Journal of Pharmaceutical and Health Sciences 2016;4(1), 15-21.

Original Article

JPHS

Functionalized fullerene materials (fullerol nanoparticles) reduce brain injuries during cerebral ischemia-reperfusion in rat

Open Access

Mahsa Sarami Foroshani[1], Mohammad Taghi Mohammadi[2*]

[1]*Department of Nanotechnology, School of New Sciences and Technology, Islamic Azad University of Pharmaceutical Sciences Branch, Tehran, Iran*

[2]*Department of Physiology and Biophysics, School of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran*

Abstract

Aim: Oxidative stress plays a crucial role in the pathophysiology of ischemic stroke. Since water-soluble fullerene derivatives act as the potent scavenger of oxygen free radicals in biological systems, we aimed to investigate the possible protective effects of fullerol nanoparticles on brain infarction and edema in transient model of focal cerebral ischemia in rat.

Materials & Methods: Experiment was performed by three groups of rats (each group; n=8): sham, control ischemia (IR) and ischemia treated rats with fullerol. Brain ischemia was induced by 90 min middle cerebral artery occlusion (MCAO) followed by 24 hours reperfusion. Treated rats received fullerol at dose of 1 mg/kg 30 min before induction of MCAO. The brains were processed for histochemical triphenyltetrazolium chloride (TTC) staining and quantitation of the ischemic infarct. Finally, the brain hemispheres were weighed as an index of brain edema.

Results: MCAO induced brain infarction in large areas of cortex (261± 23 mm3) and subcortex (138± 23 mm3). Treatment with fullerol significantly reduced the infarct volume both in cortex and subcortex by 64.75% and 52.17%, respectively. Induction of MCAO significantly increased the weights of right hemispheres in IR group (0.77± 0.01 g) compared with sham rats (0.59± 0.01 g). Treatment with fullerol decreased the weights of ischemic hemispheres in IR treated group (0.69± 0.03 g) compared to IR non-treated rats.

Conclusion: Our findings indicate that fullerol nanoparticles are able to reduce the ischemia-induced brain injury and edema possibly through their scavenging properties.

Keywords: Fullerene, Infarction, Edema, Stroke, Ischemia-reperfusion

***Corresponding author**: Mohammad Taghi Mohammadi, PhD Associated Professor of Physiology
Department of Physiology & Biophysics, School of Medicine Baqiyatallah University of Medical Sciences, Tehran, Iran
Tel/Fax: +98 21 26127257 Mob: +98 9127713583 Fax +98 21 26127257
Email address: Mohammadi.mohammadt@yahoo.com Mohammadimohammadt@bmsu.ac.ir

Introduction

Stroke is second leading cause of mortality in worldwide (Woodruff et al., 2011). Approximately 45% of ischemic strokes are caused by small or large artery thrombus, 20% are embolic in origin, and others have an unknown cause (Hinkle and Guanci, 2007). When an ischemic stroke occurs the blood supply to the brain is interrupted and brain cells are deprived of the glucose and oxygen that they need to function (Hinkle and Guanci, 2007). The pathophysiology of stroke is complex, and involves excitetoxicity mechanisms, inflammatory pathways, oxidative damage, ionic imbalances and apoptotic signals (Deb et al., 2010). The final consequence of these ischemic cascades initiated by acute stroke is neuronal death along with an irreversible loss of neuronal function (Rodrigo et al., 2013). There are currently relatively few treatment options available to minimize neuronal damage and death following stroke (Woodruff et al., 2011).

Nanoparticles are the third natural allotropic variation of carbon and the materials with overall dimensions in the nanoscale, ie, under 100 nm. In recent years, these materials have emerged as important players in modern medicine, with clinical applications ranging from contrast agents in imaging to carriers for drug and gene delivery into tumors (Murthy, 2007). Water-soluble derivatives of fullerenes are also shown to be a potent free radical scavenger that makes this class of compounds attractive tools for regulation of free radical processes and for reducing the severity of oxidative stress (Zeynalov et al., 2009, Tong et al., 2011, Andrievsky et al., 2009). Hydroxylated fullerenes are employed as potent neuroprotective agents because they effectively scavenge free radicals in biological environments (Fluri et al., 2015). Based on previous findings, C60 fullerenes in low doses may be considered as a novel antioxidant agent, which substantially diminishes the harmful effects of ionizing radiation (Andrievsky et al., 2009). Also, it is reported that polyhydroxylated fullerene derivatives at low concentrations protect against oxidative stress in RAW cells and ischemic lung (Chen et al., 2004).

