Effects of Ketanserin on Endotoxic Shock and Baroreflex Function in Rodents

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Background. Ketanserin, a 5-hydroxytryptamine receptor antagonist, is clinically used as an antihypertensive agent and could enhance baroreflex function. The present work tested the hypothesis that restoration of baroreflex function is an effective treatment for lipopolysaccharide (LPS)–induced shock.

Methods. Kunming mice were injected with LPS (30 mg/kg; intraperitoneal) to induce endotoxic shock. Ketanserin (0.3, 1, 3, or 10 mg/kg; intraperitoneal) was administered immediately after LPS injection. Survival time was monitored, and serum cytokines were analyzed after the onset of LPS. Effects of ketanserin were also examined in IL-10–deficient mice and mice with sinoaortic denervation. Finally, effects of ketanserin on blood pressure, heart rate, and baroreflex sensitivity were examined in Wistar–Kyoto (WKY) rats with endotoxic shock.

Results. Ketanserin significantly increased survival time and decreased serum levels of tumor necrosis factor α and interleukin (IL) 1β in mice with endotoxic shock. At a dose of 10 mg/kg, ketanserin also significantly increased serum IL-10 concentration. The antishock effect of ketanserin was also apparent in IL-10–knockout mice. In mice with sinoaortic denervation, however, ketanserin had little antishock effects. In WKY rats, ketanserin significantly prevented the baroreflex impairment induced by LPS and prolonged the survival time.

Conclusions. Ketanserin could ameliorate endotoxic shock by restoring baroreflex function.

Endotoxic shock is caused by lipopolysaccharide (LPS)–producing gram-negative bacteria and represents a serious clinical condition with high mortality [1]. LPS initiates a cascade of events, including release of inflammatory mediators such as nitric oxide, interleukin (IL) 1 and tumor necrosis factor (TNF). Activation of the coagulatory, fibrinolytic, and complement systems decreases tissue perfusion and eventually causes multiple organ failure [2, 3].

Arterial baroreflex (ABR) is one of the body’s homeostatic mechanisms for maintaining blood pressure. It provides a negative feedback loop in which an elevated blood pressure reflexively causes heart rate and thus blood pressure to decrease. Its function, expressed by baroreflex sensitivity (BRS), can be determined by using a pharmacologic method [4]. The ABR function can be abolished by interrupting the ABR arc, as by sinoaortic denervation (SAD) in animal studies [5, 6]. Recently, the pathologic importance of ABR function has attracted the attention of many investigators. BRS is impaired in aging, hypertension, and many other cardiovascular diseases [4, 7]. ABR dysfunction is correlated to poor prognosis in patients with acute myocardial infarction, heart failure, or ischemic stroke [8–10]. In our previous studies, we demonstrated that diminishing ABR function with SAD decreases survival time in both LPS-induced and cecal ligation and puncture (CLP)–induced lethal shock [11, 12]. These findings imply that restoring impaired ABR function might be useful in the treatment of endotoxic shock.

Ketanserin is a selective 5-hydroxytryptamine (5-HT2A) antagonist with minor effects on α1 adrenergic receptors. Clinically, it is used as an antihypertensive agent. A study conducted in this laboratory demonstrated that ketanserin could enhance BRS in hypertensive rats or rats...
with acute myocardial infarction via central 5-HT₃A receptors [13]. The present work was designed to test the hypothesis that restoring baroreflex with ketanserin could ameliorate endotoxic shock.

MATERIALS AND METHODS

Animals and Agents
Male Kunming mice (25–30 g) were purchased from Sino-British SIPPR/BK Laboratory Animal. IL-10–deficient mice were purchased from Jackson Laboratory (B6.129P2-Il10tm1Cgn, 002251). Wistar–Kyoto (WKY) rats were provided by the Experimental Animal Center of Second Military Medical University in Shanghai, China. All animals were maintained at 22°C under a 12-hour light-dark cycle and had free access to water and a standard rodent diet. All experimental procedures were in accordance with institutional animal care guidelines and approved by the local ethics committee. Ketanserin was obtained from Janssen Pharmaceutica. LPS from Escherichia coli serotype 0111:B4 was purchased from Sigma Chemical.

