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***Experimental Study** / Deneysel Çalışma*

A comparison of the vasodilatory effects of verapamil, papaverine and nitroglycerin on isolated rat aorta

Verapamil, papaverin ve nitrogliserinin izole sıçan aortu üzerindeki vazodilatör etkilerinin karşılaştırılması

Turan Ege,[1] Ümit Halıcı,[1] Özcan Gür,[2] Selami Gürkan,[2] Demet Özkaramanlı Gür,[3] Enver Duran[1]

[1]Department of Cardiovascular Surgery, Medical Faculty of Trakya University, Edirne, Turkey
[2]Department of Cardiovascular Surgery, Medical Faculty of Namık Kemal University, Tekirdağ, Turkey
[3]Department of Cardiology, Tekirdag State Hospital, Tekirdağ, Turkey

Background: This study aims to compare the vasodilatory effects of verapamil, papaverine and nitroglycerin on rat aortic preparations in *in vitro* isolated tissue bath system and to evaluate the role of vascular endothelium on vasodilatory responses of the isolated rat aorta samples.

Methods: The thoracic aorta segments collected from 30 male Wistar rats (20 endothelialized and 20 de-endothelialized vascular rings for each drug in 2 mm wide strips, total number of 120 vascular rings) were suspended into the Krebs solution of the isolated tissue bath system. Phenylephrine was used to induce isometric contraction and tissue samples were treated with verapamil, papaverine and nitroglycerin separately to draw concentration-response curves of isometric vasodilatory responses. This procedure was repeated for de-endothelialized aorta samples.

Results: Papaverine and verapamil induced vasodilatatory responses starting from the concentration of 10^{-8} M and reached its maximum at concentration of 10^{-3} M, while nitroglycerin induced vasodilation at lower concentrations starting from a concentration of 10^{-12} M, reaching its maximum at 10^{-6} M. Nitroglycerin was the most potent agent, followed by verapamil and papaverine. Efficacy analysis revealed that the most efficient agents were papaverine (140±6.7%), nitroglycerin (110.8±1.35%) and verapamil (99±4.14%), respectively. The results were similar in aorta samples without endothelium ($p\geq0.05$, F test).

Conclusion: In this study examining isolated rat aorta, nitroglycerin was the most potent agent, while papaverine was the most efficient agent. Our study results showed that endothelium played no role in vasodilatation responses of these drugs.

Key words: Aorta; nitroglycerin; papaverine; vasodilatation; verapamil.

Amaç: Bu çalışmada verapamil, papaverin ve nitrogliserinin sıçan aort preparatlarında oluşturduğu vazodilatör etkileri *in vitro* izole doku banyosu sistemi ile karşılaştırıldı ve izole sıçan aortu örneklerinde vasküler endotelin vazodilatör yanıtları üzerindeki rolü değerlendirildi.

Çalışma planı: Otuz adet Wistar tipi erkek sıçandan alınan torasik aort segmentleri (Her ilaç için 20 endotelli 20 endotelsiz olmak üzere; 2 mm genişliğinde toplam 120 vasküler halka) içinde Krebs solüsyonu bulunan izole organ banyosuna asıldı. Doku örnekleri fenilefrin ile izometrik kasılma sağlandıktan sonra verapamil, papaverin ve nitrogliserinin her biri ile ayrı ayrı izometrik gevşeme yanıtları konsantrasyon-yanıt eğrisi oluşturacak şekilde kaydedildi. Bu işlem endotelsiz doku preparatlarında da tekrarlandı.

Bulgular: Papaverin ve verapamilin 10^{-8} M konsantrasyondan itibaren gevşeme yanıtı oluşturmaya başladığı ve 10^{-3} M konsantrasyonda maksimuma çıktığı, nitrogliserinin ise daha düşük konsantrasyonda 10^{-12} M'de etki göstermeye başladığı ve maksimum etkisini 10^{-6} M konsantrasyonda gösterdiği saptandı. Nitrogliserin en potent ilaç iken, verapamil ile papaverin bunu takip etmekteydi. Etkinlik yönünden değerlendirildiğinde ise, en etkili ilaçlar sırasıyla papaverin (%140.5±6.7), nitrogliserin (%110.8±1.35) ve verapamil (%99±4.14) idi. Endotelsiz aort preperatlarında yanıtlar benzerdi ($p\geq0.05$, F test).

