Neuroprotective Effect of Fullerenes

Subjects: Biophysics

Contributor: Daisy L. Wilson, Jyoti Ahlawat, Mahesh Narayan

The use of carbon nanomaterials including fullerenes, carbon nanotubes, carbon nano-onions, carbon dots and carbon quantum dots for environmental applications has increased substantially. These nanoparticles are now used in the development of sensors and switches, in agriculture as smart fertilizers and in the biomedical realm for cancer therapy intervention, as antioxidants, in gene delivery and as theranostics.

Keywords: fullerenes ; carbon nanomaterials (CNMs) ; oxidative stress

1. Potential of Fullerenes and Their Derivatives in Mitigating Amyloid-Associated Toxicity

Amyloid fibrils are formed via a complex multistep process involving self-assembly of misfolded amyloid proteins into a soluble oligomer. Oligomers, regarded as primary neurotoxic agents, can then form insoluble beta-sheet rich oligomers, protofibrils, mature fibrils and plaques. Therefore, aggregation of protein from free monomeric amyloid beta to a fibrillar state involves numerous intermediate stages including nucleation, elongation (oligomerization and protofibril formation) and saturation (fibril and plaque formation) [1][2][3][4][5][6]. Despite differences in the amyloid polypeptide precursor, the resulting amyloid fibrils share common/generic features including well-defined cross beta-sheet structures with beta sheets running parallel to the fibril axis, insolubility due to alpha-helical to beta fold transition and specific staining with thioflavin T (ThT) and Congo red dyes. More than fifty human amyloid misfolding diseases have been identified to date [1][2][3][4][5][6].

The amyloid fibrils are implicated in several disease conditions, such as AD and PD. Aggregation of these insoluble amyloids can induce toxicity or interfere with normal functioning of cells, resulting in the progression of disease [1][2][3][4][5][6]. This fibrillization process occurs due to the imbalance between the production and removal of amyloid beta in brain vasculature and parenchyma [7]. Amyloid beta deposition represents the pathological hallmark of disease and is responsible for neuronal loss, vascular damage, neurofibrillary tangle formation, and dementia. The presence of amyloid fibrils in affected tissues indicates a disease condition. [1][2][3][4][5][6]. In vitro, the fibrillization process can be affected by several factors including solution properties such as ionic strength, temperature, pH, +/− of chaperones and inhibitors. An abundance of research indicates that nanoparticles can interfere in amyloid formation. However, whether nanoparticles accelerate or decelerate the process of fibrillization is still controversial, and it depends on the physico–chemical properties of nanoparticles and stability of the protein [1][2][3][4][5][6]. For example, if the mutant (a protein that is easier to misfold or aggregate) has high intrinsic stability and a low intrinsic aggregation rate then nanoparticles will accelerate the process of fibrillization, whereas if the intrinsic stability of the mutant is low and its intrinsic aggregation rate is high, opposite trends are observed, where nanoparticles tend to retard amyloid fibril formation. Therefore, study of the biological applications of fullerenes has attracted increasing attention, which is especially promising in the field of neuroprotection [1][2][3][4][5][6].

Sun et al. conducted an atomistic simulation to study the effect of 1,2-(dimethoxymethano) fullerenes (DMF) on amyloid beta (Aβ) aggregation [8]. Their results showed that the interaction between DMF and Aβ resulted in the distortion of β hairpin structure and inter-peptide β sheets structures within the amyloid fibril. In addition to the interaction between the hydrophobic core of the Aβ, leucine–valine–phenylalanine–phenylalanine–alanine (LVFFA), the DMF also interacted with the aromatic residues, namely, phenylalanine 4, tyrosine 10 and C-terminal hydrophobic stretch isoleucine 31–valine 40. Hence, the results obtained from the simulation provide information about the possible interactions between the DMF and Aβ fibril that might be responsible for the inhibition of amyloid aggregation [8].

