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Vasorelaxant Effect of Sildenafil on Aorta and Pulmonary Artery in Rabbits

[1]Niyazi Güler, [2]Hanefi Özbek and [1]Beyhan Eryonucu
[1]Department of Cardiology,
[2]Department of Pharmacology, Yüzüncü Yıl University, School of Medicine, Van, Turkey

Abstract: This *in vitro* study was designed to determine the direct vasorelaxant effect of the sildenafil on isolated rabbit pulmonary artery and compare it with the response of isolated rabbit aorta. Endothelium intact aortic and pulmonary artery rings from seven domestic rabbits were suspended in organ chambers containing 15 mL Krebs solution aerated with 95% O_2, 5% CO_2. In both phenylephrine and potassium chloride (KCl) precontracted vessels, relaxant responses of sildenafil were recorded by strain gauge transducers connected to a polygraph. Sildenafil (10^{-9} to 3×10^{-5} M) induced a dose–dependent vasodilation of phenylephrine precontracted aorta and pulmonary artery; 55 and 95% relaxations were obtained, respectively, at a concentration of 3×10^{-5} M. Sildenafil also caused a dose-dependent vasodilation of KCl-precontracted aorta and pulmonary artery, but this vasodilation was significantly lesser. Sildenafil-induced relaxations were higher in pulmonary arteries when compared to aortic rings precontracted with either phenylephrine or KCl. We concluded that sildenafil induces a dose-dependent vasodilation on phenylephrine and KCl-precontracted rabbit aorta and pulmonary artery. This vasodilatory effect is more potent in pulmonary arteries than in aortic rings.

Key words: Sildenafil, isolated organ bath, aorta, pulmonary artery

INTRODUCTION

As a potent and selective inhibitor of cyclic guanosine monophosphate (cGMP) specific phosphodiesterase (PDE) type 5, sildenafil citrate is safe and effective in men with erectile dysfunction of diverse etiologies[1]. It was reported that Sildenafil induces arterial vasodilation via a Nitric Oxide (NO) dependent mechanisms[2]. Nitric oxide, released by nonadrenergic-noncholinergic neurons and arteriolar endothelial cells, diffuses into vascular smooth muscle cells; activates soluble guanylate cyclase, which increases the concentration of cGMP and initiates a cascade of events that results in smooth muscle relaxation[2]. Sildenafil, originally investigated as a potential antianginal agent, is a potent competitive inhibitor of PDE5 and is selective over PDE1 to 4 and retinal PDE6[2]. In addition to its high concentration in the corpora cavernosa, PDE5 is abundant in vascular, tracheal and visceral smooth muscle and in lung[3,4]. However, the function of PDE5 in the vasculature remains largely unknown.

In hemodynamic studies, sildenafil produced small decreases in systemic and pulmonary blood pressure, but caused no adverse cardiovascular effects in specific populations of men with coronary heart disease[2,5]. The majority of adverse events due to sildenafil were related to vasodilation such as headache, flushing, nasal congestion, dyspepsia and abnormal vision[1]. Sildenafil has been shown to cause vasodilation on the isolated rabbit aorta, human coronary, mammary, radial and penile artery *in vitro*[6-8]. In many *in vivo* studies, it has been shown that sildenafil is a selective pulmonary vasodilator in pulmonary hypertension[9-14]. However, the effect and the extent of sildenafil on the isolated pulmonary artery is not known. Although clinical evidence suggests that sildenafil may be useful in patients with pulmonary hypertension, studies comparing the direct action of sildenafil on aorta and pulmonary artery are lacking. We thus studied the vasorelaxant effect of sildenafil on isolated rabbit aorta and pulmonary artery precontracted with potassium chloride (KCl) or phenylephrine (Phe).

MATERIALS AND METHODS

Seven domestic rabbits, weighing 2 to 2.5 kg, were used for the experiment. The rabbits were anesthetized with pentobarbital sodium (40 mg kg^{-1}). A segment of thoracic aorta and main pulmonary artery just distal to the bifurcation was removed immediately following respiratory arrest through mid-sternotomy. Rabbit aorta (n=7) and pulmonary arteries (n=7) were dissected from

Corresponding Author: Dr. Hanefi Özbek, Department of Pharmacology Medical School, University of Yüzüncü Yıl, 65300 Van, Turkey Fax: +90 432 216 83 52

the connective tissue and cut into rings approximately 3 mm wide. Precaution was taken not to damage the endothelium. 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 (COMMAT Iletişim Ltd., FDT10-A isometric transducer, Ankara, Turkey), which was linked to an amplifier and a computerized chart recorder (COMMAT Iletişim Ltd., TDA 97, Polygraph Systems, Ankara, Tukey) and vascular responses were recorded on the polygraph. 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 6.75 g for aorta or 3.75 g for pulmonary artery. Prior to the beginning of experiment, in order to confirm the presence of endothelium, the rings were precontracted with Phe (3×10^{-6} M) and challenged with acetylcholine (10^{-5} M). Relaxations evoked by acetylcholine and greater than 50% of maximal relaxation (maximal relaxation represented complete return to the resting tension from the Phe precontraction) indicated a structurally 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 Phe (3×10^{-6} M). Cumulative concentration-response curves of sildenafil were obtained by adding increasing concentrations of sildenafil in logarithmic increments (10^{-9} to $3\times10^{-}$ M), when contractile responses reached a plateau. Then the rings were washed until complete recovery of the resting tension was obtained. Another hour was allowed for equilibration and the submaximal precontraction of the vessels was again elicited by KCl (3×10^{-2} M) or Phe (3×10^{-6} M) alternatively, using the drug opposite to prior agent and the same protocol with sildenafil was applied.

