Effects of Nitric Oxide Scavenger, Carboxy-PTIO on Endotoxin-Induced Alterations in Systemic Hemodynamics in Rats

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ABSTRACT—The present experiments were conducted to clarify the mode of cardiovascular action of carboxy-2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (carboxy-PTIO), a nitric oxide (NO) scavenger, during rat endotoxic shock by determining cardiac output and systemic arterial tone simultaneously. Lipopolysaccharide (LPS) (10 mg/kg, i.v.) decreased systemic blood pressure and cardiac output with transient increases in hematocrit and total vascular resistance. Administration of carboxy-PTIO (1.7 mg·kg⁻¹·min⁻¹, i.v. for 60 min) at 90 min after LPS attenuated further decline in blood pressure and cardiac output without affecting changes in hematocrit or total vascular resistance. It is concluded that carboxy-PTIO attenuates endotoxin-induced hypotension predominantly by maintaining cardiac output in rat experimental endotoxic shock.

Keywords: Endotoxic shock, Nitric oxide, Carboxy-PTIO

Though a number of endogenous mediators were shown to be involved in the pathogenetic sequence in endotoxic shock, overproduction of nitric oxide (NO) by inducible NO synthase (iNOS) has been considered to play a pivotal role in the pathogenesis of septic shock (1). For instance, in rodent models of experimental endotoxic shock, although a high dose of a nonselective NOS inhibitor elicited a progressive peripheral vasoconstriction as a result of inhibition of constitutive NOS (2), the appropriate choice of NOS inhibitor in terms of dosage, selectivity and timing of administration, offered beneficial effects, possibly due to blockade of excessive NO production by iNOS (1, 3). Furthermore, the administration of lipopolysaccharide (LPS) to mice deficient in iNOS showed markedly attenuated hypotension and reduced mortality, clearly demonstrating the pathological significance of the overproduction of NO by iNOS in a rodent model of endotoxic shock (4). However, it remains to be elucidated how NO is involved in the systemic hemodynamic changes seen during endotoxic shock.

The purpose of this study was to elucidate the role of overproduced NO in the endotoxin-induced systemic hemodynamic alterations by using a new class of NO scavenger, carboxy-2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (carboxy-PTIO). When administered therapeutically, carboxy-PTIO attenuated hypotension, renal dysfunction and markedly improved survival rate in rats given LPS intravenously at a dose of 10 mg/kg (5). Thus, we used the rat endotoxic shock model and studied the hemodynamic changes in detail to characterize the mode of action of carboxy-PTIO on systemic hemodynamics in endotoxic shock.

All experiments were conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society. Male Sprague-Dawley rats (Charles River Japan, Inc., Osaka) weighing 200–240 g were anesthetized with thiopental sodium (120 mg/kg, i.p.; Tanabe Pharmaceuticals, Osaka), and rectal temperature was controlled at 37–37.5°C by a heating blanket and a lamp (ATB-110D; Nihon Kohden, Tokyo). The trachea was cannulated to facilitate respiration. The left femoral artery and vein were cannulated for blood sampling and monitoring the systemic blood pressure and heart rate and for infusions of saline or drug solutions at the rate of 4.5 ml·kg⁻¹·h⁻¹, respectively. We measured cardiac output using the thermodilution technique (Cardiotherm 500-AC-R; Columbus Instruments, Columbus, OH, USA) as described previously (6). Total peripheral resistance was calculated by dividing the mean arterial pressure by the cardiac output.

After measuring control parameters, LPS (E. coli 0127: B8; Difco Laboratories, MI, USA) dissolved in saline (10
mg/ml was slowly injected intravenously (1 min) at a dose of 10 mg/kg. At 90 min after LPS administration, second measurements of each parameter were made and then intravenous infusion of carboxy-PTIO (Wako, Osaka) dissolved in phosphate-buffered saline (PBS) (1.7 mg·kg⁻¹·min⁻¹, 3 ml/h for 60 min) was started (n = 5) with consecutive measurements of cardiac output and hematocrit every 30 min until the end of the study (5 h after LPS administration). As a corresponding control group, vehicle (PBS) instead of carboxy-PTIO was administered to rats receiving LPS (n = 6). The dose and the administration protocol of carboxy-PTIO was chosen since it effectively prevented LPS-induced hypotension and improved survival rates with effective scavenging action of NO (5). In a different set of experiments, effects of carboxy-PTIO (n = 4) and its vehicle (PBS) (n = 5) were examined without LPS treatment.

