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Protection of *l*-arginine against oxygen free radicals-injured rabbit aortic endothelium[1]

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ABSTRACT This study was to investigate the protective effect of *l*-arginine, a precursor of endothelium-derived relaxing factor (EDRF), against damages due to endogenous or exogenous oxygen free radicals (OFR) on the aortic endothelium. The superfusion cascade bioassay of rabbit thoracic aorta was used. Endogenous OFR were generated by diethyldithiocarbamate (DETC) to deplete the cytosolic Zn-Cu form of superoxide dismutase (SOD). Exogenous OFR were generated by electrolysis of Krebs' solution. Acetylcholine (ACh) was infused through the donor aortic segment and relaxation of detector aortic ring was used as an indicator of the release of EDRF. The content of malondialdehyde (MDA) in the donor aorta was assayed biochemically. Both DETC and electrolysis inhibited vasodilator responses to ACh and increased MDA content in the aortic segment. Inhibition of DETC was abolished by exogenous SOD. *l*-Arginine improved impairment of endothelium-dependent relaxation and reduced elevation of MDA content by DETC or electrolysis. These results suggest that *l*-arginine presents a protective effect of endothelium against damage due to endogenous or exogenous OFR, and that the protective effect of *l*-arginine may be correlated with reduction in lipid peroxidation.

KEY WORDS arginine; diethyldithiocarbamate; nitric oxide; electrolysis; reactive oxygen species; thoracic aorta

Endothelium - derived relaxing factor (EDRF) released by vascular endothelium has been identified as nitric oxide (NO) and is derived from the terminal guanidine nitrogen atom of *l*-arginine[1,2]. NO activates soluble guanylate cyclase, with a subsequent elevation of intracellular concentrations of cGMP, resulting in relaxation of vascular smooth muscle. Many pathologic processes, including atherosclerosis, ischemia-reperfusion, hypertension, and diabetes, are associated with attenuated endothelium-dependent relaxation and excessive generation of oxygen free radicals (OFR). Superoxide anions and other oxyradical species can inactivate NO[3]. Exogenous OFR generated by electrolysis of Krebs' solution inhibited the endothelium - dependent vasodilation of isolated rabbit thoracic aorta[4]. Diethyldithiocarbamate (DETC), an inhibitor of superoxide dismutase (SOD), evoked accumulation of endogenous OFR and inhibited endothelium-dependent vasodilation of rabbit thoracic aorta. The effect of DETC was negated by exogenous SOD[5].

It has been suggested that *l*-arginine improves the impairment by hypercholesterolemia of endothelium dependent relaxation[6] and that the toxicity of hypercholesterolemia may be due to generation of OFR[7]. *l*-Arginine is a precursor of NO which is a super antioxidant[8,9]. This suggests that the protection of *l*-arginine may be secondary to antioxidation of NO. In the present study, we in-

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vestigated the protective effects of *l*-arginine against damages due to both endogenous and exogenous OFR in a superfusion cascade system of rabbit thoracic aorta.

MATERIALS AND METHODS

Cascade superfusion A cascade bioassay system was prepared using a modification of the method described previously[4]. Briefly, rabbits (2.0 ± *s* 0.3 kg) of either sex were anesthetized with pentobarbital sodium (60 mg·kg^{-1} iv). The thoracic aorta was cut into an intact endothelial segment as EDRF donor and a denuded endothelial ring about 4 mm wide as bioassay detector. The denudation was confirmed by demonstrating the absence of relaxation to direct application of ACh (1 μmol·L^{-1}). Both ends of the donor aorta were cannulated by stainless steel cannulae, and the donor aorta was mounted vertically in organ bath and perfused intraluminally with Krebs' solution (5 ml·min^{-1}) at 37 ℃, gassed with 95% O_2 + 5% CO_2 (pH 7.4). The release of NO was detected by the detector aortic ring which was suspended directly beneath the donor aorta. The tension of detector ring was monitored by an isometric force transducer and recorded by a polygraph. Both donor aortic segment and the detector ring were equilibrated by superfusion with Krebs' solution for 90 min before the ring was loaded with a tension of 6.5 g. Then the detector aortic ring was made to precontract with norepinephrine (0.2 μmol·L^{-1}) infused into the perfusion stream by another pump. At the peak stable contraction of the ring, ACh (1 μmol·L^{-1}) was infused. When infused directly over the ring, ACh did not induce relaxation, indicating denudation of endothelium. When infused through the donor aorta, ACh evoked relaxation, indicating the release of NO from the donor aorta. The ratio of the relaxation was calculated as a percentage of the contraction to norepinephrine.

Generation of OFR Exogenous OFR were generated by electrolysis of Krebs' solution as described previously[10]. Two platinum wire electrodes were placed into the flow tract just above the donor vessel. The anode was placed 4 cm above the upper end of the donor vessel and the cathode was 8 cm apart. A direct current of 5 mA generated by an electronic stimulator (SEN-320 Nihon Kohden, Japan) was applied to the Krebs' solution for 2 min. For generation of endogenous OFR, the donor vessel was exposed to DETC (5 mmol·L^{-1}) for 20 min to deplete the cytosolic SOD.

