Punica granatum L. Juice Attenuates Experimental Cerebral Vasospasm in the Rabbit Subarachnoid Hemorrhage Model: A Basilar Artery Morphometric Study and Apoptosis

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Abstract

Background This study investigated the effect of *Punica granatum* L. (pomegranate) juice on the rabbit basilar artery in an experimental subarachnoid hemorrhage (SAH) model.

Methods Eighteen adult male New Zealand white rabbits were randomly divided into three groups: a control group ($n = 6$), SAH group ($n = 6$), and SAH + treatment group ($n = 6$). Basilar artery diameter was measured with magnetic resonance angiography (MRA) in all groups at the beginning of the study. Experimental SAH was created by injecting autologous arterial blood into the cisterna magna. In the treatment group, the subjects were administered a daily dose of 30 ml/kg pomegranate juice via gastric gavage for 4 days after the SAH. The SAH group and SAH + treatment group underwent cerebral MRA after 72 hours. After a neurologic score assessment, all the animals were killed. The wall thickness and lumen area of the basilar artery were measured histometrically in all groups, and the apoptotic cell percentage in the artery was identified. The mean diameter of the basilar artery during MRA was measured.

Keywords
- subarachnoid hemorrhage
- pomegranate
- vasospasm
- treatment
- rabbit

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Results Pomegranate improved neurologic functions compared with the SAH group ($p < 0.01$). The mean basilar artery diameter on MRA in the SAH + treatment group was larger than in the SAH group and smaller than in the control group ($p < 0.01$ and $p < 0.05$, respectively). The mean vessel wall thickness value in the SAH + treatment group was lower than in the SAH group ($p < 0.01$), whereas there was no difference between the control and the SAH + treatment group ($p > 0.05$). The apoptotic cell rate in the SAH + treatment group was significantly lower than in the SAH group ($p < 0.001$). Evaluation of the basilar artery luminal area showed no difference between the three groups ($p > 0.05$).

Discussion Pomegranate was shown to have a vasospasm- attenuating effect on the basilar artery in the rabbit SAH model for the first time in our study.

Introduction

Subarachnoid hemorrhage (SAH) has a high mortality and morbidity mostly due to the ensuing cerebral vasospasm and early brain damage. Vasospasm is a multifactorial and complex process. Many experimental and clinical studies have been conducted and many active substances tried to treat this problem in the last 3 decades. Unfortunately, the etiology has not been fully elucidated, and there is no effective treatment.

Punica granatum L. (pomegranate) is a fruit rich in polyphenol and also contains pectin and ascorbic acid.[1] A polyphenol-rich diet has been shown to provide a neuroprotective effect in animal models from focal brain ischemia, periventricular white matter injury, and Alzheimer disease.[2–6] Pomegranate extract contains antioxidant,[7] anti-inflammatory,[8] antimicrobial,[9] and anticancer[10,11] characteristics. Its effect on cerebral vasospasm is unknown. In this study, we investigated the effects of pomegranate on vasospasm in the rabbit basilar artery in an experimental SAH model.

Materials and Method

The experimental part of the study was conducted at the Ankara Training and Research Hospital laboratory after obtaining consent from the Ankara Training and Research Hospital Animal Experiments Local Ethics Committee (Decision no. 233, August 10, 2013). The material was prepared and the microscopic basilar artery diameter measurement and histopathologic investigations performed at the Ankara University Medical Faculty's Histology-Embryology Department. The subjects underwent the magnetic resonance angiography (MRA) investigations at Dışkapı Y.B. Training and Research Hospital's Radiology Department. We used 18 New Zealand white rabbits weighing 2.5 to 3.0 kg in the study. All subjects were kept under stable and standard environmental conditions during the experiment and were fed standard animal food and water.

Preparation of Pomegranate Juice

Pomegranate was obtained from local commercial sources. Pomegranate juice was obtained by squeezing the edible portion of the pomegranate and filtering it to remove the residue. All the samples were used within 1 hour after they were squeezed and filtered.

Groups

Control Group

There was no SAH and treatment procedure performed during the experiment. A total of 3 mL cerebrospinal fluid (CSF) was aspirated under negative pressure, and the same CSF was reinjected into the cisterna magna of the rabbit within 2 minutes. Overall, 90 mL/day (30 mL/kg/day) of water was administered through gastric gavage for a total of 3 days.