In another study, water-soluble fullerenol $C_{60}(OH)_{16}$ was synthesized to prevent Aβ fibrillation. The inhibition of amyloid formation was followed using the thioflavin T (ThT) assay and atomic force microscopy (AFM) images [9]. Simulation studies were performed to study the possible interactions between the amyloid and the fullerenol. The simulation results show that the electrostatic interactions between the hydroxyl group of fullerenol and the carboxyl group of the amino acids

and the hydrophobic interaction between fullerenol and C-terminal of the peptide were responsible for preventing the self-assembly of the peptide and for the structural disruption of the Aβ fibril. To assess the toxicity profile of the fullerenol, a cell viability assay was performed on neuroblastoma cells with no observed significant toxicity. Thus, the results show the potential of fullerenol as a therapeutic drug for AD [9].

In a different study, the potential of hydrophobic fullerene to inhibit formation of β-sheet rich oligomers was evaluated using an atomistic simulation study [10]. The results showed that fullerene was able to prevent β-sheet rich fibril formation, composed of glycine–asparagine–asparagine–glutamine–glutamine–asparagine–tyrosine (GNNQQNY), by strongly interacting with the nonpolar amino acids N3, Q4 and Q5, thus increasing the exposure of the peptide backbone to water and hence preventing the inter-peptide N3–Q4, Q4–Q4 and Q4–Q5 interactions that are crucial for the β-sheet formation and oligomerization. Hence, from the obtained results, it can be concluded that fullerenes can act as potential therapeutic candidates against amyloidosis [10].

Melchor et al. studied the ability of diethyl fullerenemalonates to inhibit $A\beta_{42}$ aggregation [11]. The dose-dependent inhibitory activity of as-synthesized fullerenes was studied using the ThT assay and transmission electron microscopy (TEM). The cytotoxicity of the drug was tested on neuroblastoma cells, which displayed no significant toxicity, thus rendering the fullerenes biocompatible. Hence, from the obtained results, it can be concluded that fullerenemalonates can serve as a future therapy to treat AD and other types of dementia [11].

In another study, fullerenol of variants C_{60} (hydrophobic), $C_{60}(OH)_{24}$ (amphiphilic) and $C_{60}(OH)_{40}$ (hydrophilic) was tested for its inhibitory activity against amyloid aggregation [12]. Due to their aggregative properties, and thus reduced surface area, the C_{60} hydrophobic fullerenes were not able to prevent self-assembly and hence aggregation of the amyloidogenic core region of the non-amyloid-β component in alpha-synuclein (NACore). Despite the formation of aggregates in the $C_{60}(OH)_{24}$ amphiphilic fullerenol, hydroxyls on the surface still allowed for interaction with the peptide backbone of the amyloid, thus interrupting the formation of β-sheet rich aggregates. On the other hand, the $C_{60}(OH)_{40}$ hydrophilic fullerenol, although effective at reducing the formation of amyloid aggregation, did not significantly interact with the backbone peptides, indicating that an increase in hydroxyls does not necessarily enhance the interaction with peptides to reduce amyloid aggregation. As a result, both β-sheet rich aggregates and β-barrel intermediates were significantly impacted and hence suppressed, unlike in the case of hydrophobic fullerenes. The observed interaction between the polar regions of the fullerenol and the peptide backbone of the amyloid provide invaluable insight that could be essential in the future development of theranostics. This inhibition or suppression was followed using ThT assay, TEM, FT-Infrared spectroscopy (FTIR), and computational studies. Hence, it can be suggested that fullerenol C_{60} $(OH)_n$ with n = 0, 24 and 40 can be used as an anti-amyloid inhibitor to treat PD [12].