Sonuç: İzole sıçan aortunda yapılan çalışmada potensi en yüksek olan ilaç nitrogliserin, etkinlik yönünden en güçlü ilaç ise papaverin idi. Çalışma bulgularımız endotelin bu ilaçların oluşturduğu vazodilatasyon yanıtında rol oynamadığını gösterdi.

Anahtar sözcükler: Aort; nitrogliserin; papaverin; vazodilatasyon; verapamil.

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Correspondence: Özcan Gür, M.D. Namık Kemal Üniversitesi Tıp Fakültesi Kalp ve Damar Cerrahisi Anabilim Dalı, 59100 Tekirdağ, Turkey.
Tel: +90 282 - 250 52 49 e-mail: ozcangur@hotmail.com

The aorta is a pulsatile structure which equalizes cardiac ejection and permits continuous blood flow to the peripheral organs through arterial-ventricular coupling. The tone of the aorta is adjusted not only by the elastic and muscular layer but also by the endothelial cells that secrete various hormones. In addition, the endothelium serves as a barrier and preserves the muscular layer from contact with various vasoactive agents.[1-5]

Various vasoactive agents, such as papaverine, verapamil, and nitroglycerin are used to control blood pressure and vascular tone in hemodynamic perturbations.[6-12] Verapamil, a calcium (Ca) channel blocker, papaverine, a phosphodiesterase inhibitor, and nitroglycerin, an activator of guanylate cyclase, are widely used both topically to relieve free arterial graft spasms and systemically to decrease blood pressure and heart rates (with verapamil) in the perioperative period.

In this study, we aimed to study the potency and efficacy of verapamil, papaverine, and nitroglycerin and assess the role of the vascular endothelium in the vasodilatation of isolated rat aortae.

MATERIALS AND METHODS

After obtaining the approval of the local ethics committee, 30 male Wistar rats were anesthetized with ether and then euthanized. After their deaths, a maximum length of the thoracic aorta in each of the rats was isolated and removed. The dissected vessel was immediately placed in Krebs solution [pH 7.4, 95% oxygen (O_2) and 5% carbon dioxide (CO_2), containing 122 mM sodium chloride (NaCl), 5 mM potassium chloride (KCl), 25.0 mM sodium bicarbonate ($NaHCO_3$), 1.2 mM magnesium sulfate ($MgSO_4$), 11.5 mM glucose, and 1.25 mM calcium chloride ($CaCl_2$)].

After excision of the aorta from the adhering connective tissue and fat, two pairs of 2 mm wide aortic samples from the proximal ascending aorta were prepared (a total number of four aortic samples from each rat). One pair of rings was kept intact, but the other was de-endothelialized by simply rubbing the endothelial layer with a cotton bud.

The vascular rings were then suspended in a classic tissue bath system on steel hooks attached to an FDT 10-A force displacement transducer (COMMAT Ltd., Ankara, Turkey). As suggested in the literature, the tissue samples were first entrained and adapted to the tissue bath system to reach a level of equilibrium. This procedure was mandatory in order to provide reproducible and correct outcomes. The tissue samples were treated with KCl to depolarize the rings and cause constriction. This was done both to test the viability of the tissue and determine the optimal tension under which the vascular rings could be suspended during the experiments. The KCl was then washed thoroughly, and the rings were suspended under the determined preload for the next 60 minutes to reach equilibrium.

During this period, the Krebs solution in the tissue bath reservoir was continuously oxygenated with 95% O_2 and 5% CO_2 at 37 °C and changed every 20 minutes to keep the tissues alive.

After 60 minutes, 10^{-6} M of phenylephrine HCl (Merck, İstanbul, Turkey) was added to the tissue bath system to induce submaximal vasoconstriction of the vascular rings so that any added vasodilatory effect of the drug to be added could be elucidated. The tissues were treated with one of the study drugs only after the aforementioned procedure was completed.

Nitroglycerin (Perlinganit ampule 10 mg / 10 ml, Adeka, İstanbul, Turkey) was added to the tissue bath system cumulatively starting with a concentration of 10^{-12} M, and this was increased by half logarithmic increments every two minutes until a concentration of 10^{-6} M was reached. In contrast, verapamil (Isoptin ampule 5 mg / 2 ml, Abbott Laboratories, İstanbul, Turkey) and papaverine (Papaverine HCl amp 0.5 gr, Biofarma Pharmaceutical Industry Co. Inc., İstanbul, Turkey) were added to the system cumulatively starting with a concentration of 10^{-8} M, and the amounts were increased by half logarithmic increments every two minutes until achieving a concentration of 10^{-3} M. The dose-response data was obtained via a TDA-97 transducer data acquisition system (COMMAT Ltd., Ankara, Turkey) and recorded using the Polwin 97 software (Commat Ltd., Ankara, Turkey), which was also used to construct the dose-response curves. The responses were calculated as percentages of maximal vasodilatation.

