Journal of Neurological Sciences [Turkish] **29:(4)**# 33; 722-731, 2012
http://www.jns.dergisi.org/text.php3?id=592
Research Article

Edaravone Leads To Increased Internal Luminal Vascular Circumference Following Subarachnoid Hemorrhage in An Animal Model of Vasospasm

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Summary

Purpose: Cerebral vasospasm is the leading cause of morbidity and mortality following subarachnoid hemorrhage. Although a number of factors have been examined in clinical and experimental studies, the agent(s) responsible for developing and diminishing vasospasm remain poorly understood. Here, the role of edaravone, an antioxidant agent, was evaluated for its ability to diminish vasospasm in an animal model of subarachnoid hemorrhage.
Materials and Methods: A rat basilar artery subarachnoid hemorrhage model was used. Rats were divided into three groups: sham (n=7; Group 1), subarachnoid hemorrhage (n=7 Group 2), and subarachnoid hemorrhage plus edaravone (4 mg/kg intraperitoneally, n=7; Group 3). At the end of the seventh day, the rats were sacrificed, their brains were removed, and sections were taken from the basilar artery. These were examined using a light microscope, comparing the internal luminal circumference of the basilar artery of each group.
Results: The circumference was largest in Group 1, followed by Group 3 and then Group 2. That of Group 3 was 2% higher than that of Group 2, but this difference was not statistically significant.
Conclusion: This animal model for vasospasm suggests that edaravone helps enlarge internal luminal circumference following vasospasm caused by subarachnoid hemorrhage. It may do this by blocking lipid peroxidation and thereby reducing the effects of oxyhemoglobin and reactive oxygen species.

Key words: Basilar artery; Edaravone; Subarachnoid hemorrhage; Vasospasm

Edaravone Hayvan Vazospazm Modelinde Subaraknoid Kanama Sonrası Damar İç Lümen Çevresinde Genişlemeye Yol Açmaktadır

Özet

Amaç: Serebral vazospazm subaraknoid kanamada mortalite ve morbiditenin önemli nedenlerinden biridir. Klinik ve laboratuar çalışmalarda bir çok faktör denenmesine rağmen, vazospazm gelişiminde etkili ajanlar tam olarak anlaşılamamıştır. Bu çalışmada antioksidan ajan olan edaravone'un, hayvan subaraknoid kanama modelinde vazospazm üzerindeki olası azaltıcı etkisi sunulmuştur.
Gereç ve yöntemler: Çalışmada rat baziler arter subaraknoid kanama modeli kullanıldı. Ratlar 3 grubu ayrıldı. Grup-1: Şam grubu (n=7 rat), Grup=2: Subaraknoid kanama grubu (n=7 rat), Grup=3: Subaraknoid kanama + Edaravone grubu (4 mg/kg intraperitoneal) (n=7 rat). Yedinci günde rat beyinleri çıkarılıp baziler arter ışık mikroskobisi altında incelendi. Baziler arter iç lümen çevresi her üç grup için karşılaştırıldı.

Bulgular: Damar iç lümen çevresi en geniş grup 1 de olup bunu grup 3 ve grup 2 takip etti. Grup 3 ün iç lümen çevresi grup 2 ye göre %2 daha fazla olmasına rağmen istatistiksel olarak anlamlı değildi.
Sonuç: Bu hayvan vazospazm modeli, edaravone'un subaraknoid kanama sonrasında oluşan vazospazmda damar iç lümen çevresini genişlettiğini göstermiştir.Edaravone un bu etkisi, lipid peroksidasyonunu engelleyerek oksihemoglobin ve reaktif oksijen ürünlerinin etkisini azaltmasına bağlı olabilir.

Anahtar Kelimeler: Baziler arter; Edaravon; Subaraknoid kanama; Vazospazm

INTRODUCTION

Early brain injury, rebleeding, and vasospasm are among the leading causes of mortality and morbidity in patients with subarachnoid hemorrhage (SAH; 1) but the pathogenesis of cerebral vasospasm is multifactorial and remains unclear. For this reason, many clinical and experimental studies have been conducted, but an optimal treatment course has yet to be established despite vasospasm being one of the most serious complications associated with SAH.

Following SAH, the lysis of erythrocytes in subarachnoid clots increases oxyhemoglobin levels. This, in turn, releases reactive oxygen species (ROS) and instigates lipid peroxidation in the erythrocyte membrane. ROS, especially hydroxyl radicals, induce vasospasm by depleting nitric oxide and resulting in the contraction of blood vessels[9,17]. Several therapeutic options have been implemented in an attempt to prevent cerebral vasospasm associated with SAH with nimodipine and Triple-H therapy being the most preferred treatments[6,19,21]. Other options include monoclonal antibodies[5], caspase inhibitors[29], ebselen[10], octreotid acetate[7], melatonin[4], and recombinant tissue plasminogen activator (r-tpa)[27]. However, the therapeutic effects of these free radical scavengers are not yet at the desired level.