Baseline hemodynamic parameters were not statistically different between the vehicle group and PTIO group before administration of LPS. Table 1 shows the time-course effects of LPS on systemic hemodynamics (vehicle group). Blood pressure was decreased with LPS. As cardiac output was more profoundly decreased, the calculated total peripheral resistance was elevated with LPS administration, suggesting that LPS caused systemic arterial vasconstriction. Heart rate was increased after LPS administration and remained elevated until the end of the study. Hematocrit was increased by approximately 5% at 90 min after LPS (when infusion of the vehicle for carboxy-PTIO started) but started to decline thereafter. Two of 6 rats in this group died following LPS administration: one by 180 min and another by 270 min after LPS. When the dead rats were excluded from the analysis, both blood pressure and cardiac output decreased progressively from 95 ± 8 mmHg (90 min) to 78 ± 12 mmHg (300 min) and from 28.3 ± 2.9 ml·min⁻¹·100 g⁻¹ to 22.9 ± 3.5 ml·min⁻¹·100 g⁻¹, respectively (Fig. 1). These results suggest that LPS-induced hypotension was caused solely by a reduction in cardiac output but not by systemic arterial vasodilation. In contrast, when rats were treated with carboxy-PTIO, all of 5 rats receiving LPS survived during the entire course of the experiments and carboxy-PTIO significantly attenuated the further decrease in blood pressure (Fig. 1), thus confirming the previous report by Yoshida et al. (5). Furthermore, carboxy-PTIO effectively attenuated the reduction in cardiac output observed in the latter phase following LPS administration (Fig. 1). As elevated total peripheral resistance by LPS was not affected by carboxy-PTIO, the present results provide clear evidence that attenuated hypotension was solely attributable to the improved cardiac output.

How did carboxy-PTIO attenuate the LPS-induced decrease in the cardiac output? As the LPS-induced change in heart rate was not modified by carboxy-PTIO treatment (Fig. 1), stroke volume per se was improved by carboxy-PTIO. Such an action of carboxy-PTIO may arise from removal of the cardiodepressant action of nitric oxide. In fact, the in vivo role of NO in parasympathetic inhibition of β-adrenergic myocardial contractility was reported (7). Alternatively, carboxy-PTIO may have improved the venous return. In this respect, two possible sites of action of carboxy-PTIO should be considered. First, carboxy-PTIO blocked preload reduction due to extravasation of plasma. However, this possibility is unlikely since the increase in the hematocrit following LPS administration was not affected by carboxy-PTIO (Fig. 1). Another possibility is that carboxy-PTIO blocked venous dilatation and decrease in venous return due to blood pooling in the capacitance vessels. In canine acute endotoxic shock, we previously reported that LPS decreased cardiac output due to, at least in part, venodilatation (8). Furthermore, NO is

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Blood pressure (mmHg)</th>
<th>Cardiac output (ml·min⁻¹·100 g⁻¹)</th>
<th>Total peripheral resistance (mmHg·min·100 g⁻¹)</th>
<th>Heart rate (beats/min)</th>
<th>Hematocrit (%)</th>
<th>Numbers of rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>107 ± 4</td>
<td>43.0 ± 1.2</td>
<td>2.49 ± 0.04</td>
<td>360 ± 19</td>
<td>47.4 ± 0.8</td>
<td>6</td>
</tr>
<tr>
<td>Lipopolysaccharide (10 mg/kg)</td>
<td></td>
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</tr>
<tr>
<td>90 min</td>
<td>85 ± 8*</td>
<td>24.3 ± 3.1*</td>
<td>3.61 ± 0.27*</td>
<td>420 ± 14*</td>
<td>52.1 ± 0.8*</td>
<td>6</td>
</tr>
<tr>
<td>120 min</td>
<td>94 ± 9</td>
<td>24.1 ± 2.3*</td>
<td>3.96 ± 0.29*</td>
<td>423 ± 10*</td>
<td>48.5 ± 0.6*</td>
<td>6</td>
</tr>
<tr>
<td>150 min</td>
<td>93 ± 10</td>
<td>23.3 ± 2.6*</td>
<td>4.03 ± 0.23*</td>
<td>419 ± 13*</td>
<td>47.3 ± 0.8*</td>
<td>6</td>
</tr>
<tr>
<td>180 min</td>
<td>99 ± 6</td>
<td>25.4 ± 2.7*</td>
<td>3.98 ± 0.23*</td>
<td>423 ± 17*</td>
<td>46.2 ± 1.2*</td>
<td>5</td>
</tr>
<tr>
<td>210 min</td>
<td>91 ± 9*</td>
<td>23.9 ± 2.5*</td>
<td>3.83 ± 0.23*</td>
<td>422 ± 18*</td>
<td>45.9 ± 1.9*</td>
<td>5</td>
</tr>
<tr>
<td>240 min</td>
<td>84 ± 12*</td>
<td>23.4 ± 3.7*</td>
<td>3.61 ± 0.21*</td>
<td>420 ± 20*</td>
<td>45.9 ± 1.9*</td>
<td>5</td>
</tr>
<tr>
<td>270 min</td>
<td>90 ± 9*</td>
<td>23.6 ± 3.1*</td>
<td>3.84 ± 0.30*</td>
<td>436 ± 20*</td>
<td>44.1 ± 1.3*</td>
<td>4</td>
</tr>
<tr>
<td>300 min</td>
<td>78 ± 12*</td>
<td>22.9 ± 3.5*</td>
<td>3.40 ± 0.17*</td>
<td>435 ± 21*</td>
<td>44.3 ± 1.6*</td>
<td>4</td>
</tr>
</tbody>
</table>

