

The Effect of Nebivolol on Subarachnoid Hemorrhage-induced Vasospasm in the Rabbit

Tavşanlarda Oluşturulan Subaraknoid Kanama Sonrası Gelişen Vazospazmda Nebivololün Etkisi

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Abstract

Objective: This study aimed to investigate the vasorelaxation effect of nebivolol on vasospasm in the rabbit model of subarachnoid hemorrhage (SAH).

Method: Single-hemorrhage model in the rabbit SAH was employed. SAH was induced in animals by cisterna magna injection of 4 mL autologous blood. Thirty-two animals were categorized into four groups: 1) control group (no SAH), 2) SAH group, 3) SAH + solvent infused group and 4) SAH + nebivolol treatment group. Forty-eight hours after SAH-induction, rabbits in group 3 and in 4 were received solvent or nebivolol, respectively. Nebivolol (0.073 mg/kg) was administered via the vertebral artery in 5 minutes. Digital subtraction angiography was performed at forty-nine hour following SAH-induced groups. The diameters of basilar arteries in four groups were measured at three points, and the average of the measurements was accepted as a consecutive result.

Results: SAH-induced rats demonstrated severe vasospasm on day 2. Angiographic vasospasm was present in group 2 (SAH only), and in 3 (SAH plus solvent). Animals in group 4 (SAH plus nebivolol) and group 1 (control), respectively, demonstrated the largest diameters of basilar arteries. Animals treated with nebivolol has reached eighty-eight percent of the value in the control group 1. There was no statistical difference between the control group and SAH plus nebivolol treatment group ($p>0.05$). However, the difference was obtained between the groups SAH plus solvent and SAH plus nebivolol treatment ($p<0.01$).

Conclusion: Vasospasm of the rat basilar arteries were significantly reversed by delivery of nebivolol directly into the constricted basilar artery. That drug used in cardiovascular disease may serve as a new treatment in the management of SAH patients.

Keywords: Nebivolol, subarachnoid hemorrhage, rabbit, vasospasm

Öz

Amaç: Günümüzde, subaraknoid kanama (SAK) sonrası ortaya çıkan vazospazm halen etkin olarak tedavi edilememektedir. β -1 reseptör antagonisti olan nebivololün, deneysel ve klinik araştırmalarda vazodilatasyon etkisi kanıtlanmıştır. Bu çalışmada, nebivololün SAK sonrası gelişen deneysel vazospazmın tedavisindeki etkinliği araştırılmıştır.

Yöntem: Çalışmada, 32 adet Yeni Zelanda türü tavşan eşit olarak 4 gruba ayrıldı: 1) Kontrol grubu, 2) SAK grubu, 3) SAK + solvent verilen grup ve 4) SAK + nebivolol tedavi grubu. İkinci, 3. ve 4. gruptaki tavşanlarda tek kanamalı SAK modeli uygulandı. Tüm deneklere dijital anjiyografi yapıldı. Üçüncü ve 4. gruptaki tavşanlarda SAK sonrası 48. saatte transfemoral yöntemle Arteria vertebralis içine yerleştirilen kateterle 1 mL solvent (nebivololün içinde çözünmesinde kullanılan solüsyon) veya 1 mL hacim içinde 0.073 mg/kg dozda nebivolol infüzyonu yapıldı. SAH uygulanan 2., 3. ve 4. grup deneklere SAK uygulamasının 49. saatinde ve 1. gruptaki deneklere dijital anjiyografi yapıldı.

Bulgular: Baziler arter ortalama çapları grup 1'de 0,64 mm, grup 2'de 0,33 mm, grup 3'te 0,37 mm ve grup 4'te 0,56 mm olarak saptandı. İkinci gruptaki deneklerde SAK sonrası 48. saatte yapılan dijital anjiyografi incelemesinde ağır vazospazm geliştirdiği saptandı. Baziler arter çapları dikkate alındığında, istatistiksel olarak grup 1 ve 4 sonuçları arasında anlamlı fark saptanmadı ($p>0,05$). Üçüncü grupta solvent verilen deneklerde ağır vazospazm oluşumu saptanmasına rağmen, dördüncü grupta nebivololün SAK'ye bağlı vazospazm gelişimini etkin olarak tedavi ettiği saptandı ($p<0,01$).