SAH Group

Autologous arterial blood was administered into the animals with a cisterna magna puncture to create SAH. A total of 90 mL/day (30 mL/kg/day) of water was administered through gastric gavage for a total of 3 days starting 2 hours after the creation of SAH.

SAH + Treatment Group

The rabbits in the treatment group underwent cisterna magna puncture and autologous arterial blood administration. Pomegranate juice 90 mL/day (30 mL/kg/day) was administered for a total of 3 days starting 2 hours after the creation of SAH.

Anesthesia and Surgical Procedure

The rabbits were administered 50 mg/kg ketamine hydrochloride (Ketalar vial [Pfizer, New York, United States]) and 10 mg/kg Xylazine (Alfazyne 2% vial [Ege-Vet, Turkey]) intramuscularly before the surgical procedure. General anesthesia was ensured, and they were left to their spontaneous respiration. No ventilator support was used during the experiment.

The occipital and posterior neck region of the rabbits was shaved and the region prepared in accordance with asepsis and antisepsis rules. After entering subarachnoid distance by cisternal puncture method, 3 mL CSF were removed. In the control group, 3 mL CSF was aspirated under negative pressure, and the same CSF was reinjected into the cisterna magna of the rabbit within 2 minutes. The auricular arteries of the subjects in the SAH and SAH + treatment groups were cannulated with a 24G catheter, and 3 mL fresh blood was drawn. The blood was slowly injected into the rabbits in the SAH and SAH + treatment groups from the cisterna magna as autologous blood in ~ 2 minutes, and the rabbits were held upside

down for ~ 15 minutes to ensure the spread of blood to the basal cisterns. The rabbits in the control and SAH group were administered water 90 mL/day (30 mL/kg/day) (based on a person weighing 70 kg taking in an average of 2,000 mL of fluid a day) through gastric gavage for 3 days. The water was administered on the second hour after SAH in the SAH group. SAH was clinically observed to develop in the subjects after the procedure. The rabbits in the SAH + treatment group were administered 90 mL/day (30 mL/kg/day) pomegranate juice through gastric gavage for 3 days starting on the second hour after SAH. The physiologic parameters were measured with an auricular artery catheter.

All of the animals had similar conditions during the experiment. The mean arterial blood pressure of the animals was 100 ± 2 mm Hg; heart rates were 167 ± 4 beats/minute. Body temperatures of the rabbits were 37 ± 0.5°C with a heater. The animals were kept at a temperature of 23 to 25°C afterward. The rabbits in all groups underwent thoracotomy under general anesthesia, and the thoracic and abdominal spaces were opened on the third day of the experiment. The descending aorta was clamped, and the heart was cannulated by ventricular puncture so as to extend to the aorta. Perfusion of physiologic saline was started, and fixation was performed with formaldehyde. The brain, brainstem, and upper cervical region were removed after fixation. All tissues were fixed for 96 hours in 10% buffered formalin solution.

Neurologic Evaluation

The rabbits were observed and assessed neurologically using the Endo et al grading system for the experimental rabbit SAH model during 3 days.[12] Endo et al noted grade 1, no neurologic deficit (normal); grade 2, a minimal or suspected neurologic deficit; grade 3, a mild neurologic deficit without abnormal movements; and grade 4, a severe neurologic deficit with abnormal movements.

Angiography

All subjects were administered 10 mg/kg ketamine hydrochloride (Ketalar vial) intramuscularly (before any surgical procedure was performed) for sedation on the first day of the study. Oxygen saturation, heart rate, and systolic, diastolic, and mean blood pressure were evaluated and recorded before the MRA. They then underwent cerebral 1.5-T MRA (Philips Healthcare, Eindhoven, Netherlands). At the end of the third day (before the experiment ended), subjects in the SAH group and the SAH + treatment group underwent a second cerebral MRA. All MRA images were recorded and measured digitally. Measurements were performed to pass through each basilar artery in five separate segments in the vertical plane and were then evaluated statistically.

Histologic Study

Tissue Preparation

The control group was killed 72 hours after the injection of saline, and the experimental groups were killed 72 hours after creating the SAH. The perfusion-fixation method was used under general anesthesia. Following this procedure, the brain and brainstem were removed and fixed in 10% buffered formalin. The entire basilar artery was removed from each animal and sectioned into five segments, each 2 mm in length to assess vasospasm. Basilar artery samples were dehydrated in a graded ethanol series, cleared in xylene, and embedded in paraffin. Sections were cut in 5-µm thickness using a microtome (Leica RM 2125RT [Leica, Wetzlar, Germany]) and stained with hematoxylin and eosin (H&E). Slides were examined and photographed using an Axio Scope-A1 (Carl Zeiss, Oberkochen, Germany) microscope at ×100 magnification.

