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The Effect of Quinapril on the Aortic Contractile Response of Streptozotocin-Diabetic Rats

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ABSTRACT

Angiotensin-converting enzyme (ACE) inhibitors appear to correct many of the abnormalities associated with the vascular dysfunction found in diabetic patients. In this respect, quinapril is a unique ACE inhibitor with multiple protective effects. The present study was carried out to investigate the effect of intraperitoneal administration of quinapril on the aortic reactivity of streptozotocin (STZ)-diabetic rats. For this purpose, male Wistar rats received one injection of streptozotocin (STZ), 60 mg/kg, to induce diabetes. Three days after STZ injection, rats were treated with quinapril (2 mg/kg/day) for 4 weeks, after that aortic reactivity to vasoactive agents were compared with those of untreated diabetic rats or non-diabetic control rats. For this purpose, contractile response to phenylephrine (PE) was obtained from aortic rings. Concentration-response curves from quinapril-treated diabetic rats to PE in the presence and absence of endothelium were attenuated as compared to vehicle-treated diabetics. Therefore, the 4-week treatment of diabetic rats with quinapril could prevent the functional changes in vascular reactivity in diabetic rats. *Iran. Biomed. J. 7 (4): 173-177, 2003*

Keywords: Quinapril, Aortic reactivity, Diabetes mellitus, STZ, Rat

INTRODUCTION

The incidence of diabetes and its complications is increasing to staggering proportions [1]. Diabetes mellitus (DM) has been identified as a primary risk factor for cardiovascular disorders [2] and alters the vascular responsiveness to several vasoconstrictors and vasodilators [3]. For patients with DM, cardiovascular abnormalities encompass macro-vascular diseases, with heart attacks, strokes, and gangrene; and microvascular disease, with retinopathy, nephropathy, and neuropathy (somatic and autonomic). Diabetic arteriopathy, which encompasses endothelial dysfunction, inflammation, hypercoagulability, changes in blood flow, and platelet abnormalities, contributes to the early evolution of other threatening complications.

Efforts are under way to determine interventions that may have the potential to prevent or halt the devastating complications of DM [4]. Strategies that interrupt the renin-angiotensin system, especially those capable of inhibiting angiotensin-converting enzyme (ACE) reduce cardiovascular disease mortality and morbidity in high-risk persons such as those with DM [5]. The usefulness of treatment with an angiotensin-converting enzyme-inhibitor (ACE-inhibitor) in normotensive patients with type 1 diabetes is still controversial [6]. The mechanisms underlying pharmacological effects of ACE inhibitors are not fully understood. Various experimental evidences support the involvement of hemodynamic effects and/or the stimulation of cytoprotective prostaglandins [7]. The enhancement of bradykinin-induced relaxation, augmented release of endothelium-derived nitric oxide, decreased production of endothelin-I and a free radical scavenging action have also been postulated [8]. Other findings confirm the role of oxidative stress in the development of nephropathy already at the early stages of diabetes development and point to the possible antioxidative and nephroprotective action of ACE inhibitors [9].

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Quinapril is a non-sulfhydryl ACE inhibitor that has been studied extensively in a variety of *in vitro* and *in vivo* animal models. Quinapril inhibits the contractile and pressor effects of angiotensin I in rabbit aorta and in rats, respectively, and lowers blood pressure in both high- and normal-renin rodent and diuretic-treated dog models of hypertension [10]. Quinapril treatment in elderly patients was efficacious and well tolerated, and quinapril appears to be an effective antihypertensive drug devoid of untoward effects on metabolic risk factors for cardiovascular disease [11]. Quinapril also produces favorable hemodynamic changes, and improves ventricular and endothelial function in patients with various cardiovascular disorders; these effects are mediated through the binding of quinapril to both tissue and plasma ACE [12].

Although ACE inhibitors like quinapril have been shown to enhance conduit artery endothelial function in animal experiments and in patients with established coronary atherosclerosis, their precise effect in insulin-dependent diabetes mellitus has not been well known and their efficacy is still controversial [13]. Therefore, the present study was carried out to investigate the effect of a 4-week administration of quinapril on the aortic contractile response of streptozotocin (STZ)-diabetic rats.