Edaravone (3-methyl-1-phenyl-2-pyrasoline-5-one) is widely used in East Asian countries as a novel free radical scavenger and neuroprotective agent for both in vivo and in vitro studies.[15] This drug exerts antioxidant effects on hydroxyl radicals as well as iron-dependent lipid peroxidation and is thought to inhibit vascular endothelial cell injury, an aggravation of brain edema caused by free radical-induced lipid peroxidation[17]. Due to these characteristics, edaravone is used in patients with crescendo transient ischemic attacks[8], ischemic strokes, and reperfusion injury with acute myocardial infarction[26]. Clinical studies with edaravone have evaluated SAH and its association with vasospasm, ischemic and traumatic spinal cord injury, intra-cerebral hemorrhage, and neuronal dysfunction[3,9,20,18,25,28]. Experimental studies with edaravone have also been successful.

The present study investigated whether edaravone administration results in morphometric and histopathological changes in the basilar artery of rats with SAH.

MATERIAL AND METHODS

After obtaining consent from the Local Animal Ethics Committee of Ege University Faculty of Medicine (25.09.2010-No: 2009–138), surgeries to remove brain sections from the subjects were performed in the animal laboratory of the same university. Animal rights were protected during the study. Histopathological and morphometric analyses were performed in the pathology department of Izmir Ataturk Training and Research Hospital. For this study, 21 Wistar Albino rats weighing 200–300 g were used. The animals were housed under standard conditions with a 12/12 h light/dark cycle, 22±2°C ambient

temperature, and 60±5% humidity. Subjects were allowed free access to food and water during the study. Animals were excluded from analyses if death occurred during the experiment.

The rats were divided into three groups: sham (n=7; Group 1), subarachnoid hemorrhage (n=7 Group 2), and subarachnoid hemorrhage plus edaravone (4 mg/kg intraperitoneally, n=7; Group 3). The rats were anesthetized with 2 mg/kg kethiaminehydrochloride (Ketalar vial 50mg/mL, Pfizer) intraperitoneally and autologous cardiac blood was used to induce SAH. The atlanto-occipital region of the head was brought to hyperflexion and povidine-iodine (Baticon solution, 10%) was applied. Rats were put in a lurch position in an inclined plane, or a 50° angle to the floor. To avoid compression of the trachea during inclination, the head was suspended at a 90° angle to the body over the edge of the table. After suspension of the head, the occipital ledge and posterior arcus of the atlas were identified and used to enter the cisterna magna with a 24 G needle.

In Group 1, sham operations were performed and 0.1 cc cerebrospinal fluid (CSF) was removed. In Group 2, after cerebrospinal puncture, 0.1 cc autologous cardiac blood was injected into the cisterna magna over a period of 2 min. In Group 3, rats received an intraperitoneal injection of edaravone (4 mg/kg) 2 h and 24 h after injection of the autologous blood into the cisterna magna. On the seventh day after surgery, the brains were removed and immediately placed in 10% formaldehyde. The basilar artery specimens were marked with methylene blue and all coronal brain tissue slices were passed through a tissue-tracking device with formaldehyde for fixation. Then the samples were placed in high-grade alcohol (70%, 95%, and 100% alcohol; 3× each for 30 min each) followed by a xylene phase for dehydration (three different xylenes; each for 30 min). After dehydration, the tissue samples were put through three different phases of paraffin (30 min each) before the final phase, which included paraffin and incentive vacuum (30 min). This step-up treatment lasted a total of 8 h. At the end of this process, the paraffin-embedded tissue samples were frozen into paraffin blocks, sectioned at 5 µm thickness with a microtome, and then deparaffinized in an incubator at 60°C for 1 h. The deparaffinization process continued with a xylene phase (3×) before the samples were rehydrated in high grade alcohol (70%, 95%, and 100% alcohol) and then washed with water and stained with hematoxylin eosin. Preparations were examined using an Olympus Brand Microscope with 100×, 200×, and 400× magnifications. Morphometric measurements of intravascular lumen length were conducted with photo images taken with an Olympus DP 20 mark (high resolution; 1600×1200) and measured with a BSW software Olympus DP-2 program under 40× magnification. Following analysis, the circumferences of the intravascular lumens were compared. These values were statistically analyzed and compared using the Kruskal-Wallis test. A p value of <0.05 was accepted as statistically significant.

