Effects of carvedilol pretreatment on mortality and inflammatory responses to septic shock induced by cecal ligation and puncture in rats

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Abstract

Purpose: Carvedilol, a nonselective beta-adrenoceptor and selective alpha 1-adrenoceptor blocker, has high efficacy and is widely used in hypertensive and/or cardiac failure patients. However, few studies have examined the effects of carvedilol on septic shock. The present study evaluated the effects of carvedilol pretreatment in cecal ligation and puncture (CLP)-induced septic shock in rats.

Methods: Male Sprague Dawley rats (n = 90) were randomly assigned to one of the following three groups (n = 30 per group): control group (no medication), high-dose carvedilol group (10 mg/kg/day, orally administered for 5 days), and low-dose carvedilol group (2 mg/kg/day, orally administered for 5 days). Septic shock was induced by CLP, and the animals received no other therapies. Mortality was calculated for 5 days after CLP. Arterial blood gases and plasma cytokine concentrations were measured in 36 additional male rats 12 hr after CLP.

Results: Mortality rates 5 days after CLP were 76.7%, 86.7%, and 86.7% in the control, high-dose, and low-dose groups, respectively; there were no significant differences among the three groups. Furthermore, observed decreases in base excess, increases in lactate concentrations, and increases in cytokine concentrations in the carvedilol-treated groups did not differ from those in the control group.

Conclusions: The present findings indicate that orally administered carvedilol has no beneficial effects on mortality and inflammatory responses in CLP-induced septic shock in rats, suggesting that carvedilol may not contribute to recovery from septic shock.

Key word: beta-blocker, carvedilol, sepsis, shock, cytokine

Introduction

Carvedilol is a nonselective beta-adrenoceptor and selective alpha 1-adrenoceptor blocker that is widely used in hypertensive and/or chronic heart failure patients. Unlike classic beta-blockers, carvedilol has additional endothelium-dependent vasodilatory effects[1)~3)]. Several recent studies have reported that carvedilol has beneficial effects in patients with cardiogenic failure and shock[4)~6)]. The carvedilol or metoprolol European trial demonstrated that carvedilol protected better against vascular events such as myocardial infarction and stroke than metoprolol in patients with chronic heart failure[5)]. The findings of the carvedilol post-infarct survival control trial indicated that carvedilol treatment offers an early benefit in clinically stabilized patients with post-myocardial infarction left ventricular dysfunction[6)]. Furthermore, beta-blocker therapy during the perioperative period may improve morbidity and mortality rates in high-risk patients with ischemic heart disease[7)8)].

Several studies have shown that carvedilol and beta-blockers generally suppress lipopolysaccharide (LPS)-induced cytokine and tissue factor production

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Abbreviations: CLP, cecal ligation and puncture; IL, interleukin; TNF, tumor necrosis factor; LPS, lipopolysaccharide; BE, base excess; Lac, lactate

in vitro and attenuate myocardial dysfunction in septic animals[9)~11)]. An open-label randomized phase 2 study in clinical practice indicated that in patients with septic shock, administration of the short-acting beta-blocker esmolol helped in maintaining the heart rate and decreased the risk of death significantly (esmolol group: 49.4%; control group: 80.5%; $p < 0.01$)[12)]. Moreover, the study suggested that the use of esmolol in patients with septic shock might improve myocardial movement and oxygen consumption efficiency. However, few studies have investigated the effect of beta-blockers on septic shock in patients who were previously treated with a beta-blocker. Therefore, in the present study, we aimed to evaluate the effects of carvedilol pretreatment on mortality and inflammatory responses in rats with cecal ligation and puncture (CLP)-induced sepsis.

Materials and Methods

The experimental protocol in this study was approved by the Animal Care Committee of our institute (#143309), and the animals were cared for and handled in accordance with the National Institutes of Health guidelines.