2. Potential of Fullerenes and Their Derivatives in Mitigating Oxidative Stress

Many neurodegenerative disorders arise due to the imbalance in the production and removal of ROS and reactive nitrogen species (RNS) or due to alteration in the functionality of the antioxidant defense system of the cells. These alterations can arise either from mutations in radical scavenging enzymes such as superoxide dismutase (which catalyzes the conversion of superoxide radical into hydrogen peroxide), glutathione peroxidase and catalase (which catalyzes the conversion of hydrogen peroxide to water molecules) or due to exposure to environmental toxicants such as pesticides [13][14][15]. The brain is vulnerable to oxidative damage and consumes 20% of all oxygen and 25% of all glucose intake in the body. The main source of ROS is the electron transport chain (ETC) in the mitochondrial membrane where ATP is generated. These superoxide and nitric oxide radicals can also originate from overexcited glutamic acid receptors, astrocytes, and microglia. The ROS/RNS can be produced via Fenton chemistry, as the brain has a high content of redox active metals. These free radicals can attack biological molecules such as DNA, RNA, lipids, carbohydrates and protein, causing their oxidation. These modifications in their external structure can then produce more potent oxidants. Oxidation of DNA or RNA introduces nucleic acid strand break, which can affect crucial gene replication, transcription, and translation. Oxidation or carbonylation of proteins can lead to protein misfolding and biologically unfunctional protein, which can initiate diseases such as amyloidosis [13][14][15]. The brain consumes a high content of polyunsaturated fatty acids (PUFA) and these are sensitive to oxidation. Therefore, oxidation of lipids (peroxidation of PUFA) can impact structural integrity of the cell membrane which can result in cell apoptosis. Hence, fullerenes, due to their sp^2 hybridized architecture and therefore their ability to act as free radical sponges, have demonstrated promising behavior in this field [13][14][15].

In another study, pentoxifylline-C_{60} (PTX-C_{60}) nanoparticles were synthesized to overcome the $A\beta_{25–35}$ associated cytotoxic effects in Neuro-2A cells. The formulation significantly reduced the $A\beta_{25–35}$ induced neuronal death by rescuing

the cells from oxidative stress, decreasing the ROS levels and maintaining the mitochondrial membrane potential (MMP) [16].

In another study, PEGylated-C_{60} was prepared as a radical sponge to mitigate the neuronal apoptosis induced by $A\beta_{25–35}$. Endoplasmic reticulum (ER) stress response genes and antioxidant related genes were analyzed to study the response of the C_{60} against $A\beta_{25–35}$ induced cytotoxicity. The results showed the protective activity of C_{60} against $A\beta_{25–35}$ treatment in Neuro-2A cells [17].

In a different study, carboxylic acid functionalized C_{60} were synthesized as free radical scavengers of cultured cortical neurons against N-methyl–D-aspartate and alpha-aminoso-3–hydroxy-5–methyl-4–isoxazolepropionic acid, thus pointing towards the potential of these water-soluble C_{60} derivatives as active therapeutic agents against several acute or chronic neurodegenerative diseases [7]. The protective efficiency of C_{60} against $A\beta_{25–35}$ induced neurotoxicity upon intrahippocampal microinjection in mice was analyzed in a different study. The results were impressive, as the introduction of buckyball before the causative agent's introduction was able to prevent any disturbances to protein synthesis, thus pointing towards the possibility of developing an anti-amyloid drug with free radical scavenging capability and anti-aggregative capabilities [18].

