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α_1-Adrenoceptor antagonist profile of doxazosin and its enantiomers in isolated rabbit blood vessels

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Abstract: **AIM**: To investigate the different selectivity of α_1-adrenoceptor antagonist *R*-doxazosin and *S*-doxazosin in the rabbit thoracic aorta and carotid artery as potentially therapeutic agent for benign prostatic hyperplasia. **METHODS** Isometric contractile responses to norepinephrine(NE) in the rabbit thoracic aorta and carotid artery were observed, and the pA_2 values of doxazosin and its enantiomers were calculated from the Schild plots. **RESULTS** rac-Doxazosin, *R*-doxazosin or *S*-doxazosin at 0.03, 0.1 and 0.3 $\mu mol \cdot L^{-1}$ produced parallel shifts to the right of the concentration-response curves for NE without significant decrease in the E_{max} values in the rabbit thoracic aorta and carotid artery. The slope of the Schild plot for rac-, *R*- or *S*-doxazosin was not significantly different from unity, indicating that three agents competitively inhibited the concentration-response curves for NE. The rank order of the α_1-adrenoceptor antagonist pA_2 was *R*-doxazosin > rac-doxazosin > *S*-doxazosin in the rabbit thoracic aorta and carotid artery. **CONCLUSION** To be contrary to the previously reported results in the human prostate, the selectivity of *S*-doxazosin against α_1-adrenoceptor is significantly lower than that of rac-doxazosin and *R*-doxazosin in the rabbit thoracic aorta and carotid artery.

Key words: doxazosin; enantiomers; receptors, adrenergic, α; aorta, thoracic; carotid arteries; rabbits

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Radioligand-binding study indicates that the densities of α_1- and α_2-adrenoceptors are similar in human prostate adenomas[1-3], however, isolated organ experiments demonstrate that α_1-adrenoceptors are the primary α- adrenoceptors mediating the contraction of human prostate[1,2,4]. At present, α_1-adrenoceptor antagonists are usually considered as the first-line therapy for benign prostatic hyperplasia(BPH)[5], which decreases urethral pressure and resistance, and improves the urethral obstruction symptoms of BPH[6,7].

It is well known that the blockade of α_1-adrenoceptors in cardiovascular system in the treatment of BPH produces several side effects, which limits the clinical use of α_1-adrenoceptor antagonists. Comparative binding and functional studies have provided the most compelling evidence that the tension of human prostatic smooth muscle is mediated primarily by the α_{1A}-adrenoceptors[8,9], and Williams, *et al*[10] suggest that high affinity antagonists on α_{1A}-adrenoceptors appear to exhibit only weak cardiovascular effects when compared to standard non-subtype-selective α_1-adrenoceptor antagonists. Recently, the properties of α-adrenoceptor antagonist doxazosin and its enantiomers were characterized using human prostate tissue, and it was demonstrated that they were highly selective α_1-adrenoceptor antagonists, and there were no significant differences in the pA_2 values among racemic-doxazosin (rac-doxazosin), *R*-doxazosin and *S*-doxazosin in the human prostate[11].

In the anesthetized cat, we observed that rac-doxazosin reduced the urethral pressure increased

by the hypogastric nerve stimulation[12]. Furthermore, we prepared *R*-doxazosin and *S*-doxazosin by chiral mobile phase HPLC[13]. Since the α_1-adrenoceptor antagonist properties of the enantiomers of doxazosin in the blood vessels are not known, we attended to characterize the properties of doxazosin and its enantiomers in the rabbit thoracic aorta and carotid artery. Differential pharmacological properties of enantiomers of doxazosin in blood vessels, if have, might give some hits for the development of chiral drugs in the medical therapy of BPH.

1 MATERIALS AND METHODS

1.1 Animals

Male New-Zealand white rabbits(2.5 – 3.5 kg) were obtained from the Experimental Animal Center of Hebei Medical University (Certificate No. 0059).

1.2 Reagents

rac-Doxazosin methane sulphonate synthesized by Dr NIE Xin-Yong and LIU Yu-Ting (Hebei Drug Research Institute) is a white crystalline powder[12]. *R*-Doxazosin hydrochloride and *S*-doxazosin hydrochloride were prepared by HPLC by us(Center for Drug Research and Development, North China Pharmaceutical Corporation)[13]. Desmethylimipramine hydrochloride, propranolol hydrochloride, deoxycorticosterone acetate and (–)-norepinephrine bitartrate (NE) were obtained from Sigma Chemical Co. All chemicals were dissolved in distilled water except deoxycorticosterone acetate that was dissolved in 1,2-propanediol.

