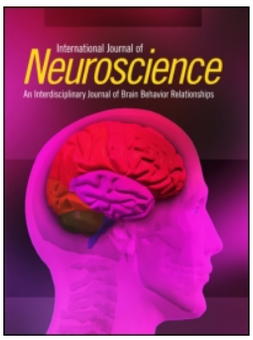

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ORIGINAL ARTICLE

The effect of pentoxifylline on cerebral vasospasm following experimental subarachnoid hemorrhage

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ABSTRACT

Objects: Cerebral vasospasm is an important event that occurs following subarachnoid hemorrage which has significant mortality and morbidity. The goal in this study was to investigate the effect of pentoxifylline on vasospasm in an experimental subarachnoid hemorrhage model.

Methods: In this study, 20 male New Zeland White rabbits weighing 3000–3500 g were assigned randomly to four groups. Animals in group 1 served as controls. Animals in group two received only intravenous pentoxifylline injection 3 times in 12 h intervals. In group 3, SAH was induced and no injection was given. Animals in group 4 received intravenous pentoxifylline (6 mg/kg) injections 3 times at 12th, 24th and 36th hours after subarachnoid hemorrhage induction. All animals were sacrificed and basilar arteries were removed at 48th hour. Basilar artery vessel diameters, wall thicknesses and luminal section areas were measured with Spot for Windows version 4.1. Statistical analysis was performed using ANOVA and Kruskall–Wallis tests.

Results: Mean basilar artery luminal section areas and luminal diameters in group 4 were significantly higher compared to group 3 ($p < 0.05$). Basilar artery wall thicknesses and were found to be higher in group 3 than in other groups and this was also statistically significant ($p < 0.05$).

Conclusion: Our study demonstrated that intravenous administration of pentoxifylline significantly decreases vasospasm after subarachnoid hemorrhage.

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pentoxifylline; subarachnoid hemorrhage; cerebral vasospasm; basilar artery

Introduction

Cerebral vasospasm is a cascade of pathological events which involves slow but prominent narrowing of large cerebral arteries and cerebral ischemia or infarction [1]. Following subarachnoid hemorrhage (SAH), cerebral vasospasm begins as a result of inflammatory response during first 48 hours, however it is rarely detected angiographically in the first 3 days [2]. It is mostly documented between 5th and 14th days of subarachnoid hemorrhage and resolves gradually between 2nd and 4th weeks [3, 4]. Among patients who suffer from SAH, 20-30% of them show clinical manifestations of vasospasm, whereas 70% of patients are shown to have cerebral vasospasm angiographically [5].

Despite significant amounts of research in treatment of cerebral vasospasm, a successful treatment modality hasn't been reached yet. Recent modalities focused on therapeutic agents which effect inflammatory cascade to prevent narrowing of arteries and thus, improving cerebral blood flow. Pentoxifylline (PTX), a derivative of metylxanthine, is a potent nonselective inhibitor of phosphodiesterases (PDE). It has immunomodulatory and anti-inflammatory properties at low dosages. While classically used for peripheral artery diseases and obstructive pulmonary diseases for its modulatory effects on smooth muscle relaxation, it was shown to be effective against ischemic injury of brain and intestine as well as some other disorders due to its anti-inflammatory effects [6–8].

In this study, we aimed to investigate the effect of systemic PTX on cerebral vasospasm following experimental SAH.

Materials and methods

Animal model

This study was approved by the Hacettepe University Animal Research Committee. Twenty male New Zeland White rabbits weighing 3,000–3,500 g were randomly assigned to one of 4 groups. Number of animals used in the experiment is determined by resource equation method based on Festing's report and kept low enough to limit amount of sacrificed animals, yet high enough to be appropriate [9]. Animals in group 1 ($n = 5$) served as control group. Animals in group 2

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($n = 5$) were not subjected to SAH, but were administered intravenous 6 mg/kg PTX (Trental, Aventis Pharma Sanayi ve Ticaret Ltd., Turkey) three times with 12 h intervals. In Group 3 ($n = 5$) SAH was induced by protocol, but no treatment was given. Rabbits in group 4 ($n = 5$) was subjected to SAH followed by intravenous administration of 6 mg/kg PTX at 12th, 24th and 36th hours. All procedures were performed by two investigators that are not blinded to treatment groups during surgery and euthanasia. Vascular measurements were performed by a pathologist in a blinded fashion.