CLP Model
CLP was performed as described elsewhere [14]. In brief, male Kunming mice (14 weeks of age) were anesthetized by intraperitoneal administration of a combination of ketamine and diazepam. The cecum was exposed through a 1.0–1.5-cm abdominal midline incision and ligated at 6 mm from the cecal tip, followed by a single puncture with a G23 needle. A small amount of intestinal content was manually expelled from the punctures to ensure patency. The cecum was returned to the peritoneal cavity, and the abdominal incision was closed. In sham-operated mice, the cecum was mobilized but not ligated or punctured. Survival time after CLP was monitored for 72 hours.

Determination of TNF-α, IL-1β, and IL-10 Levels
TNF-α, IL-1β and IL-10 levels in serum were measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit (R&D system).

Histologic Analysis
The liver 24 hours after LPS injection was excised and rinsed with saline solution, fixed in 4% paraformaldehyde for 24 hours, and embedded in paraffin. Tissue sections (2 μm) were stained with hematoxylin-eosin according to a routine protocol and examined by light microscopy.

Blood Pressure and Heart Rate Measurements
Rats were anesthetized with a combination of ketamine and diazepam. A polyethylene catheter was inserted into the lower abdominal aorta via the left femoral artery for blood pressure measurement. Another catheter was inserted into left femoral vein for drug administration. After a 2-day recovery period, the animals were placed in individual cylindrical cages for blood pressure recording. Food and water were available during the recording. The aortic catheter was connected to a transducer via a rotating swivel that allowed the animals to move freely in the cage. After 4-hour habituation, the blood pressure signal was digitized by a microcomputer. Systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate values were determined online [4, 6].

SAD Operation
SAD was performed according to a method described elsewhere [15, 16]. Briefly, the mice were anesthetized with chloral hydrate (350 mg/kg; intraperitoneal). After a midline neck incision and bilateral isolation of the neck muscles, bilateral aortic baroreceptor denervation was carried out by cutting the superior laryngeal nerves near the vagi, removing the superior cervical ganglia, including a small section of the sympathetic trunk, and severing the aortic depressor nerves. Baroreceptors in the carotid sinus were denervated bilaterally by stripping the carotid bifurcation and its branches, followed by the application of 1% phenol (in 95% ethanol) to the external, internal, and common carotid arteries and the occipital artery. Mice in which we could not isolate the aortic nerve with confidence were excluded from this study. Sham-operated mice received midline neck incision and bilateral isolation of the neck muscles. Experiments were conducted 2 weeks after SAD.

BRS Measurement
BRS was measured as described elsewhere [4, 17]. The principle of this method is to measure the prolongation of heart period (in ~60,000/heart rate) in response to an elevation of SBP. Briefly, a bolus injection of phenylephrine (1–2 μg/kg) was used to induce an elevation of SBP (20–40 mm Hg). The delay between SBP elevation (stimulus) and heart period prolongation (response) was ~1 second. Heart period was plotted against SBP for linear regression analysis for 2–8 shifts (calculated by computer). The slope with the largest correlation coefficient (r) of heart period/SBP was taken as the measure for BRS (ms/mm Hg). The mean of 2 measurements was taken as the final result.

Experimental Protocols
Experiment 1: Effects of Ketanserin on Survival Time in Mice With LPS-Induced Shock and Mice With CLP. Mice received 30 mg/kg LPS (intraperitoneal), followed by vehicle or ketanserin (0.3–10.0 mg/kg; intraperitoneal; n = 20 per group). In another set of experiments, mice received vehicle or ketanserin (0.3–10.0 mg/kg; intraperitoneal; n = 20 per group) after CLP operation. Survival time was monitored for 72 hours.

Experiment 2: Effects of Ketanserin on Serum Cytokines in Mice With LPS-Induced Shock. Blood samples were collected at 90 minutes after LPS injection for TNF-α, and at 4 hours for IL-1β and IL-10. Serum samples were analyzed using a standard protocol for ELISA cytokine assays (R&D Systems).

Experiment 3: Effects of Ketanserin on Hepatic Injury in Mice With LPS-Induced Shock. Mice were injected with LPS...
(30 mg/kg; intraperitoneal), followed by vehicle or ketanserin (3.0–10.0 mg/kg; intraperitoneal; n = 9). Liver tissue specimens were excised at 24 hours. A group of mice that received vehicle only was used as normal controls.

