Protective effect of enalapril on vascular reactivity of the rat aorta

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Received 12 September 2003; received in revised form 12 September 2003; accepted 24 February 2004

Abstract

Cardiovascular complications are the major cause of morbidity and mortality in patients with diabetes mellitus (DM). Strategies that interrupt the renin–angiotensin system have been shown to reduce the ensuing threatening risk factors. The present study was carried out to investigate the effect of subchronic administration of enalapril on the aortic reactivity of streptozotocin (STZ)-diabetic rats. For this purpose, STZ-diabetic rats received enalapril (10 and 20 mg/kg ip) daily for 2 months. Contractile responses to phenylephrine (PE) and relaxation responses to acetylcholine (Ach) and isosorbide dinitrate (ISD) were obtained from aortic rings. Concentration–response curves from enalapril-treated diabetic (ED) rats to PE were attenuated as compared to vehicle-treated diabetics (VD), especially at a dose of 20 mg/kg for enalapril. In addition, endothelium-dependent relaxation responses induced by Ach was significantly higher in ED rats as compared to diabetic ones. The endothelium-independent relaxation responses for ISD were also found not to be significantly different among the groups. Therefore, subchronic treatment of diabetic rats with enalapril in a dose-dependent manner could prevent the functional changes in vascular reactivity in diabetic rats.

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Keywords: Aortic reactivity; Diabetes mellitus; Enalapril; Streptozotocin; Rat

1. Introduction

Diabetes mellitus (DM) has been identified as a primary risk factor for cardiovascular disorders (Uemura et al., 2001) and alters the vascular responsiveness to several vasoconstrictors and vasodilators (Senses et al., 2001). Extensive efforts are under way to determine interventions that may have the potential to prevent or halt the devastating complications of DM (Vinik and Vinik, 2003). 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 the insulin resistance syndrome and DM (Kirpichnikov et al., 2002).

The ACE inhibitors, including captopril and enalapril, have been widely used for the treatment of hypertension, myocardial infarction and congestive heart failure for many years. They also retard the development of chronic renal failure and diabetic nephropathy (Omata et al., 1996) and atherosclerosis (Chobanian et al., 1990) in experimental models and may be advantageous for the improvement of endothelial dysfunction in patients with coronary artery disease (Mancini et al., 1996). 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 (Van Gilst et al., 1986). The enhancement of bradykinin-induced relaxation (Gohlke and Unger, 1995), augmented release of endothelium-derived nitric oxide (NO; Auch-Schwelk et al., 1995), decreased production of endothelin-I (Itoh et al., 2002) and a free radical scavenging action (Chopra et al., 1992) have also been postulated. Renoprotective effects of the ACE inhibitors in streptozotocin (STZ)-induced diabetes have also been reported in rats (Kalender et al., 2002). 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 (Kedziora-Kornatowska et al., 2000). Although ACE inhibitors 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 DM has not been well known and their efficacy is still controversial (Mullen et al., 1998). Therefore, the present study was carried out to investigate the effect of subchronic administration of enalapril on the aortic reactivity of STZ-diabetic rats.

2. Materials and methods

2.1. Animals

Male albino Wistar rats (Pasteur’s institute, Tehran, Iran) weighing 205–235 g (7–9 weeks old) were housed in an air-conditioned colony room on a light/dark cycle at 21 °C and supplied with standard pellet diet and tap water ad libitum. Procedures involving animals and their care were conducted in conformity with the institutional guidelines of Iran University of Medical Sciences (Tehran, Iran) and in accordance with the NIH guidelines for the care and use of laboratory animals.

The animals were randomly divided into four experimental groups; i.e., vehicle-treated control (VC, n=8) receiving 0.9% saline, enalapril-treated control in two equal-sized subgroups (EC, n=8 for each subgroup), saline-treated diabetic (VD, n=8), and enalapril-treated diabetic in two equal-sized subgroups (ED, n=8 for each subgroup). Diabetes was induced by a single intraperitoneal injection of STZ (60 mg kg⁻¹) dissolved in cold 0.9% saline immediately before use. Enalapril was administered from Day +3 thereafter at a dose of 10 and 20 mg/kg. All of the experimental groups received the treatments daily and intraperitoneally for a period of 2 months. Serum glucose level and body weight were monitored at the start and at 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).