Based on previous studies, water-soluble fullerene derivatives have powerful scavenging properties for oxygen free radicals in biological systems. Therefore, we aimed to examine the possible protective effects of fullerol nanoparticles on brain infarction and edema in transient model of focal cerebral ischemia in rat.

Materials and Methods

Animals

Male Wistar rats (280-320 g) were obtained from the animal house facility of the University of Baqiyatallah Medical Sciences. All protocols of the study were approved by the institutional animal ethics committee of the University of Baqiyatallah Medical Sciences, which followed the NIH Guidelines for care and use of animals. Animals were housed in standard cages in a room with controlled temperature (22-24°C), humidity (40-60%) and light period (07.00-19.00), while access to rat chow and water ad libitum.

Middle cerebral artery (MCA) occlusion

Animals had been fasted overnight prior to use without deprivation of water. The rats were anesthetized with 2.5% isoflorane (Forane, UK) and placed in dorsal recumbent. Core temperature was continuously recorded by a rectal probe connected to a thermistor and maintained at 37±1ºC with a heating pad and lamp.

Middle cerebral artery occlusion (MCAO) of the right cerebral hemisphere was carried out by intraluminal filament method described by Longa et al. (Longa et al., 1989). In brief, the right common carotid artery was exposed through a midline neck incision, and then, via external carotid artery, a 4-cm Poly-L-Lysine-

coated nylon thread (3-0) was inserted into the internal carotid artery and gently advanced up until feeling a resistance and seeing a sharp decline in the blood flow trace. MCAO was maintained for 90 min, and then the thread was gently taken out to reestablish blood flow to the ischemic region. Finally, all the incisions were sutured, the animals were allowed to recover from anesthesia, and returned to a warm cage for recuperation during reperfusion period.

Experimental protocols and groups

In sham group (n=8), the rats underwent the surgery at the neck region and received a single intraperitoneal (i.p.) injection of the vehicle (1 mL/kg, normal saline) without being exposed to MCAO. Surgery was performed at the neck region of control ischemic group (IR, n=8) same as sham. These rats received a single i.p. injection of the vehicle (1 mL/kg, normal saline) 30 min before MCAO. After 10 min rest, brain ischemia was achieved by 90 min MCAO followed by 24 hours reperfusion. The rats of ischemia pretreated group (Fullerol, n=8) received a single i.p. injection of 1 mg/kg fullerol (Sigma, Germany) in 1 mL normal saline 30 min before induction of MCAO and other procedures were followed same as control group. Other procedures were followed same as control group. The number of animals presented for each group is the number of rats that survived during 24 hours reperfusion period. The collected data of the animals that died during 24 hours reperfusion period were excluded.

Evaluation of cerebral lesions

Brain infarction was measured according to the method of Swanson et al. (Swanson et al., 1990). In brief, after induction of deep anesthesia with sodium thiopental, the animals were slaughtered. Then, their brains were removed, cleaned, and solidified by immersing in pre-cooled normal saline (4oC) and keeping in the refrigerator for 5 min. The prepared slices were stained with 2% 2, 3, 5-triphenyltetrazolium chloride (TTC, Sigma) and fixed in 10% buffered formalin solution. After staining, the color of the ischemic areas was white and of non-ischemic areas was red. The slice images were digitized by using a Cannon camera. Images of the stained sections were taken. Grossly visible infarction zones were quantified using image analysis software (NIH Image Analyzer) and finally cerebral infarct volume was calculated.

Brain weight as an index of brain edema

Twenty four hours after reperfusion the rats were killed and the brains were removed. The brains divided into two hemispheres, and then, olfactory bulb and brain stem were removed to determine the weights of hemispheres.

Statistical analysis

All values are presented as mean±SEM. The comparison of data between groups were performed by analysis of variance (ANOVA) followed by Tukey post-hoc test. All states, $P<0.05$ was considered as significance.

Result

Effects of fullerol on brain infarction

As shown form the images of the TTC-stained brain sections (Fig. 1), the infarcted brain tissue appeared white, whereas the normal regions appeared red. No infarction was observed in the sham-operated group and an extensive infarction on cortical (261± 23 mm3) and on subcortical (138± 23 mm3) areas were observed in the IR group. Administration of fullerol (1 mg/kg) before induction of MCA occlusion significantly decreased the cortical and subcortical infarction by 64.75% and 52.17%, respectively ($P<0.01$), (Fig. 2).