Sildenafil (1-[4-ethoxy-3-(6,7-dihidro-1-methyl-7-ox-3-propyl-1H-pyrazolo (4,3-d) pyrimidin-5-yl) phenylsulphonyl]-4-methyl-piperazine) was provided by Fako Ilaçları AŞ. and all other chemicals utilized in this research were of analytical grade from commercial sources (Sigma Co., Merck Co.). Sildenafil was dissolved in distilled water and the final concentration of sildenafil used in these studies ranged from 10^{-9} to 3×10^{-5} M.

Data were analyzed by a software (Polwin 97, MAY). Mean of the negative logarithmic concentrations causing 50% inhibition of the maximum contraction was expressed as pD_2. All values are expressed as the mean ±SEM. The relaxations are expressed as the percentage decrease in tension obtained by Phe or KCl precontraction. Statistical differences between two means were determined by Student's t-test for paired or unpaired observations where appropriate. Statistical comparisons were performed using analysis of variance for repeated measures, followed by Student-Newman-Keuls post hoc testing for multiple comparisons. Statistically significance was defined as $p<0.05$.

RESULTS AND DISCUSSION

Sildenafil (10^{-9} to 3×10^{-5} M) induced a dose-related vasodilation in isolated rabbit pulmonary artery precontracted with either Phe or KCl (Fig 1 and Table 1). The magnitude of relaxation was significantly less in KCl-precontracted rings than that of Phe-precontracted rings (Fig. 1). At a concentration of 3×10^{-5} M, sildenafil caused almost 95% relaxation of the pulmonary arterial rings precontracted with Phe as compared to 75% relaxation in KCl-precontracted ones.

Similarly, sildenafil also caused vasodilation in the Phe- and or KCl-precontracted isolated rabbit aortic rings in a concentration-dependent manner. The magnitude of relaxation was significantly less in KCl-precontracted rings than that in Phe-precontracted aortic rings at the same doses of sildenafil (Fig. 1). At a concentration of 3×10^{-5} M, sildenafil produced almost 55% relaxation of the aortic rings precontracted with Phe as compared to 40% relaxation in KCl-precontracted ones.

Sildenafil caused more relaxation in pulmonary arteries than aortic rings independent of the nature of precontraction (Table 1).

Five different isoforms of PDEs (1, 2, 3, 5 and 9) that can metabolize cGMP are reported present in lung tissue[4,15]. Phosphodiesterase type-5 hydrolyzes cGMP in the lung, thereby modulates NO/cGMP-mediated pulmonary vasodilation. Several studies have demonstrated pulmonary vasodilation in response to PDE5 inhibitors such as zaprinast, dypyridamole, T-1032 and DMPPO[16-19]. Present *in vitro* experiments provide the first evidence for the vasodilatating action of the sildenafil on rabbit pulmonary artery. In consistent with the present study, Prasad *et al.*[9] proposed that oral sildenafil may be beneficial as a selective pulmonary vasodilator in patients with primary pulmonary hypertension. Abrams *et al.*[10] also suggested a potential role for sildenafil in the management of pulmonary hypertension. Weimann *et al.*[11] found that sildenafil causes selective pulmonary vasodilation in awake sheep with acute pulmonary hypertension induced by a tromboxane analog.

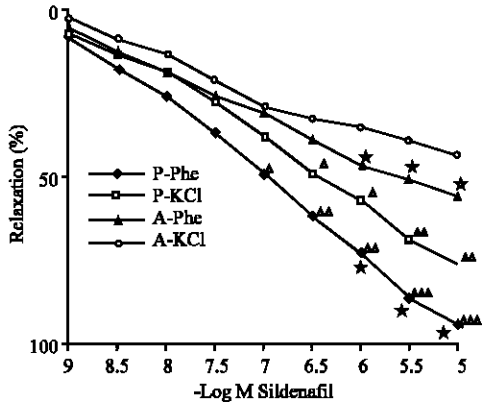

Fig. 1: Concentration-response curves to sildenafil on KCl or phenylephrine precontracted rabbit pulmonary arterial and aortic rings. Data are presented as mean±SEM of 7 individual rings. P-Phe, phenylephrine precontracted pulmonary artery; P-KCl, KCl precontracted pulmonary artery; A-Phe, phenylephrine precontracted aorta; A-KCl, KCl precontracted aorta; △-p<0.05, △△-p<0.01, △△△-p<0.001 aorta versus pulmonary artery (phenylephrine or KCl precontracted); ☆-p<0.05 between phenylephrine-precontracted vs KCl precontracted