MDA determination At the end of bioassay, the donor aorta was quickly frozen until assay for MDA. The content of thiobarbituric acid reactive substance, reflecting level of lipid peroxidation, in homogenate was measured by a spectrofluorometer[11] and expressed as the amount of MDA.

Experimental procedures Experiments were performed in 6 groups (4—6 rabbits in each group); 1) the control group, the same procedures were followed except without DETC pretreatment and electrolysis; 2) DETC; 3) SOD (200 u·ml^{-1}) was perfused for 20 min after DETC treatment; 4) *l*-arginine (0.5, 1.0, 2.5 mmol·L^{-1}) in the presence of DETC was perfused for 20 min and remained in the perfusate for 20 min through the donor aorta; 5) electrolysis; and 6) *l*-arginine (1 mmol·L^{-1}) was perfused for 5 min before electrolysis and for 20 min further.

Drugs and chemicals DETC and *l*-arginine (Sigma); thiobarbituric acid (Fluka chemika); norepinephrine and ACh (Shanghai Tian Feng Pharmaceutic Factory and Shanghai Xin Zhong Chemical Factory, China); SOD (Changsha Biological Pharmaceutic Factory, China). All drugs were dissolved and diluted in Krebs' solurion.

Statistical analysis All data were expressed as $\bar{x} \pm s$. The differences between groups were tested by analysis of variance.

RESULTS

Effect of *l*-arginine on relaxation In the cascade bioassay, ACh perfused through the donor aorta induced a marked relaxation of the detector ring, while ACh perfused over the ring directly did not cause any effect. DETC almost completely inhibited the endothelium-dependent relaxation to ACh. To examine the role of superoxide anion in the inhibition of vascular relaxation, SOD (a scavenger of superoxide anion) was used. Inhibition by DETC of relaxation to ACh was reversed by SOD 200 u·ml^{-1} (Tab 1).

Similarly, *l*-arginine abolished the inhibition by DETC of relaxation to ACh in a

Tab 1. Effect of *l*-arginine (1 mmol·L^{-1}) or SOD (2000 u·ml^{-1}) on impairment by DETC (5 mmol·L^{-1}) of endothelium-dependent relaxation to ACh in rabbit thoracic aorta. Con: control; *l*-Arg: *l*-arginine. $\bar{x}\pm s$. $^cP<0.01$ *vs* control. $^eP<0.05$, $^fP<0.01$ *vs* DETC.

	n	Relaxation index
Con	6	1.00±0.09
DETC	5	0.12±0.05^c
DETC+SOD	4	1.02±0.16^e
DETC+*l*-Arg	5	0.82±0.07^f

concentration dependent manner (Tab 2).

Tab 2. Effect of *l*-arginine on impairment by DETC of endothelium-dependent relaxation to ACh in rabbit thoracic aorta. $\bar{x}\pm s$.

l-Arg/mmol·L^{-1}	*n*	Relaxation index
0.5	4	0.68±0.04
1.0	5	0.82±0.07
2.5	4	1.08±0.29

Exposure of the donor aortic segment to electrolyzed Krebs' solution for 2 min induced impairment of endothelium-dependent relaxation to ACh. *l*-Arginine also attenuated the impairment by electrolysis of vasodilator responses to ACh (Tab 3).

Tab 3. Effect of *l*-arginine (1 mmol·L^{-1}) on impairment by electrolysis of endothelium-dependent relaxation to ACh in rabbit thoracic aorta. Els: electrolysis. $\bar{x}\pm s$. $^cP<0.01$ *vs* control. $^fP<0.01$ *vs* electrolysis.

	n	Relaxation index
Con	6	1.00±0.09
Els	6	0.27±0.08^c
l-Arg+Els	5	0.85±0.04^f

Effect of *l*-arginine on content of MDA

DETC or electrolysis increased the level of MDA in the aortic tissue. *l*-Arginine attenuated the elevation of MDA induced by DETC or electrolysis. The elevation of MDA content by DETC was also reversed by SOD (Tab 4).

Tab 4. Effect of *l*-arginine (1 mmol·L^{-1}) or SOD (2000 u·ml^{-1}) on elevation of MDA by DETC (5 mmol·L^{-1}) or electrolysis in rabbit thoracic aorta. $\bar{x}\pm s$. $^bP<0.05$ *vs* control, $^eP<0.05$ *vs* electrolysis, $^bP<0.05$ *vs* DETC.

	n	MDA, nmol/g wet wt
Con	6	17.02±6.29
Els	6	43.83±12.76^b
Els+*l*-Arg	5	22.33±9.01^e
DETC	5	44.13±12.98^b
DETC+SOD	4	21.51±6.66^b
DETC+*l*-Arg	5	23.46±4.95^b

DISCUSSION

This study shows that *l*-arginine improves the impairment of endothelium-dependent relaxation and inhibits the elevation of MDA content by both DETC and electrolysis in isolated rabbit aorta. These results suggest that *l*-arginine possesses a protective effect on the endothelium against damages due to both endogenous and exogenous OFR.

