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Original Investigation

# The Effects of Intravenous Cilostazol and Nimodipine on Cerebral Vasospasm after Subarachnoid Hemorrhage in an Experimental Rabbit Model

## Deneysel Tavşan Subaraknoid Kanama Modelinde İntravenöz Cilostazol ve Nimodipine Etkilerinin Araştırılması

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### ABSTRACT

**AIM:** Our aim in this study was to investigate the efficacy of intravenous administration of cilostazol and compare these effects with intravenous usage of nimodipine in subarachnoid hemorrhage model.

**MATERIAL and METHODS:** Twenty-five male New Zealand White rabbits were assigned randomly to 1 of 5 groups. Animals in group 1 (n=5) served as controls, group 2 (n=5) was the SAH-only group, group 3 (n=5) was treated with intravenous 10 mg/kg cilostazol, group 4 (n=5) was treated with 0.05 mg/kg intravenous nimodipine, and group 5 (n=5) served as the vehicle group and treated with a mixture of dimethyl sulfoxide and phosphate buffer solution. Basilar arteries were removed from the brain stems and analyzed. The vessels were measured using computer-assisted morphometry (SPOT for Windows Version 4.1). Statistical comparisons were performed using the Kruskall-Wallis and Mann-Whitney U tests.

**RESULTS:** Basilar artery wall thicknesses in group 3 and 4 were smaller than the group 2 and this was statistically significant at $p<0.05$. The mean arterial cross-sectional areas in group 3 and 4 were higher than group 2 and this was also statistically significant at $p<0.05$.

**CONCLUSION:** Our results demonstrate that intravenous administration of both cilostazol and nimodipine significantly attenuates cerebral vasospasm after SAH.

**KEYWORDS:** Cilostazol, Nimodipine, Subarachnoid hemorrhage, Cerebral vasospasm, Basilar artery

### ÖZ

**AMAÇ:** Bu çalışmada, biz subaraknoid kanamada intravenöz olarak uygulanan cilostazolün etkisini incelemeyi ve yine bu ilacın etkilerini intravenöz olarak kullanılan nimodipin ile karşılaştırmayı amaçladık.

**YÖNTEM ve GEREÇ:** 25 adet erkek Yeni Zelanda beyaz tavşanı kullanıldı ve hayvanlar 5 gruba ayrıldı. Grup 1 (n=5) kontrol, grup 2 (n=5) sadece SAK grubu, grup 3 (n=5) SAK sonrası intravenöz 10 mg/kg cilostazol uygulanan grup ve grup 4 (n=5) SAK sonrası 0.05 mg/kg intravenöz nimodipin alan grup ve grup 5 (n=5) dimetil sülfoksit ve fosfat buffer solüsyonu karışımı uygulanan taşıyıcı grup olarak belirlendi. Baziller arterler beyin sapından ayrılarak analiz edildi. Damar ölçümleri bilgisayar programı kullanılarak (Windows versiyon 4.1 SPOT) yapıldı. İstatistiksel analizde Kruskall-Wallis ve Mann-Whitney U testleri kullanıldı.

**BULGULAR:** Baziller arter damar kalınlığı grup 3 ve 4'te grup 2'ye göre daha küçük olarak bulundu ve bu istatistiksel olarak anlamlı idi $p<0.05$. Ortalama damar alanı ise grup 3 ve 4'te grup 2'den daha yüksek bulundu ve bu da istatistiksel olarak anlamlı idi $p<0.05$.

**SONUÇ:** Elde edilen sonuçlara bakıldığında hem cilostazolün hemde nimodipin'in intravenöz kullanımı, SAK sonrasında gelişen serebral vazospazm da etkili bulundu.

**ANAHTAR SÖZCÜKLER:** Cilostazol, Nimodipine, Subaraknoid kanama, Serebral vazospazm, Baziller arter

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## INTRODUCTION

Despite its clinical significance, cerebral vasospasm is still an important clinical entity and its exact pathogenesis is unknown. Morbidity and mortality of cerebral vasospasm after subarachnoid hemorrhage is still high (1,6,13,21). The amount of subarachnoid blood is associated with the development of vasospasm and it leads to an inflammatory response during the first 48 hour (7). The treatment modalities for cerebral vasospasm are aimed to improve cerebral blood flow by dilatation of effected vessels. Studies on prevention and reversal of cerebral vasospasm are focused on various therapeutic agents.

