective photosensitizer for killing cancer cells by the rapid induction of apoptosis after illumination [65].

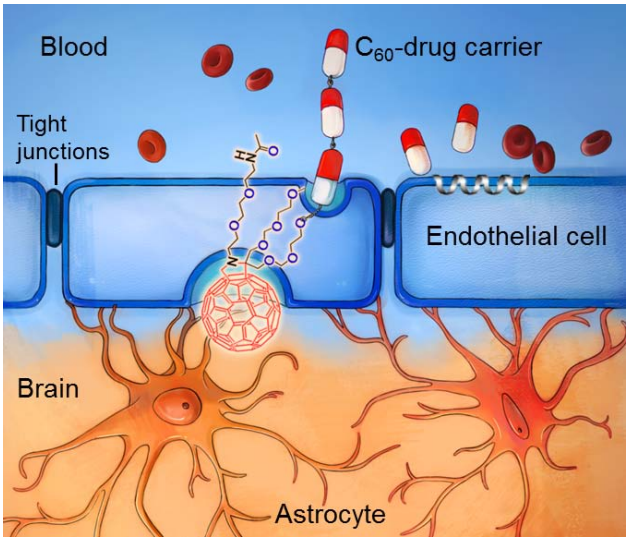

Fig. (2). The diagram for C_{60} fullerene drugs to transport across the blood brain barrier (BBB). (This photo is designed by Siao-Min Hu, Department of Fine Arts, National University of Tainan, Taiwan).

HIV INHIBITION

Several antiviral compounds can suppress the replication of the HIV. These are effective in preventing or detaining the commencement of acquired immunodeficiency syndrome (AIDS). Fullerenes (C_{60}) and their derivatives have promising antiviral activity, and this has strong implications for the treatment of HIV infection [47]. Fullerene derivatives can inhibit and form complexes with HIV protease (HIV-P) [66-67]. Amino acid derivatives of fullerene C_{60} (ADFs) were found to inhibit HIV and human cytomegalovirus replication [68]. "Cationic-, anionic-, and amino acid-type fullerene derivatives inhibit HIV-reverse transcriptase and hepatitis C virus replication. Anionic fullerene derivatives present antioxidant properties, whereas cationic derivatives have antibacterial and antiproliferative activities. Amino acid-type derivatives were found to be the most active of all the fullerene derivatives [47, 69]."

FULLERENE DYAD FOR NEURODEGENERATIVE DISEASES

Many nanostructures, including polymeric nanoparticles, polymeric nanospheres and nanosuspensions, polymeric nanogels, carbon nanotubes and nanofibers, polymeric nanomicelles, and polymeric nano-liposomes, have been applied to the development of nano-enabled drug delivery systems to treat neurodegenerative disorders [57]. Polymeric nanoparticles were used for the delivery of several drugs to the central nervous system (CNS). Compositions comprising C_{60} fullerene for ameliorating, treating and reversing CNS disorder has been developed [70]. Calvo (2001) [71] PEGylated long-circulating PHDCA nanoparticles and found they could penetrate into the brain to a larger extent than all the other tested formulations. The results given in Alyaudtin (2001) [72], supported the hypothesis that the mechanism of blood-brain barrier transport of drugs by polysorbate 80-coated nanoparticles was one of endocytosis followed by possible transcytosis. At concentrations of PBCA nanoparticles and polysorbate-80 that achieved significant drug delivery to the brain, specific mechanisms of delivery to the CNS rather than a simple disruption of the BBB, which allowed a diffusional drug entry, is suggested [73]. A doxorubicin bound to nanoparticle study showed that the therapy offered a therapeutic potential for the treatment of human glioblastoma [74]. Nanogel is a hydrophilic nanosized carrier based on a crosslinked network of branched polyethylenimine (PEI) and poly(ethylene glycol) (PEG) molecules. It was demonstrated as a drug delivery system to combine molecules such as oligonucleotides, small-interfering RNA, DNA, proteins, and low-molecular-mass drugs [75]. Polymeric nano-micelles have a coreshell architecture with a hydrophobic core and a shell of hydrophilic polymer blocks. The shell stabilizes the nano-micelles and covers the drug from interactions with serum proteins and untargeted cells. Polymeric nano-micelles are versatile and have been shown to deliver DNA molecules efficiently *in vitro* and *in vivo*, although no study on their successful delivery to the CNS has been reported [76-78]. A fullerene core and glutamate receptor ligand residues were also used as neuroprotective agents [79]. The C_{60} fulleropyrrolidine-thalidomide dyad (CLT) is an effective agent for suppressing the release of nitric oxide (NO) and tumor necrosis factor (TNF)-α by lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages [80]. The fulleropyrrolidine-xanthine dyad Fig. (**3**) can effectively suppress LPS-induced NO production [81]. These water-soluble C_{60}-fullerene-xanthine dyads aggregate in water, producing an average nanoscale dimension of approximately 78.9 nm in diameter. Huang *et al.*, [80-81] have prepared and explored the usability of novel water-soluble fullerenes which can endure variety of known anti-inflammatory agents Fig. (**4**). The concept of C_{60} hybridizing a biologically active moiety dyad that utilizes a synergistic effect is consistent with our previous report that the C_{60} fulleropyrrolidine moiety with the xanthine moiety is more efficient in suppressing inflammation in LPS-stimulated macrophages than is the fulleropyrrolidine moiety alone. A C_{60} fullerene derivative incorporating poly(ethylene glycol) (PEG), PEG-C_{60}-3, reduced β-amyloid $(Aβ)_{25\text{-}35}$-induced cytotoxicity and increased cell viability in cells co-treated with C_{60} and $Aβ_{25\text{-}35}$. Moreover, intracellular ROS accumulation caused by Aβ-treated Neuro-2A cells was reduced by PEG-C_{60}-3 co-treatment. Aβ treated cells triggered the signaling cell cycle, which stimulated repair and protected cells from apoptosis through changes in gene expression. Microarray analysis elucidated the cytoprotective effect of PEG-C_{60}-3 that might result from intracellular oxidative stress alleviation, ion-channel and transporter homeostasis, cell cycle regulation, and modulation in transcription factor activity, thus, protecting the cell from apoptosis [82]. Lee *et al.*, [83] further discovered the use of the dyads, PEG-C_{60}-3 (incorporating PEG) and PTX-C_{60}-2, as novel agents to fight neurodegenerative diseases. They found that cytoprotection by PEG-C_{60}-3 and PTX-C_{60}-2 was partially lessened by an autophagy inhibitor, showing that the elicited autophagy and antioxidative activities protected cells from Aβ damage. PTX-C_{60}-2 was more effective than