**Experiment 4: Effects of Ketanserin on Serum TNF-α in IL-10–Knockout Mice.** IL-10–deficient mice (10-week old) were treated with 20 mg/kg LPS (intraperitoneal), followed by vehicle or ketanserin (10 mg/kg; intraperitoneal; n = 6 per group). Blood samples were collected at 90 minutes for ELISA TNF-α assay. Age- and sex-matched C57/BL6 controls were used as wild-type controls.

**Experiment 5: Effects of Ketanserin on Hemodynamics and Survival Time in Rats With LPS-Induced Shock.** At 30 minutes after collection of baseline mean arterial pressure (MAP; 1/3 SBP + 2/3 DBP [mm Hg]) and heart period, rats received LPS (50 mg/kg; intraperitoneal), followed by vehicle or ketanserin (1.0 mg/kg; intraperitoneal; n = 10). Blood pressure and survival time were recorded for the ensuing 500 minutes. MAP was calculated during the first 140 minutes, because deaths started to occur at 150 minutes.

**Experiment 6: Effects of Ketanserin on BRS in Rats With LPS-Induced Shock.** At 30 minutes after the collection of baseline blood pressure and heart period, rats received LPS (20 mg/kg; intraperitoneal), followed by vehicle or ketanserin (1.0 mg/kg; intraperitoneal; n = 10). BRS was examined at 2 hours.

**Experiment 7: Effects of Ketanserin on Survival Time in Mice With SAD.** Mice with SAD received LPS (20 mg/kg; intraperitoneal), followed by vehicle or ketanserin (1.0 mg/kg; intraperitoneal; n = 21). Survival time was monitored for 72 hours. A parallel set of control experiments with the same design was conducted in sham-operated mice.

**Statistical Analysis**
Data were analyzed with 1-way analysis of variance (ANOVA), followed by Student t test for experiments involving only 2 groups, and Dunnett t test for experiments involving >2 groups. Serial data of MAP was analyzed with ANOVA of repeated measures. For survival time analysis, Kaplan-Meier analysis was used, followed by a Cox regression test. All data are expressed as means ± standard deviations. Statistical significance was set at P < .05.

**RESULTS**

**Effects of Ketanserin on Survival Time in Mice With LPS-Induced Shock**
In response to a lethal dose of LPS, all mice displayed decreased activity, piloerection, periocular discharge, and diarrhea. The survival rate at 72 hours was 14%. Ketanserin increased the survival rate in a dose-dependent manner. Similar results were obtained in mice with CLP (Figure 1). We included 30 mg/kg ketanserin in the LPS-induced survival experiment but observed similar protective effects with 10 mg/kg ketanserin, possibly because of a ceiling effect (Supplemental Figure 1).

**Effects of Ketanserin on Serum Cytokines in Mice With LPS-Induced Shock**
At 0.3–10.0 mg/kg, ketanserin significantly decreased serum TNF-α levels in mice with LPS-induced shock (P < .05 vs vehicle control for all ketanserin doses) (Figure 2A). Serum IL-1β was significantly decreased (vs vehicle control for all ketanserin doses; P < .05) (Figure 2B). At 10.0 mg/kg, ketanserin also

**Figure 1.** Effects of ketanserin on survival time in mice with lipopolysaccharide (LPS)–induced shock and mice with cecal ligation and puncture (CLP). A, Mice received LPS (30 mg/kg; intraperitoneal) and then vehicle or ketanserin (0.3–10.0 mg/kg; intraperitoneal) after CLP operation. Colors indicate LPS (red) or LPS plus ketanserin by dosage (0.3 [purple], 1.0 [green], 3.0 [yellow], or 10.0 [blue] mg/kg; n = 20 per group). **P < .01 (vs LPS); ***P < .001 (vs LPS); *P < .05 (vs vehicle).
significantly increased serum IL-10 ($P < .05$ vs vehicle control) (Figure 2C).

Effects of Ketanserin on Hepatic Injury in Mice With LPS-Induced Shock
LPS induced significant damage (Figure 3B vs Figure 3A for normal controls). The observed changes included widened portal areas, thin fibrous septa throughout the hepatic parenchyma, and inflammatory cell infiltration. It showed bridging or septal fibrosis connecting portal areas and central veins in a portal to portal, portal to central, and/or central to central pattern. Collagen fibers (stained red by Sirus) were clearly visible in the septa. At 3.0 mg/kg, ketanserin ameliorated LPS-induced damage, with less infiltration by inflammatory cells, less putrescence of hepatic cells, and hyperplasia of Kupffer cells (Figure 3C). In mice that received 10 mg/kg ketanserin, LPS-induced inflammation was minimal (Figure 3D).