In the present study, Both DETC and electrolysis inhibited vasodilator responses to ACh, and the effect of DETC was reversed by SOD, a scavenger of superoxide anion. This suggests that impairment by DETC of endothelium-dependent relaxation to ACh is due to the generation of superoxide anion.

NO induces relaxation of smooth muscle via activation of guanylate cyclase and stimulation of cGMP production, and the superoxide anion, besides inactivating NO, inhibits the cGMP production through inhibition of catalase which activates guanylate cyclase[12,5]. In

addition, superoxide anion and other oxyradicals, such as hydrogen peroxide and hydroxyl radical, induce the peroxidation of membrane lipid. The present study showed that the content of MDA was enhanced by electrolysis or DETC. Our results suggest that the impairment by DETC or electrolysis of vasodilator responses to ACh may be due to inactivation of NO and reduction of synthesis and/or release of NO through elevation of lipid peroxide.

l-Arginine can restore cholinergic relaxation of aorta and other arteries in the hypercholesterolemic humans and rabbits[6,13]. Supplementation with dietary *l*-arginine also improves endothelium dependent relaxation and lessens the histomorphological changes of atherosclerosis in hypercholesterolemic rabbits[13,14]. In the present study, *l*-arginine improved impairment by both DETC treatment and electrolysis of endothelium-dependent relaxation in the isolated aorta of rabbits. These results support the proposal that *l*-arginine possesses protection of endothelium against OFR-induced functional injury.

l-Arginine is a precursor for NO synthesis. NO is an antioxidant, and has broad antioxidant activity[8,9]. In the present study, *l*-arginine markedly inhibited the elevation of MDA content by DETC or electrolysis, suggesting that protection of *l*-arginine against endothelium might be correlated with its antioxidation.

In conclusion, the present study suggests that *l*-arginine can protect the aortic endothelium against damages induced by both endogenous and exogenous OFR. The protection of *l*-arginine might be secondary to antioxidant effect of NO.

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左旋精氨酸对氧自由基损害兔胸主动脉内皮的保护作用

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左旋精氨酸 自由基 胸主动脉

摘要 用离体兔胸主动脉淋浴式灌注方法探讨左旋精氨酸对内、外源性 OFR 损伤血管内皮功能的保护作用. 结果：用二乙二硫氨基甲酸盐(DETC)产生的内源性 OFR 与电解缓冲液产生的外源性 OFR 均可明显抑制血管内皮依赖性扩张，并使血管壁 MDA 含量增加. 左旋精氨酸能对抗内、外源性 OFR 所致 MDA 增加与内皮依赖舒血管功能损害.

关键词 精氨酸；二乙基二硫代氨甲酸酯；一氧化氮；电解；活性氧；胸主动脉

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Sympatholytic effect of captopril in regression of cardiovascular remodeling in spontaneously hypertensive rats[1]

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ABSTRACT Fifty-eight spontaneously hypertensive rats (SHR) at 12 wk of age were divided into 3 groups: A) captopril (Cap) 20 mg · kg^{-1} · d^{-1}; B) clonidine (Clo) 30 μg · kg^{-1} · d^{-1}; C) Clo 30 μg · kg^{-1} · d^{-1} + Cap 20 mg · kg^{-1} · d^{-1} orally for 24 wk. Concomitant administration of Cap and Clo did not result in more lowering of the systolic blood pressure (SBP) than that by Cap alone. Regression of left ventricular hypertrophy (LVH) were remarkable in Groups A and C, but not to the extent in that of WKY. No significant difference between these two groups was found. Cap alone resulted in a greater decrease of myocardial norepinephrine (NE) than that of Groups B and C. The wall/lumen ratio and the number of smooth muscle cell (SMC) layers of renal artery decreased in Groups A and C, but little difference was found between them. It seemed that combined blockade of renin-angiotensin-aldosterone (RAA) system and sympathetic nervous system (SNS) did not produce more significant BP reduction and reversal of cardiovascular remodeling than Cap alone did. The sympathetic inhibitory effect of angiotensin converting enzyme inhibitor (ACEI) was not enhanced by sympatholytic treatment.

KEY WORDS inbred SHR rats; inbred WKY rats; captopril; clonidine; blood pressure; myocardium; norepinephrine; calcium; hydroxyproline; renal artery

Cardiovascular remodeling always accompanies hypertension[1-2]. Both sympathetic nervous system (SNS) and renin-angiotensin-aldosterone (RAA) system were involved in the process of cardiovascular remodeling, besides the hemodynamic effect of hypertension. Hypertensive cardiovascular remodeling regressed after angiotensin converting enzyme inhibitor (ACEI) treatment, which blocked RAA directly or inhibited the SNS at different levels indirectly[1-3]. Sympatholytic drugs prevented or regressed the LVH[1,2,4], but it is un-

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