Sonuç: Bu çalışmada elde edilen sonuçlar, β -1 reseptör antagonisti olan nebivololün SAK sonrası ortaya çıkan vazospazmın tedavisinde yeni bir seçenek olabileceğini ortaya koymuştur.

Anahtar kelimeler: Nebivolol, subaraknoid kanama, tavşan, vazospazm



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Cite this article as: Güleç İ, İzgi AN. The Effect of Nebivolol on Subarachnoid Hemorrhage-induced Vasospasm in the Rabbit. Bagcilar Med Bull 2019;4(2):42-48

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Bagcilar Medical Bulletin published by Galenos Publishing House.

Introduction

The patients with cerebral vasospasm is a disturbance of the cerebral arteries following subarachnoid hemorrhage (SAH). A prolonged narrowing of the arteries encased in blood clots causes decreasing of blood flow in the distribution of arterial narrowing (1,2). Developing delayed ischemic neurological deficit (DIND) can be permanent, or transient. Nimodipine is the only agent to reverse limitedly vasoconstriction-induced cerebral vasospasm and related DIND. Nowadays, the patients suffered DIND have a poor outcome as almost 50% ratio (1).

β -blockers called as β -adrenergic blocking agents have been using in the treatment of some cardiovascular diseases. They reduce sympathetic nervous system activity through blockade of adrenergic receptor subtypes including β_1 , β_2 , and β_3 (3). Based on historical development, there are 3 different available generations of β -blockers. The first one is non-selective β -blockers (eg, propranolol, sotalol), the second one is β -1-selective ("cardioselective") (eg, metoprolol, bisoprolol, atenolol), and the third generation shows β -1-selective activity and vasorelaxant effects (eg, carvedilol and nebivolol).

Substantial evidence showed that nebivolol could have significantly vasomotor property on the vasculature in different animal species and in humans (4,5,6,7,8). Carvedilol has vasorelaxant effect mediated by α -adrenoreceptor (AR) blockade (3). The vasodilatative effect might be organ/tissue specific, and unrelated to β -1 receptor and α -AR mediated action (9,10). *In vitro* studies demonstrated that the mechanism was associated with endothelium-dependent (6,8) or endothelium-independent (7,11). Molecular studies have been investigating to identify signaling pathway networks. It was obtained that endothelial nitric oxide synthase (eNOS), β_3 -AR, reactive oxygen species (ROS), asymmetric dimethyl arginine (ADMA), ATP stimulated P2Y-receptors and platelet-derived growth factor- β (PDGF- β) could modify the cell-signaling (8,11,12,13,14).

The purpose of the current study was to investigate whether the influence of nebivolol on the vasospasm following SAH. We thought that if vasoconstriction subsequent to SAH might be ameliorated with this agent, it would be the chance of a new treatment.

Material and Methods

Animals

Thirty-two, male or female New Zealand white rabbits, weighing from 2.9-3.6 kg were used in this study.

Animals were housed at 22°C with lights on from 07:00 to 19:00 daily. All animals had free access to food and water. All surgical procedures were performed under sterile conditions at the experimental laboratory of the Department of Neurosurgery at İstanbul Faculty of Medicine.

Experimental groups

The experimental rabbit population was randomly divided into four groups: the control group (n=8), the SAH group (n=8), the SAH + solvent group (n=8) and the SAH + nebivolol (in the solvent) treatment group (n=8). The control and SAH group has not received any treatment while the last two groups received solvent or nebivolol (in the solvent) treatment, respectively.

Treatments in the last two groups were begun at 48th following SAH induction. By a catheter placed in the A.vertebralis, the solvent or nebivolol infusion was continued for 5 minutes. After fifty-five minutes break, digital subtraction angiography (DSA) was performed in SAH only and SAH plus treated groups at the 49th hour.

