Curcumin increases vasodilatory effect of cilostazol in diabetic rat aorta

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Increased generation of oxidants and (or) reduced endogenous antioxidant defense mechanisms are associated with the etiology of diabetic vascular complications. The aim of the present study was to evaluate whether curcumin supplementation increases the vasodilatory effect of cilostazol in streptozotocin induced diabetic rat aorta. Cumulative addition of cilostazol caused concentration-dependent relaxations of thoracic aorta rings. The sensitivity and the maximal response to cilostazol were significantly higher in control than those in diabetic animals. Treatment with curcumin in control rats increased the sensitivity to cilostazol. Further, in aortic rings from diabetic rats treated with curcumin, the responses to cilostazol were significantly increased in comparison to the response in aorta from untreated diabetic rats. It can be conclude, that curcumin increases the cilostazol-induced vasodilation in diabetic rat aorta.

Keywords: Aorta, Cilostazol, Curcumin, Diabetes

Cardiovascular disease is one of the most common complications in diabetic patients and causes more than half of diabetes-related mortality. Hyperglycemia increases the expression of adhesion molecules, such as vascular cell adhesion molecule (VCAM)-1 in endothelial cells and smooth muscle cells, resulting in inflammation and vascular dysfunction. Thus, the prevention of the adhesion molecule expression in aorta may be an important therapeutic strategy for the treatment of cardiovascular disease in diabetic and nondiabetic patients as well.

In recent years, cilostazol, a quinolinone derivative, a potent phosphodiesterase III and adenosine uptake inhibitor, has drawn a great interest because of its inhibitory effect on the overexpression of VCAM-1. Cilostazol suppresses adhesion molecule expression in human umbilical vein endothelial cells in vitro. Its anti-platelet, vasodilatory and anti-proliferative effects are utilized in treating peripheral arterial disease. Its anti-atherosclerotic effects have also been reported. Further, recently, cilostazol has been increasingly prescribed because of its protective effect against vascular inflammation in diabetic patients, and attenuation of the increase in carotid artery intimal thickness in patients with type 2 diabetes.

Curcumin, a naturally occurring phenolic compound isolated as a yellow pigment from turmeric (dry rhizomes of Curcuma longa) which is commonly used as a spice and food colorant. This compound has been reported to possess a variety of biological and pharmacological activities, including antioxidant, anti-inflammatory, anticarcinogenic and antidiabetic. Since it is widely accepted that oxidative stress and inflammation contribute to atherogenesis, effects of curcumin on cardiovascular systems have also received much attention recently.

Increased oxidative stress has been associated with the pathogenesis of chronic diabetic complications, including cardiomyopathy. The nitric oxide (NO) pathway is suggested to be involved in augmenting oxidative stress. NO is produced by a set of three nitric oxide synthase (NOS) isozymes: endothelial NOS (eNOS), inducible NOS (iNOS), and neuronal NOS (nNOS). These enzymes convert L-arginine to L-citrulline, leading to the generation of the free radical NO. eNOS and iNOS play an essential role in the pathogenesis of cardiovascular complications in diabetes. As curcumin has a striking anti-oxidant property and has been shown to down-regulate NOS and reduce NO oxidation by sequestering the reaction...
intermediate, nitrogen dioxide\textsuperscript{28}, it may have preventive effective on the development of cardiovascular complications in diabetes. By increasing intracellular cyclic adenosine monophosphate (cAMP), cilostazol activates protein kinase A, which activates eNOS\textsuperscript{29,30}. Further, cilostazol increases interstitial adenosine concentrations by inhibiting the reuptake of adenosine into cells\textsuperscript{31}.

Though the in vitro effects of curcumin and cilostazol have been reported, to date, no experimental studies have assessed the effects of both cilostazol and curcumin in diabetes. The purpose of the present study is to investigate the effects of curcumin supplementation the response to cilostazol of diabetic rat aortas.

Materials and Methods

**Animals**—Male Wistar rats (6-8 weeks old) were divided randomly into 4 groups of 6 rats each. The experimental groups included normal healthy (Gr I), curcumin (200 mg/kg/day, po, for 4 weeks)-treated (Gr II), pathologic; diabetic (DM) (Gr III), and DM-curcumin-treated (Gr IV). All experiments were carried out with the approval of Local Animal Use Ethical Committee of Selçuk University. Diabetes was induced by a single injection of streptozotocin (STZ, 50 mg/kg, ip) that was prepared in a physiological saline solution, pH 4.5. Plasma glucose levels were determined from tail vein blood samples (Acura Ac 1018) 72 h after STZ injection. Rats with blood glucose concentration of 300 mg/dL or more 72 h after STZ injection were considered diabetic. Control rats were injected with saline only. After diabetes was verified, curcumin (200 mg/kg/day) was given for 4 weeks in the DM-curcumin-treated group. Body weights of rats were measured in all groups before and for 4 weeks after diabetes induction. All rats were kept under identical conditions for 4 weeks with free access to food and water before the experiments were conducted. After 4 weeks of curcumin treatment, rats were anaesthetised with sodium thiopenthal (40 mg/kg, ip).