MATERIALS AND METHODS

Animals. Male albino Wistar rats (the Pasteur institute of Iran, Tehran), weighing 210-250 g (7-9 weeks old) were housed in an air-conditioned colony room on a light/dark cycle at 21 ± 3°C and supplied with standard pellet diet and tap water ad libitum. The animals were randomly divided into four experimental groups; i.e. control (VC, n = 7) receiving 0.9% saline, quinapril-treated control (QC, n = 7), saline-treated diabetic (VD, n = 7), and quinapril-treated diabetic (QD, n = 7). Diabetes was induced by a single intraperitoneal injection of STZ (60 mg/kg) dissolved in cold 0.9% saline immediately before use. Quinapril was administered from day +3 thereafter at a dose of 2 mg/kg. All of the experimental groups received the treatments daily and intraperitoneally for a period of 4 weeks. Serum glucose level and body weight were monitored at the start and end of the experiment. Diabetes was verified by a serum glucose level higher than 250 mg/dl [14] using glucose oxidation method (glucose oxidase kit, Zistchimie, Tehran, Iran).

Experimental protocol. At the end of the experiment, the rats were anesthetized with diethyl ether, decapitated, and after opening the abdomen, descending thoracic aorta was carefully excised and placed in a Petri dish filled with cold Krebs solution containing (in mM): NaCl 118.5, KCl 4.74, $CaCl_2$ 2.5, $MgSO_4$ 1.18, KH_2PO_4 1.18, $NaHCO_3$ 24.9, and glucose 10.0. The aorta was cleaned of excess connective tissue and fat and cut into rings of approximately 4 mm in length. One ring of each pair was left intact, and in the other ring, endothelium was mechanically removed by gently rotating it on a glass rod. Aortic rings were suspended between the bases of two triangular-shaped wires. One wire was attached to a fixed tissue support in a 50 ml isolated tissue bath containing Krebs solution (pH 7.4) maintained at 37°C and continuously aerated with a mixture of 5% CO_2 and 95% O_2. The other end of each wire was attached by a cotton thread to a F60 isometric force transducer connected to MK-IV-P physiograph (Narco Biosystems, USA). In all experiments, special care was taken to avoid damaging the luminal surface of endothelium. The rings were allowed to equilibrate for 90 min under a resting tension of 2 g before experiments were begun. This had been shown in preliminary experiments to be the optimal resting tension for all groups. During equilibration period, the rings were washed every 30 min.

At the end of the equilibration period, dose-response curves were obtained with phenylephrine (PE) in aortic rings. PE was added in a cumulative manner until a maximum response was achieved. After the addition of each dose, a plateau response was obtained before addition of a subsequent dose. Consecutive concentration-response curves were taken at 30-min intervals, during which the Krebs solution was changed at least 3 times. After each experiment, aortic rings were blotted, weighed, and the cross-sectional area (csa) was calculated using the following formula: Cross-sectional area (mm^2) = weight (mg) × [length (mm) × density (mg/mm^3)]$^{-1}$. The density of the preparations was assumed to be 1.05 mg/mm^2 [14]. The mean weight of the blotted aortic rings was recorded as 10.4 mg for control and as 9.8 mg for diabetic rats.

Drugs and chemicals. Phenylephrine-HCl and STZ were purchased from Sigma Chemical (St. Louis, Mo., USA). All other chemicals were purchased from Merck (Germany). All drugs except STZ were dissolved in Krebs' solution. STZ was freshly dissolved in 0.9% saline solution.

Table 1. Body weight, serum glucose level, and aortic cross-sectional area of control, diabetic, and quinapril-treated diabetic rats.