Preparation of stock solutions

The solvent is consist of a mixture of distilled water 80% and polyethylene glycol 20%. The stock solution of nebivolol is prepared in a solvent as 0.2 mg/mL. One mL of nebivolol in the solvent solution or 1 mL of the solvent solution were given in to via the vertebral artery and perfused at a flow rate of 0.2 mL/min.

Rabbit model of SAH

Anesthesia in the rabbit was induced with ketamine (50 mg/kg, intramuscularly) and xylazine (10 mg/kg, intramuscularly). After that, rabbits were mechanically ventilated (SW-ventilator, GE Palmer, London, England). Inspired gas was containing 21% O₂ and 79% with room air and with a tidal volume of 12-18 mL, respiratory rate of 12-14 breaths/minute. Oxygen saturation (SpO₂), blood pressure and exhaled CO₂ (EtCO₂) were monitored.

The auricular artery was catheterized (with 20 G vascular catheter), for blood pressure monitoring and arterial blood sampling. In SAH group, the atlantooccipital membrane was exposed through an occipitocervical midline incision. After the withdrawal of 1 mL CSE, 4 mL of fresh autologous arterial blood was injected into the cisterna magna. Thereafter, rabbits were placed in a head-down prone position for 15 minutes. Then, the incision was sutured and returned to the cage box.

Cerebral angiography and evaluation of vasospasm

The basilar artery was assessed by DSA in all groups. Animals were anesthetized with using the same method as described above. DSA was performed in angiography unit of Radiology Department in İstanbul Faculty of Medicine (Philips Integris V. 3000). The femoral artery was catheterized with a no. 4.0 French (Cordis, Johnson and Johnson, Florida, USA). Thereafter, continued with a no. 3.0 French of microcatheter (tracker, Boston scientific, California, USA). Under fluoroscopy control, the tip of microcatheter was placed into the left subclavian artery and 1 mL of contrast medium injected Iopamidol (Iopamiro 300 mg iodine/mL, Bracco, s.p.a, Milano; ITALY). For measurements of the basilar artery's diameter, a radio positive metal was positioned under the animal's head as a reference marker of magnification. Serial angiographic imaging was collected. The diameter of the basilar artery was measured at three points including upper, medial and lower portion and recruited average value (Figure 1).

Statistical Analysis

The data in this research was measured as the differences of mean values (arterial diameter) analyzed with one-way variance analysis (One-way ANOVA) and post-hoc Tukey test. "P" value of less than 0.05 was expressed statistically significant.

Results

Two animals (6.25%) have died from anesthesia-induced complications in an early stage. The same number of animals were added.

The diameter of the basilar arteries was 0.64 mm in group 1 (no SAH), 0.33 mm in group 2, 0.37 mm in group 3 and 0.56 mm in group 4 (Figure 2). The average decreases in basilar arteries compared to the control group was in the last three groups as 49, 43 and 13%, respectively. Effective vasospasm was produced in group 2 (SAH only) and 3 (SAH + solvent group). Regarding arterial narrowing, there was no statistical difference between group 2 and 3. The solvent solution had no effect on reversing of induced vasospasm. There was no statistical difference between group control and group SAH plus nebigivolol treatment ($p>0.05$). However, the difference was obtained between group SAH plus solvent and group SAH plus nebigivolol treatment ($p<0.001$). So, there was no doubt that impact was belonged to nebigivolol, not to the solvent solution.

Mean blood pressure (MAP) in group 4 was decreased ~15%, started forty-five minutes after infusion of nebigivolol, normalized at the third hour. Heart rate (HR) in group 4 was decreased 12%, started fifteen minutes after infusion of nebigivolol, normalized at the sixth hour. No significant changes in MAP and HR were observed in other groups. We have preferred short time infusion of nebigivolol. In our