1.3 Arterial preparation

Rabbits were sacrificed with an overdose of pentobarbitone sodium injected *via* the ear vein, then exsanguinated. The thoracic aorta and common carotid artery were excised and cleaned of excess connective tissue and fat. In order to avoid the possible involvement of endothelium-derived relaxing factor in the mechanical response, the vascular endothelium was removed by gently rubbing the lumen with a scored polythene cannula, the external diameter of which was slightly smaller than the internal diameter of the vessels[14]. Ring segments(4 mm in length) without endothelium were mounted horizontally in a 10 mL organ bath by carefully inserting a tungsten wire through the lumen of the vessel ring and anchoring it to a stationary support. Another wire similarly inserted, was connected to an isometric tension transducer, and responses were recorded on a polygraph(ERT-884, Youlin Electron Co, Kaifeng).

Preloads of 4.0 g and 3.0 g were applied to the thoracic aorta and carotid artery rings, respectively. The preparations were allowed to equilibrate for 1.5 h in physiological solution containing ($mmol \cdot L^{-1}$)[15]: NaCl 133, KCl 4.7, NaH_2PO_4 1.35, $NaHCO_3$ 16.3, $MgSO_4$ 0.61, glucose 7.8 and $CaCl_2$ 2.52, pH 7.2. The solution was maintained at 37℃ and aerated with 95% O_2 and 5% CO_2. A successful removal of the arterial endothelium was confirmed by the loss of relaxation response to acetylcholine ($1\ \mu mol \cdot L^{-1}$) in NE precontracted arterial rings[16].

1.4 Drug administration

Desmethylimipramine ($0.1\ \mu mol \cdot L^{-1}$), deoxycorticosterone ($5\ \mu mol \cdot L^{-1}$) and propranolol ($1\ \mu mol \cdot L^{-1}$) were added to the bath solution to block neuronal and extra neuronal uptake of NE and to block β-adrenoceptors, respectively[17]. Cumulative concentration-response curves for NE were constructed for 6 times in each of arterial rings, and the first and second sets of concentration-response curve were not used in the present study. rac-Doxazosin, *R*-doxazosin or *S*-doxazosin at three concentrations(0.03, 0.1 and 0.3 $\mu mol \cdot L^{-1}$) were respectively added to the organ bath 30 min before the fourth, fifth and sixth concentration-response curves for NE. The competitively antagonistic activities were expressed as pA_2 values that were calculated from the Schild plots[18] with a computer program of PHARM/PCS-Version 4.

1.5 Statistical analysis

Data were expressed as $\bar{x} \pm s$. The EC_{50} values were calculated with the equation: $\lg[E/(E_{max} - E)] = \lg C - \lg K$, in which, E:

response; E_{max}: maximal response; C: NE concentration; K: equilibrium dissociation constant. Dunnett multiple comparisons test (with GraphPat InStat V2.05a) was used to evaluate any differences between more than two groups. P values less than 0.05 were considered statistically significant.

2 RESULTS

2.1 Reproducibility of the concentration-response curves for norepinephrine in the thoracic aorta and carotid artery

Cumulative concentration-response curves for NE were constructed for 6 times in each of arterial rings as time-control preparations. The values of E_{max} or EC_{50} obtained from the third to the sixth concentration-response curves for NE were reproducible without significant differences ($P > 0.05$, $n = 5$) in the rabbit thoracic aorta with the E_{max} values (g) of 7.1 ± 0.5, 7.5 ± 0.7, 7.6 ± 0.6, 7.7 ± 0.5 and EC_{50} values ($\mu mol \cdot L^{-1}$) of 0.22 ± 0.03, 0.26 ± 0.05, 0.25 ± 0.04, 0.22 ± 0.06, and in the rabbit carotid artery with the E_{max} values (g) of 3.14 ± 0.26, 3.16 ± 0.15, 3.27 ± 0.27, 3.52 ± 0.21 and EC_{50} values ($\mu mol \cdot L^{-1}$) of 0.12 ± 0.04, 0.11 ± 0.03, 0.10 ± 0.04, 0.12 ± 0.03, respectively.

