Quinapril Attenuates the Effect of Long-Term L-NAME Administration on the Vascular Reactivity of Diabetic Rats

Farshad Roghani Dehkordi1, Mehrdad Roghani*2 and Tourandokht Baluchnejadmojarad3

1Dept. of Cardiology and Internal Medicine, School of Medicine, Shahrekord University of Medical Sciences, Shahrekord; 2Dept. of Physiology, School of Medicine, Shahed University; 3Dept. of Physiology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

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ABSTRACT

Angiotensin-converting enzyme (ACE) inhibitors including quinapril could exert a protective effect on cardiovascular system through endothelial system in normoglycemic and diabetic rats. The present experimental work was designed to study the vascular reactivity of aortic ring segments isolated from streptozotocin (STZ)-diabetic rats treated for 4 weeks with nitro-L-arginine-methyl ester (L-NAME; 50 mg/100 ml) or L-NAME plus quinapril (10 mg/100 ml) in drinking water. The results showed that quinapril treatment significantly attenuated the augmented contractile response to phenylephrine and KCl in diabetic rats. In addition, quinapril treatment partially restored the reduced contractile response in diabetic animals treated chronically with L-NAME. It can be concluded that quinapril could partly counteract the effect of long-term L-NAME administration on vascular reactivity in STZ-diabetic rats.

INTRODUCTION

Angiotensin-converting enzyme (ACE) inhibitors including quinapril exert some beneficial and protective effects on cardiovascular system through improving endothelial ability to release vasorelaxant agents [1]. It has previously been reported that quinapril inhibits the contractile and presser effects of angiotensin I in rat aorta and lowers blood pressure in both high- and normal-renin rodent and diuretic-treated dog models of hypertension [2]. Furthermore, quinapril treatment appears to be an effective anti-hypertensive drug devoid of undesirable effects on metabolic risk factors for cardiovascular disease [3]. Quinapril also produces favorable hemodynamic changes and may improve ventricular and endothelial function in patients with various cardiovascular disorders [4]. Although ACE inhibitors like quinapril have been shown to enhance conduit artery endothelial function in animals and in patients with established coronary atherosclerosis, their precise mechanism of effect on vascular system in insulin-dependent diabetes mellitus has not been well established [5]. Since the attenuating effect of quinapril on phenylephrine (PE)-induced contractile responsiveness of aortic rings has previously been reported in streptozotocin (STZ)-diabetic rats [6], this study was undertaken to investigate the effect of quinapril on the reactivity of thoracic aorta in STZ-diabetic rats following chronic administration of nitro-L-arginine-methyl ester (L-NAME).

MATERIALS AND METHODS

Animals. Male Albino Wistar rats, weighing 230-275 g and 8-10 weeks old, were obtained from the Pasteur institute of Iran (Tehran) and housed in an air-conditioned colony room on a light/dark cycle at 21 ± 2°C and then were supplied with standard rat chow and tap water ad libitum. The animals were randomly divided into five experimental groups: control (n = 8), untreated diabetic (n = 7), quinapril-treated diabetic (QD, n = 6), L-NAME-treated diabetic (LD, n = 6) and quanipril plus L-NAME-treated diabetic groups (QDL, n = 10). Diabetes was...
induced by a single intraperitoneal injection of STZ (60 mg/kg) and dissolved in cold 0.9% saline immediately before use. Inhibitor of nitric oxide (NO) synthesis (L-NAME) was given in drinking water for 4 weeks at a concentration of 50 mg/100 ml of tap water. Other rats were given L-NAME (50 mg/100 ml) plus quinapril (10 mg/100 ml). All of the experimental groups received the treatments for a period of 4 weeks. Serum glucose level and body weight were monitored at the start and the end of the experiment. Diabetes was verified by a serum glucose level higher than 250 mg/dl 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 and decapitated. Then, 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; CaCl2, 2.5; MgSO4, 1.18; KH2PO4, 1.18; NaHCO3, 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. 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% CO2 and 95% O2. 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. In preliminary experiments, this had been shown to be the optimal resting tension for all groups. During equilibration period, the rings were washed every 30 min. For examining the endothelial integrity, pre-constricted rings with phenylephrine (PE, 1 µM) were exposed to a single addition of acetylcholine (ACh, 10 µM). Only those endo-thelium-intact rings exhibiting more than 50% relaxation in response to ACh were used for further experiments. In all experiments, after addition of each dose, a plateau response was obtained before addition of a subsequent dose. After an initial equilibration, the aortic rings were allowed to achieve maximal tension by cumulative addition of KCl (10-50 mM) or phenylephrine (10⁻⁹-10⁻⁵ M) to the bath solution.

**Drugs and chemicals.** Phenylephrine-HCl was obtained from Darupakhsh Co. (Tehran, Iran). L-NAME 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.