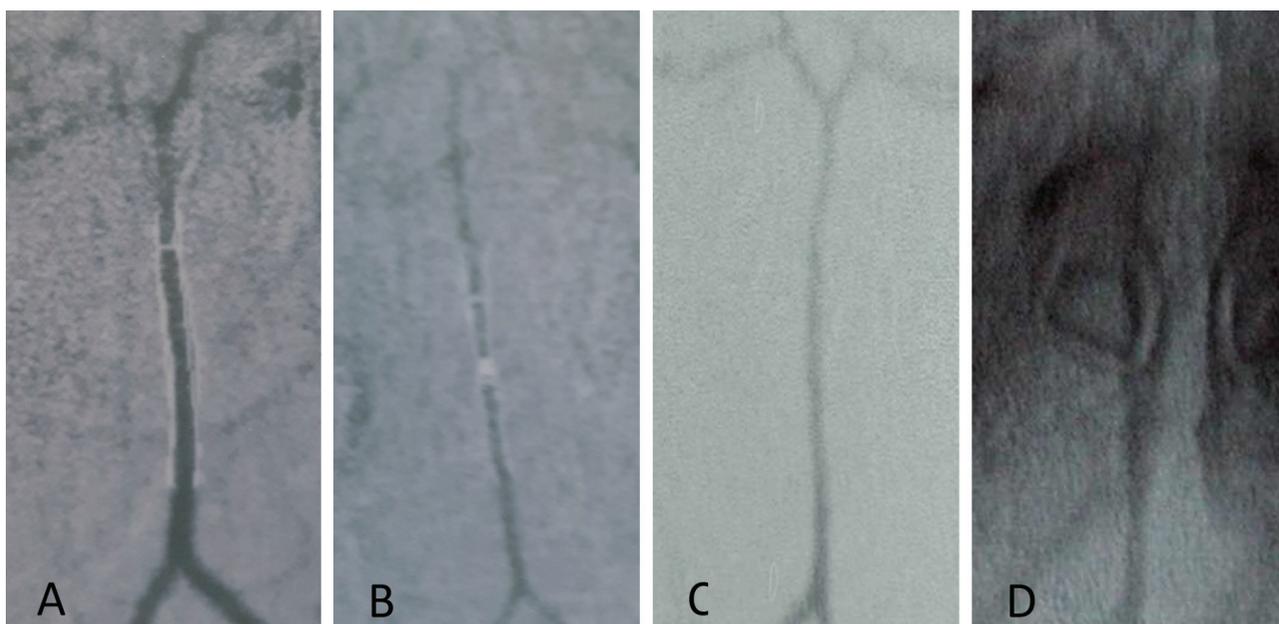


Figure 1. Digital subtraction angiography: A) Control, B) SAH, C) SAH plus the solvent, D) SAH plus nebigivolol. Right the vertebral artery injection revealed severe vasospasm in the group SAH and SAH plus the solvent, and mild vasospasm in group SAH plus nebigivolol

SAH: Subarachnoid hemorrhage

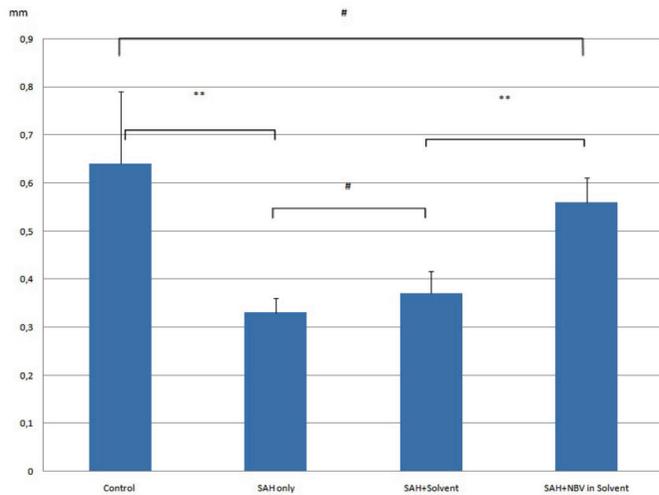


Figure 2. The diameters of the basilar arteries measured by angiography. The diameter of the basilar arteries was 0.64 mm in group 1 (no SAH), 0.33 mm in group 2, 0.37 mm in group 3 and 0.56 mm in group 4. There was no statistical difference between group control and group SAH plus nebivolol treatment ($p>0.05$). Group of SAH plus nebivolol treatment's result is significantly different from group 2 and 3 [(#non-significant ($p>0.05$), **very significant ($p<0.001$)]

experiment which prevented to occur a big issue. If infusion time is prolonged in the future, that problem needs to be resolved.