RESULTS

In all groups, basilar artery sections were examined under light microscopy and the internal luminal circumferences were measured. Histopathological preparations were stained with hematoxylin eosin and examined under a microscope at 100×, 200×, and 400× magnification. Figures 1, 2, and 3 show cross-sectional views of a basilar artery from each group, with the internal luminal circumference indicated by a yellow arrow.

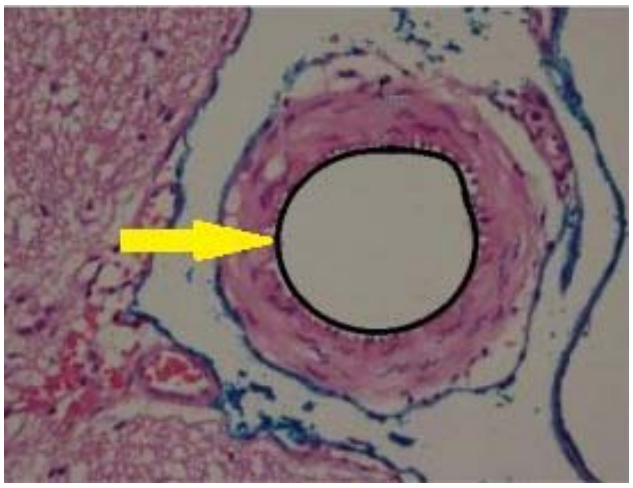

Figure 1: *Sham group.*

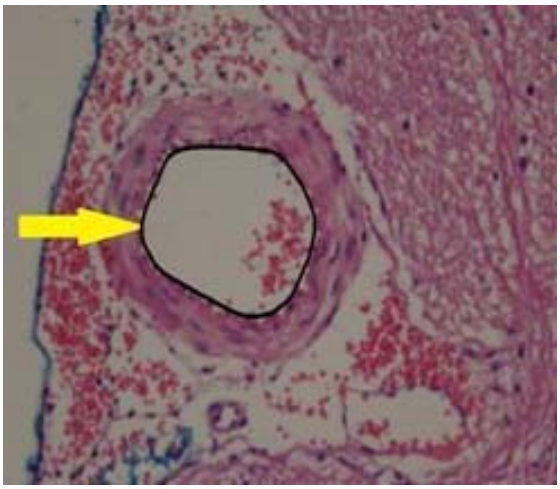

Figure 2: *Subarachnoid group.*

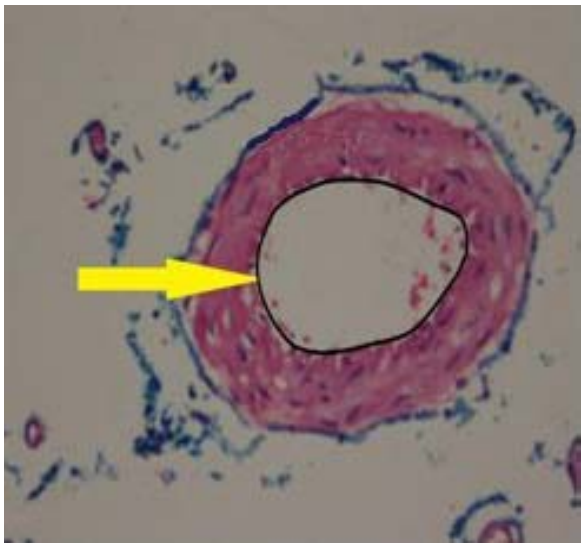

Figure 3: *Subarachnoid + edaravone group.*

Morphometric Analysis

Table 1 lists the average internal luminal circumference of each group, and Table 2 summarizes the minimum, maximum, and median values. The values were compared using a Kruskal-Wallis test; there were no statistically significant differences among the groups (p=0.374). Figure 4 shows the comparison of mean arterial internal luminal circumference among all groups.

Table 1. Average internal luminal circumference according to group.

Number of subjects	Internal luminal circumference (micron) Sham	Subarachnoid hemorrhage	Subarachnoid hemorrhage + Edaravone
1	362	429	367
2	354	379	400
3	496	327	362
4	379	358	395
5	398	373	382
6	398	374	380
7	397	373	380

Table 2. Descriptive statistics for the sham group, SAH group, and SAH + edaravone group.