Histomorphometry Analysis of the Basilar Artery

The wall thickness and luminal area of the basilar artery were measured using the AxioVision (AxioVision, Oberkochen, Germany) software program at the five predetermined segments. The wall thickness was measured at four points (3, 6, 9, and 12 o'clock) of each segment of the basilar artery between the endothelium of the intima and the external border of the tunica media. The luminal area was calculated from the luminal border of each segment. The obtained measurement results were recorded and evaluated statistically.

TUNEL Staining

Immunohistochemical detection of cells undergoing DNA fragmentation was performed using a terminal deoxynucleotidyl transferase (TdT) labeling (TUNEL) method with a commercial in situ apoptosis detection kit (ApopTag Peroxidase in Situ Apoptosis Detection Kit, Millipore [Darmstadt, Germany], S7100). The 5-µm-thick sections were stained according to the manufacturer's protocol for the Peroxidase in Situ Apoptosis Detection Kit. Diaminobenzidine (DAB) was used as a chromogen, and counterstaining was performed using methyl green.

Assessment of Apoptotic Cell

In the light microscopic examination, the apoptotic cell percentage was calculated by comparing TUNEL-positive stained cells with the complete cell count including the entire circumferential vessel wall. The examination was done by a histologist who was blind to the study.

Immunohistochemistry Staining

For immunohistochemistry analysis, sections were deparaffinized and rehydrated by using xylene and a graded series of ethanol, followed by 5-minute washes in phosphate buffered saline (PBS). Antigen retrieval was performed in trypsin at 37°C for 30 minutes and washed in PBS for three times for 5 minutes. Afterward, the sections were incubated for 5 minutes in 3% hydrogen peroxide in methanol to block endogenous peroxidase activity, washed in PBS three times for 5 minutes, blocked at room temperature for 30 minutes by using blocking solution (Histostain-Plus Kit [Massachusetts, United States], 85–9043, USA) and incubated in a humidified chamber 1 hour at 37°C with the primary antibodies anti-Cleaved Caspase-3 (1:200 dilution; Cell Signaling Technology, USA). Sections were washed in PBS (three times for 5 minutes each) and incubated at room temperature for 1 hour with the secondary antibodies (Histostain-Plus Kit, 85–9043. After a

wash with PBS (3 times for 5 minutes), the sections were incubated with ready-to-use streptavidin peroxidase at room temperature for 30 minutes and well rinsed with PBS. Colors were developed with a DAB kit. The sections were then counterstained with hematoxylin, dehydrated, and mounted. Negative controls were prepared by substituting PBS for the primary antibodies.

Statistical Analysis

Data are expressed as mean plus or minus standard deviation. Statistical differences between the control and other groups were compared by one-way analysis of variance (ANOVA) and then the Bonferroni multiple comparison procedure, if a significant difference had been determined by ANOVA. The clinical behavior scores were compared by the Fisher exact test. Severity of vasospasm (100-[diameter at 72 hours post-SAH]/[diameter before SAH], %) on MRA was calculated in each animal in the groups and compared statistically among the three groups by the Mann-Whitney U test. A probability value of $p < 0.05$ was considered statistically significant.

Results

Neurologic Evaluation

There was a significant difference between the distribution of groups into neurologic deficit grade categories ($p < 0.001$). There was no neurologic deficit in the control group (grade 1). All animals showed a progressive deficit after SAH in the SAH group. Three animals were detected with grade 3 and three animals were detected grade 4 in the SAH group 72 hours after the experimental procedure. Neurologic deficit was observed after the SAH procedure in the SAH + treatment group on the first day. The neurologic deficits gradually improved and 72 hours after SAH, four animals presented grade 1 and two animals presented a grade 2 deficit. In the SAH group, the distribution of grade categories was significantly higher than the control ($p = 0.002$), and SAH + treatment ($p = 0.002$) groups. There was no difference between the control and SAH + treatment groups ($p = 0.455$) (►**Table 1**).