**Drugs**—Cilostazol was provided by Abdi İbrahim (Istanbul, Turkey). Other chemicals used in experiments were obtained from Sigma Chemical (St. Louis, MO, USA). Cilostazol was dissolved in dimethylsulfoxide and curcumin in corn oil.

**Experimental design**—The descending thoracic aorta was quickly isolated, cleaned and sectioned into 3- to 4-mm-long rings. The rings were then placed in 25 mL organ baths containing Krebs-Henseleit solution (KHS, mM: NaCl 119, KCl 4.70, MgSO\textsubscript{4} 1.50, KH\textsubscript{2}PO\textsubscript{4} 1.20, CaCl\textsubscript{2} 2.50, NaHCO\textsubscript{3} 25, Glucose 11), which were thermoregulated at 37 °C and aerated (95% O\textsubscript{2} and 5% CO\textsubscript{2}). Changes in isometric tension were recorded by a force-displacement transducer (BIOPAC MP36, Santa Barbara, California, USA) connected through amplifiers to a ITBS08 Integrated Tissue Bath System (Commat, Ankara, Turkey). The rings were equilibrated for 60 min under a resting tension of 1 g before experiments began. After equilibration, the rings were contracted with 5-hydroxytryptamine (5-HT; 10\textsuperscript{-6} M, 0.1 mL). At the peak of contraction, a cumulative concentration-response curve for cilostazol (10\textsuperscript{-8}-3x10\textsuperscript{-4} M) was obtained on each ring. The same procedure was determined in all groups.

The influence of nitric oxide on relaxations to cilostazol was specifically addressed by pre-treating the control aortic rings with the nitric oxide synthase inhibitor N\textsuperscript{G} nitro-L-arginine methyl ester (L-NAME, 10\textsuperscript{-4} M, 0.1 mL).

**Statistical analysis**—Relaxation to cilostazol was expressed as a percentage decrease of the 5-HT-induced contraction. Data are presented as group means ± SE. Maximal responses and IC\textsubscript{50} values for curves were compared by using Student’s t test. Statistical significance was set at $P<0.05$.

Results

**General characteristics of the Rats**—The initial and final body weights and blood glucose levels for all animals of the four treatment groups are shown in Table 1. There was no difference in the initial weights in the animals from the four groups but the diabetic rats lost significantly ($P<0.05$) more weight than the control rats (Gr I). Curcumin treatment (Gr II) did not significantly affect the final weight of either control rat. After diabetes induction, the DM-curcumin-treated, diabetic and diabetic-curcumin-treated rats lost significantly ($P<0.05$) more weight than the control group (Gr I).

<table>
<thead>
<tr>
<th>Body Weight (g)</th>
<th>Blood glucose level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>After 6 weeks</td>
</tr>
<tr>
<td>Control</td>
<td>270 ± 5.0</td>
</tr>
<tr>
<td>Curcumin</td>
<td>272 ± 2.3</td>
</tr>
<tr>
<td>DM</td>
<td>275 ± 3.2</td>
</tr>
<tr>
<td>DM+</td>
<td>270 ± 2.5</td>
</tr>
<tr>
<td>Curcumin</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} $P<0.05$; compared to control group

Table 1—Body weight, and blood glucose levels of control, curcumin-treated, diabetic and diabetic-curcumin-treated rats [Values are mean ± SE of 6 animals]
(Gr-I) or diabetic (Gr-III) rats. All of the STZ-treated rats (Gr-III) exhibited significantly elevated blood glucose levels and curcumin treatment did not affect those levels in either control (Gr-I) or diabetic rats (Gr-III).

**Vascular relaxation**—Cumulative addition of cilostazol ($10^{-8}$-$3 \times 10^{-4}$ M) to the isolated organ bath resulted in concentration-dependent relaxations of aortic rings precontracted with 5-HT ($10^{-6}$ M) in all groups (Fig. 1). Treatment with L-NAME decreased the vasodilatory effect of cilostazol, pIC$_{50}$ values were found as 5.8±0.2 in control and 4.5±0.3 with L-NAME, respectively. The sensitivity and maximum relaxation to cilostazol was significantly reduced in diabetic rats (Gr-III) in comparison to the control rats (Gr-I). Treatment with curcumin (200 mg/kg/day) for 4 weeks in control rats increased the sensitivity to cilostazol. Further, in aortic rings from diabetic rats treated with curcumin, the responses to cilostazol were significantly increased in comparison to the response in aorta from untreated diabetic rats (Fig. 1, Table 2).