Table 1: pD_2 (-log EC_{50}) values for the vasorelaxant effect of sildenafil in the rabbit isolated pulmonary artery and aorta precontracted with phenylephrine (Phe) or potassium chloride (KCl). Data are given as mean±SEM of 7 individual vessel rings

	Pulmonary artery		Aorta	
	Phe-precontracted	KCl-precontracted	Phe-precontracted	KCl-precontracted
pD_2	5.93±0.59 *,§	5.06±1.22 #	4.11±0.62 *	3.78±1.67

*p<0.05 Phe-precontracted versus KCl-precontracted
§p<0.001 Pulmonary artery versus aorta (Phe-precontracted)
#p<0.001 Pulmonary artery versus aorta (KCl-precontracted)

Similarly, Ichinose *et al.*[12] reported that nebulized sildenafil is a selective pulmonary vasodilator in lambs with acute pulmonary hypertension. Kleinsasser *et al.*[13] reported that mean pulmonary artery pressure of 24 anesthetized pigs was decreased after high-dose sildenafil administration. Since nitric oxide is an effective, selective pulmonary vasodilator in a variety of conditions associated with increased pulmonary vascular tone including pulmonary hypertension of the newborn, ARDS, pulmonary hypertension secondary to left ventricular dysfunction and mitral valve disease[20-23], pulmonary vasodilator effect of sildenafil may involve in nitric oxide-mediated dilation.

In a recent study, Medina *et al.*[7] have demonstrated that sildenafil causes significant relaxation in the human internal mammary artery, radial artery and forearm vein; whereas it had a modest relaxant effect in the coronary artery only at the highest concentration. In our study, sildenafil had a modest relaxant effect only at the highest concentration in the rabbit aortic rings. On the contrary, Wallis *et al.*[6] reported that sildenafil had minimal effect on rabbit aortic rings. However, sildenafil potentiated the vasorelaxant effects of glyceryl trinitrate on rabbit isolated aortic rings[6]. The discrepancy between studies may rise from the differences in the resting tensions applied to the vessels. The initial resting tension applied to the rabbit aortic rings in the latter study was 2 g. However, the resting tension of each ring in our study was adjusted to 6.75 g for aorta or 3.75 g for pulmonary artery based on a previous study of the optimal resting tension in rabbit pulmonary arteries[24].

Another finding of this study is that when aortic and pulmonary arterial rings are compared, relatively more relaxation was observed in the latter. Similarly, infusion of U46619 in previous studies caused greater vasoconstriction of the pulmonary than the systemic vasculature[25,26]. It is possible that PDE5 enzyme activity may be greater in pulmonary rather than systemic vascular smooth muscle, contributing to a greater impact of the sildenafil on the former.

In this study, when the rings were precontracted with Phe or KCl, sildenafil induced concentration-dependent relaxation. It is assumed that sildenafil, a potent and selective inhibitor of PDE5, elicits these effects as a consequence of enhancing cGMP levels within the vessel wall. However, relaxations of KCl-precontracted aorta and pulmonary artery in response to sildenafil in our study can be interpreted that sildenafil possess some Ca^{2+} channel blocking activities, because it is known that KCl induces contraction of the blood vessel by increasing Ca^{2+} influx through voltage operated channels following membrane depolarization. In consistent with this, Medina *et al.*[8] reported that in human penile arteries and veins, sildenafil relaxes norepinephrine-contracted vessels and inhibits sympathetic contraction in the absence of an endogenous and pre-existing activation of the NO-cGMP system. Medina *et al.*[8] also demonstrated that relaxation does not involve the intervention of the L-arginine–NO pathway; because L-NAME, an inhibitor of NO synthase, did not modify this effect. Thus, the relaxation induced by sildenafil observed in the vessels does not support the proposal that the action of sildenafil is only dependent on pre-existing activation of the NO-cGMP levels. On the other hand, the fact that sildenafil produced significantly much more relaxation on Phe–precontracted than KCl-precontracted vessels indicates that the major mechanism of its vasodilatory effect may be attributable to direct

inhibition of the release of Ca^{2+} from the sarcoplasmic reticulum, or directly doing so by decreasing Ca^{2+} influx through its Ca^{2+} channel–blocking effect, which in turn attenuates Ca^{2+} induced Ca^{2+} release.

In conclusion, this study has showed that sildenafil causes dose-dependent vasodilation of isolated rabbit aorta and pulmonary artery rings precontracted with Phe and KCl. The results of the current study indicate that sildenafil is a potent vasodilator and its vasodilatory effect is more potent on pulmonary artery than aorta. Further studies are needed to elucidate the exact mechanism and extend of vasorelaxant effect of sildenafil on different vasculature.

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