	Body weight (g)		Serum glucose (mg/dl)		Cross-sectional area (mm^2)
	Week 0	Week +4	Week 0	Week +4	
Control					
VC	213.0 ± 6.7	242.6 ± 5.2	107.4 ± 5.9	110.8 ± 5.1	0.99 ± 0.02
QC	207.8 ± 6.3	254.3 ± 5.2	109.2 ± 4.2	114.3 ± 4.8	0.98 ± 0.02
Diabetic					
VD	217.2 ± 5.1	174.3 ± 3.9*	99.7 ± 4.8	371.2 ± 13.2*	0.84 ± 0.02**
QD	218.6 ± 7.1	187.7 ± 4.6*	103.5 ± 5.3	358.5 ± 11.8*	0.92 ± 0.02

Data are represented as mean ± SEM. *P<0.001 (Compared to VC); **P<0.05 (Compared to VC).

Data and statistical analysis. All values were given as mean ± SEM. Contractile response to PE was expressed as grams of tension per cross-sectional area of tissue. Statistical analysis was carried out using student's paired *t*-test and one-way analysis of variance (ANOVA) followed by Tukey post-hoc test. Statistical P<0.05 was considered significant.

RESULTS

Body weight, Serum glucose, and cross-sectional area. No marked alteration in body weight or food or water intake was observed following 4-week administration of quinapril (2 mg/kg/day) in QC group compared to VC group. Body weight, serum glucose level, and cross-sectional area of the aorta have been shown in Table 1. After 4 weeks, the weight of the vehicle-treated diabetic rats was found to be significantly decreased compared to control rats (P<0.001). Untreated diabetic rats had also an elevated serum glucose level over those of control rats (P<0.001). Treatment of diabetic rats with quinapril did not cause any significant change in the above parameters. Furthermore, a significant reduction (P<0.05) in cross-sectional area of aortic rings of VD group was noted in comparison with VC group, showing the slow development of some structural changes in the wall of vascular system following diabetes induction.

Vascular reactivity. Cumulative addition of PE (10^{-9}-10^{-4} M) to the isolated organ bath resulted in concentration-dependent contractions in aortas of all groups (Fig. 1). The contractile responses to PE at concentrations higher than 10^{-7} M in the aortas from vehicle-treated diabetic rats in the presence and absence of endothelium were found to be significantly (P<0.001) greater than vehicle-treated control rats. Furthermore, concentration-response curves of aortas from quinapril-treated diabetic rats to PE were attenuated compared to vehicle-treated diabetics, especially at concentrations greater than 10^{-6} for endothelium-intact rings and their responses were closer to those of vehicle-treated controls. In addition, aortic rings with intact endothelium from quinapril-treated control group showed a significant decrease (P<0.05) in contractile response to PE only at concentrations higher than 10^{-5} when compared to vehicle-treated control.

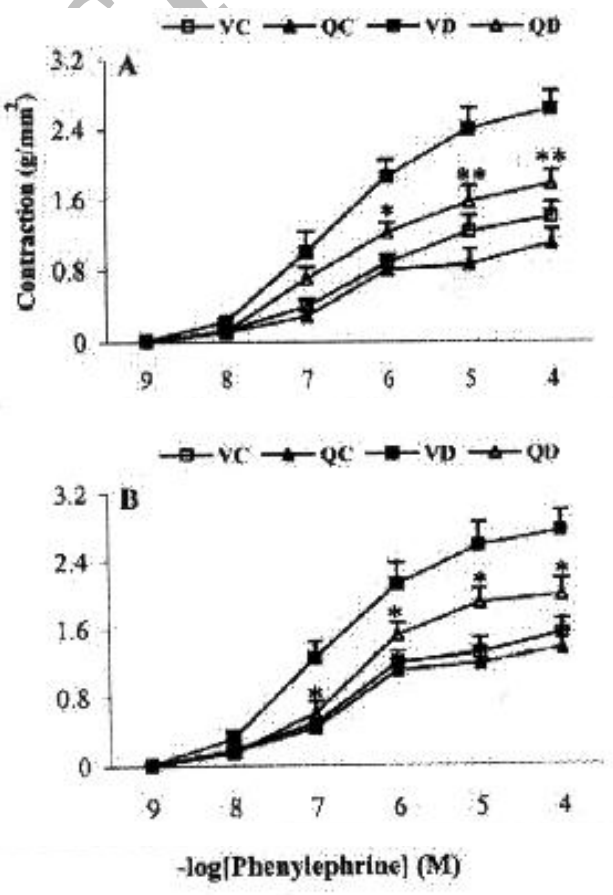

Fig. 1. Cumulative concentration-response curves for phenylephrine in aortic preparations four weeks after experiment in the presence **(A)** and absence **(B)** of endothelium. Contractile responses are expressed as grams of tension per cross sectional area (mm^2). Data are shown as means ± SEM. *P<0.05, ** P<0.01 (Compared to VD)(VC, QC, VD, and QD stand for vehicle-treated control, quinapril-treated control, vehicle-treated diabetic, and quinapril-treated diabetic, respectively).