Effects of Ketanserin on Serum TNF-α in IL-10–Knockout Mice
In comparison with levels in their wild-type littermates, serum TNF-α levels were significantly higher in IL-10–deficient mice ($P < .05$) (Figure 4). Serum TNF-α levels were significantly decreased in wild-type mice ($P < .01$) as well as in IL-10-deficient mice ($P < .05$) after treatment with ketanserin (10 mg/kg).

Effects of Ketanserin on Hemodynamics and Survival Time in Rats With LPS-Induced Shock
LPS increased heart rate and elicited a rapid reduction in MAP, followed by a rebound and then a secondary decrease (Figure 5A). Ketanserin did not alter the effects of LPS on MAP and heart rate (Figure 5B) ($P > .05$) but significantly prolonged the survival time (Figure 5C) ($P < .05$). In another set of experiment, rats were challenged with 20 mg/kg LPS, and the protective effects of ketanserin were strong and long-lasting (Supplemental Figure 2).

Effects of Ketanserin on BRS in Rats With LPS-Induced Shock
LPS significantly decreased BRS (0.28 ± 0.08 vs 0.74 ± 0.16 ms/mm Hg; $P < .01$) (Figure 6A). In rats treated with 1.0 mg/kg ketanserin, LPS also decreased BRS (0.44 ± 0.18 vs 0.78 ± 0.25 ms/mm Hg; $P < .01$) (Figure 6B). However, ketanserin significantly attenuated the depression of BRS induced by LPS ($-0.21 ± 0.12$ vs $-0.43 ± 0.14$ ms/mm Hg; $P < .01$) (Figure 6D).

Effects of Ketanserin on Survival Time in Mice With SAD
In sham-operated mice, ketanserin (1.0 mg/kg) significantly reduced LPS-induced mortality (52% vs 90%; $P < .05$) (Figure 7A). Such an effect was attenuated by SAD (Figure 7B).

DISCUSSION
Our previous studies in rats demonstrated that baroreflex dysfunction could aggravate shock and that ketanserin is a very effective drug for restoring baroreflex function [13, 15]. The present study confirmed that restoring baroreflex, by using ketanserin, could alleviate endotoxic shock. First, ketanserin significantly decreased the mortality induced by a lethal dose of LPS and prolonged the survival time dose dependently at a range of 0.3–10.0 mg/kg in mice. Similar findings were observed also in rats. Second, ketanserin decreased the levels of TNF-α and IL-1β. Third, ketanserin prevented organ damage induced by LPS, as exemplified by results in the liver.
IL-10 modulates the transcription of many genes and suppresses inflammation in many diseases [18, 19]. In our experiments, ketanserin significantly increased serum levels of IL-10, but only at a high dose of 10 mg/kg. Moreover, the beneficial effect of ketanserin on serum TNF-α was barely altered in IL-10–knockout mice, suggesting that increasing IL-10 is not a major action of ketanserin that produces its protective effects on LPS-induced shock.

A series of studies in our laboratory have clearly demonstrated that ketanserin could enhance BRS in spontaneously hypertensive rats, stroke-prone spontaneously hypertensive rats, and Sprague-Dawley rats with cardiac infarction [20–22]. In the present work, we found significantly decreased BRS after LPS exposure. Ketanserin significantly attenuated in the effect of LPS on BRS.

Interruption of the ABR arc may occur at 3 levels: (1) receptor and afferent level, SAD; (2) central level, by destroying the nucleus tractus solitarius; and (3) efferent level, by blocking of sympathetic and parasympathetic systems. It is generally accepted that SAD is the best approach with the highest overall selectivity and operability. Removal of the carotid sinus and aortic arch baroreceptors directly and selectively diminishes the ABR function [23]. Consistent with our previous studies [11, 12], results from the current study showed that SAD dramatically reduced the survival rate after LPS challenge in mice (5% vs 52%). In mice with intact ABR, ketanserin increased the survival rate after LPS challenge by 38%. In mice with SAD, the increase was only 10%. These results confirmed that the beneficial effect of ketanserin on endotoxic shock is mediated primarily by BRS enhancement. The small but statistically significant increase of survival time by ketanserin in mice with SAD suggested the presence of minor mechanisms other than BRS enhancement.