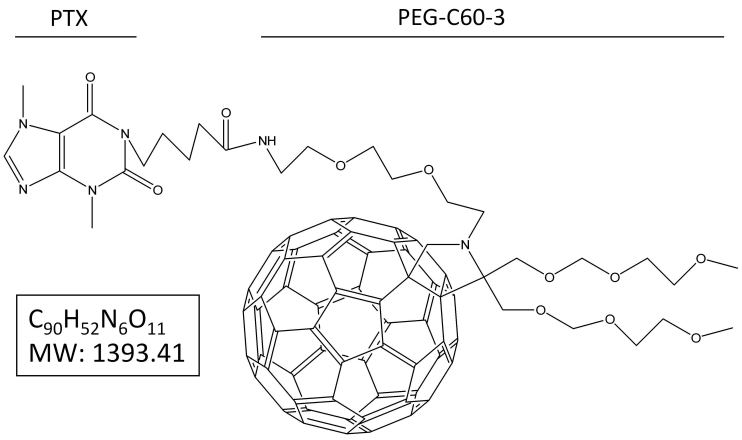

Fig. (3). Structure of the C_{60} fulleropyrrolidine-xanthine hybrid particle. Reproduced with permission from Lee CM, Huang ST, Huang SH *et al.* 2011.

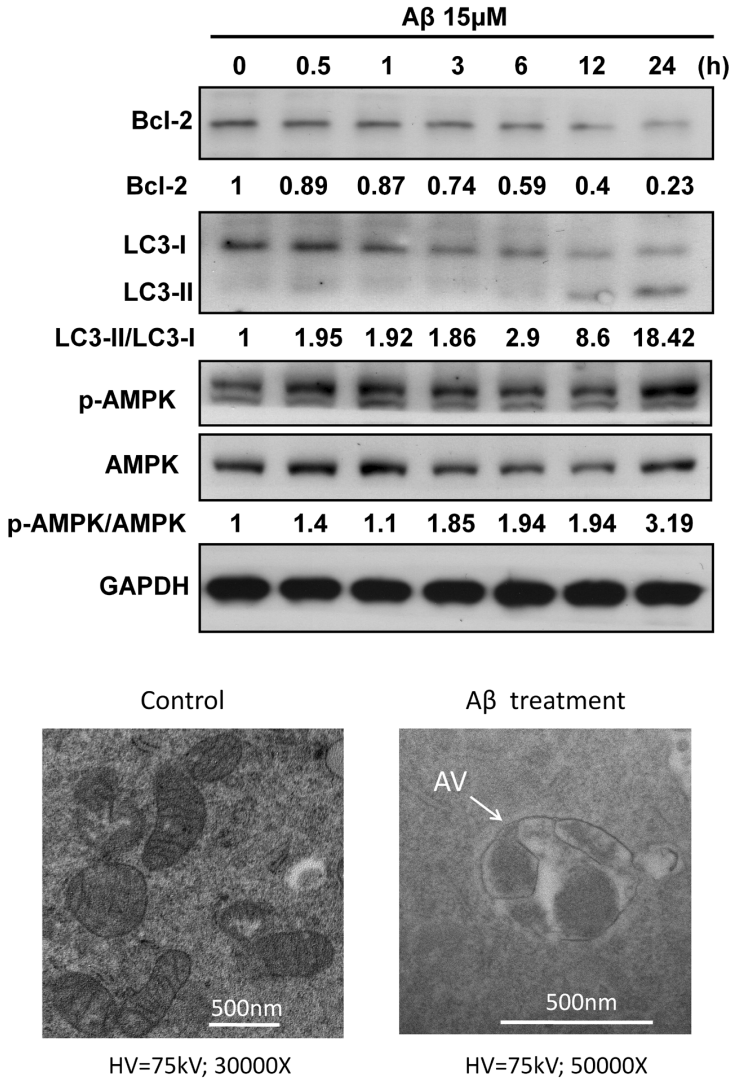

Fig. (4). Effects of β-amyloid $(Aβ)_{25-35}$ on autophagy elicitation represented by LC3 conversion and AMPK activation (**A**). Cells were treated with 10 μM $Aβ_{25-35}$ for 0, 0.5, 1, 3, 6, 12, and 24 h, and immunoblotting was performed to probe proteins with respective antibodies against Bcl-2, LC3-I, LC3-II, AMPK and then phosphorylated (p)-AMPK. GAPDH levels served as an internal control. (**B**) The ultrastructure of cell organelles shown using a transmission electron microscopy with 10 μM $Aβ_{25-35}$ treatment for 24 h. Bar = 500 nm. AV, autophagic vacuole; HV, high voltage. Reproduced with permission from Lee CM, Huang ST, Huang SH *et al.* 2011.

$PEGC_{60}$-3 at sustaining the induced autophagy. They believe fulleropyrrolidine moiety in PTX-C_{60}-2 acting as an antioxidant is not the sole molecular mechanism of these compounds for rescuing cells from Aβ-induced cell cytotoxicity. An agent combining antioxidative activities with the ability to cross the BBB would be expected to have great synergy in medicinal applications in combating brain diseases. Moreover, Kepley (2011) [84] invented methods for treating inflammatory disorders or for inhibiting the build-up of arterial plaque by applying a therapeutically effective amount of a synthetically modified fullerene. These results suggest the promising use of C_{60} fullerene or fullerene dyads as drug treatment to support the pharmacological and non-pharmacological therapy of neurodegenerative diseases.

Graphene is the structural element of fullerenes that consists a single- or few-layered sheet of Sp2-bonded carbon atoms. The non-porous carbon materials wherein the carbon material has a smallest dimension of less than 100 nanometers are employed for medical use. In preferred aspects, the material is topically used on wounds, orally administered as sorbent for various toxins, or employed as a sorbent in hemodialysis [85]. This 2D nanomaterial was reported compatible with blood [86], thus the graphene-based drug loading and delivery systems were explored and ultra-high drug loading efficiency was achieved owing to its extremely large surface area. Even there are some unresolved issues and challenges in graphene-based nanomedicine, the unique physical and chemical properties of graphene, are intensively discussed for its novel applications in biological sensing, cancer therapies and potentially biomedical imaging [5].

