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Vasorelaxant Effects of Sildenafil and Verapamil on Isolated Rat Aorta with and without Intact Endothelium

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Abstract: This study was designed to compare vasorelaxant effects of the sildenafil and verapamil on isolated rat aorta. Endothelium intact and denuded aortic rings were suspended in organ chambers. Sildenafil (10^{-10} to 10^{-4} M) induced a dose-dependent vasodilation of phenylephrine precontracted aortic rings. Relaxation of endothelium intact and denuded aortic rings caused by 10^{-4} M sildenafil was about 96 and 79%, respectively. Verapamil (10^{-10} to 10^{-4} M) induced a dose-dependent vasodilation of phenylephrine precontracted aortic rings. Relaxation of endothelium intact and denuded aortic rings caused by 10^{-4} M verapamil was about 99 and 98%, respectively. In the phenylephrine precontracted aortic rings, pD2 values for sildenafil were 4.93±0.59 and 4.11±0.62 in the presence and absence of endothelium, respectively. The pD2 values for verapamil were not different in the presence and absence of endothelium (5.15±1.05 vs 4.96±1.14). Verapamil and sildenafil showed a similar degree of vasorelaxant effect in the intact aortic rings, although there was a significant difference in the degree of relaxation in the absence of endothelium (98 vs 79%). Sildenafil induced both endothelium-dependent and independent vasorelaxation on the aortic rings. Although, there was no significant difference in the degree of relaxation induced by verapamil and sildenafil in aortic rings with intact endothelium, verapamil has more relaxing effect in the denuded aortic rings.

Key words: Sildenafil, isolated organ bath, rat aorta, endothelium, verapamil

INTRODUCTION

In human corpus cavernosum, the release of the Nitric Oxide (NO) from the non-adrenergic, non-cholinergic nerves and/or the endothelium activates guanylyl cyclase and increases intracellular cyclic guanosine monophosphate (cGMP) levels[1,2]. Owing to its effects on the NO-cGMP pathway, sildenafil has been evaluated as an antianginal therapy in the early phases of its clinical development. Sildenafil, which is the first orally active therapy for erectile dysfunction, enhances the increase of cGMP levels by phosphodiesterase type-5 (PDE-5) inhibition in corpus cavernosum smooth muscle cells. The increase in intracellular cGMP modulates intracellular calcium and in turn regulates smooth muscle contractility and erectile function[3]. In addition to its high concentration in the corpora cavernosa, PDE type 5 is abundant in vascular and visceral smooth muscles[4]. It has been well documented with vascular preparations that sildenafil causes vasodilation *in vitro*[4-6]. Although PDE-5 occurs throughout the systemic vasculature, other PDEs appear to play a greater role in regulating the breakdown of cGMP in the vascular smooth muscle cells that mediate blood pressure effects[7]. Thus, in studies healthy subjects, single doses of sildenafil (25-50 mg) produced mean maximum decreases in systolic and diastolic blood pressures of 8 and 6 mmHg, respectively[8]. All these studies were performed on the intact endothelium and the exact mechanism has not been delineated. The vasodilatory effects of sildenafil have been most commonly attributed to endothelial dependent generation of NO and subsequent relaxation of the vascular smooth muscle. However, a recent study[9] suggested that high concentrations of sildenafil had additional vasorelaxant effect(s) such as Ca^{2+}-channel antagonistic-like effect. In the light of these findings, this study was designed to compare the vasorelaxant effects of sildenafil and verapamil on the isolated rat aorta with intact endothelium and denuded endothelium and further discuss its mechanism of action *in vitro*.

MATERIALS AND METHODS

This study was approved by the Medical Research Ethics Committee of Yüzüncü Yıl University Research Hospital and conducted in 2002. Twelve rats, weighing

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150 to 250 g, were used for the experiment. The rats were anesthetized with pentobarbital sodium, 40 mg kg^{-1}. A segment of thoracic aorta was removed immediately following respiratory arrest through midsternotomy. Thoracic aorta was dissected from the connective tissue and cut into rings approximately 3 mm wide. Precaution was taken not to damage the endothelium. Two aortic rings (one intact and one denuded) were obtained from each animal. Both were tested under the same conditions for comparison. The deendothelization was performed by gently rubbing the intimal surface with forceps. Ring preparations were mounted between two stainless steel triangles in an organ chamber containing 15 mL Krebs solution (37°C, pH of 7.4) aerated with 95% O_2, 5% CO_2. The solution consisted of 118 mM NaCl, 0.8 mM KCL, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, 1.18 mM KH_2PO_4, 1.19 mM $MgSO_4$, 11 mM D-glucose. The upper end of each ring was attached to an isometric force transducer, which was linked to an amplifier and a computerized chart recorder (MAY COMMAT, TDA 97, Polygraph Systems, FDT10-A) for recording the isometric responses[10]. Preparations were allowed to equilibrate for 60 min in Krebs solution. During this period the organ baths were washed with fresh (37°C) buffer solution every 15 min. The initial resting tension of each ring was set at 2 g. Prior to the beginning of the experiments, in order to confirm the presence or successful denudation of endothelium, the rings were precontracted with 3×10^{-6} M phenylephrine and challenged with acetylcholine (10^{-5} M). Relaxations greater than 50% of maximal relaxation evoked by acetylcholine (maximal relaxation represented complete return to the resting tension from the contraction in response to phenylephrine) indicated an intact endothelium. Once the presence of a functional endothelium had been confirmed, baseline conditions were reestablished by washing the tissues in Krebs solution.