Effects of fullerol on hemisphere weight

In the present study, we used the hemispheres

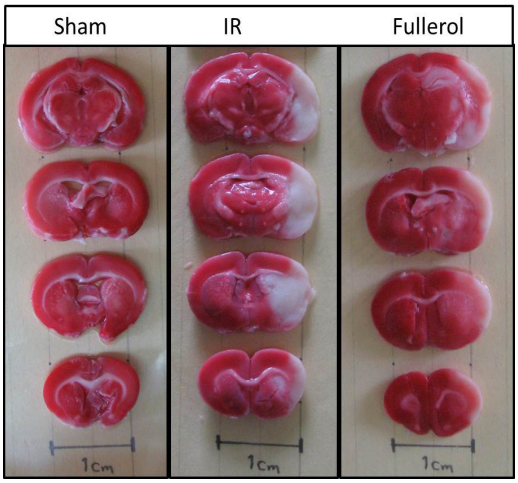

Figure 1: Photograph is showing the coronal sections of rat's brains stained with triphenyltetrazolium chloride (TTC) in sham, ischemic (IR) and ischemic treated (fullerol) groups. Non-ischemic areas are colored red, whereas ischemic areas are white.

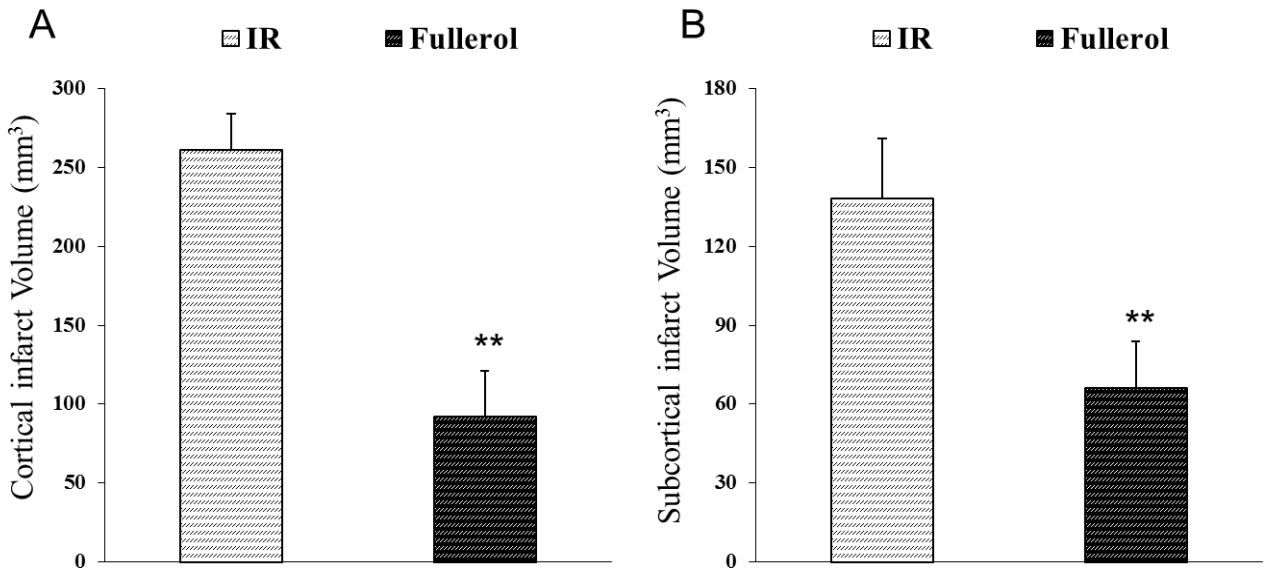

Figure 2: Effects of fullerol on cortical (A) and subcortical (B) infarct volume in ischemic (IR) and ischemic treated (fullerol) groups. All values are presented as mean±SEM. **Significant difference compared to IR group (P<0.01)

weights of brain as an index of brain edema. As shown in Fig. 3, the right hemisphere in the IR group (0.77± 0.01 g) contained more fluid than the corresponding left hemisphere (0.67± 0.03 g), and the mean weight of right hemispheres was significantly increased in the IR group (0.77± 0.01 g) compared with sham group (0.59± 0.01 g) (P<0.01). Administration of fullerol before induction of MCA occlusion significantly reduced the mean value of right hemispheres weights in ischemic treated rats (0.69± 0.03 g) compared to ischemic non-treated animals (P<0.05).