Cilostazol is a potent inhibitor of phosphodiesterase 3. It relaxes vascular smooth muscles with this effect and causes vasodilatation. It is also used for the treatment of ischemic neurological deficits and for the prevention from the recurrent cerebral infarction (12).

Our aim in this study was to investigate the effects of intravenous usage of selective phosphodiesterase 3 inhibitor cilostazol on cerebral vasospasm after SAH and compare these effects with the intravenous administration of nimodipine.

## MATERIALS and METHOD

### Animal Model

The experimental protocol was approved by the Hacettepe University Animal Research Committee. Twenty-five male New Zealand White rabbits weighing 2500-3000 were assigned randomly to 1 of 5 groups. The intravenous (iv) form of cilostazol (Pletal, Abdi Ibrahim, Turkey) was prepared by using dimethyl sulfoxide and phosphate buffer solution. Animals in group 1 (n=5) served as controls, group 2 (n=5) was the SAH only group, group 3 (n=5) was treated with an intravenous 30 mg/kg cilostazol (1) 3 times at 12 hours, 24 hours and 36 hours after the SAH induction, group 4 (n=5) was treated with an 0.05 mg/kg intravenous nimodipine (8) (Nimotop, Bayer, Germany) for 6 times at 6,12,18, 24, 30 and 36 hours, and group 5 (n=5) served as a vehicle group and treated with a mixture of dimethyl sulfoxide and phosphate buffer solution after SAH induction. All procedures were performed by 2 investigators who were not blinded to the treatment group during surgery and euthanasia. Vascular measurements were performed in a blinded fashion.

### Induction of Experimental SAH

All animals subjected to experimental SAH were anesthetized by intramuscular injection of a mixture of ketamine 50 mg/kg (Ketalar, Parke-Davis, Eczacibasi, Istanbul, Turkey) and xylazine 10 mg/kg (Rompun, Bayer, Istanbul, Turkey), and all animals breathed spontaneously throughout the procedures. A 23-gauge butterfly needle was inserted into the cisterna magna after exposing of the atlanto-occipital membrane with a small incision at the occipitocervical junction. After withdrawal of 1.0 mL of CSF, 1 mL volume of nonheparinized autologous blood from the central ear artery was injected into the cisterna magna over 2 minutes. The animals were then placed in a head-down position at 30° for 30 minutes to hold the blood in the basal cisterns. Arterial blood gases were analyzed during the surgical procedure and maintained within the physiological range. After recovering from anesthesia, the rabbits were observed for possible neurological deficits and then returned to the vivarium.

### Perfusion – Fixation

All animals subjected to experimental SAH were euthanized by perfusion-fixation 48 hours after SAH induction. The animals were anesthetized as described above. The ear artery was catheterized for monitoring blood pressure and for blood gas analysis. When satisfactory respiratory parameters were obtained, a thoracotomy was performed, the left ventricle cannulated, the right atrium opened widely, and the abdominal aorta clamped. After perfusion of a flushing solution (Hanks' balanced salt solution [Sigma Chemical Co], pH 7.4. at 37°C, 300 mL), a fixative was perfused (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. Animals in the control group were killed using the same procedure. The brains were then removed and stored in formaldehyde fixation solution at 4°C overnight.

### Morphometric Analysis of the Basilar Artery

Basilar arteries were removed from the brain stems and arterial segments from the proximal third of the artery were dissected for analysis. The vessels were embedded in epoxy resin, and cross-sections were cut at a thickness of 0.5 $\mu$m. The sections were mounted onto glass slides and stained with hematoxylin 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 and arterial wall thickness were taken by an investigator who was blinded to the identity of the group to which the

animals belonged. Five cross-sections of each vessel were selected randomly for measurement, and the average of these measurements were calculated.