Table 1 Neurologic evaluation of groups[a,b]

Variables	Control	SAH	SAH + treatment	*p*
Neurologic deficit, *n* (%)				
Grade 1	6 (100)	0 (0)	4 (66.7)	**0.002**
Grade 2	0 (0)	0 (0)	2 (33.3)	
Grade 3	0 (0)	3 (50)	0 (0)	
Grade 4	0 (0)	3 (50)	0 (0)	

Abbreviation: SAH, subarachnoid hemorrhage.
[a]Grade 1, no deficit; grade 2, minimal or suspected deficit; grade 3, mild deficit without abnormal movements; grade 4, severe deficits with abnormal movements.
[b]Statistical differences between SAH and SAH + treatment groups were compared with the Fisher exact test.

Angiography

The mean artery diameter on MRA was different in the three groups ($F(2, 15) = 21{,}129$; $p < 0.001$). The mean MRA basilar artery diameter was found to be 0.105 ± 0.007 cm in the control group, 0.071 ± 0.011 cm in the SAH group, and 0.091 ± 0.003 cm in the SAH + treatment group. In the control group, the mean artery diameter was found to be higher than in the SAH and SAH + treatment groups ($p < 0.001$ and $p < 0.05$, respectively). The mean artery diameter on MRA for the SAH group was significantly lower than the SAH + treatment group ($p < 0.01$) (►**Fig. 1** and ►**Table 2**). To detect the severity of vasospasm on MRA, when rate change (percentage) was compared between the SAH and SAH + treatment groups, in the SAH group there was a vasospasm at the rate of 37.8% (17.5–47.5%). In the SAH + treatment group, this ratio was 18.9% (13.5–23.08%). The difference was statistically significant ($p < 0.05$). ►**Table 3** shows the degree of vasospasm (percentage of the baseline vessel diameter) on MRA.

Histologic Assessment

Widespread SAH was observed macroscopically at the ventral surface of the brain in all SAH and SAH + treatment groups. Histomorphometric assessment was performed on samples with H-E, TUNEL staining, and Caspase-3 staining.

H&E Staining

In the control group, three layers of the basilar artery wall, tunica intima, tunica media, and tunica adventitia, were observed at normal appearance (►**Fig. 2A**). In the SAH group, narrowed basilar artery lumen, shrunken endothelial cells, corrugated internal elastic membrane, thickened vessel wall, and contracted smooth muscle cells were seen (►**Fig. 2D**). In the SAH + treatment group, a basilar arterial lumen was larger and vessel wall was thinner than in the SAH group. The internal elastic membrane was smooth, endothelial cells were normal shaped, and smooth muscle cells were elongated (►**Fig. 2G**).

Evaluation of basilar artery wall thicknesses showed a significant difference between the three groups ($F(2, 15) = 9{,}273$; $p < 0.01$). The mean vessel wall thickness was found to be 18.460 ± 4.390 µm in the control group, 32.04 ± 6.457 µm in the SAH group, and 22.91 ± 5.660 µm in the SAH + treatment group. In the control group, the mean of the wall thickness was statistically significantly lower than the SAH group ($p < 0.01$), whereas there was no difference between the control and the SAH + treatment group ($p > 0.05$). On the other side, the mean wall thickness for the SAH group was higher than the SAH + treatment group ($p < 0.05$) (►**Fig. 2** and ►**Table 2**).

There was no difference between the three groups in terms of the mean of the luminal area ($F(2, 15) = 1{,}924$; $p > 0.05$).The mean luminal area of the basilar artery was found to be 159743.31 ± 195712 µm^2 in the control group, 80653.76 ± 43234 µm^2 in the SAH group, and

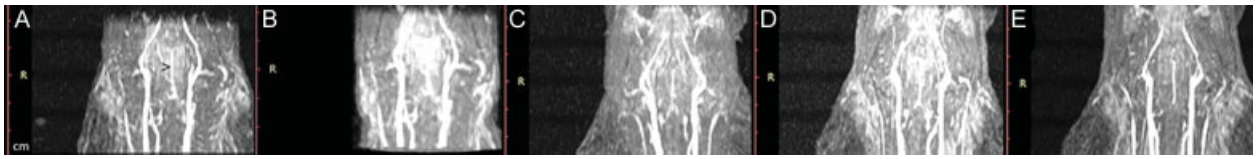

Fig. 1 (A) Basilar artery appearance in a control group rabbit (indicated by black arrow). (B) Basilar artery appearance of the rabbit from the subarachnoid hemorrhage (SAH) group on magnetic resonance angiography (MRA) obtained before SAH. (C) Basilar artery appearance of the same rabbit after SAH from the SAH group. (D) Basilar artery appearance of the rabbit from of the SAH + treatment group on MRA obtained before SAH. (E) Basilar artery appearance of the same rabbit after SAH and treatment with pomegranate.