2.2. Experimental protocol

At the end of the experiment, the rats were anesthetized with diethyl ether, decapitated, and through 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

Table 1

<table>
<thead>
<tr>
<th>Body weight (g)</th>
<th>Serum glucose (mg/dl)</th>
<th>Csa (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VC 205±7.5</td>
<td>247.6±4.4</td>
<td>119.4±6.6</td>
</tr>
<tr>
<td>EC 217.8±5.1</td>
<td>261.4±4.8</td>
<td>107.2±4.9</td>
</tr>
<tr>
<td>Diabetic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VD 212.3±5.2</td>
<td>170.3±4.2**</td>
<td>98.7±5.2</td>
</tr>
<tr>
<td>ED 230.5±4.1</td>
<td>199.1±6.1**</td>
<td>101.5±4.7</td>
</tr>
</tbody>
</table>

Data are represented as mean±S.E.M.

* P<.05 (compared to VC).
** P<.005 (compared to VC).
*** P<.001 (compared to VC).

Fig. 1. Cumulative concentration–response curves for PE in aortic preparations 2 months after experiment in the presence (A) and absence (B) of endothelium. Contractile responses are expressed as grams of tension per csa (mm²). Data are shown as means±S.E.M. *P<.05 (EC as compared to VD). (VC, EC, VD, and ED stand for vehicle-treated control, enalapril treated control, vehicle-treated diabetic, and enalapril (10 mg/Kg)-treated diabetic rats respectively).
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 addition of each dose, a plateau response was obtained before addition of a subsequent dose. To evaluate acetylcholine (Ach; $10^{-9} – 10^{-4}$ M) - and isosorbide dinitrate (ISD; $10^{-9} – 10^{-4}$ M) - induced vasodilation, rings with endothelium for Ach and without endothelium for ISD were preconstricted with a submaximal concentration of PE (which produced 70–80% of maximal response) to reach a stable plateau, and then, the cumulative concentration–response curves were obtained to evaluate endothelium-dependent and -independent relaxations, respectively. Consecutive concentration–response curves were taken at 30-min intervals, during which the Krebs’ solution was changed at least three times. The sensitivity to the agonists was evaluated as pD2, which is the negative logarithm of the concentration of the drug required to produce 50% of maximum response.

After each experiment, aortic rings were blotted, weighed, and the cross-sectional area (csa) was calculated using the following formula: 
\[
\text{csa (mm}^2\text{)} = \frac{\text{weight (mg)} \times \text{length (mm)} \times \text{density (mg mm}^{-3}\text{)}}{\text{density (mg mm}^{-3}\text{)}}\]
\[
= \text{weight (mg)} / \text{density (mg mm}^{-3}\text{)} \times \text{length (mm)} \times 10^{-3}\text{.}
\]
The density of the preparations was assumed to be 1.05 mg mm$^{-2}$ (Abebe et al., 1990).

### 2.3. Drugs and chemicals

PE–HCl, Ach chloride and STZ were purchased from Sigma (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.

### 2.4. Data and statistical analysis

All values were given as mean±S.E.M. Contractile response to PE was expressed as grams of tension per csa of tissue. Relaxation responses for Ach and ISD were expressed as a percentage decrease of the maximum contractile response induced by PE. Statistical analysis was

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**Fig. 2.** See legend of Fig. 1. *P<.05, **P<.01 (ED as compared to VD). (VC, EC, VD, and ED stand for vehicle-treated control, enalapril treated control, vehicle-treated diabetic, and enalapril (20 mg/Kg)-treated diabetic rats respectively).**

**Fig. 3.** Cumulative concentration–response curves for Ach (A) and ISD (B) in aortic rings precontracted with PE 2 months after experiment. Relaxation responses are expressed as a percentage. Data are shown as means±S.E.M. *P<.05 (compared to VD). (VC, EC, VD, and ED stand for vehicle treated control, enalapril (10 mg/Kg)-treated control, vehicle-treated diabetic, and enalapril-treated diabetic respectively).**
carried out using Student’s paired t test and one-way analysis of variance (ANOVA) followed by Tukey post hoc test. Statistical P value less than .05 was considered significant.