**Figure 3.** Effects of ketanserin on hepatic injury in mice challenged with lipopolysaccharide (LPS). A, Normal control. Light micrographs show hepatic tissue with normal appearance. B, LPS injection resulted in widened portal areas, thin fibrous septa throughout the hepatic parenchyma, and inflammatory cell infiltration. C, D, LPS plus ketanserin at 3.0 (C) or 10 (D) mg/kg. Ketanserin treatment produced less infiltration by inflammatory cells, less putrescence of hepatic cells, and hyperplasia of Kupffer cells (n = 9 per group; magnification, ×200). White arrows, vessels; black arrows, array of hepatocytes.

**Figure 4.** Effects of ketanserin on serum tumor necrosis factor (TNF) α in interleukin 10–knockout (KO) mice challenged with lipopolysaccharide (LPS). Knockout mice or age- and sex-matched wild-type (WT) mice received LPS (20 mg/kg; intraperitoneal) and then vehicle or ketanserin (10 mg/kg; intraperitoneal). Blood samples were obtained 90 minutes after LPS administration (n = 6 per group). #P < .05 (vs wild-type mice); **P < .01 (vs LPS); *P < .05 (vs LPS). Abbreviation: Con, control group.
Figure 5. Effects of ketanserin on hemodynamic and survival time in rats challenged with lipopolysaccharide (LPS). Rats received LPS (50 mg/kg; intraperitoneal) and then vehicle or ketanserin (1.0 mg/kg; intraperitoneal). A, Prototypic tracing of mean arterial pressure (MAP). B, Changes in MAP and heart rate. C, Effects of ketanserin on survival time in rats receiving LPS (red) or LPS followed by ketanserin (blue) (n = 10 per group); *P < .05.

Figure 6. Effects of ketanserin on baroreflex sensitivity (BRS) in rats challenged with lipopolysaccharide (LPS). A, BRS in normal control versus LPS group (n = 12 per group). B, Effects of ketanserin. (n = 9 per group). C, Average BRS in A and B; **P < .01. D, Difference between white column and black column (ΔBRS) in C; **P < .01 (vs control). Abbreviation: NS, normal saline.
Ketanserin is an antagonist for 5-HT$_{2A}$ receptors. However, it has broad effects on G protein receptors signaling beyond 5-HT$_{2A}$ receptors, most importantly its antagonistic effect on α$_1$ receptors. Our previous studies showed that the baroreflex enhancement induced by ketanserin in spontaneously hypertensive rats is mainly mediated by central 5-HT$_{2A}$ receptors [13, 21]. Ritanserin is a central serotonic system was destroyed, the effect of ketanserin on BRS disappeared [13].

Recently, the cholinergic anti-inflammatory pathway has been increasingly implicated in inflammatory diseases, including endotoxic shock [24, 25]. Because the α7 nicotinic acetylcholine receptor (α7 nAChR) is a key point in this cholinergic pathway [26–28], we speculated that ketanserin enhances baroreflex function, and then parasympathetic activity and increasing α7 nAChR function. This was confirmed by our recent findings showing that parasympathetic tension, endogenous transmitter acetylcholine, and the expression of α7 nAChR are all decreased in SAD rats.

The present studies showed that ketanserin is used as a tool to enhance BRS without affecting blood pressure. However, translating such an idea eventually into clinical practice is a long process. Agents that enhance BRS without potential effects on blood pressure may be needed. With regard to ketanserin, we believe that it may be clinically useful if titrated properly and carefully. Overall, our results indicate that ketanserin could alleviate endotoxic shock, mainly by restoring baroreflex function.

**Supplementary Data**

Supplementary materials are available at The Journal of Infectious Diseases online (http://www.oxfordjournals.org/our_journals/jid/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

**Acknowledgments.** G. F. Z., C. L., and W. H. L. conducted all experiments in mice; S. W. S. and G. J. C. conducted experiments in rats; D. F. S. and C. Y. M. designed the study.

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