This procedure was repeated for each drug in both the intact and de-endothelialized vascular rings for each aortic specimen. Hence, two intact and two de-endothelialized tissues were studied for each of the aortic specimens obtained from the rats. A total of 120 vascular rings (40 tissue samples for each drug) were studied, half of which had an endothelium (n=20) and half did not (n=20).

Statistical analysis

The responses obtained from the endothelialized and de-endothelialized aorta segments treated with

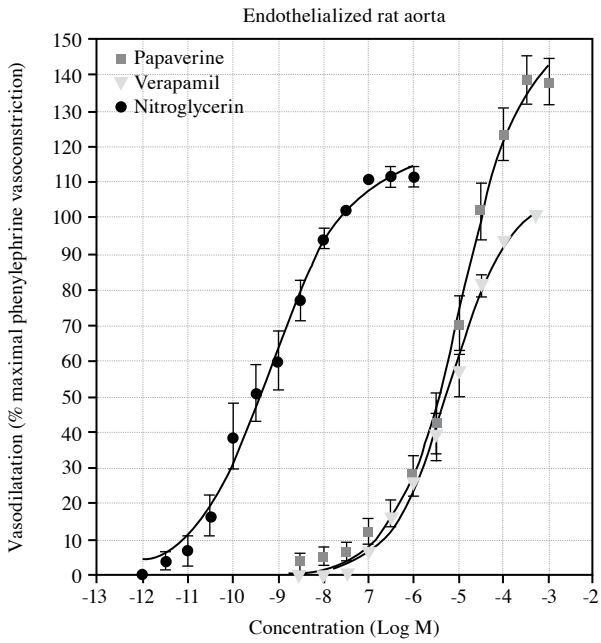

Figure 1. Concentration-vasodilatation curve in the endothelialized rat aortae.

the three drugs were compared using the Graphpad Prism version 4.00 for Windows software (Graphpad Software Inc., La Jolla, CA, USA). The dose-response curves obtained with each drug were constructed by non-linear regression, and comparisons between the curves for the parameters of log(EC50) and maximum relaxion (Emax) were performed by the extra sum of squares on an F-test. A value of $p<0.05$ represented the cut-off point for significance.

RESULTS

The papaverine and varapamil had similar vasodilatation responses which started from concentrations of 10^{-8} M and reached their peak at concentrations of 10^{-3} M. Nitroglycerin, on the other hand, induced vasodilatation at lower concentrations that began at a concentration of 10^{-12} M and reached their maximum at 10^{-6} M (Figure 1).

Nitroglycerin was the most potent of the three drugs in terms of their log(EC50) values followed by verapamil and papaverine, respectively, and papaverine (140.5±6.7%) had the highest efficacy followed by nitroglycerin (110.8±1.4%) and verapamil (99±4.1%). The results were similar for the aorta samples without the endothelium ($p\geq0.05$; F-test) (Figure 2).

Figure 3 shows a comparison of the effectiveness of the three drugs with regard to the endothelialized and de-endothelialized rat aortae.

DISCUSSION

We also evaluated whether the presence or absence of an endothelium had any effect on the action of these drugs and found that it did not.

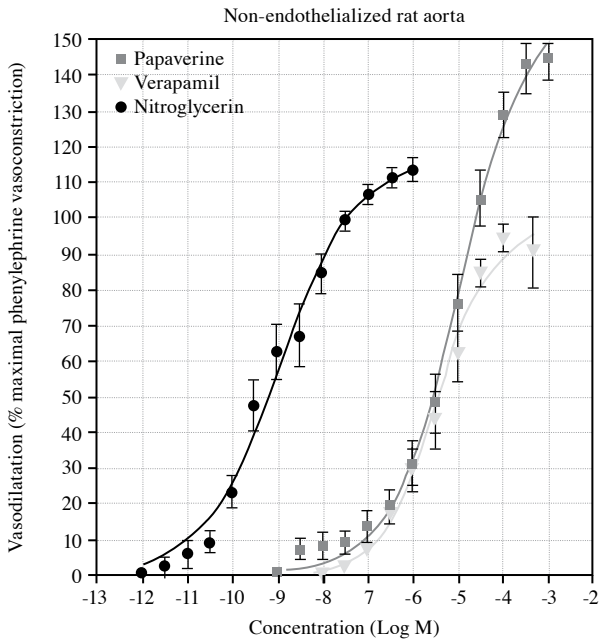

Figure 2. Concentration-vasodilatation curve in non-endothelialized rat aortae.