Following stabilization, the submaximal precontraction of the vessels was randomly induced by either KCl (3×10^{-2} M) or phenylephrine (3×10^{-6} M)[10].

The rings were washed until complete recovery of the resting tension was obtained. After equilibration, the submaximal precontraction of the vessels was again elicited by KCl (3×10^{-2} M) or phenylephrine (3×10^{-6} M). When the contraction reached to plateau, cumulative concentration response curves of sildenafil and verapamil were determined in the same manner. In the aortic rings with endothelium, when the influences of N^G-nitro-L-arginine methyl ester (L-NAME) on the sildenafil-induced relaxation were evaluated, L-NAME (10^{-2} M) and indomethacin (10 μM) were added to the organ chamber 20 min before addition of phenylephrine.

Data were analyzed by a computer (Polwin 97, MAY). pD_2 represents negative logarithm of the concentration causing 50% inhibition of the maximum contraction. All values are expressed as the mean±SEM. The relaxations are expressed as the percentage decrease in tension from the phenylephrine or KCl precontraction. Statistical differences between two means were determined by Student's t-tests. Statistical comparisons were performed using analysis of variance for repeated measures, followed by Student-Newman-Keuls post hoc testing for multiple comparisons. The difference was considered statistically significant when $p<0.05$.

RESULTS

The vasorelaxant effects of sildenafil and verapamil in isolated aortic rings with and without intact endothelium are shown in Fig. 1 and Table 1. In the phenylephrine precontracted aortic rings with endothelium, sildenafil (10^{-10} to 10^{-4}M) and verapamil (10^{-10} to 10^{-4}M) induced a similar degree of dose-dependent vasodilation; about 96 and 99% relaxations were obtained at a concentration of 10^{-4}M sildenafil and verapamil respectively (Table 2). However, verapamil caused higher endothelium independent relaxation of denuded aortic rings precontracted with phenylephrine than sildenafil (98 vs 79%, $p<0.01$).

In the aortic rings with endothelium denuded, the magnitude of relaxation was significantly less in KCl-precontracted (66%) rings than that in phenylephrine-precontracted (79%) arteries at the same doses of sildenafil ($p<0.05$). However, verapamil induced a similar degree of dose dependent vasodilation (99%) in KCl-precontracted rings. Vasodilatation induced by verapamil was significantly higher in the endothelium-denuded rings compared to that induced by sildenafil ($p<0.01$). However,

Table 1: Vasorelaxant effects of the sildenafil and verapamil on phenylephrine or KCl-precontracted rat aorta with and without endothelium

	Endothelium intact		Endothelium denuded	
pD_2 values	Phe-precontracted	KCl-precontracted	Phe-precontracted	KCl-precontracted
Sildenafil	4.93±0.59	4.86±1.22†	4.11±0.62	4.02±1.67
Verapamil	5.15±1.05	5.08±0.82	4.96±1.14*	5.02±0.96*

Data are presented as mean±SEM of 12 individual rings, † $p<0.01$, Intact endothelium versus denuded endothelium in KCl-precontracted rings phenylephrine-precontracted, * $p<0.01$, verapamil versus sildenafil

Table 2: Vasorelaxations induced by sildenafil and verapamil at various concentrations in phenylephrine-precontracted isolated aortic rings

	Endothelium-intact preparation		Endothelium-denuded preparation	
Concentration (M)	Sildenafil (%)	Verapamil (%)	Sildenafil (%)	Verapamil (%)
10^{-10}	2.7±6.4	11.4±7.5**	2.2±8.6	8±6.4**
10^{-9}	10.5±8.2	36.1±11.2**	13.8±9.7	34.3±10.7**
10^{-8}	25.3±6.9	60.4±7.8**	27.3±9.1	56.4±9.7**
10^{-7}	55.7±11.4	67.3±9.6*	54.5±10.1	63.9±8.6*
10^{-6}	67.8±13.1	73.4±10.6*	62.4±11.4	76.6±11.8*
10^{-5}	82.4±11.3†	85.9±10.7	70.9±13.8	84.7±10.9*
10^{-4}	95.9±10.7†	99.2±7.6	79.1±12.5	98.3±11.1*