3. Results

3.1. Body weight, serum glucose and csa

No marked alteration in body weight or food or water intake was observed following 2-month daily administration of enalapril (10 and 20 mg/kg) in EC group compared to VC group. Body weight, serum glucose level, and csa of the aorta have been shown in Table 1. After 2 months, the weight of the vehicle-and enalapril (20 mg/kg)-treated diabetic rats was found to be significantly decreased as compared to control rats (P<.005). Untreated diabetic rats had also an elevated serum glucose level over those of control rats (P<.001). Treatment of diabetic rats with enalapril (20 mg/kg) did not cause any significant change in the above parameters. Furthermore, a significant reduction (P<.05) in csa of aortic rings of VD group was noted in comparison with VC group.

3.2. 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 (Figs. 1 and 2). The contractile responses to PE at concentrations higher than 10^{-7} M in the aortas from VD rats in the presence and absence of endothelium were found to be significantly (P<.001) greater than VC rats (Figs. 1 and 2). However, in endothelium-denuded diabetic aortic rings, the maximum contractile response to PE was 10.7% greater in comparison with endothelium-intact diabetic ones. Furthermore, concentration–response curves of aortas from enalapril (20 mg/kg)-treated diabetic rats to PE were attenuated as compared to VD, especially at concentrations greater than 10^{-5} for endothelium-intact rings and their responses were closer to those of VC. In addition, aortic rings with intact endothelium from enalapril (20 mg/kg)-treated control group showed a significant decrease (P<.05) in contractile response to PE only at concentrations higher than 10^{-6} when compared to VC (Fig. 2).

Addition of Ach resulted in concentration-dependent relaxations in all aortic rings precontracted with PE (Figs. 3A and 4A). As it was expected, endothelium-dependent relaxation responses induced by Ach was significantly lower in VD rats in relation to VC. Meanwhile, the existing difference between enalapril (20 mg/kg)-treated and VC rats was only significant (P<.01) at concentrations higher than 10^{-6} M (Fig. 4A). Although there existed a marked and greater relaxation response to Ach in enalapril (20 mg/kg)-treated controls compared to VC, the existing difference did not reach a significant level. The endothelium-independent relaxation responses for ISD were also found not to be significantly different in the VC, EC, VD and ED rats (Figs. 3A and 4A).

Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>pD2</th>
<th>(E_{\text{max}})</th>
<th>pD2</th>
<th>(E_{\text{max}})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PE</td>
<td>Ach</td>
<td>PE</td>
<td>Ach</td>
</tr>
<tr>
<td></td>
<td>E+</td>
<td>E−</td>
<td>E+</td>
<td>E−</td>
</tr>
<tr>
<td>VC</td>
<td>6.48±0.28</td>
<td>6.89±0.12</td>
<td>1.45±0.17</td>
<td>1.53±0.17</td>
</tr>
<tr>
<td>EC</td>
<td>6.59±0.29</td>
<td>7.21±0.19</td>
<td>0.98±0.16</td>
<td>1.18±0.12</td>
</tr>
<tr>
<td>VD</td>
<td>6.72±0.21</td>
<td>6.69±0.18</td>
<td>2.51±0.21</td>
<td>2.69±0.24</td>
</tr>
<tr>
<td>ED</td>
<td>6.67±0.17</td>
<td>6.54±0.13</td>
<td>1.83±0.15*</td>
<td>1.98±0.17*</td>
</tr>
</tbody>
</table>

Data are expressed as mean±S.E.M. For PE and Ach, maximum responses (\(E_{\text{max}}\)) are presented as g/mm² and percentage decrease of the maximum contractile response induced by PE, respectively.

* P<.01 (significantly different from VD).
Maximum responses ($E_{\text{max}}$) of aortic rings from control, diabetic and enalapril (20 mg/kg)-treated diabetic rats and their sensitivity, expressed as $pD_{2}$, to abovementioned vasoactive agents have been shown in Table 2. In this respect, there was no significant difference among the groups regarding $pD_{2}$.