**Discussion**

In the present work, the effects of curcumin supplementation were studied on cilostazol-induced vasodilation of aorta from diabetic rats. To the best of our knowledge the present study is the first to show the responses to cilostazol in aortic rings from curcumin supplemented diabetic rats.

The present results showed that cilostazol induced concentration dependent relaxation of rat thoracic aorta. Cilostazol is an inhibitor of phosphodiesterase III and therefore prevents the breakdown of cAMP by blocking its hydrolysis. Drugs that increase the level of cAMP, are thought to dilate blood vessels by increasing the concentration of cAMP in vascular smooth muscle cells, subsequently activating protein kinase A and reducing the level of intracellular calcium. Recent preclinical studies have demonstrated that cilostazol also possesses the ability to inhibit adenosine uptake, a property that may distinguish it from other phosphodiesterase III inhibitors. Elevation of interstitial and circulating adenosine levels by cilostazol has been found to potentiate the cAMP-elevating effect of phosphodiesterase III inhibition in platelets and smooth muscle, thereby augmenting antiplatelet and vasodilatory effects of the drug. It was suggested that cilostazol-induced NO release is involved in endothelium-dependent relaxation in rat aorta and the inhibition of high glucose-mediated endothelial-neutrophil adhesion in human endothelial cells. Hashimoto et al. reported that cilostazol induces NO production. In the present study, it was also observed that pre-incubation with the nitric oxide synthase inhibitor N$^G$ nitro-L-arginine methyl ester (L-NAME) decreased the vasodilatory effect of cilostazol. On the other hand, Birk et al. demonstrated that cilostazol produced a vasodilation of guinea pig basilar arteries which was not dependent on an intact endothelium. Further, it is also reported that in rabbit cerebral penetrating arterioles, cilostazol produces vasodilation independent of the presence or activation of the endothelium. This discrepancy may depend on species and tissues differences.

Diabetes mellitus (DM) is characterized by chronic hyperglycemia and develops diabetic complications,

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**Table 2**—IC$_{50}$ and $E_{\text{max}}$ values for cilostazol-induced relaxations of aortic rings from rats

<table>
<thead>
<tr>
<th>Cilostazol</th>
<th>IC$_{50}$($\times 10^{-6}$)</th>
<th>$E_{\text{max}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.7 ± 0.3</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>Curcumin</td>
<td>0.4 ± 0.2</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>DM</td>
<td>6.4 ± 0.1*</td>
<td>93 ± 0.3*</td>
</tr>
<tr>
<td>DM+Curcumin</td>
<td>3.6 ± 0.2b</td>
<td>96± 0.7b</td>
</tr>
</tbody>
</table>

$P<0.05$; compared to acontrol, bdiabetic
in particular, macroangiopathy and microangiopathy. These pathophysiological complications are often responsible for a decreased quality of life in diabetic patients. Hyperglycemia can produce reactive oxygen species (ROS) production by a series of cellular events and that further leads to diabetic complications due to oxidative stress. In the present study, in aortic rings from diabetic rats, cilostazol-induced relaxations were impaired as compared to control responses. Recently, cilostazol has been increasingly prescribed by endocrine physicians because of its protective effect against vascular inflammation in diabetic patients. Suzuki et al. demonstrated that cilostazol activates AMP-activated protein kinase (AMPK) in endothelial cells, resulting in increased NO production and restoration of endothelium-dependent vasodilation of diabetic rats. The investigators also reported that this endothelium-dependent effect of cilostazol may be due to eNOS activation. Oida et al. reported that cilostazol not only dilates blood vessels, but also suppresses platelet aggregation and smooth muscle cell proliferation. Therefore, cilostazol should improve endothelial dysfunction.

Anti-oxidant and anti-inflammatory properties of curcumin have been well documented. In the present study, treatment with curcumin (200 mg/kg/day) for 4 weeks in control rats significantly increased the sensitivity to cilostazol. Further, in aortic rings from diabetic rats treated with curcumin, the relaxant response to cilostazol was significantly increased in comparison to the response in aorta from untreated diabetic rats (Fig. 1, Table 2). Diabetes is known to increase vascular ROS production, resulting in decreased NO bioavailability. Agrawal et al. reported that like other phosphodiesterase inhibitors, cilostazol may suppress the formation in platelets and endothelial cells and improves cellular redox status. Rungseesantivanon et al. reported that the supplementation of curcumin improves diabetes-induced endothelial dysfunction, as shown by the increase in ACh-activated vasodilation, through its ability to decrease one of the most important ROS; superoxide anion.

In conclusion, the present results suggest that curcumin supplementation significantly improves the vasodilatory effect of cilostazol in diabetic rat aorta. This study demonstrates for the first time that curcumin treatment may increase the cilostazol-induced responses of diabetic rat aorta.

**Acknowledgement**

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**References**