DISCUSSION

The objective of the present study was to investigate the beneficial effect of 4-week administration of quinapril on the aortic reactivity of STZ-diabetic rats. The results of the present study demonstrated that aortas from 4-week STZ-diabetic rats are more responsive to the contractile effect of α_1-adrenoceptor agonist like PE both in the presence and absence of endothelium than those from corresponding controls. Although the actual responsible mechanisms have not been completely understood, some possible factors that could have been involved in the increased vascular smooth muscle responsiveness to α_1-adrenoceptor agonist in diabetic rats are as follows: 1) deficient endothelial activity; 2) enhanced phosphoinositide, (PI) metabolism; 3) enhanced sensitivity of calcium channels; 4) increased sensitivity to adrenergic agonists; and 5) enhanced oxidative stress due to excessive production of oxygen-free radicals and decreased antioxidant defense systems. Therefore, the oxidative stress in diabetic animals might be responsible for augmented ontractility ogether with deficient endothelial activity [15, 16].

The results also showed that daily i.p. administration of quinapril for 4 weeks could partially attenuate the increased responsiveness of aortic rings to the vasoconstrictor agent PE, especially in endothelium-intact rings. The beneficial effect of sub-chronic quinapril treatment on contractile responses was not limited to aortas of diabetic rats, because quinapril-treated control rats also showed a significant lower contractile response to PE as compared to VC group. Although ACE inhibitors are commonly used in clinical practice for the treatment of some cardiovascular abnormalities, but the mechanisms mediating their beneficial effects are not clear [17].

Several likely mechanisms could explain the protective effect of quinapril on the functional abnormalities observed in the diabetic rat aorta. The results of previous studies have shown that acute *in vitro* administration of quinapril could decrease vascular responsiveness to alpha-adrenergic agonist, possibly as a result of decreased degradation of the bradykinin. In addition, chronic treatment of normotensive rats with ACE inhibitors is reported to attenuate significantly contractile responses to both norepinephrine and phenylephrine [18]. It has also been shown that ACE inhibitors potentiate the effects of bradykinin on endothelial cells by a local mechanism, probably independent of the degradation of bradykinin [17]. Other findings reported that the effect of ACE inhibitors is not only via inhibition of the formation of vasoconstrictory angiotensin II, but also by accumulation of bradykinin that stimulates the synthesis of vasodilatory nitric oxide (NO). In this respect, ACE inhibitors could not alter NO synthesis by vascular smooth muscle cells under basal and interleukin-1 beta-stimulated conditions in cultured rat vascular smooth muscle cells [19]. The other explanation is that ACE inhibitors have shown a free radical scavenging action and may lower lipid peroxidation in vascular system. Thus, since diabetes mellitus itself is a state of increased free radical activity, antioxidant activity of quinapril and its lipid-peroxidation-lowering effect could exert reversal effect on the contractility of rat aorta [20]. It is also possible that ACE inhibitors like quinapril can directly affect Ca^{2+} handling in aortic smooth muscle cells [21]. In addition, these compounds may also reduce the vascular expression of plasminogen activator inhibitor-1 (PAI-1), an important regulator of fibrinolysis and extracellular matrix turnover vascular PAI-1 expression [22].

Taken together, this is the first study to report that treatment of diabetic rats with quinapril could prevent the functional changes in vascular reactivity observed in diabetic rats. Therefore, it is suggested that ACE inhibitors like quinapril in addition to standardized hypoglycemic agents may be administered as a beneficial therapeutic regimen for diabetic patients.

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