References

1. Marsagishvili, L.G.; Bobylev, A.G.; Shpagina, M.D.; Troshin, P.A.; Podlubnaya, Z.A. Effect of fullerenes C 60 on X-protein amyloids. Biophysics 2009, 54, 135–138.
2. Cabaleiro-Lago, C.; Szczepankiewicz, O.; Linse, S. The effect of nanoparticles on amyloid aggregation depends on the protein stability and intrinsic aggregation rate. Langmuir 2012, 28, 1852–1857.
3. Mahmoudi, M.; Kalhor, H.R.; Laurent, S.; Lynch, I. Protein fibrillation and nanoparticle interactions: Opportunities and challenges. Nanoscale 2013, 5, 2570–2588.
4. Kim, J.E.; Lee, M. Fullerene inhibits β-amyloid peptide aggregation. Biochem. Biophys. Res. Commun. 2003, 303, 576–579.
5. Huy, P.D.Q.; Li, M.S. Binding of fullerenes to amyloid beta fibrils: Size matters. Phys. Chem. Chem. Phys. 2014, 16, 20030–20040.
6. Guo, J.; Li, J.; Zhang, Y.; Jin, X.; Liu, H.; Yao, X. Exploring the influence of carbon nanoparticles on the formation of β-sheet-rich oligomers of IAPP22–28 peptide by molecular dynamics simulation. PLoS ONE 2013, 8, e65579.
7. Dugan, L.L.; Turetsky, D.M.; Du, C.; Lobner, D.; Wheeler, M.; Almli, C.R.; Shen, C.K.-F.; Luh, T.-Y.; Choi, D.W.; Lin, T.S.; et al. Carboxyfullerenes as neuroprotective agents. Proc. Natl. Acad. Sci. USA 1997, 94, 9434–9439.
8. Sun, Y.; Qian, Z.; Wei, G. The inhibitory mechanism of a fullerene derivative against amyloid-β peptide aggregation: An atomistic simulation study. Phys. Chem. Chem. Phys. 2016, 18, 12582–12591.
9. Bednarikova, Z.; Huy, P.D.Q.; Mocanu, M.M.; Fedunova, D.; Li, M.S.; Gazova, Z. Fullerenol C 60 (OH) 16 prevents amyloid fibrillization of Aβ 40–in vitro and in silico approach. Phys. Chem. Chem. Phys. 2016, 18, 18855–18867.
10. Lei, J.; Qi, R.; Xie, L.; Xi, W.; Wei, G. Inhibitory effect of hydrophobic fullerenes on the β-sheet-rich oligomers of a hydrophilic GNNQQNY peptide revealed by atomistic simulations. RSC Adv. 2017, 7, 13947–13956.
11. Melchor, M.H.; Susana, F.G.; Francisco, G.S.; Norma, R.F.; Gustavo, B.I. Fullerenemalonates inhibit amyloid beta aggregation, in vitro and in silico evaluation. RSC Adv. 2018, 8, 39667–39677.
12. Sun, Y.; Kakinen, A.; Zhang, C.; Yang, Y.; Faridi, A.; Davis, T.P.; Ding, F. Amphiphilic surface chemistry of fullerenols is necessary for inhibiting the amyloid aggregation of alpha-synuclein NACore. Nanoscale 2019, 11, 11933–11945.
13. Da Ros, T.; Prato, M. Medicinal chemistry with fullerenes and fullerene derivatives. Chem. Commun. 1999, 8, 663–669.
14. Christen, Y. Oxidative stress and Alzheimer disease. Am. J. Clin. Nutr. 2000, 71, 621S–629S.
15. Butterfield, D.A.; Halliwell, B. Oxidative stress, dysfunctional glucose metabolism and Alzheimer disease. Nat. Rev. Neurosci. 2019, 20, 148–160.
16. Lee, C.M.; Huang, S.T.; Huang, S.H.; Lin, H.W.; Tsai, H.P.; Wu, J.Y.; Lin, C.M.; Chen, C.T. C60 fullerene-pentoxifylline dyad nanoparticles enhance autophagy to avoid cytotoxic effects caused by the β-amyloid peptide. Nanomed. Nanotechnol. Biol. Med. 2011, 7, 107–114.
17. Lu, T.Y.; Kao, P.F.; Lee, C.M.; Huang, S.T.; Lin, C.M. C60 Fullerene Nanoparticle Prevents?—Amyloid Peptide Induced Cytotoxicity in Neuro 2A Cells. J. Food Drug Anal. 2011, 19, 17.

18. Makarova, E.G.; Gordon, R.Y.; Podolski, I.Y. Fullerene C60 Prevents Neurotoxicity Induced by Intrahippocampal Microinjection of Amyloid-β Peptide. J. Nanosci. Nanotechnol. 2012, 12, 119–126.