Fullerenol Nanoparticles Decrease Blood-Brain Barrier Interruption and Brain Edema during Cerebral Ischemia-Reperfusion Injury Probably by Reduction of Interleukin-6 and Matrix Metalloproteinase-9 Transcription

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Background: The present study aimed to examine the protective role of fullerenol nanoparticles against blood-brain barrier (BBB) interruption and brain edema during cerebral ischemia-reperfusion injury probably by reduction of interleukin-6 (IL-6) and matrix metalloproteinase-9 (MMP-9) transcription. *Methods:* The male Wistar rats (weighting 280-320 g) were randomly assigned into four groups as follows: sham, control ischemic, pretreated ischemic, and posttreated ischemic groups. Cerebral ischemia-reperfusion (IR) injury was performed by occlusion of middle cerebral artery (MCA) for 90 minutes followed by twenty-four hours reperfusion. Rats were administered fullerenol 5 mg/kg, intraperitoneally, 30 minutes before induction of IR in pretreated ischemic group and immediately after termination of MCA occlusion in posttreated ischemic group. After twenty-four hours reperfusion, the method of Evans blue dye extravasation (EBE) and RT-PCR were used for determination of BBB permeability and mRNA expression levels of MMP-9 and IL-6, respectively. Neuronal deficit score (NDS) and edema of the ischemic hemispheres were also evaluated. *Results:* MCA occlusion increased NDS in control ischemic rats (3.16 ± 0.16) with concomitant increase in EBE (15.30 ± 3.98μg/g) and edema (3.53 ± 0.50%). Fullerenol in both pretreated and posttreated ischemic groups reduced NDS (36% and 68%, respectively), EBE (89% and 91%, respectively) and edema (53% and 81%, respectively). Although MCA occlusion increased the mRNA expression levels of MMP-9 and IL-6 in ischemic hemispheres, fullerenol in both treatment groups noticeably decreased the mRNA expression levels of these genes. *Conclusion:* In conclusion, fullerenol nanoparticles can protect BBB integrity and attenuate brain edema after cerebral ischemia-reperfusion injury possibly by reduction of IL-6 and MMP-9 transcription.

Key Words: Blood-brain barrier—Ischemic stroke—Matrix metalloproteinase-9—Interleukin-6—Fullerenol

Ischemic stroke is one of the major reasons of death and disability in worldwide.[1] According to the increasing evidence, oxidative damage and inflammation are the main mechanisms of brain damage in ischemic stroke.[2, 3]

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Received January 3, 2018; revision received June 25, 2018; accepted June 30, 2018.

Financial Disclosure: No conflicts of interest, financial or otherwise, are declared by the authors.

Ethical Approval: None to be declared.

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1052-3057/$ - see front matter

https://doi.org/10.1016/j.jstrokecerebrovasdis.2018.06.042

Also, disruption of blood-brain barrier (BBB) integrity is commonly associated with brain ischemia. An unavoidable consequence of BBB disruption is an increase in the permeability of the damaged microvasculature, which results in vasogenic brain edema.[4] A variety of biochemical changes are involved in the delayed BBB breakdown observed after ischemic stroke.[5] Previous findings indicate a robust correlation between inflammatory cytokines and intensity of BBB breakdown after brain ischemia.[3, 6] An increase in brain levels of cytokines including interleukin-6 (IL-6), interleukin-1β, interleukin-10, interleukin-12, and tumor necrosis factor-α (TNF-α) has been reported in the experimental and clinical situations of ischemic stroke.[7, 8] Also, matrix metalloproteinases (MMPs) are other factors that play a crucial role in alteration of the cerebral microvessels permeability after brain ischemia.[5] According to the previous findings, deletion of MMP-9 gene protects the BBB integrity in the experimental model of ischemic stroke.[9] Additionally, it has been demonstrated that an increase in the levels of reactive oxygen species (ROS) triggers expression of a number of inflammatory cytokines and activation of MMP-9 after brain ischemia.[3, 10]