2.2 Effects of rac-doxazosin, *R*-doxazosin and *S*-doxazosin on the vasoconstriction induced by norepinephrine in the thoracic aorta

The values of E_{max} or EC_{50} obtained from the third concentration-response curve for NE in the rabbit isolated thoracic aorta before the treatment by rac-doxazosin, *R*-doxazosin or *S*-doxazosin were not significantly different in three groups ($P > 0.05$, Tab 1). rac-Doxazosin, *R*-doxazosin or *S*-doxazosin at 0.03, 0.1 and 0.3 $\mu mol \cdot L^{-1}$ produced parallel shifts to the right of the concentration-response curves for NE without significant decrease in the E_{max} values ($P > 0.05$, Fig 1). The slope of the Schild plot for rac-, *R*- or *S*-doxazosin was not significantly different from unity ($P > 0.05$, Tab 2), indicating that three agents competitively inhibited the concentration-response curves for NE in the thoracic aorta. The pA_2 value of *R*-doxazosin was significantly higher than that of rac-doxazosin, and the pA_2 value of *S*-doxazosin was significantly lower than that of rac-doxazosin (Tab 2).

Tab 1. Vasoconstrictive responses to norepinephrine (NE) in the rabbit isolated arteries in the absence of antagonists

Group	E_{max}/g	EC_{50}/$\mu mol \cdot L^{-1}$
Thoracic aorta		
rac-Doxazosin	7.4 ± 1.0	0.36 ± 0.18
S-Doxazosin	7.0 ± 0.5	0.21 ± 0.04
R-Doxazosin	8.1 ± 1.5	0.17 ± 0.09
Carotid artery		
rac-Doxazosin	3.5 ± 0.5	0.15 ± 0.05
S-Doxazosin	3.4 ± 0.4	0.10 ± 0.03
R-Doxazosin	3.6 ± 0.3	0.17 ± 0.07

NE was given cumulatively at the concentration of 0.03 – 3000 $\mu mol \cdot L^{-1}$ to the organ bath. $\bar{x} \pm s$, $n = 5$. There was no significant difference ($P > 0.05$) compared with one another in thoracic aorta or carotid artery by Dunnett multiple comparisons test.

Tab 2. The pA_2 values of rac-doxazosin, *R*-doxazosin and *S*-doxazosin in the rabbit isolated arteries

Group	pA_2	Slope
Thoracic aorta		
rac-Doxazosin	8.24 ± 0.14	1.0 ± 0.4
S-Doxazosin	$7.82 \pm 0.13^{**}$	1.2 ± 0.3
R-Doxazosin	$8.57 \pm 0.11^{**}$	1.2 ± 0.3
Carotid artery		
rac-Doxazosin	8.25 ± 0.16	1.2 ± 0.3
S-Doxazosin	$7.73 \pm 0.09^{**}$	1.10 ± 0.20
R-Doxazosin	$8.52 \pm 0.09^{**}$	1.18 ± 0.21

rac-Doxazosin, *R*-doxazosin and *S*-doxazosin at three different but increasing concentrations (0.03, 0.1 and 0.3 $\mu mol \cdot L^{-1}$) were respectively added in the organ bath 30 min before the fourth, fifth and sixth concentration-response curves for NE. $\bar{x} \pm s$, $n = 5$. $^{**}P < 0.01$, compared with corresponding rac-doxazosin group by Dunnett multiple comparisons test.

2.3 Effects of rac-doxazosin, *R*-doxazosin and *S*-doxazosin on the vasoconstriction induced by norepinephrine in the carotid artery

The E_{max} or EC_{50} values of the third concentration-response curve for NE in the rabbit carotid