	Internal luminal circumference (micron) Sham	Subarachnoid hemorrhage	Subarachnoid hemorrhage + Edaravone
Number of subjects	7	7	7
Minimum	354	327	362
Maximum	496	429	400
Median	397	373	380

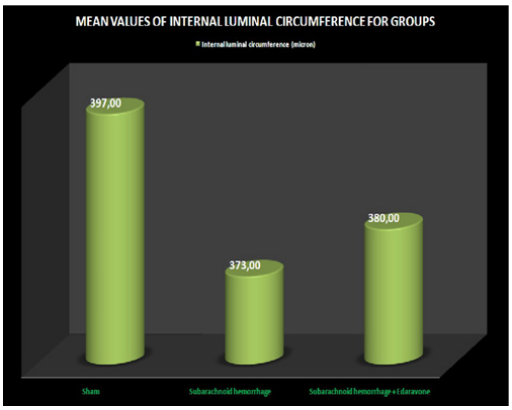

Figure 4: *Comparison of mean arterial internal luminal circumference among all groups*

DISCUSSION

Intermittent administration of edaravone (4 mg/kg intraperitoneally) subsequent to SAH increased the internal lumen circumference of the basilar artery in a rat vasospasm model. Following SAH, products of erythrocyte breakdown are important in the development of vasospasm, particularly oxyhemoglobin, which has been identified as a potent spasmogen with a central role in this pathogenesis[2]. Oxyhemoglobin releases superoxide anions, generates hydroxyl radicals, and induces lipid peroxidation in the erythrocyte membrane[17]. Edaravone is a free radical scavenger that promotes antioxidant action through the enhancement of prostacyclin production, the inhibition of lipoxygenases, the metabolism of arachidonic acid by trapping hydroxyl radicals, the inhibition of lipid peroxidation, and the quenching of active oxygen, which leads to the protection of various cells, such as endothelial cells and myocardial cells, against damage by ROS[11].

Cerebral vasospasm is a reversible narrowing of the intra-dural subarachnoid arteries within the Circle of Willis, which occurs 4–14 days following SAH and may be divided into two primary types. The radiological detection of vasospasm by digital subtraction angiography is called angiographic vasospasm[14]. Symptomatic, or clinical, vasospasm is the deterioration of one's neurological condition during the course of treatment objectively defined as an alteration in Glasgow Coma Scale score by more than two points for at least 1 h[22]. The link between angiographic vasospasm and neurological outcomes may be associative rather than causative. It is known that cerebral vasospasm is seen angiographically in 70% of patients with SAH and clinically in 20–30% of patients[12,22]. While the presence of angiographic vasospasm does not necessarily lead to symptomatic vasospasm, the latter may occur in the absence of the former. Symptoms and signs probably do not develop unless there is an angiographic diameter reduction greater than 50%[14]. Moreover, treating angiographic vasospasm does not always lead to improvements in clinical and functional outcomes[22].

Nimodipine, a calcium channel antagonist, is a common therapeutic option for patients with aneurysmal SAH[8,9,14,22]. The oral administration of nimodipine decreases brain oxygen concentrations in the absence of changes in other parameters, such as cerebral perfusion pressure[14], and reduces the risk of poor outcome and secondary ischemia after SAH[8,9,14]. An uncontrolled calcium concentration associated with cerebral ischemia leads to irreversible cell destruction and death in the central nervous system. Although all of the beneficial effects of nimodipine following SAH remain unclear, this drug exerts its neuroprotective action by blocking calcium influx at a neuronal level after tissue ischemia. However, the beneficial effects of nimodipine do not extend to ischemia stroke or traumatic brain injury[22]. Another popular treatment following SAH is hemodynamic therapy, also known as Triple-H therapy. This method includes the

use of hypertension, hypervolemia, and haemodulition[9,14,22] and may be done prophylatically or therapeutically. Hypervolemia successfully elevates cardiac filling pressure but has no effect on cerebral blood flow measurements or the incidence of clinical vasospasm[14]. Despite the fact that Triple-H therapy remains the mainstay of neurocritical care management, much of the literature supports its prophylactic use. The favorable effects of prophylactic hypervolemia are likely due to prevention of hypovolemia because hypotension can lead to neurological deficits in patients after cerebrovascular accidents[14,22]. However, potentially harmful effects on the cardiopulmonary function of patients with heart disease limit the use of this treatment[19]. Yet another therapy commonly utilized following SAH is tissue plasminogen activator (tpa), which is effective due to fibrinolytic activity but can induce hemorrhagic transformation and may, in fact, exacerbate brain edema[27]. Other therapies may be just as useful for the reduction of cerebral vasospasm following SAH in different ways.For instance, octreotid acetate can inhibit the synthesis of endothelin[7], melatonin and ebselen can inhibit lipid peroxidation[4,29], caspase inhibitors lead to apoptosis in the endothelium[28], and monoclonal antibodies prevent the action of cell adhesion molecules[5].