All values are shown as the mean ± S.E.M. *P<0.05 compared to control (randomized block ANOVA followed by Duncan’s new multiple range test). Two rats died by 180 and 270 min, respectively.
a potent venodilator as NOS blockade with Nω-nitro-L-arginine evoked a venoconstriction (9). Thus, overproduction of NO elicited by LPS may have provoked venodilatation and subsequently decreased cardiac output. This notion is supported by the report that L-canavanine, a putative iNOS inhibitor, attenuated the decline in the mean circulatory filling pressure in endotoxemic rats (3). Nevertheless, the precise site of action of carboxy-PTIO to improve cardiac output remains to be elucidated in the present experimental setting.

In humans, septic shock is characterized by intensive vasodilation and reduced perfusion pressure. Cardiac output rather increases as a consequence of a compensatory mechanism, a characteristic of hyperdynamic shock (10). In such a situation, NO is believed to be responsible for the sustained vasodilatation and critical hypotension with reduced vascular responsiveness to endogenous and exogenous vasoconstrictor agents. In fact, Nω-monomethyl-L-

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Fig. 1. Effects of carboxy-PTIO on LPS-induced alterations in systemic hemodynamics. Rats were given lipopolysaccharide (LPS) at a dose of 10 mg/kg intravenously at time 0. At 90 min after LPS, carboxy-PTIO (1.7 mg·kg⁻¹·h⁻¹) (closed circle n=5) or its vehicle (phosphate buffered saline 3 ml/h) (open circle n=4) was intravenously administered. Two rats given vehicle following LPS died before the end of the study were excluded for analysis and data only from survived rats were shown in this figure. Values just prior to carboxy-PTIO or vehicle administration were assigned as 100% and data were shown as percent changes from the values. Values are expressed as mean ± S.E.M. *P<0.05 vs vehicle group (the Student’s t-test). N.S.: no statistically significant difference.
arginine and $N^\omega$-nitro-$l$-arginine methylester could normalize peripheral vasodilation, which is attributable to the blockade of vasodilator action of nitric oxide (11). Rats receiving LPS at the same dose as those used in the present study also developed reduced pressor responses to nor-epinephrine and hypotension that was reversed by NOS inhibitors (12). However, the beneficial effects of carboxy-PTIO seen in the present experiments could not be attributable to the prevention of LPS-induced decrease in the pres- sor response to norepinephrine and subsequent loss in the vascular tone of the resistance vessels. First, LPS elicited a rise in the total peripheral resistance, indicating systemic arterial vasoconstriction, an opposite situation to those found in human sepsis. Second, carboxy-PTIO had no effect on the response of total peripheral resistance to LPS. Thus, the site of hypotensive action of NO in endotoxic shock is considerably different between human and rat.

In rat endotoxic shock models, intravenous administra- tion of LPS at a dose of approx. 10 mg/kg is the one frequently used by other investigators (3, 6, 13) and one used in the present experiments. With this maneuver, expression of iNOS became apparent approx. 3 h after LPS adminis- tration (14). It is also well established that LPS treatment elicits overproduction of NO through induction of iNOS. Thus, selective blockade of iNOS would diminish the deleterious action of excess NO. However, although both $l$-canavanine and aminoguanidine are selecte iNOS blockers and attenuated rat endotoxic shock (3, 13), the mechanism of action was different; the former improved cardiac output and the latter increased systemic vascular resistance without affecting cardiac output. Such differences may be derived from the difference in selectivity to NOS isoforms. In the latter study, aminoguanidine also inhibited constitutive NOS in addition to iNOS inhibition since it increased blood pressure before LPS administration. In this context, carboxy-PTIO did not affect any hemodynamic parameters in naïve rats (data not shown). This fact together with the potent NO scavenger action of carboxy-PTIO (5, 15) suggest that hemodynamic improve- ment seen with carboxy-PTIO treatment resulted from elimination of excessively produced NO by iNOS following LPS treatment.

In summary, we demonstrated here that carboxy-PTIO given therapeutically to endotoxemic rats attenuated sys- temic hypotension by improving cardiac output. Systemic resistance vessels were not the site of action of carboxy-PTIO in preventing the delayed hypotension. These results indicated that main hemodynamic action of NO in rat endotoxic shock was to reduce cardiac output.

Acknowledgments

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REFERENCES