**Statistical Analysis**

Data are expressed as mean ± SD. Statistical comparisons were performed using a Kruskall-Wallis and Mann-Whitney U tests. Statistical significance was accepted at p 0.05.

## RESULTS

The value of the basilar artery wall thickness was 48.4 ± 2.70 μm in the control group (group 1) and 73.0 ± 7.41 μm in the SAH group (group 2). The two treatment groups after SAH induction, SAH+cilostazol (group 3) and SAH+nimodipine (group 4), had average values of 49.0 ± 11.4 μm and 49.6 ± 9.98 μm respectively. For the vehicle group (group 5), the average value was 71.0 ± 1.87 μm (Figure 1).

The mean cross-sectional areas were 115823.8 ± 18048.14 μm2 in group 1, 10491.00 ± 3652.47 μm2 in group 2, 38487.00 ± 14437.02 μm2 in group 3, 36735.00 ± 9973.57 μm2 in group 4 and 10467.6± 2726.73 μm2 in the vehicle group (Figure 2).

Mean basilar artery cross-sectional areas (CSA) and arterial wall thickness (AWT) values are provided in (Table I).

Basilar artery wall thicknesses in group 3 and 4 were smaller than for group 2 and this was statistically significant at p<0.05. The mean arterial cross-sectional areas in group 3 and 4 were higher than in group 2 and this was also statistically significant at p<0.05.