147956.88 ± 77497 μm^2 in the SAH + treatment group (►**Table 2**). Evaluation of the basilar artery luminal area showed no difference between the three groups ($p = 0.180$).

TUNEL Staining

There were not any TUNEL (+) endothelial cells in the control groups (►**Fig. 2B**). The TUNEL (+) endothelium was localized on the inner surface of the corrugated internal elastic membrane in the SAH groups (►**Fig. 2E**). In the SAH + treatment group, positive TUNEL staining was obtained in endothelial cells less than the SAH group (►**Fig. 2H**).

IHC (Cleaved Caspase-3) Staining

The endothelial cells cytoplasm and nucleus showed positive cleaved Caspase-3 immunoreactivity.

The means of the apoptotic cell percentage were different from each other in three groups ($F(2, 15) = 326{,}889$; $p < 0.001$).The apoptotic cell percentages were found to be 1.83 ± 0.05 in the control group, 83.16 ± 1.25 in the SAH group, and 62.50 ± 3.80 in the SAH + treatment group (►**Table 2**). In the control group, the mean of the apoptotic cell percentage was significantly lower than the SAH and SAH + treatment groups ($p < 0.001$ and $p < 0.001$, respectively). In the SAH group, the means of the apoptotic cell percentage was significantly higher than the SAH + treatment group ($p < 0.001$) (►**Table 2**).

Discussion

Cerebral vasospasm is the reversible contraction of the arteries in the brain usually due to the mechanical and physiologic stimuli that appear following SAH and is seen in 30 to 70% of these patients. It typically starts 3 to 5 days after the bleeding and resolves within 2 to 4 weeks. The most common reason for mortality and morbidity after SAH is the ischemia that develops after cerebral vasospasm and early brain damage.

Table 2 Results of measurements[a]

Variables	Control	SAH	SAH + treatment	*p*
Artery diameter on MRA, mm, mean ± SD	0.105 ± 0.007	0.071 ± 0.011	0.091 ± 0.003	<**0.001**
Wall thickness, μm, mean ± SD	18.460 ± 4.390	32.040 ± 6.457	22.915 ± 5.660	**0.002**
Luminal area measurement, μm^2, mean ± SD	159743.31 ± 95712.71	80653.76 ± 43234.22	147956.88 ± 77497.83	0.180
Apoptotic cell percentage, %, mean ± SD	1.833 ± 0.983	82.500 ± 3.082	62.500 ± 9.311	<**0.001**

Abbreviations: MRA, magnetic resonance angiography; SAH, subarachnoid hemorrhage; SD, standard deviation.
[a]Statistical differences between the control and other groups were compared by one-way analysis of variance and then the Bonferroni multiple comparison procedure.

Table 3 Evaluation of vasospasm: percentage change on magnetic resonance angiography

Variables	Control	SAH	SAH + treatment	*p*
Before, mean ± SD Median (Min–Max)	0.10 ± 0.007 0.10 (0.09–0.11)	0.11 ± 0.02 0.11 (0.08–0.13)	0.11 ± 0.005 0.11 (0.10–0.12)	
After, mean ± SD Median (Min–Max)	–	0.07 ± 0.01 0.065 (0.06–0.09)	0.09 ± 0.003 0.09 (0.086–0.096)	
Rate of change, % Median (Min–Max)	–	37.8 (17.5–47.5)	18.9 (13.5–23.08)	**0.026**

Abbreviations: Max, maximum; Min, minimum; SAH, subarachnoid hemorrhage; SD, standard deviation.