Induction of experimental SAH

Animals in group 3 and group 4 were anesthetized by intramuscular injection of a mixture of ketamine (Ketaset, 50 mg/kg) and xylazine (Rompun, 10 mg/kg) and all animals breathed spontaneously during the procedures. 1 cm midline vertical incision is made at the occipitocervical junction and atlanto-occipital membrane is exposed which is followed by insertion of a 27-gauge insulin needle into the cisterna magna. Following withdrawal of 1.0 ml of cerebrospinal fluid (CSF), 3 ml of non-heparinized blood taken from central ear artery was injected into cisterna magna. The SAH induced animals were then placed in a trandelenburg position at 30° for 30 min in order to keep the blood in the basal cisterns. After recovery from anesthesia, all the rabbits were observed for possible neurological deficits and returned to vivarium. We detected no mortality within the time period of the experiment.

Perfusion-fixation

All experimental animals including the control group were sacrificed 48 h after the interventions and perfusion-fixation was performed. The animals were anesthetized as described in animal model section above. The ear artery was catheterized for monitoring blood pressure and blood gas analysis. When satisfactory respiratory parameters were obtained, thoracotomy was performed, the left ventricle of the heart was cannulated, the right atrium was widely opened and the abdominal aorta was clamped. After perfusion of a flushing solution (Hanks' balanced salt solution [Sigma Chemical Co], pH 7.4. at 37 °C, 300 mL), circulation was perfused with a fixation of 2% paraformaldehyde. 2,5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, at 37 °C, 200 mL. Perfusion was performed at a standard height of 100 cm from the chest. The brains were then excised and stored in fixation solution at 4 °C overnight.

Morphometry and statistical analysis

Basilar arteries were harvested from brain stems and segments from the proximal one-third of the artery were excised for analysis. The segments were embedded in paraffin and cross-sections were cut at a thickness of 0.5 μm. Then, the sections were mounted onto glass slides and stained with hematoxylin and eosin for light microscopic analysis. The vessels were measured using computer-assisted morphometry (SPOT for Windows Version 4.1). Automated measurements of the cross-sectional area of the arterial sections, arterial wall thickness and luminal diameter were taken by an investigator blinded to the identity of the group. Four cross-sections of each artery were selected randomly for measurement and a single value for each rabbit is obtained by averaging the measurements.

Statistical analysis was performed using Kruskal–Wallis H test to assess difference between groups. In the presence of difference, Mann Whitney U test was performed to evaluate binary comparison. Statistical significance was accepted at $p < 0.05$.

Results

Physiologic parameters such as body weight, arterial blood pressure, and arterial blood gas values showed no significant difference among four groups ($p > 0.05$). Therefore they were not considered as a variable in statistical analysis.

In gross examination, a subarachnoid blood clot over the basal surface of the brain stem was noted in animals that were subjected to experimental SAH. Elastic lamina was folded and corrugated resulting in significant narrowing of arterial lumina. Besides the accumulation of erythrocytes and inflammatory cells around tunica adventitia, tunica media was also vacuolized in animals subjected to SAH (Figure 1).

Regarding basilar artery wall thickness, control and vehicle groups did not differ significantly. SAH and treatment groups had significantly higher wall thickness compared to other groups. However, treatment group had significantly thinner arterial walls than SAH group (Table 1).

Similarly, basilar artery lumens in SAH and treatment groups had significantly smaller cross-sectional areas compared to control and vehicle groups. The SAH group had the smallest cross-sectional area compared to all other groups (Table 1).