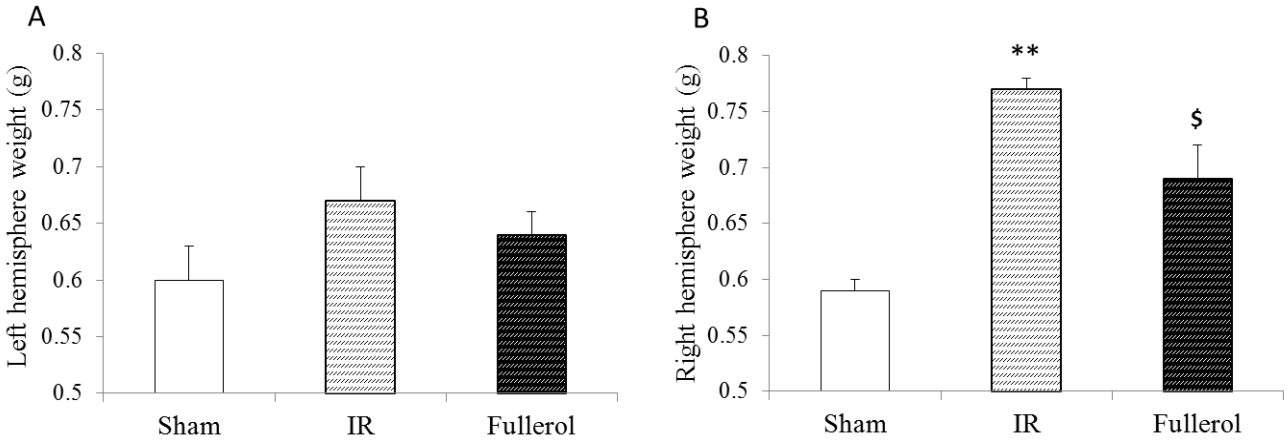

Figure 3: Effects of fullerol on the values of left (A) and right (B) hemispheres weights in sham, ischemic (IR) and ischemic treated (fullerol) groups. All values are presented as mean±SEM. **Significant difference compared to sham group (P<0.01), $Significant differen

Discussion

It is well known that water-soluble fullerene derivatives have robust neuroprotective effects against oxidative stress, inflammation and apoptotic cascades in several pathological states (Cai et al., 2008, Fluri et al., 2015). These nanoparticles are capable to eliminate both oxygen and nitrogen free radicals as well as lipid peroxidation in vitro and in vivo studies (Cai et al., 2008, Injac et al., 2013). Our results showed that fullerol (polyhydroxylated fullerenes) nanoparticles decrease the brain infarction after cerebral ischemia-reperfusion injury. We also observed the attenuation of ischemia-induced brain edema by these nanoparticles after induction of MCA occlusion followed by 24 hours reperfusion. Therefore, our findings indicate the neuroprotective effects of fullerol nanoparticles against ischemic stroke.

The findings of present study indicate that occlusion of MCA induced brain injury in cortical and subcortical areas of ischemic hemispheres. Different neurodegenerative cascades are activated during cerebral ischemia and mediate the neuronal death and injury (Woodruff et al., 2011). It is reported that overproduction of different free radicals and inflammation are crucial factors in mediating neuronal death in cerebral ischemia-reperfusion injury (Jin et al., 2010). ROS generation may play an important role in neuronal damage and also contribute to enlargement of the infarct size during brain ischemia (Hong et al., 2006). Overproduction of oxygen free radicals (ROS) damages to the cell constituents such as membranes (lipolysis), mitochondria and DNA (Chen et al., 2011). ROS also induces the formation of inflammatory mediators, which activate microglia and lead to the invasion of blood-borne inflammatory cells (leukocyte infiltration) via upregulation of endothelial adhesion molecules (Dirnagl et al., 1999). Moreover, free radicals exert their deleterious actions on brain edema during cerebral ischemia-reperfusion (Heo et al., 2005).

Based on mentioned results, inhibition of ROS overproduction or scavenging of these free radicals would be useful for attenuation of neurodegenerative cascades during brain ischemia. In the present study, administration of fullerol nanoparticles before occlusion of MCA could reduce the infarction in cortical and subcortical regions. Since the antioxidant and also anti-inflammatory effects of fullerol nanoparticles have well known, it is concluded that these nanoparticles have attenuated the brain infarction possibly through their

scavenging properties. Previous reports have demonstrated the neuroprotective effects of fullerol nanoparticles. Fullerol nanoparticles overwhelm the cellular apoptosis by enhancing the gene expression of anti-oxidative enzymes and decreasing the level of ROS (Liu et al., 2013). These nanoparticles, hydroxylated fullerenes, are neuroprotective because they scavenge free radicals (Cai et al., 2008, Injac et al., 2013). Fullerol may protect against oxidant-mediated inflammation and tissue damage by virtue of its ability to scavenge free radicals and by its ability to inhibit the activation of nuclear factor kappaB (NF- B), (Fluri et al., 2015). Furthermore, fullerol inhibits the glutamate channels, which results in a decrease in glutamate-induced intracellular calcium and cell death (Fluri et al., 2015).
In conclusion, it is suggested that fullerol nanoparticles can be considered as a powerful scavenger for different free radicals, which are produced by cerebral ischemia-reperfusion injury. Consequently, fullerol has the potential to serve as a novel therapeutic agent because it exerts multiple neuroprotective effects on ischemic stroke.