Effect of carvedilol on mortality in septic shock rats

Ninety male Sprague Dawley rats were randomly allocated to one of the following three groups (n = 30 each): control (no medication), high-dose carvedilol (10 mg/kg/day) for 5 days, or low-dose carvedilol (2 mg/kg/day) for 5 days. These doses were determined according to the findings of the past studies[13)]. In our preliminary study, the animals received carvedilol with either food or water, and its administration with food was shown to decrease heart rate markedly. Therefore, in the present study, carvedilol was administered with food by crushing and mixing it well into the rat chow. The rats were bred freely in cages, and water and food were freely available. The rats ate 25 mg of food per day, and carvedilol was administered at doses of 4 mg/day (high-dose group) or 0.8 mg/day (low-dose group). Each rat ate all of the allowed food during the 5-day period.

After pretreatment with carvedilol for 5 days, all rats were anesthetized by the intraperitoneal injection of pentobarbital 40 mg/kg. Then, a laparotomy was performed, and the cecum immediately distal to the ileocecal valve was ligated. An appendix ligation puncture model in which two punctures were made with an 18-gauge needle was used in this study. The mortality up to 5 days post-CLP was observed.

Effects of carvedilol on inflammatory responses in rats with septic shock

An additional 36 male Sprague Dawley rats were randomly assigned to one of the following three groups: control (no medication, n = 15), high-dose carvedilol (10 mg/kg/day orally administered for 5 days, n = 10), and low-dose carvedilol (2 mg/kg/day, orally administered for 5 days, n = 11). All animals were anesthetized with an intraperitoneal injection of pentobarbital, and CLP was performed as described above.

At 12 hr after CLP, arterial blood samples (0.25 ml) were collected to measure pH, arterial carbon dioxide tension ($PaCO_2$), arterial oxygen tension (PaO_2), and base excess (BE) and lactate (Lac) concentrations. Additional arterial blood samples (1.5 ml) were collected to measure plasma cytokine levels, including tumor necrosis factor (TNF)-alpha, interleukin (IL)-1beta, and IL-6, at 12 hr after CLP. All of the cytokine concentrations were measured using enzyme-linked-immunosorbent assay kits (BioSource, Camarillo, CA, USA).

Statistical analysis

Statistical power calculated[14)] using a one-sided design with a significance level of 5% and a probability of 80% showed that a minimum of 30 rats was needed in each group to detect a difference of at least 30% between the control and treatment groups[13)].

Data are presented as mean ± standard deviation. Mortality rates among the groups were compared using the Kaplan-Meier and Mantel-Cox tests. Differences between groups were analyzed by unpaired *t* test. Significance was defined as values of $p < 0.05$. The statistical analysis was performed using StatView software (version 5.0 for Macintosh; Abacus Concepts, Berkeley, CA, USA).

Results

Mortality rates

A total of 30 rats received high-dose carvedilol, another 30 received low-dose carvedilol, and the remaining 30 received no medication (control group). Thereafter, CLP was performed and the survival status for 5 days was observed. As shown in Fig. 1, the first death occurred the next day in the control group, and 23 of 30 rats died by the fifth day; hence, the mortality rate was 76.7%. On the other hand, in the high- and low-dose groups, 26 of 30 rats died by the fifth day, and the mortality rates were 86.7% in both conditions. The statistical analysis of these

survival rates using the Kaplan-Meier and Mantel-Cox tests revealed that the p value was 0.5434 (i.e., no statistically significant difference).

Blood gases

Ten rats received high-dose carvedilol and another 11 rats received low-dose carvedilol for 5 days. The remaining 15 animals were included in the control group. CLP was performed in these rats and BE and Lac concentrations were measured 12 hr later. As shown in Fig.2, BE concentrations were -4.07 ± 5.38 mmol/l, -2.39 ± 2.97 mmol/l, and -1.57 ± 1.7 mmol/l in the control, high-dose, and low-dose groups, respectively. There were no significant differences among the three groups (p = 0.3786, control versus high-dose group; p = 0.1518, control versus low-dose group; and p = 0.4432, high-dose versus low-dose group). Similarly, Lac concentrations were 4.85 ± 3.96 mmol/l, 7.40 ± 6.15 mmol/l, and 2.57 ± 1.03 mmol/l for the control, high-dose, and low-dose groups, respectively. There were no significant differences among the three groups (p = 0.2269 control versus high-dose group, p = 0.2502 control versus low-dose group, and p = 0.1018 high-dose versus low-dose group). In other words, there were no significant differences in BE and Lac concentrations in all the groups at 12 hr after CLP, regardless of the dose of carvedilol.