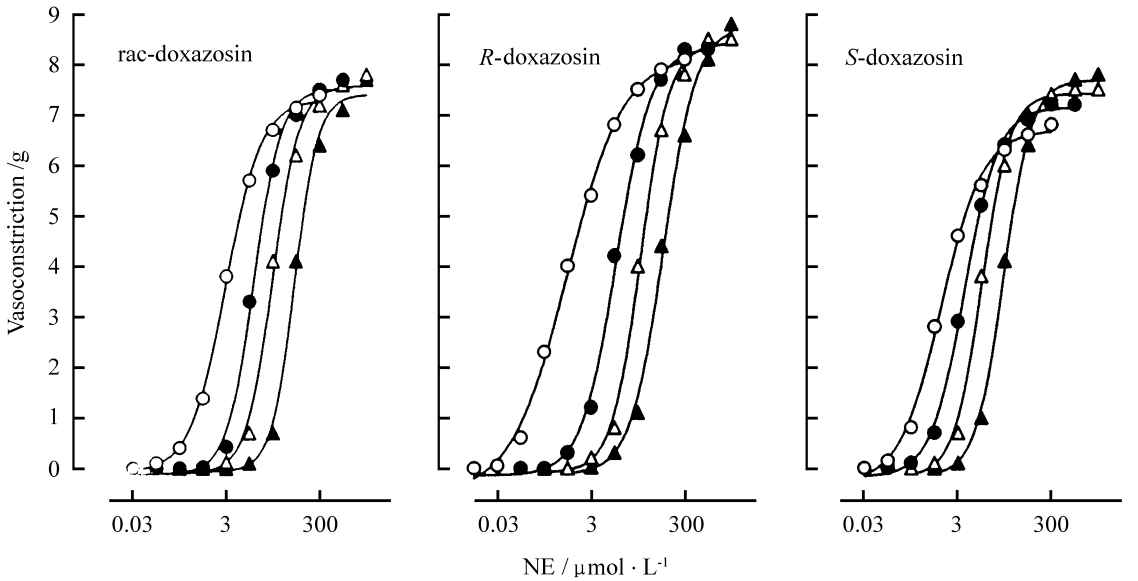

Fig 1. Antagonistic effects of rac-doxazosin, *R*-doxazosin and *S*-doxazosin on the contractile response to norepinephrine in the rabbit thoracic aorta. (○) control, (●, △, ▲) 0.03, 0.1, 0.3 μmol·L^{-1}, respectively. $n = 5$.

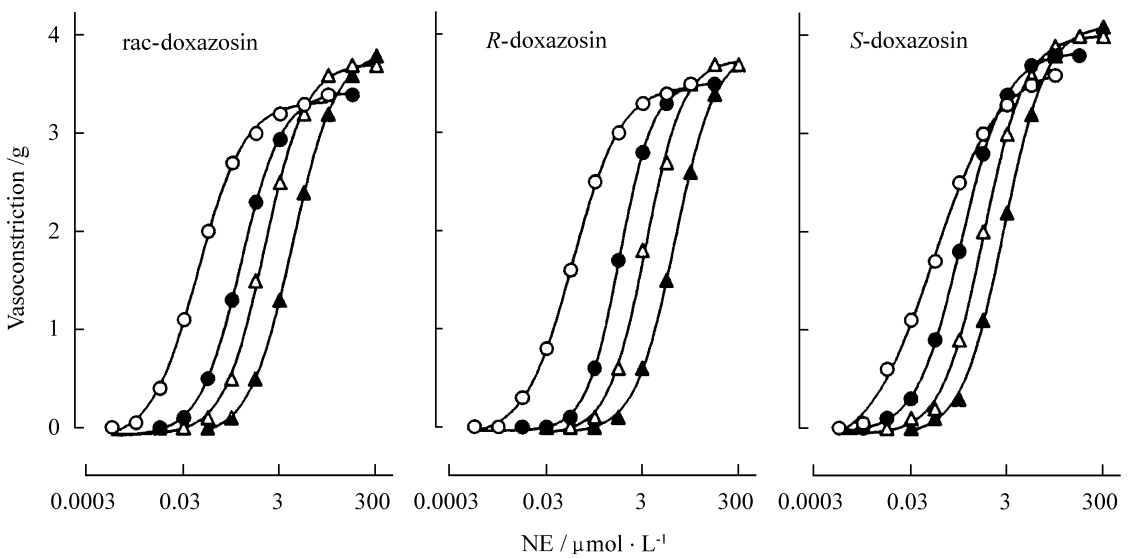

Fig 2. Antagonistic effects of rac-doxazosin, *R*-doxazosin and *S*-doxazosin on the contractile response to norepinephrine in the rabbit carotid artery. (○) control, (●, △, ▲) 0.03, 0.1, 0.3 μmol·L^{-1}, respectively. $n = 5$.

artery before the treatment with α-adrenoceptor antagonist were not significantly different in the three groups ($P > 0.05$, Tab 1). rac-Doxazosin, *R*-doxazosin or *S*-doxazosin at 0.03, 0.1 and 0.3 μmol · L^{-1} shifted the concentration-response curves for NE to the right in a parallel manner, and did not affect the E_{max} values of NE significantly ($P > 0.05$, Fig 2). Each slope of the Schild plot for rac-, *R*- or *S*-doxazosin was not significantly different from unity ($P > 0.05$, Tab 2). The pA_2 value of *R*-doxazosin was significantly higher than that of rac-doxazosin, and the pA_2

value of *S*-doxazosin was significantly lower than that of rac-doxazosin(Tab 2).

3 DISCUSSION

It has been demonstrated that the contraction of human prostatic smooth muscle is mediated primarily by the α_{1A}-adrenoceptors[8,9], and rac-doxazosin, *R*-doxazosin and *S*-doxazosin antagonize the contractile responses to phenylephrine in the human prostate without significant difference in their pA_2 values[11]. However, the present study demonstrated that the pA_2 value of *S*-doxazosin antagonizing α_1-adrenoceptors was significantly lower than that of rac-doxazosin or *R*-doxazosin with a potency order of *S*-doxazosin < rac-doxazosin < *R*-doxazosin in the rabbit isolated thoracic aorta and carotid artery.