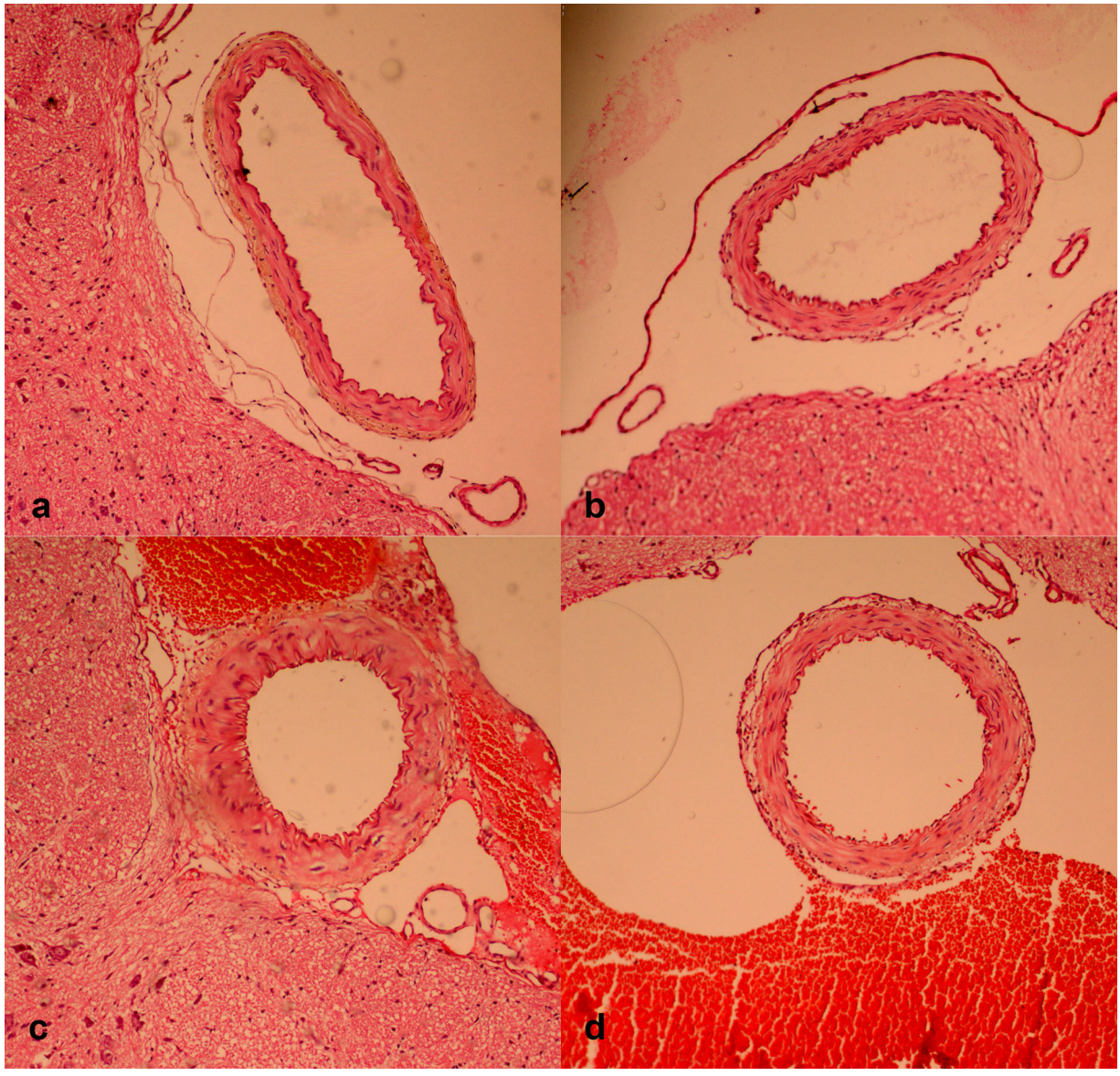

Figure 1. Basilar artery luminal cross-sectional areas and arterial wall thicknesses of study groups are shown with hematoxylin and eosin staining at 20× magnification: a) control group; b) vehicle group, c) SAH group, d) treatment group.

Table 1. Basilar artery wall thickness, cross sectional area of basilar artery lumen and basilar artery lumen diameter among groups.

Groups	Basilar artery wall thickness (μm) Mean ± SD	Cross sectional area of basilar artery lumen (μm^2) Mean ± SD	Basilar artery lumen diameter (μm) Mean ± SD
Group 1 (Control)	42.6 ± 0.76*	48300 ± 2722.2*	419.8 ± 9.56*
Group 2 (Vehicle)	42.2 ± 1.52	45518 ± 217.0	420.0 ± 9.57
Group 3 (SAH)	66.2 ± 0.88*	29220 ± 760.3*	249.8 ± 19.41*
Group 4 (Treatment)	51.8 ± 1.82*	39990 ± 930.9*	343.0 ± 37.12*

*$p < 0.05$.

Finally, basilar artery lumen diameters in control and vehicle groups were significantly greater than others, but similar to each other. On the other hand, basilar artery lumen diameters of rabbits in group 3 were significantly smaller than those in group 4 (Table 1).

Discussion

In this study PTX limited the extent of cerebral vasoconstriction when administered at 12, 24 and 36 h following SAH. Nontreated animals had narrower cerebral vessel lumens compared to PTX administered

animals. It is also found that PTX had no effect on cerebral vessels in the absence of SAH.