4. Discussion

The objective of the present study was to investigate the beneficial effect of 2-month administration of enalapril on the aortic reactivity of STZ-diabetic rats. For this purpose, contractile and relaxation responses of aortic rings to vasoactive chemicals were investigated. There are three major conclusions to be drawn from the obtained results.

First, the results of the present study demonstrated that aortas from 2-month STZ-diabetic rats are more responsive to the contractile effect of PE, especially in endothelium-intact rings, and also diminish the reduction in endothelium-dependent relaxation responses to Ach in diabetic rats. The beneficial effect of subchronic enalapril treatment on contractile responses was not limited to aortas of diabetic rats, because EC rats also showed a significant lower contractile response to PE as compared to VC group. The results also indicated that enalapril did not modify the sensitivity ($pD_{2}$) of vascular smooth muscle of diabetic rats to PE and Ach. Although ACE inhibitors are commonly used in clinical practice for the treatment of cardiovascular complications, the mechanisms mediating their beneficial effects are not clear (Auch-Schwelk et al., 1995). In this study, several possible mechanisms could explain the protective effect of enalapril on the functional abnormalities observed in the diabetic rat aorta. The results of previous studies have shown that acute in vitro administration of ACE inhibitors could decrease vascular responsiveness to $\alpha$-adrenergic agonists and produce increased relaxation responses, possibly as a result of decreased degradation of the bradykinin (Kikta and Fregly, 1982). In addition, chronic treatment of normotensive rats with ACE inhibitors is reported to attenuate significant contractile responses to both norepinephrine and PE (Kikta and Fregly, 1983). Contrary to our findings, it has been previously demonstrated that following enalapril administration (10 mg kg$^{-1}$ once daily) for 2 weeks, there is no improvement in the impaired endothelium-dependent and -independent relaxant responses in the isolated rat aorta (Duarte et al., 1999). The inability of enalapril to prove efficacy in that study could be attributed to its lower dosage being administered for a shorter time period and the model of diabetes that was investigated. In addition, it has been demonstrated that ACE inhibitors could diminish contractile responses to PE in the presence of a functional endothelium in normal rats that is not possibly mediated through bradykinin receptors (Lemos et al., 2002). In this respect, it has also been shown that these compounds potentiate the effects of bradykinin on endothelial cells by a local mechanism, probably independent of the degradation of bradykinin (Auch-Schwelk et al., 1995). Furthermore, ACE inhibitors may also stimulate prostacyclin (PGI2) synthesis in arterial tissue and that this effect may be secondary to changes in the activity of the kinin system (Hoffmann et al., 1990). Another explanation for the beneficial effect of enalapril may be attributed to its free radical scavenging action (Wojakowski et al., 2000). In this respect, reactive oxygen species (ROS) are known to be involved in the pathogenesis and progression of various cardiovascular diseases and ACE inhibitors may act as an antioxidant in addition to their hemodynamic effects (Sand et al., 2001). It is also possible that ACE inhibitors can directly affect Ca$^{2+}$ handling in aortic smooth muscle cells (Zhu et al., 1993). In this respect, it has been demonstrated that these compounds could block KCl- and noreadrenaline-induced increase of intra-
cellular calcium in smooth muscle cells via a voltage-dependent Ca\(^{2+}\) channel (Qi et al., 1996). Finally, they may reduce the vascular expression of plasminogen activator inhibitor-1 (PAI-1), an important regulator of fibrinolysis and extracellular matrix turnover vascular PAI-1 expression (Hamdan et al., 1996).

In conclusion, to the best of our knowledge, this is the first study to report that in vivo treatment of diabetic rats with enalapril in a dose-dependent manner could prevent the functional changes in vascular reactivity observed in diabetic rats. Therefore, it is suggested that ACE inhibitors, like enalapril, in addition to hypoglycemic agents may be administered as an auxiliary beneficial therapeutic regimen for diabetic patients.

Acknowledgements

This study was supported by a grant-in-aid from the IUMS University (Tehran, Iran). We also gratefully appreciate personnel of the Department of Physiology of IUMS University for their excellent technical assistance.

References