In the present experiments, the E_{max} and EC_{50} values of NE in the rabbit isolated thoracic aorta or carotid artery before the treatment with doxazosin and its enantiomers were not significantly different from each other. After incubating the preparations with rac-doxazosin, *R*-doxazosin or *S*-doxazosin, the concentration-response curves for NE were moved to the right in a parallel manner, and the slope of the Schild plot for rac-, *R*- or *S*-doxazosin was not significantly different from unity, indicating that three agents competitively inhibited the concentration-response curves for NE *via* α_1-adrenoceptors in the two kinds of arteries.

Competitive α_1-adrenoceptor antagonist such as terazosin, prazosin and alfuzosin have been shown to be effective in relieving urinary outflow obstruction and reducing symptom scores in patients with BPH. However, their dose-limiting cardiovascular effects including postural hypotension, particularly with initial dosing, offset the usefulness of α_1-adrenoceptor antagonists in BPH. Recently, the interest has focused on the role of the α_{1A}-adrenoceptor subtype in BPH, as a result of studies demonstrating that this subtype predominates in the urethra and prostate of man[19]. Doxazosin is a quinazoline derivative structurally related to prazosin and terazosin, and a long-acting α_1-adrenoceptor antagonist[20]. Several controlled clinical studies have demonstrated that doxazosin is an effective, safe and well-tolerated drug for the treatment of symptomatic BPH[20].

Using the human prostatic tissue, Hatano, *et al*[11] demonstrated that the mean pA_2 values of rac-, *R*- and *S*-doxazosin against the phenylephrine-induced contraction of prostate smooth muscles were 8.43 ± 0.28, 8.58 ± 0.40, 8.75 ± 0.38, respectively. In the present study, the mean pA_2 values of rac-doxazosin(8.24 – 8.25) and *R*-doxazosin (8.52 – 8.57) against NE-induced vasoconstriction in the isolated rabbit thoracic aorta and carotid artery were similar to those in human prostate, however, the mean K_i value (antagonist equilibrium dissociation constant) of *S*-doxazosin(pA_2 = 7.73 – 7.82) in the isolated rabbit arteries was 10 times as big as that in human prostate. Therefore, it is reasonable to suggest that *S*-doxazosin is an agent preferentially acting on the prostatic tissue with minor cardiovascular side effects, in comparison with rac-doxazosin and *R*-doxazosin. In conclusion, the selectivity of *S*-doxazosin against α_1-adrenoceptor is significantly lower than that of rac-doxazosin and *R*-doxazosin in the rabbit thoracic aorta and carotid artery, which is contrary to the previous results reported in the human prostate.

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多沙唑嗪及其手性对映体对离体兔血管 α_1 受体的拮抗特性

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摘要: 目的　分析 α_1 肾上腺素受体阻断药多沙唑嗪手性对映体对兔胸主动脉和颈总动脉的选择性作用,以探讨作为良性前列腺增生症治疗药物的可能性。**方法**　测定去甲肾上腺素(NE)诱发兔离体胸主动脉和颈总动脉收缩反应,并采用 Schild 作图法计算 rac-多沙唑嗪、*R*-多沙唑嗪和 *S*-多沙唑嗪的 pA_2 值。**结果**　在兔胸主动脉和颈总动脉,0.03, 0.1 和0.3 $\mu mol \cdot L^{-1}$的 rac-多沙唑嗪、*R*-多沙唑嗪和 *S*-多沙唑嗪均使 NE 诱发的血管收缩反应量效曲线平行右移,E_{max}不变;由 Schild 作图法计算得到的多沙唑嗪及其手性对映体的斜率值,经统计学分析符合竞争性拮抗。3 种拮抗剂 pA_2 值的强度顺序为: *R*-多沙唑嗪 > rac-多沙唑嗪 > *S*-多沙唑嗪。**结论**　与多沙唑嗪及其手性对映体对人前列腺组织作用的报道结果不同,*S*-多沙唑嗪对兔胸主动脉和颈总动脉 α_1 肾上腺素受体拮抗作用的选择性显著低于 rac-多沙唑嗪和 *R*-多沙唑嗪。

关键词: 多沙唑嗪; 对映体; 受体, 肾上腺素, α; 主动脉, 胸; 颈动脉; 兔

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