Plasma cytokine concentrations

TNF-alpha, IL-1 beta, and IL-6 levels were measured in the rats described above. As shown in Fig.3, TNF-alpha levels were 7.0 ± 3.9 pg/ml, 5.8 ± 5.3 pg/ml, and 10.3 ± 6.8 pg/ml for the control, high-dose, and low-dose groups, respectively. There were no significant differences among the three groups (p = 0.3336, control versus high-dose group; p = 0.4066, control versus low-dose group; and p = 0.1965, high-dose versus low-dose group). Similarly, IL-1 beta levels were 135 ± 29 pg/ml, 146 ± 16 pg/ml and 149 ± 13 pg/ml for the control, high-dose, and low-dose groups, respectively. There were no significant differences among the three groups (p = 0.3054, control versus high-dose group; p = 0.1543, control versus low-dose group; and p = 0.6124, high-dose versus low-dose group). Furthermore IL-6 levels were 44.5 ± 6.9 pg/ml, 44.1 ± 3.4 pg/ml and 44.4 ± 2.6 pg/ml for the control, high-dose, and low-dose groups, respectively. There were no significant differences among the three groups (p = 0.8532, control versus high-dose group; p = 0.9686, control versus low-dose group; and p = 0.7880, high-dose versus low-dose group).

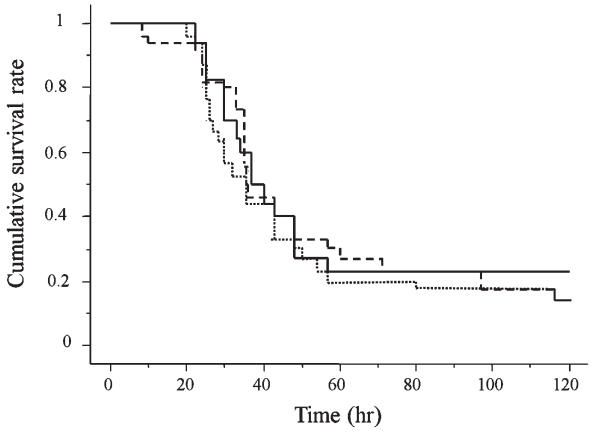
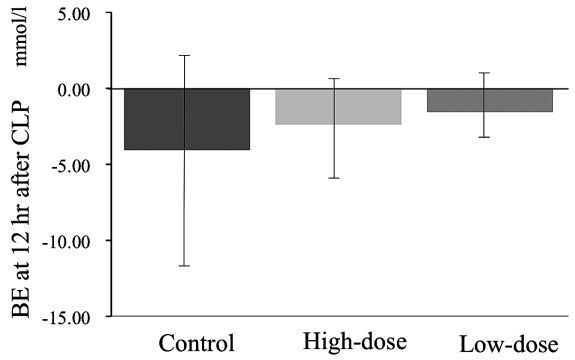


Fig. 1. Survival curves for the control, high-dose carvedilol, and low-dose carvedilol groups after cecal ligation and puncture.
- - - - - High-dose group,
..... Low-dose group,
——— Control group



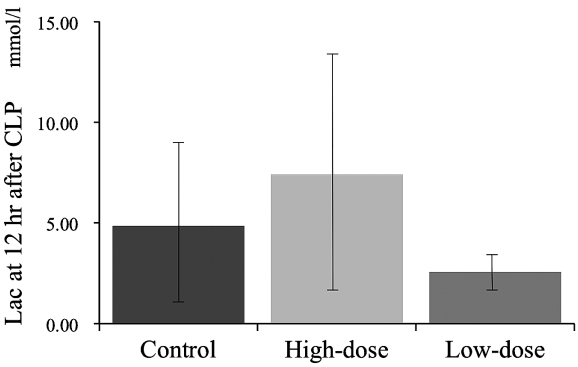


Fig. 2. Base excess (BE) (top) and plasma lactate (Lac) (bottom) concentrations 12 hr after the cecal ligation and puncture. The mean BE levels were -4.07 ± 5.38, -2.39 ± 2.97, and -1.57 ± 1.7 mmol/l in the control, high-dose, and low-dose groups, respectively, while the mean Lac levels were 4.85 ± 3.96, 7.40 ± 6.15, and 2.57 ± 1.03 mmol/l in the control, high-dose, and low-dose groups, respectively.