Acknowledgements

We would like to offer our special thanks to the research council of Baqiyatallah University of Medical Science for their support of this study.

References:

Andrievsky GV, Bruskov VI, Tykhomyrov AA, Gudkov SV. Peculiarities of the antioxidant and radioprotective effects of hydrated C60 fullerene nanostuctures in vitro and in vivo. Free Radic Biol Med 2009;47:786-93.

Cai X, Jia H, Liu Z, et al. Polyhydroxylated fullerene derivative C(60)(OH)(24) prevents mitochondrial dysfunction and oxidative damage in an MPP(+) -induced cellular model of Parkinson's disease. J Neurosci Res 2008;86:3622-34.

Chen H, Yoshioka H, Kim GS, et al. Oxidative stress in ischemic brain damage: mechanisms of cell death and potential molecular targets for neuroprotection. Antioxid Redox Signal 2011;14:1505-17.

Chen YW, Hwang KC, Yen CC, Lai YL. Fullerene derivatives protect against oxidative stress in RAW 264.7 cells and ischemia-reperfused lungs. Am J Physiol Regul Integr Comp Physiol 2004;287:R21-6.

Deb P, Sharma S, Hassan KM. Pathophysiologic mechanisms of acute ischemic stroke: An overview with emphasis on therapeutic significance beyond thrombolysis. Pathophysiology 2010;17:197-218.

Dirnagl U, Iadecola C, Moskowitz MA. Pathobiology of ischaemic stroke: an integrated view. Trends Neurosci 1999;22:391-7.

Fluri F, Grunstein D, Cam E, , et al. Fullerenols and glucosamine fullerenes reduce infarct volume and cerebral inflammation after ischemic stroke in normotensive and hypertensive rats. Exp Neurol 2015;265:142-51.

Heo JH, Han SW, Lee SK. Free radicals as triggers of brain edema formation after stroke. Free Radic Biol Med 2005;39:51-70.

Hinkle JL, Guanci MM. Acute ischemic stroke review. J Neurosci Nurs 2007;39:285-93, 310.
Hong H, Zeng JS, Kreulen DL, Kaufman DI, Chen AF. Atorvastatin protects against cerebral infarction via inhibition of NADPH oxidase-derived superoxide in ischemic stroke. Am J Physiol Heart Circ Physiol 2006;291:H2210-5.

Injac R, Prijatelj M, Strukelj B. Fullerenol nanoparticles: toxicity and antioxidant activity. Methods Mol Biol 2013;1028:75-100.
Jin R, Yang G, Li G. Inflammatory mechanisms in ischemic stroke: role of inflammatory cells. J Leukoc Biol 2010;87:779-89.

Liu Q, Jin L, Mahon BH, Chordia MD, Shen FH, Li X. Novel treatment of neuroinflammation against low back pain by soluble fullerol nanoparticles. Spine (Phila Pa 1976) 2013;38:1443-51.

Longa EZ, Weinstein PR, Carlson S, Cummins R. Reversible middle cerebral artery occlusion without craniectomy in rats. Stroke 1989;20:84-91.

Murthy SK. Nanoparticles in modern medicine: State of the art and future challenges. Int J Nanomedicine 2007;2:129-41.

Rodrigo R, Fernandez-Gajardo R, Gutierrez R, et al. Oxidative stress and pathophysiology of ischemic stroke: novel therapeutic opportunities. CNS Neurol Disord Drug Targets 2013;12:698-714.

Swanson RA, Morton MT, Tsao-Wu G, Savalos RA, Davidson C, Sharp FR. A semiautomated method for measuring brain infarct volume. J Cereb Blood Flow Metab 1990;10:290-3.

Tong J, Zimmerman MC, Li S, et al. Neuronal uptake and intracellular superoxide scavenging of a fullerene (C60)-poly(2-oxazoline)s nanoformulation. Biomaterials 2011;32:3654-65.

Woodruff TM, Thundyil J, Tang S-C, Sobey CG, Taylor SM, Arumugam TV. Pathophysiology, treatment, and animal and cellular models of human ischemic stroke. Mol Neurodegener 2011;6:11-11.

Zeynalov EB, Allen NS, Salmanova NI. Radical scavenging efficiency of different fullerenes C60–C70 and fullerene soot. Polymer Degradation and Stability 2009;94:1183-9.