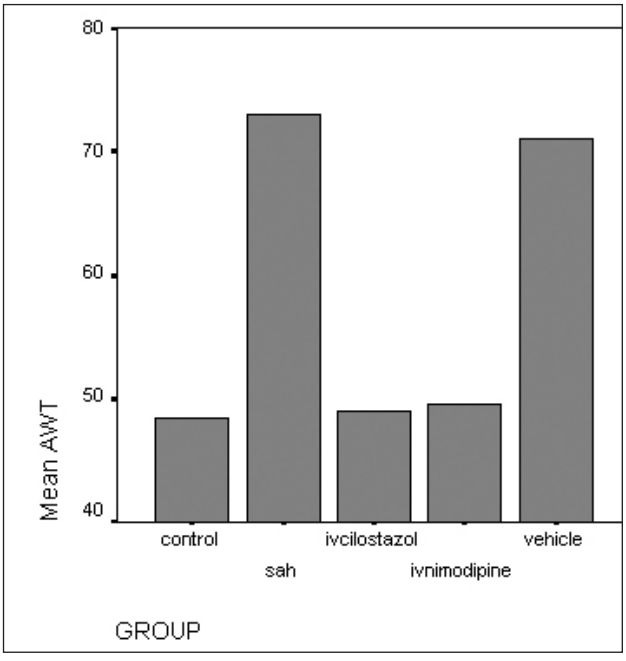


***Figure 1:*** *Mean basilar artery wall thicknesses.*

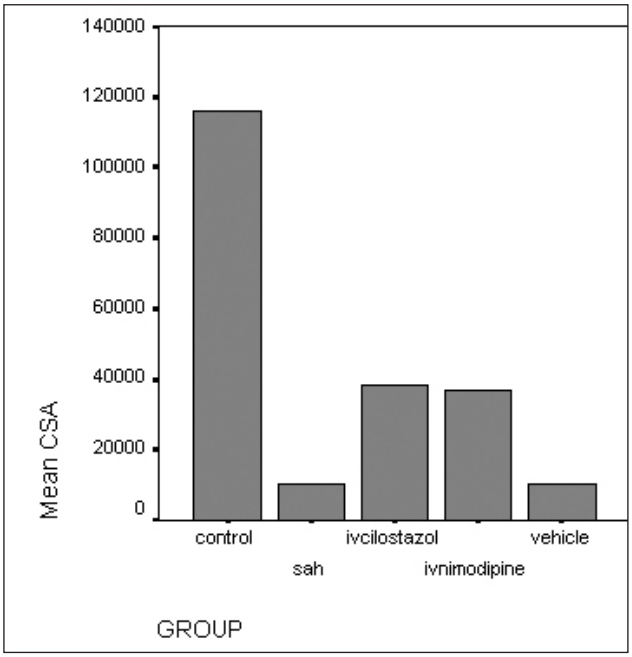


***Figure 2:*** *Mean basilar artery cross-sectional areas.*

**Table I:** Mean Basilar Artery Cross-Sectional Area (CSA) and Arterial Wall Thickness (AWT) Values

| Group | CSA (μm2) | AWT (μm) |
|---|---|---|
| Control | 115823±18048.1 | 48.4±2.70 |
| SAH | 10491.00±3652.4 | 73.0±7.41 |
| SAH + Iv cilostazol | 38487.00±14437.03 | 49.0±11.40 |
| SAH + Iv nimodipine | 36735.00±9973.5 | 49.6±9.98 |
| SAH + Vehicle | 10467.6±2726.73 | 71.0±1.87 |

Results are expressed as mean ± SD.

A subarachnoid clot over the basal surface of the brain stem was seen in histopathological examination in animals that were subjected to SAH. When compared with the control group, narrowing at the diameter of arteries with folding and corrugation of lamina elastica, accumulation of red and inflammatory cells around the outer adventitia and the vacuolization of the tunica media were seen at animals that were subjected to SAH. (Figure 3) represents the cross-sectional areas of basilar arteries of different groups.

Physiological parameters of the rabbits revealed no significant changes in body weight, arterial blood gas values and blood pressures among the five groups (Table II).

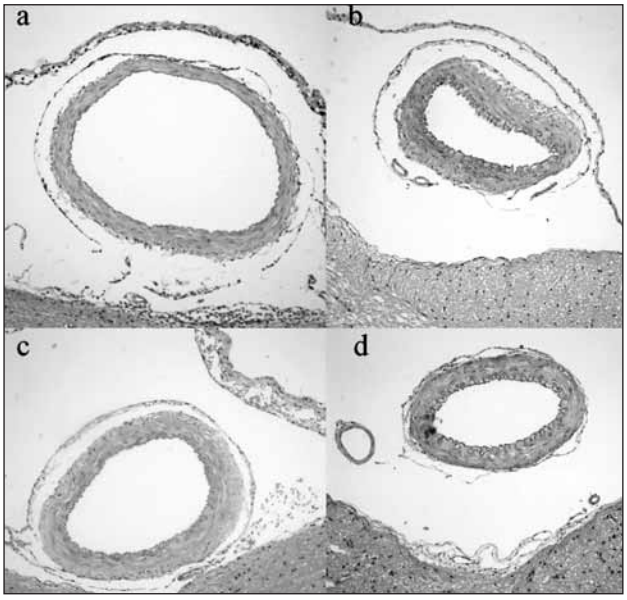


***Figure 3:*** *Basilar artery cross-sectional areas and arterial wall thicknesses are shown in different groups.* ***A****: Control group;* ***B****: SAH;* ***C****: SAH + iv cilostazol;* ***D****: SAH + iv nimodipine (at 20x magnification)*

## DISCUSSION

Cerebral vasospasm is an important clinical problem and its pathophysiology is still not fully understood. The number of patients who develop symptomatic vasospasm is between 20-30% while radiographic vasospasm can be seen in 70% of patients without any clinical consequences (13). The major problem produced by cerebral vasospasm is ischemic neurological deficit and the treatment strategies are focused on these parameters.

The pathogenesis of cerebral vasospasm is complex, multi-factorial and not completely elucidated. One of the major possible mechanisms of cerebral vasospasm depends on nitric oxide (NO) metabolism. NO is a potent vasodilator and has an important role in the development of cerebral vasospasm. Its main effect is the relaxation of vascular smooth muscle cells. The activation of soluble guanylyl cyclase by nitric oxide results in dephosphorylation of myosin light chains, activation of potassium channels and closure of voltage-dependent calcium channels. This reaction produces smooth muscle relaxation (21,23). Another factor that plays an important role on pathogenesis of cerebral vasospasm is bilirubin oxidation products. These products occur after free radical oxidation of bilirubin and produce BOXes. The effects of BOXes are on vascular smooth muscle cells and produce vasoconstriction (6,22). Clark et al. have shown in their studies that the concentration of BOXes in cerebrospinal fluid correlates with the clinical vasospasm in patients with SAH (6). Another potent vasoconstrictor is Endothelin-1. It has two receptor subtypes ETA and ETB. ETA receptors are found on smooth muscle cells and mediate vasoconstriction. ETB receptors are located on both endothelial cells and venous smooth muscle cells. They mediate the release of relaxing factors by acting on endothelial cells and mediate vasoconstriction by acting on venous smooth muscle cells (5,19).