Discussion

Rats, cats, rabbits, dogs, and primates are used as the model for SAH-induced vasospasm (2). SAH is induced with different volumes of blood by using different surgical methods. The majority of animal models of SAH can create an effective degree of vasospasm. However, they did mostly not mimic pathological and pathophysiological changes seen in human (2). So, all models can provide a limited amount of data extrapolated to human. Although suitable animal for this purpose is dog and primate. Using those animal models are getting harder because of ethic and costing problems. In this experiment, we have chosen the rabbit model of SAH. This model exerts biphasic pattern of vasospasm (15) and morphological changes in constricted arteries (16) similar to observed in humans. Another advantage is to be easier for performing DSA. In our current study, we have created angiographic vasospasm in the rabbit model of SAH to test the nebivolol's effect.

From the literature review, nebivolol has an impact as the widening of constricted vasculature (4,6,9). Mechanisms of the action of nebivolol-induced vasodilation have occurred

in several pathways including the endothelial-dependent and -independent manner. The main mechanism due to the action of nebivolol is accepted by evoking NO-induced vasorelaxation. *In vitro* studies, nebivolol-induced vasodilation effect was abolished by NOS inhibitor or by removal of or damage to the endothelium (6). Its effect was could not be reversed by the α -receptor inhibitor, the cyclooxygenase inhibitor or by serotonin inhibitor. Another study showed that there was a sustained increase in eNOS expression resulted in induced NO-dependent relaxation (8). Similar results were obtained as *in vivo* studies that NOS inhibitors blocked NO-mediated venous relaxation of the forearm (4), and of forearm arterial system (5) in humans.

ATP stimulated P2Y-receptor and β 3-AR activation may be one of the underlying vasodilation by NO-induced. Exposure of cultured human endothelial cells to nebivolol and addition of external ATP to the medium results in NO release (14). Moreover, ATP produces fast-acting NO release than nebivolol's. ATP-activated P2Y receptor leads to activation of guanyl cyclase by the eNOS (14). If the P2Y receptor activity were tightly blocked by the selective antagonist, the availability of NO was significantly decreased. Therefore, it is criticized that ATP output from endothelial cells has a major role in increasing of nebivolol-induced NO (17).

Asymmetric dimethyl arginine (ADMA) is the endogenous inhibitor of NOS. Elevation of ADMA is associated with low levels of NO leading to endothelial dysfunction (18). Accumulation of ADMA cause to decrease NO amount, consequently. It might contribute to the vasospasm following SAH. Nebivolol induces the dimethyl arginine dimethyl amino hydrolase 2 (DDAH2) activity that increases protein breakdown and thereby reduce the production of ADMA (19). That pathway may be partially complementary to the occurrence of the vasoactive impact. In this regard, additional studies are needed.

Newly identified mechanism of vasorelaxant effect by nebivolol is the β 3-AR pathway. It was revealed that β 3-AR agonists have produced vasodilation in different animal species (9,10). Many studies were performed to characterize the vasorelaxant effect of β 3-AR elicited endothelial-dependent or -independent manner. In an endothelial-dependent manner, the endothelium is critically required for its vascular response. β 3-AR-induced relaxation was substantially decreased after endothelium denied which suggests that those receptors were mainly in vascular endothelium (11). Administration of the β 3-AR antagonist disrupted the enzymatic function of eNOS

activity and inhibited NO release. The endothelium-dependent effect occurs via the NOS/NO/cGMP signaling pathway, respectively. The other pathway is the endothelium-independent signaling road. In rat thoracic aorta, the source of NO generated from the endothelium-independent mechanism could not be directly related to NOS (9). Because NOS inhibitors have failed an increase of NO production while achieved with selective β 3-AR inhibitors. AR-induced relaxation of rat abdominal aorta has mostly used cyclic adenosine monophosphate (cAMP) in an independent pathway (7). Recent studies have shown that K⁺ channel-mediated vasodilatation including BKCa, KATP, and KV is activated by the β 3-AR (7,11). In human endothelium, the NO synthase - independent relaxation was completely inhibited by K⁺ channel inhibitors (11). In another study, dose-dependent relaxation effect was encountered in the canine arterial rings with intracellular high-level cAMP levels in K⁺ channel studies. Regarding the study of mechanism, the adenylate cyclase/cAMP pathway is used in endothelium-independent mechanism related β 3-AR. It should be another pathway of NO production by β 3-ARs.