The administration of edaravone in rat models mediates the effects of SAH on brain edema, neurologic deficit, brain injury[9,18,20,28], and spinal cord injury[3,25]. It also reduces iron and thrombin, which may induce brain injury[18] while reducing caspase-3 and increasing superoxide[9] and eNOS[20,28] activity. Clinical research has verified that edaravone has neuroprotective effects and anti-ischemic activities[8,24] and it also has been used to ameliorate reperfusion injury in myocardial infarction[26].

Edaravone also has a positive effect on cerebral ischemia[1,13,23]. Ahmad et al.[1] occluded the middle cerebral artery in male Wistar rats with edaravone (10 mg/kg) administered intraperitoneally 30 min before the onset of ischemia and 1 h after reperfusion. Following reperfusion, the neurobehavioral activities of the rats were evaluated before they were sacrificed; infarct volume was also investigated. Edaravone treatment significantly reduced ischemic lesion volume and improved neurological deficits. Lu et al.[13] treated gerbils with edaravone (3 mg/kg) intraperitoneally 30 min before transient forebrain ischemia induced by occluding the bilateral common carotid artery for 5 min. The effects of edaravone were examined by measuring neuronal damage and behavioral deficits. Treatment significantly inhibited lipid and DNA oxidative damage, decreased neuronal alterations, and significantly ameliorated locomotor activity deficits and memory impairments 72 h after ischemia. Furthermore a clinical study by Sharma et al.[23] found that edaravone effectively improved functional outcomes in 25 patients with acute ischemic stroke when administered twice daily (30 mg) for 14 days by infusion.

Several clinical and experimental studies, primarily in East Asia, have also investigated the effects of edaravone on intracranial vasospasm following SAH. In an in vitro study, Munakata et al.[16] injected edaravone (0.6 mg/kg) into the central ear vein of a rabbit twice a day; 4 days following SAH, the basilar artery was excised. The diameter of the basilar artery in the edaravone-treated group was significantly larger than that of the non-treated group. In an animal study by Nakagomi et al.[17], 32 mongrel dogs with SAH were examined angiographically after the administration of edaravone either by continuous infusion or bolus injection. The continuous administration of edaravone (1 mg/kg/h and 10 mg/kg/h) significantly attenuated the narrowing of the basilar

artery compared to bolus administration (3 mg/kg). Moreover, both continuous and bolus administration increased the diameter of basilar artery but the difference was not statistically significant. In a clinical study by Munakata et al.[15], edaravone was used to treat 91 patients with SAH and the incidence of delayed ischemic neurological deficits (DINDs) was assessed. While DINDs had an incidence of 21% in the control group and 10% in the edaravone-treated group, there was no statistically significant difference between groups. On the other hand, the incidence of poor outcomes caused by vasospasm was 71% in the control group and 0% in the edaravone-treated group, which was statistically significant.

In the present study, the morphometric measurements of internal luminal circumference were compared among groups using a Kruskal-Wallis test, and no statistically significant differences were found. However, the mean circumference in the edaravone-treated group was 2% higher than that of the subarachnoid group. Edaravone might influence cerebral vasospasm through antioxidant effects via both hydroxyl radicals and iron-dependent lipid peroxidation. A number of clinical and experimental studies have shown that edaravone is an effective agent for the treatment of vasospasm.

CONCLUSION

Edaravone enhanced the internal lumen circumference of the spastic basilar artery following SAH in a rat model. The mean circumference exhibited a 6% reduction in the SAH group relative to the sham group, but edaravone treatment enlarged the artery by 2% compared to the subarachnoid group, suggesting that this expansion might be a positive effect of brain feeding. For this reason, the continuous administration of edaravone might be a useful agent for the treatment of SAH. It is one of the most important candidate drugs known but further experimental and clinical trials are required to clarify its effectiveness and mechanism of action.

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Received by: 09 September 2012
Revised by: 17 October 2012
Accepted: 17 October 2012

The Online Journal of Neurological Sciences (Turkish) 1984-2012
This e-journal is run by Ege University Faculty of Medicine,
Dept. of Neurological Surgery, Bornova, Izmir-35100TR
as part of the Ege Neurological Surgery World Wide Web service.
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E-mail: editor@jns.dergisi.org
URL: http://www.jns.dergisi.org
Journal of Neurological Sciences (Turkish)
Abbr: J. Neurol. Sci.[Turk]
ISSNe 1302-1664

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