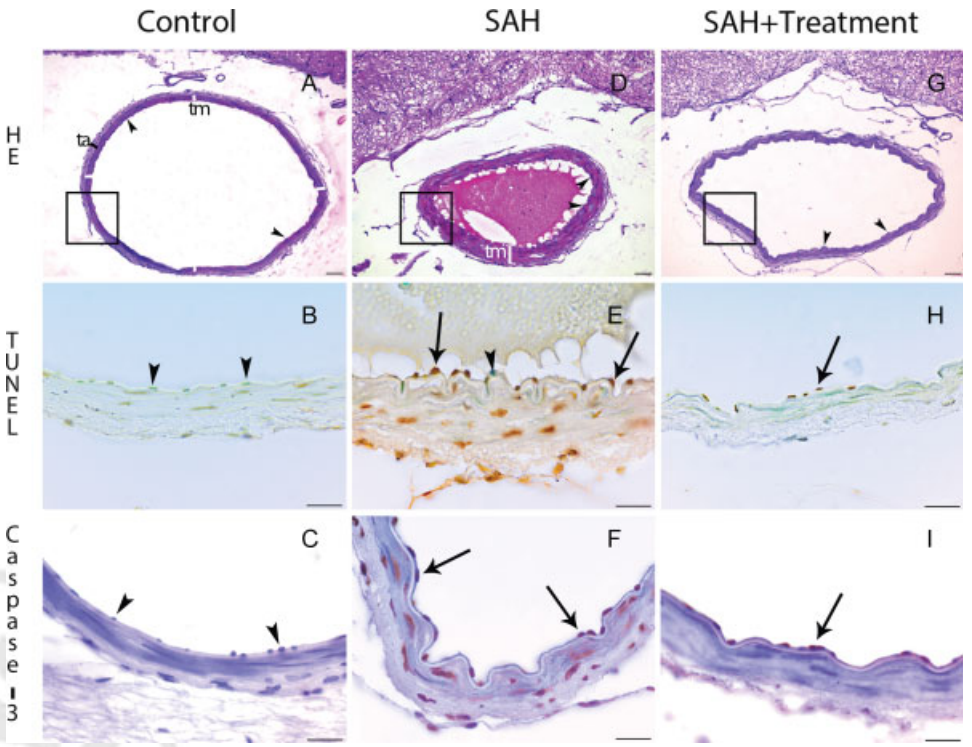

Fig. 2 Hematoxylin and eosin staining light microscopic images show basilar artery cross sections on the surface of pons. The wall thickness (white line) was measured at four points of each segment of the basilar artery between the endothelium of the intima and the external border of the tunica media (tm). Increasing arterial wall thickness and narrowing luminal area are seen prominently in the subarachnoid hemorrhage (SAH) group. (A) Control group. (D) SAH group. (G) SAH + treatment group. Arrowhead shows endothelial cell nucleus; tm, tunica media (white line); ta, tunica adventitia (black line). Scale bar: 50 µm. TUNEL staining light microscopic images. (B) Control group. Endothelial cell nuclei are stained with methyl green (arrowhead). There were no TUNEL (+) staining endothelial cell nucleus (arrow) in tunica intima. (E) SAH group. The nuclei of endothelial cells on the corrugated internal elastic lamina were stained TUNEL (+). (H) SAH + treatment group; TUNEL (+) staining endothelial cell nuclei are seen in luminal surface. Arrow shows TUNEL (+) apoptotic endothelial cell nucleus (stained with diaminobenzidine [DAB] brown color); arrowhead shows normal endothelial cell nucleus (stained with methyl green). Scale bar: 20 µm. IHC (cleaved Caspase-3) staining light microscopic images. (C) Control group. (F) SAH group. (I) SAH + treatment group. Brown cytoplasmic and/or nuclear staining was evaluated as positive. Arrow shows Caspase-3(+) endothelial cell cytoplasm and/or nucleus (stained with DAB brown color); arrowhead shows normal endothelial cell nucleus (stained with hematoxylin). Scale bar: 20 µm.