Data are presented as mean±SEM of 12 individual rings, *p<0.01, **P<0.001, verapamil versus sildenafil, † p<0.01, Intact endothelium versus denuded endothelium

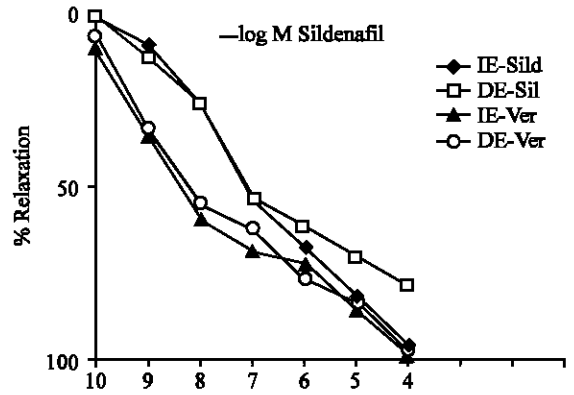

Fig. 1: Concentration-response curves of sildenafil and verapamil acting on phenylephrine precontracted isolated rat aortic rings with and without intact endothelium
IE, Phenylephrine precontracted rat aortic rings with intact endothelium
DE, Phenylephrine precontracted rat aortic rings with denuded endothelium

there was no statistically significant difference between the effects of verapamil and sildenafil in the phenylephrine precontracted aortic rings (99 vs 95%). The vasorelaxing effect of sildenafil was not affected by indomethacin (pD2: 4.84±0.9) or L-NAME (pD2: 4.79±1.2) in the intact rat aortic rings precontracted by phenylephrine.

DISCUSSION

In a recent study, Medina *et al.*[6] demonstrated that sildenafil caused significant relaxation in the human internal mammary artery, radial artery and forearm vein. On the other hand, in the same study, sildenafil had a modest relaxant effect in the coronary artery only at the highest concentration. Wallis *et al.*[4] reported that sildenafil had minimal vasorelaxant effect on rabbit aortic rings. However, our study showed that sildenafil at high doses caused highly potent vasorelaxation on rat aorta. Our findings are supported by Mochida *et al.*[9], who demonstrated that sildenafil caused almost 100% vasorelaxation in isolated rat aorta with and without endothelium. However, we found that the degree of vasorelaxation induced by sildenafil was lower in the rat aorta without intact endothelium. Although we did not explore the reason for this differences, it was previously reported that the vasodilator profile varies among different blood vessels and species[7,11,12]. In accordance, Collins *et al.*[5] found that basal endothelium derived relaxing factor activity had different effects in rabbit and rat aortic preparations.

It is assumed that sildenafil elicits vasorelaxation effect as a consequence of enhancing cGMP levels within the vessel wall. Similar to a previous study[9], present study provides the evidence for the endothelium independent vasodilatating action of sildenafil on isolated rat aorta. Since vasorelaxation was attenuated by removal of the endothelium, it may be suggested that sildenafil may also cause vasodilation on isolated rat aorta without involvement of NO-cGMP pathway. Studies[13,14] demonstrated relaxant activities of two PDE-5 inhibitors, WIN 58237 and E4021, at relatively high concentrations in vascular preparations without endothelium. Similarly, Komas *et al.*[14] reported that rolipram and denbufylline, two PDE-4 inhibitors, could relax endothelium denuded rat aortic rings. Whatever mechanism was involved to cause this effect of sildenafil, the results suggest that relaxation may not only involve the intervention of the NO-cGMP pathway because L-NAME, an inhibitor of NO synthase, did not change the relaxation induced by sildenafil in the intact endothelium rings. Also, in this study, the vasorelaxing effect of sildenafil was not affected by indomethacin in the intact rat aortic rings precontracted by phenylephrine, which suggested that the effect was not mediated through either endothelium-derived prostacyclin (PGI2).

In conclusion, our study showed that sildenafil was able to cause significant vasodilation on both phenylephrine and KCl-precontracted hypertensive rat aorta with and without endothelium, although this

vasorelaxation was attenuated by removal of the endothelium. Verapamil and sildenafil showed a similar degree of vasorelaxant effect in the intact aortic rings, although there was a significant difference in the degree of relaxation in the absence of endothelium. Although sildenafil has been shown to cause both endothelium-dependent and-independent relaxation, a precise mechanism for sildenafil-mediated relaxation has not been delineated in this study. A previous study[9] and the existence of endothelium independent relaxation on aorta in the present study led us to consider the possibility that sildenafil might be acting not only as a modulator of NO-mediated relaxation. Therefore, further studies are needed to elucidate the exact mechanism of sildenafil on the vasculature.

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