**Data and statistical analysis.** All values were given as mean ± SEM. Contractile response to PE and KCl was expressed as grams of tension (g). 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**

Phenylephrine (10⁻⁹-10⁻⁵ M) induced a concentration-dependent contraction of the aortic rings of all experimental groups with a peak tension of 1.12 ± 0.05, 1.44 ± 0.06, 1.01 ± 0.07, 1.27 ± 0.06, and 1.17± 0.06 g in the control, diabetic, LD, QD, and QDL groups, respectively (Fig. 1A). In this respect, the existing differences between control and diabetic and between diabetic and QD groups were statistically significant (*P*<0.01 and *P*<0.05 respectively). In addition, chronic administration of L-NAME in diabetic group caused a marked significant reduction in contractile response of aortic rings as compared to untreated diabetic group at PE concentrations higher than 10⁻⁶ M (*P*<0.005). Meanwhile, co-administration of quinapril and L-NAME in QDL group partially restored the attenuated contractile response to the level of quinapril-treated diabetic group at PE concentrations greater than 10⁻⁷ M.

Addition of KCl (10-50 mM) caused a dose-dependent contraction of the aortic rings in all of the experimental groups (Fig. 1B). In this regard, the existing differences between control and diabetic and between diabetic and QD groups were also statistically significant (*P*<0.005 and *P*<0.05, respectively). Furthermore, chronic administration of L-NAME in diabetic group caused a very marked significant attenuation of contractile response of aortic rings as compared to untreated diabetic group at KCl concentrations higher than 30 mM (*P*<0.001). On the other hand, co-administration of quinapril and L-NAME in QDL group partially restored this attenuated contractile response to the level of the quinapril-treated diabetic group at KCl concentrations greater than 20 mM.
Table 1 presents the body weight, serum glucose level, and maximum contractile force following KCl and PE application in the experimental groups. Regarding body weight, there was no significant differences among the groups before the experiment. In diabetic group, there was a significant reduction in body weight 4 weeks after the experiment in comparison with data before the study ($P<0.01$). Meanwhile, quinapril and L-NAME treatment individually or in combination did not significantly attenuate this reduction. Regarding serum glucose, there was also no significant differences among the groups before the experiment. In diabetic group, there was a significant increase in serum glucose 4 weeks after the experiment in comparison with data before the study ($P<0.001$). It was noteworthy that quinapril and L-NAME treatment individually or in combination did not significantly attenuate this increase in serum glucose.

**DISCUSSION**

The present study provides evidence that chronic quinapril treatment of STZ-diabetic rats could significantly attenuate the enhanced reactivity of isolated aortic rings in response to general and specific vasoconstrictors including KCl and PE. Meanwhile, chronic quinapril treatment caused a partial reversal of the reduced contractile response in L-NAME-treated diabetic group.

Although the actual responsible mechanisms for increased reactivity of aorta in diabetic state have not completely been understood, some possible factors that could have been involved in the increased vascular smooth muscle responsiveness to $\alpha_1$-adrenoceptor agonist in diabetic rats are deficient endothelial activity, enhanced phosphoinositide (PI) metabolism, enhanced sensitivity of calcium channels, increased sensitivity to adrenergic agonists and 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 contractility together with deficient endothelial activity [7-8].
In this study, chronic L-NAME administration to diabetic rats unexpectedly induced a reduction in contractile response to vasoconstrictors that was partly prevented by the angiotensin converting enzyme inhibitor quinapril. The reduced contractile response in L-NAME-treated diabetic group may be associated with a decreased vascular reactivity of the aorta in vitro to exogenously added phenylephrine and KCl, and drugs acting beyond receptor activation. In this respect, it has previously been demonstrated that consequences of acute NO blockade differs from chronic NO blockade with L-NAME. Acute L-NAME administration was associated with an increased vascular reactivity in vitro, whereas chronic L-NAME treatment induced a hyporeactivity to the same stimulus in the same vessel in vitro [9].

Chronic NO blockade might also induce a down regulation by chronic hyperstimulation of the contractile signaling pathways in aortic smooth muscle cells. This would be a response to an increased endogenous tonic contractile state in response to a normal level of contractile agonists, as previously shown [10-12]. This is supported by the observations that either angiotensin converting enzyme inhibitor or angiotensin receptor blockers can prevent L-NAME-induced hypertension [13-14]. Thus, multiple pathways might be triggered in response to chronic NO synthase inhibition. Further investigations are certainly needed to elucidate the mechanism or mechanisms involved in L-NAME–induced hyporeactivity.

In summary, the results demonstrated that chronic NO inhibition could decrease the contractile responsiveness of aortic rings from STZ-diabetic rats and chronic quinapril treatment could partially reverse this response.

REFERENCES