Food rich in antioxidants and phytochemicals has been shown to protect health in many epidemiological studies. A polyphenol-rich diet (blueberries, green tea, and apple juice) has been demonstrated to provide a neuroprotective effect in focal brain ischemia, periventricular white matter injury, and Alzheimer disease animal models.[2–6] Pomegranate is a fruit containing polyphenol and consists of 80% juice and 20% seeds. Fresh fruit juice consists of 85% water, 10% sugar, and 1.5% pectin, ascorbic acid, and polyphenolic flavonoids.[2] Polyphenol contents are anthocyanins, catechins, ellagic tannins, and gallic and ellagic acids.[2,13] The content of pomegranate has been shown to have an antioxidant activity and antiatherogenic effects in studies on rats,[2,14] and it also inhibits cyclooxygenases and lipoxygenases.[15] *P. granatum* has been reported to have antidiarrheal activity,[16] antityrosinase,[17] anti-inflammatory and antidiabetic activity,[11,18] and apoptotic and antigenotoxic[7] activity in recent articles. Loren et al reported that neonatal rats were significantly protected from hypoxic ischemic brain injury when pomegranate juice was added to the diet of the mother rats, indicating that pomegranate has a neuroprotective effect.[19] So we intended to evaluate the effect of pomegranate on the basilar artery of the rabbits exposed to SAH. Basilar artery wall thickness, luminal area of the basilar artery, apoptotic cell percentage, and MRA basilar artery diameter measurements were evaluated.

There are different experimental SAH animal models. The vessel perforation models induce SAH via intracranial puncture of the basilar or internal carotid arteries, and it has a high mortality rate. The most commonly used model is the cisternal injection model that has proven valuable for studies of the late SAH phase, and the mortality rate is low.[20] We used rabbits in this study because the rabbits have a bigger and higher visibility of the basilar artery on MRA than rats. But rabbits are vulnerable animals for experimental use. The experiment was continued for 3 days. Because of these reasons we used a cisternal injection model in this study. Different pomegranate dosages have been given to rabbits for experimental study in the literature.[21,22] The rabbits in the SAH + treatment group were administered 90 mL/day (30 ml/kg/day) pomegranate juice in this study. The dosage (30 mL/kg/day) was calculated based on a person weighing 70 kg taking in an average of 2,000 mL fluid a day.

Although a significant difference was seen between the three groups regarding basilar artery wall thicknesses in our study, the mean values of the pomegranate treatment group were lower than in the SAH group. There was a significant difference between the three groups when MRA basilar artery diameters were evaluated. When basilar artery diameter change after vasospasm was evaluated on MRA, the vasospasm-decreasing effect of pomegranate was seen clearly in the SAH + treatment group. Evaluation of statistical analysis of basilar artery luminal areas showed no difference between the three groups. We believe that histologic examination of the basilar artery luminal area is not enough to distinguish between normal artery and vasospasm. MRA scans were also performed when animals were alive, and basilar artery diameters were measured. Vasospasm was seen as more clearly distinguishable. Therefore, we believe that MRA gives more realistic results when compared with histomorphometric examination.

This study showed that pomegranate has a protective effect against apoptosis. The apoptosis rate was significantly higher in the pomegranate group than the control group and significantly less than in the SAH group in our study. This may be due to the antioxidant and anti-inflammatory effect of pomegranate juice in cells before apoptosis started. Cahill et al reported in their study on p53 inhibitors that these prevented apoptosis formation but did not stop vasospasm development, and it was necessary to inhibit the cascades that develop before the apoptotic process to treat vasospasm.[23] Park et al also prevented apoptosis and thus decreased vasogenic and cytotoxic edema in their study, but the effects of these findings on mortality were not significant. They stated it was very important to prevent the ischemic cascades causing apoptosis and hemoglobin toxicity to protect neuronal and neurovascular tissue and that preventing apoptosis had no significant effect on preventing vasospasm and neuronal tissue destruction.[24] Aoki et al reported in their study with caspase inhibitors that apoptosis only causes histologic findings and has no role in long-term vasoconstriction in vasospasm.[25] Basilar artery vasodilation was found in the SAH + treatment group and decreased the number of apoptotic cells compared with the SAH group in our study.

Clinical studies have been conducted on pomegranate in recent years after positive results were obtained from laboratory studies. Pomegranate juice added to the diet in humans was shown to decrease the risk of heart disease in a clinical study.[26] Another clinical study shows pomegranate juice consumption resulted in a 30% decrease in carotid intima-media thickness after 1 year.[27] We showed pomegranate to affect vasodilation in the basilar artery in rabbits, both with MRA analysis and histomorphometric evaluation of the basilar artery. These results show pomegranate improves neurologic function after SAH in rabbits. We think this study will create a model for neurovascular clinical studies to be conducted with pomegranate in the future.

Conclusion

Pomegranate affects many pathways of the vasospasm mechanism due to its characteristics and causes vasodilatation as a result. This study showed that pomegranate has a therapeutic effect against vasospasm in the experimental SAH model.

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