Another determinant factor in the pathophysiology of vasospasm is ROS. Intracellular ROS formation causes leading to oxidative stress, cell damage, and apoptosis (20). Increased ROS levels in the brain have been shown to be increased following SAH (21). A large amount of ROS acts scavenging of NO resulted in decreased NO availability. It may cause vascular dysfunction, which is parallel to the development of cerebral vasospasm (22). In cell cultured study, it has been shown that nebivolol is highly sensitive to act as ROS scavenger (12). In addition, nebivolol and its hepatic metabolites effectively alleviated oxidative stress, markedly decreased the ROS concentration (23). In oxidative stress condition after SAH, ROS defense mechanisms including superoxide dismutase and glutathione peroxidase were activated. The existence of an increased level of both enzymes in patients with cerebral vasospasm was found (24). Nebivolol may exert anti-vasospastic effects on cerebral vasospasm obtained current study through changing of related enzyme's activity. In some cardiovascular disease, nebivolol could also substantially upregulate some ROS related genes (25). Further studies are needed.

Cerebral vasospasm involves multiple processes including inflammation, vascular proliferation, and matrix alteration (13). Promoting to vascular remodeling, cell growth and proliferation of vascular smooth muscle

cells (VSMC) result in a restricted or a permanent stopping of blood flow. Recently, it has been reported that PDGF- β , proliferating cell nuclear antigen and α -smooth muscle actin in VSMCs was increased after SAH (13). PDGF has the ability to create vasoconstriction in cerebral arteries (26). Apoptosis and proliferation induced in coronary artery VSM and endothelial cells were significantly inhibited concentration-dependently by nebivolol in human (27). The effect of nebivolol may be due to the impairing of PDGF signaling (27,28). In addition, nebivolol substantially prevented neo-intimal thickening identified after balloon-injured carotid arteries in the rat model (29). In a study, nebivolol inhibits pro-inflammatory genes expression as VCAM-1, E-selectin, MCP-1 by modulating NF- κ B dependent genes (29). In human VSMC exposed to nebivolol, changes of proinflammatory cytokines and the increase of NO was obtained (28). Therefore, it was thought that the regulation of eNOS expression by nebivolol inhibited NF- κ B. Furthermore, the elevated neutrophil-lymphocyte ratio (NLR) is accepted as an inflammation biomarker for some vascular disease such as coronary heart disease, hypertension. Nebivolol has a strong impact on reducing NLR (30).

Platelets are activated by contact with exposed collagen and aggregate together at the injured sites leading to the formation of a thrombus. The NOS is present in platelets which is a key role for aggregation inhibition. It was shown that nebivolol exerted to prevent platelet aggregation via activating NOS which caused NO increase (31). It could be hypothesized that thrombus deposition could cause low-level production of NO that might increase the degree of vasospasm.

Conclusion

The advances in molecular biology make easier to understand the pathophysiology of SAH-induced vasospasm in human. Improving experimental SAH models provides significant contributions to the multifactorial nature of the disease. However, all experimental studies have some limitations. Unfortunately, there is still no acceptable treatment method.

The results of the current study provide important data that nebivolol treated angiographic vasospasm following SAH in rabbits. In summary, nebivolol might alleviate SAH-induced cerebral vasospasm in human and may contribute improvement of patient outcomes in the future.

Ethics

Ethics Committee Approval: This study was done as a thesis almost 20 years ago. There were not ethical committees of today in those times. The subjects used in the study was obtained from İstanbul University DETAM after the thesis had been given from the lecturer; it had been approved by the department academical committee.

Informed Consent: Experimental study.

Peer-review: Externally peer-reviewed.

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