Fullerene (C_{60}) nanoparticles are the third natural allotropic variation of carbon atom that can readily react with oxygen free radicals.[11] Water-soluble derivatives of C_{60} fullerenes are the best types of fullerenes because these types of nanoparticle are soluble in biological environments.[12] C_{60} fullerenes are candidate therapeutic agents for several pathological states of brain because they exert multiple effects on many neurodegenerative cascades.[13] Water-soluble derivatives of C_{60} fullerenes such as fullerenol ($C_{60}(OH)_{18-22}$) have been demonstrated to behave as the powerful free radical scavengers that make these classes of compounds as attractive tool for abolishing the ROS to reduce the severity of oxidative damages during ischemic states.[11, 14, 15] Liu et al reported that fullerol was able to prevent the catabolic activity of vertebral bone marrow stromal cells under inflammatory stimulus by decreasing the levels of ROS, MMPs, and TNF-α.[16] An increase in activation of the antioxidant enzymes (such as superoxide dismutase and catalase) by fullerenol has been reported by previous findings.[12] Fullerenol also inhibits activation of the different inflammatory cytokines and apoptotic signals.[17, 18] It has been reported that treatment with carboxy-fullerene, a water-soluble derivative of fullerene, protects the brain against the transient focal ischemia-reperfusion injury.[19] Furthermore, a decrease in the cellular damages and inflammation has been reported after experimental model of ischemic stroke.[13] It would appear that possible inactivation of inflammatory cytokines and MMP-9 by fullerenol can be a good strategy for BBB protection and edema formation against brain ischemia. Since the preceding studies have demonstrated the cellular protection by fullerene nanoparticles and also the principal roles of IL-6 and MMP-9 in BBB interruption during ischemic stroke, we aimed to examine whether pretreatment and posttreatment with fullerenol nanoparticles decrease permeability of BBB and mRNA expression levels of IL-6 and MMP-9 during cerebral ischemia-reperfusion injury.

Materials and Methods

Animals

The protocols of the current study were done based on the acknowledged standards of animals care and use that approved by the Institutional Care and Use of Animals Committee of Baqiyatallah University of Medical Sciences. To perform the current study, male Wistar rats, weighing approximately 280-320 g, were purchased from the animal house facility center of the University of Baqiyatallah Medical Sciences. The rats were kept in a standard situation with controlled light period (12 hours light and dark cycle), 60% relative humidity and temperature (22°C-24°C), and also *ad libitum* access to the rat chow and water.

Fullerenol (Hydroxyl fullerenes)

C_{60} fullerene or buckminsterfullerene is a spherical molecule with a van der Waals diameter about 1.1 nm.[20] To perform the current study, C_{60} fullerene was first purchased from Sigma-Aldrich (USA). Then, 18-22 OH-moieties were added to this nanoparticle to achieve fullerenol (C_{60} $(OH)_{18-22}$, hydroxyl fullerenes) in Iran University of Science & Technology.[21] Then, the micrographs from the nanoparticles in crystal form were prepared by a scanning electron microscopy (Fig. 1).

Development of Cerebral Ischemia-Reperfusion

In the current study, we used the intraluminal filament method to achieve brain ischemia-reperfusion by the middle cerebral artery occlusion (MCAO) in the right hemispheres of the ischemic rats, which previously described in detail by Longa et al[22] First, the rats were anesthetized with 2.5% isoflurane (Forane, UK). The rats were placed in the dorsal recumbent position and during the surgery the body temperature of the animals was maintained at 37±1°C. After exposing the right common carotid artery and also the right external and internal carotid arteries through a midline incision in the neck area, a 4-cm Poly-L-Lysine-coated nylon thoursead (3-0) was inserted into the internal carotid artery via the external carotid artery. The prepared filament smoothly advanced up until feeling a resistance was met and seeing a sharp decline in the blood flow trace, which was recorded using a laser Doppler flowmeter (AD Instrument, Model: ML191, Australia). There was a 75%-85% reduction in regional cerebral blood flow of ischemic zones during the MCAO (Table 3). After 90 minutes MCAO, reperfusion phase was initiated by gently taking out of the filament to reestablish the blood flow to the ischemic areas.[23] All incisions were ultimately sutured and the rats were recovered from anesthesia.