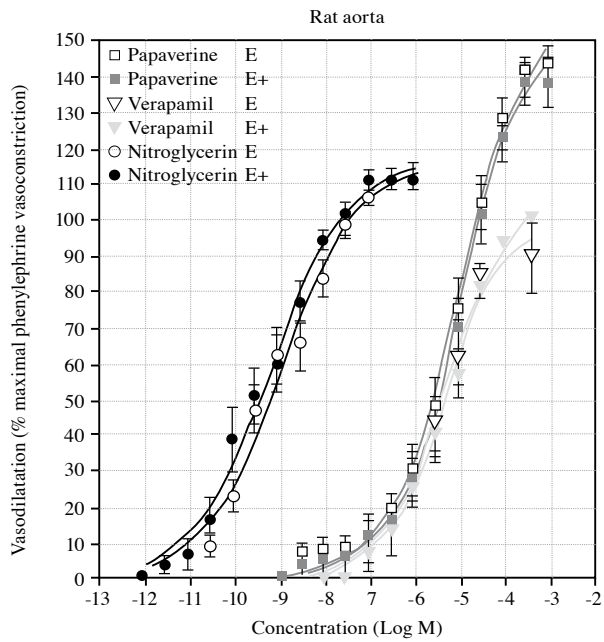

Figure 3. Concentration-vasodilatation curve in endothelialized and non-endothelialized rat aortae.

Zhou et al.[13] reported that nitroglycerin causes vasodilatation between concentrations of 10^{-8} M and 10^{-6} M. In our study, we determined that the vasodilatation response of nitroglycerin started at a concentration of 10^{-12} M, with a maximal response at 10^{-6} M. Therefore, our data indicates that even at very low doses, nitroglycerin can still cause vasodilatation, which takes place when the cyclic guanosine monophosphate (cGMP) levels increase in the smooth muscle cells.[11,14,15] Rikitake et al.[16] showed that there is no difference in the vasodilatation response between endothelialized and de-endothelialized isolated rabbit aortae when they are induced by nitrogylcerin, and our results using a similar vasodilatation process were in concordance with their study.

Papaverine, a phosphodiesterase III inhibitor, is used widely for relieving graft spasms and is commonly prescribed for patients who undergo coronary artery bypass graft (CABG) surgery to relieve the spasms of the internal mammary artery (IMA).[7,8,10,11] Endothelial injuries caused by the low pH of papaverine are known to have a negative influence on vasodilatation responses. Gao et al.[10] reported that the use of papaverine on IMA grafts increased apoptosis and caused a deterioration in graft functions. In addition, Yoshimura et al.,[17] and Gao et al.[9] found that high concentrations of papaverine ($>10^{-2}$ M) caused a deterioration in endothelial and smooth muscle functions. In our study, the most pronounced vasodilatation caused by papaverine was at a concentration of 10^{-2} M. When all of the information regarding papaverine, including our own data, is examined, it can be concluded that papaverine doses that cause maximal vasodilatation also are the ones that are the most harmful. In addition, the fact that the response to papaverine was similar in both the endothelialized and de-endothelialized aorta segments in our study indicates that the endothelium has a minor role in this process.

Verapamil, a voltage-dependent Ca channel blocker, is preferred for the treatment of angina pectoris and hypertension[18-20] and can be used in combination with other vasodilator drugs.[11,12] In our study, the vasodilatation caused by verapamil was less pronounced than that of the other two drugs. Additionally, since the plasma half-life of verapamil is longer than the other drugs, it can be used when a longer duration of vasodilatation is needed, but it should be kept in mind that it will occur at a lower rate.

Conclusion

We studied in an experimental tissue bath system by using thoracic rat aortae and determined that nitroglycerin was more potent than verapamil and papaverine with regard to the log(EC50) values. However, we determined that papaverine was more efficacious than either nitroglycerin or verapamil. Furthermore, we showed that the vascular endothelium did not influence the vasodilatation response of these drugs.

Declaration of conflicting interests

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