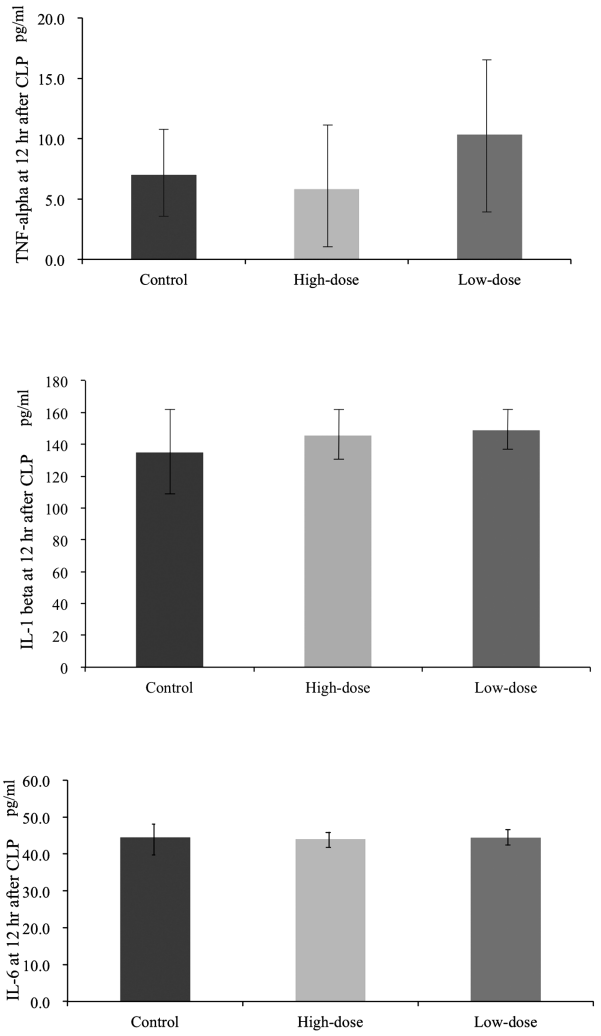


Fig. 3. Plasma tumor necrosis factor (TNF)-alpha (top), interleukin (IL)-1 beta (middle), and IL-6 (bottom) concentrations at 12 hr after cecal ligation and puncture. The mean TNF-alpha levels were 7.0 ± 3.9, 5.8 ± 5.3, and 10.3 ± 6.8 pg/ml in the control, high-dose, and low-dose groups, respectively. The mean IL-1beta levels were 135 ± 29, 146 ± 16, and 149 ± 13 pg/ml in the control, high-dose, and low-dose groups, respectively. The mean IL-6 levels were 44.5 ± 6.9, 44.1 ± 3.4, and 44.4 ± 2.6 pg/ml in the control, high-dose, and low-dose groups, respectively.

Discussion

CLP in rats is associated with hypotension and metabolic acidosis as well as elevated plasma cytokine concentrations. The mortality rate 5 days after CLP was extremely high in the control and carvedilol-treated groups. Carvedilol administration did not alleviate CLP-induced metabolic acidosis or elevate Lac concentrations. Thus, CLP-induced mortality and inflammation remained the same after the oral administration of carvedilol as that in the control group.

Numerous recent studies have demonstrated the efficacy of carvedilol for treating chronic heart failure[1)2)4)5)]. In addition, many studies have reported that carvedilol has beneficial effects in patients soon after a myocardial infarction, and beta-blocker therapy during the perioperative period showed improved morbidity and mortality rates in high-risk patients with ischemic heart disease[6)~8)]. These studies suggested that early and continuous administration of carvedilol might have beneficial effects in patients with ischemic heart disease. Recently, the number of patients receiving oral carvedilol has increased owing to its beneficial effects of carvedilol; however, some of these patients developed septic shock. On the other hand, few studies have examined the effects of orally administered carvedilol on septic shock. Therefore, in the present study, we aimed to evaluate the effects of orally administered carvedilol on mortality and inflammatory responses in rats with CLP-induced septic shock. The results of this study indicate that the oral administration of carvedilol after CLP-induced septic shock in rats has no beneficial effects on mortality and inflammatory responses. These findings suggest that carvedilol therapy may have no beneficial effects on the recovery of patients with septic shock.