**Table II:** Summary of physiological parameters of groups

| Group | MABP | pH | $pO_2$ | $pCO_2$ |
|---|---|---|---|---|
| 1 | 99±1.02 | 7.41±0.02 | 110±5.55 | 39.8±1.03 |
| 2 | 102±1.38 | 7.42±0.01 | 115±3.05 | 40.0±1.10 |
| 3 | 102±1.17 | 7.40±0.05 | 113±2.09 | 41.4±1.04 |
| 4 | 100±1.12 | 7.42±0.07 | 110±4.37 | 41.9±1.09 |
| 5 | 100±1.04 | 7.41±0.03 | 112±3.95 | 41.1±1.01 |

Results are expressed as mean ± SD

MABP: Mean Arterial Blood Pressure

Phosphodiesterase 3 is strongly expressed in platelets and vascular smooth muscle cells. This compound is responsible for the degradation of cyclic AMP (cAMP) and cyclic GMP (cGMP). These cyclic nucleotides has an important role on regulation of vascular tonus. The phosphodiesterase 3 inhibitor cilostazol increases the intracellular cAMP by blocking its hydrolysis (15). Cilostazol also lowers the intraplatelet Ca2+ and shows an antiaggregation effect on platelets and vasodilator effect on blood vessels. Tanaka et al. (25) has been reported in their experimental study that cilostazol dilates the pial arteries in cats. Birk et al. (2) reported that cilostazol dilates cerebral arteries in vitro and in another study they showed that cilostazol dilates large cerebral arteries in humans (3).

Selective phosphodiesterase (PDE) 3 inhibitor cilostazol is known as an antiplatelet, vasodilator agent and its antiplatelet effect is potentiated by prostaglandin E1 (20). Tamai et al. reported cilostazol did not prolong bleeding time besides its antiplatelet effect (24). Cilostazol also inhibits adenosine uptake. Its inhibition effect on both PDE and adenosine uptake may play a role on vascular smooth muscle relaxation and vasodilatation (17).

Kim et al. showed another inhibition mechanism of cilostazol on apoptotic death in human umbilical vein

endothelial cells (14). In their experimental study on porcine carotid artery, Kohda et al. emphasize the prevention effect of cilostazol on carotid artery thrombosis after endothelial injury (16).

Hashimoto et.al. mention that cilostazol induces nitric oxide production (10) and another effect of cilostazol about suppression of platelet/leukocyte aggregation in humans was reported by Ito et al. (11). Choi et al. reported in their studies that cilostazol has a neuroprotective effect against focal cerebral ischemia (4).

Nimodipine is a lipid soluble 1,4-dihydropyridine–derivative Ca2+ channel blocker. Its main effect is to inhibit Ca2+ influx through voltage-sensitive L-type Ca2+ channels and inhibits contractions of vascular smooth muscle (26). There are many studies on the effects of nimodipine on cerebral vasospasm (8,9,18). Our aim in this study was to investigate the effects of intravenous usage of the selective phosphodiesterase 3 inhibitor cilostazol on cerebral vasospasm after SAH and compare these effects with the intravenous administration of the calcium channel blocker nimodipine.

Our results demonstrate that intravenous administration of both cilostazol and nimodipine significantly attenuates cerebral vasospasm after SAH. When we compared the effects of these compounds, we did not find a statistically important superiority to one another. We suggest that the beneficial effects of cilostazol depend on the combination of its vasodilatory, antiapoptotic, antiinflammatory and neuroprotective effects which have previously been demonstrated. We therefore propose cilostazol as a candidate for clinical trials in the treatment of delayed cerebral vasospasm and related ischemic neurologic deficit.

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