CURRENT AND FUTURE DEVELOPMENTS

A major challenge facing neuropharmacology is to find compounds that can be delivered into the brain through the bloodstream. Fig. (**2**) shows the molecular traffic across the BBB. The large surface area of the lipid membranes of the endothelium offers an effective diffusive route for lipid-soluble agents. Versatile functionalization chemistry has been developed and various of organic reactions have been carried out with relatively small nanoscale fullerenes. Fullerene is an excellent nanocarrier for hydrophobic molecule delivery and an effective neuroprotective antioxidant. These nanoparticles possess the novel ability of selectively entering oxidation-damaged cerebral endothelial cells, rather than normal endothelial cells, and then protecting them from apoptosis. Fullerene, C_{60}, and C_{60}-drugs may answer to the special needs of modern medications. These, along with the low toxicity detected in fullerene, are sufficient to stimulate researchers to unite their efforts. Moreover, the direct application of fullerenes and their derivatives to neurodegenerative disorders is promising.

CONFLICT OF INTEREST

The authors report no conflicts of interest in relation to this work.

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ABBREVIATION

ADFs	=	Amino acid derivatives of fullerene C_{60}
AIDS	=	Acquired immunodeficiency syndrome
BBB	=	Blood brain barrier
CLT	=	C_{60} fulleropyrrolidine-thalidomide dyad
CMECs	=	Cerebral microvessel endothelial cells
CNS	=	Central nervous system
HIV	=	Human immunodeficiency virus
HIV-P	=	HIV protease
LPS	=	Lipopolysaccharide
MELDI	=	Material-enhanced laser desorption/ ionization technique
PBCA	=	Poly(butylcyanoacrylate)
PDT	=	Photodynamic therapy
PEG	=	Poly(ethylene glycol)
ROS	=	Reactive oxygen species
THF	=	Tetrahydrofuran
TOF-SIMS	=	Time-of-flight secondary ion mass spectrometry
TNF	=	Tumor necrosis factor

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