Cerebral vasospasm is considered to be one of the major determining factors of morbidity and mortality following SAH [10, 11]. Vasoactive substances that are released during vasospasm affect the course of the disorder by inflammatory, apoptotic, vasoconstrictor and other unidentified effects. One of the major possible mechanisms of cerebral vasospasm depends on nitric oxide (NO) metabolism [12]. Nitric oxide (NO) is a primary endogenous vasodilator that directly affects smooth-muscle relaxation. Hemoglobin and its breakdown products after SAH have been demonstrated to disrupt NO signaling between the endothelium and underlying smooth muscle [13, 14]. However, though narrowing of large vessel diameter has a major role in delayed ischemic injury, recent studies proposed alternative mechanisms such as early brain injury (EBI), loss of autoregulation of cerebral microcirculation and microthrombosis [15]. EBI term has been defined as the period from the onset of hemorrhage to the beginning of vasospasm suggesting that EBI also may be one of the precipitating events that eventually lead to vasospasm [16].

Nonselective PDE inhibitor PTX is known as an antiplatelet and vasodilator agent which also has antinflammatuary, antioxidant and antiapoptotic effects [7, 17, 18]. It prevents hydrolysis of cAMP and cGMP and elevates their level by acting through multiple PDEs resulting in smooth muscle relaxation [19, 20]. It also decreases the intracellular Ca^{+2} in platelets thus result in decreased platelet aggregation [14]. Furthermore attenuation of brain edema with PTX treatment was demonstrated in experimental stroke model in rats [7]. Finally, PTX was shown to reduce apoptosis and suppress tumor necrosis factor (TNF) alpha in several studies [21, 22]. The only studies regarding PTX's effect on SAH investigated its relation to EBI [23, 24]. PTX was found to exhibit neuroprotective effects through its anti-inflammatory and antiapoptotic effects. To the best of our knowledge, effect of PTX against vasospasm following subarachnoid hemorrhage hasn't been described before. Several PDE inhibitors were used in experimental SAH models for vasospasm [1, 4, 25]. Sildenafil citrate, a PDE5 inhibitor, and cilostazol, a potent PDE3 inhibitor was shown to have protective effects on vasospasm in experimental SAH models [1, 25]. Since PTX is a nonselective inhibitor of PDEs, it is able to act on both of these subtypes and results of this study are consistent with previous studies on PDE inhibitors.

There are some limitations in this study. First, apart from light microscopic evaluation, no biochemical, immunohistocehmical or neurobehavioral evaluation was performed. Neuroprotective properties as well as anti-inflammatory properties of PTX were reported in the literature prior to our study. And the study budget was not sufficient enough to accommodate additional evaluations. So, we intended to focus on previously unstudied aspect of the drug. Secondly, EBI gained attention as a more important factor determining outcome compared to vasospasm in recent years following a prospective study where treatment of vasospasm showed no significant clinical improvement. At the time of this study, publications on EBI was limited and vasospasm was still considered as major determinant of outcome, so our focus was on vasospasm rather than EBI. However, we still think this study maintains its importance. First, EBI period ends with onset of vasospasm suggesting that vasospasm may be one of the consequences of EBI, which makes it still one of the targets for treatment. Secondly, vasospasm is associated with decreased cerebral blood flow and cerebral metabolism. So, even EBI is prevented successfully, cerebral structures would still be susceptible to diminished blood flow. In fact, poor outcome following SAH may be due to a two-step injury where EBI is followed by vasospasm. Attenuating second step (vasospasm) would not effect overall outcome unless first step (EBI) is treated since the latter is a bigger determinant factor. Similarly, nontreatment of second step may limit the amount of clinical improvement achieved by treating the first step. Another limitation of the study is lack of does-effect evaluation. Experimental rabbit studies involving pentoxifylline are not abundant as other animal models and there is no universally accepted dose on rabbits. The administered dose (12 mg/kg/day) was chosen arbitrarily which may not be the optimum dose.

One advantage of agents such as PTX will be that it may act on both on EBI and vasospasm since it has both antiapoptotic, antiinflammatuary effects as well as attenuating effect against vasospasm at the same time.

Conclusion

In this study we analyzed the effect of PTX on cerebral vasospasm in an experimental rabbit model. Intravenous administration of PTX was found to decrease cerebral vasospasm significantly following subarachnoid hemorrhage *via* its vasodilatory,

antithrombotic, antiinflammatuary, antiapoptotic and neuroprotective effects. We suggest that PTX should be considered for clinical trials in the treatment of cerebral vasospasm following SAH and its consequence delayed ischemic neurologic deficit.

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This study has been presented as an oral presentation at Turkish Nueosurgical Society 33th Scientific Congress, April 11-14, 2019, Antalya, Turkey.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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