The mechanisms underlying the beneficial effects of beta-blockers on sepsis and septic shock have been reported in several studies. Sasao et al. showed that landiolol, a beta-blocker, has potent negative chronotropic effects in rabbits[15)]. Hagiwara et al. also showed that landiolol has protective effects against acute lung injury and cardiac dysfunction in a rat septic model[16)]. Sepsis causes sympathetic hypertonia. The above studies suggested that a possible mechanism underlying the effects of beta-blockers could be its inhibitory effects on sympathetic hypertonia. However, in the present study, the effect of carvedilol pretreatment was not observed in rats with septic shock, suggesting that pretreatment with beta-blockers may disturb the sympathetic nerve tone when sepsis becomes under sympathetic nerve tone suppressed condition. In addition, because carvedilol has both alpha and beta blocking agents, the alpha-blocker may inhibit to protect against organ dysfunctions.

The present findings show that orally administered carvedilol increases Lac and cytokine concentrations after CLP, and Lac response in

carvedilol-treated rats deteriorated in a manner similar to that observed in control rats. Several studies have examined the relationship among beta-blockers and cytokine and Lac concentrations[9)~11)]. Mizuochi et al.[9)] showed that carvedilol inhibits LPS-induced production of TNF-alpha and tissue factors in human monocytes *in vitro*. Suzuki et al.[10)] reported that esmolol, a beta-blocker, attenuated the elevation of TNF-alpha and Lac concentrations in septic rats. The findings of the present study differ from those of previous studies possibly due to a decrease in cardiac performance induced by carvedilol and failure to improve peripheral circulation systemically. We previously showed that the oral administration of carvedilol increases the mortality rate and exacerbates the inflammatory responses to severe hemorrhagic shock in rats[13)]. These differences suggest that administration of beta-blockers inhibits sepsis-induced increase in cardiac performance and improves the survival rate after septic shock in rats; however, further investigations are required to clarify this point.

Determining whether a dose-response relationship exists between carvedilol and outcomes is important. Therefore, the present study evaluated the effects of two different carvedilol doses (2 and 10 mg/kg/day) on mortality and the inflammatory responses after septic shock in rats. The results indicate that carvedilol has no beneficial effects on CLP-induced mortality and inflammatory responses to septic shock in rats regardless of dose. Thus, it can be suggested that dose-response carvedilol therapy may have no beneficial effects on the recovery of patients with septic shock.

Clinically, it is important to understand whether carvedilol has beneficial effects in patients who received treatment before the onset of septic shock. We previously showed that the oral administration of carvedilol increases the mortality rate and exacerbates the inflammatory responses to severe hemorrhagic shock in rats[13)], indicating that carvedilol may worsen the recovery of severe hemorrhagic shock in patients. Nevertheless, the present study showed that carvedilol does not impair the recovery from CLP-induced septic shock in rats.

There may be several important explanations for the differences in results between the present study and previous studies. First, the extent of disability caused by CLP in this study may be relatively severe. The mortality rate in all the groups 5 days after CLP was extremely high. Further studies using models with low or moderate mortality rates for the control group may be needed. Second, it is possible that the duration of carvedilol administration was relatively short. Further investigations are needed to clarify the importance of duration of carvedilol administration. Finally, the present study used normal Sprague Dawley rats. Clinically, carvedilol is generally administered to hypertensive patients; therefore, further studies are needed in hypertensive rats.

In conclusion, the present study shows that the oral administration of carvedilol increased the mortality rate and exacerbated the inflammatory responses to CLP-induced septic shock in rats in a manner similar to that observed without carvedilol administration. Although further investigations are required, the current findings suggest that carvedilol may have no beneficial effects on the recovery of patients with septic shock.

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