Effect of the Selective Adenosine A1-Receptor Antagonist KW-3902 on Lipopolysaccharide-Induced Reductions in Urine Volume and Renal Blood Flow in Anesthetized Dogs

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ABSTRACT—We investigated the effects of KW-3902 (8-noradamantan-3-yl-1,3-dipropylxanthine), a potent and selective adenosine A1-receptor antagonist, on lipopolysaccharide (LPS)-induced reduction of urine volume (UV) in anesthetized dogs, in comparison with those of furosemide. LPS was intravenously administered at a dose of 0.5 mg/kg; and the heart rate (HR), systemic blood pressure (BP), renal blood flow (RBF) and UV were measured every 15 min for 4 h. Administration of LPS continuously decreased HR, BP, RBF and UV. KW-3902, furosemide or their corresponding vehicle was given as a bolus injection 5 min after the LPS injection. Treatment with KW-3902 (1 mg/kg, i.v.) ameliorated the LPS-induced decline of UV and RBF. Furosemide (3.2 mg/kg, i.v.) tended to ameliorate the LPS-induced decline of UV but not RBF, the duration of the effect being shorter than that of KW-3902. These results suggest that KW-3902 can ameliorate the oliguria and the decrease in RBF during the early phase of LPS-induced shock. Endogenous adenosine may be involved in the endotoxin-induced oliguria via the adenosine A1-receptor.

Keywords: Lipopolysaccharide, Shock, Acute renal failure, Adenosine, Adenosine A1-receptor antagonism

Endotoxemia often causes a marked and intractable decrease in systemic blood pressure as well as a profound vasoconstriction of the renal artery, leading to septic shock and acute renal failure (ARF). The lipopolysaccharide (LPS) portion of the bacterial cell wall is thought to account for the toxicity of a gram-negative bacterial infection (1). In addition, endotoxin induces the production of secondary mediators that cause renal vasoconstriction and ARF (2–5).

A role for adenosine in the pathogenesis of endotoxin-induced ARF has been suggested by several studies. Churchill et al. (6) previously demonstrated that theophylline prevented renal failure in endotoxemic rats, and its effect was attributed to the antagonism of the adenosine receptors. The adenosine A1-receptor antagonist, 8-cyclopentyl-1,3-dipropylxanthine (CPX), prevented the LPS-induced acute renal dysfunction in rats (3). In this study, CPX was given with LPS at the same time. Recently, Nishiyama et al. (7) reported that intravenous administration of LPS significantly increased the renal interstitial concentration of adenosine and that pretreatment with FK352, a selective A1-receptor antagonist, protected against renal dysfunction during endotoxins shock. However, the therapeutic effect of the adenosine A1-receptor blockade on renal dysfunction following endotoxemia has not yet been determined.

KW-3902 (8-noradamantan-3-yl)-1,3-dipropylxanthine) is a potent and selective adenosine A1-receptor antagonist (8). In the receptor-binding study, the dissociation constant values of KW-3902 for adenosine A1-receptor and A2 receptor are 0.19 and 170 nM, respectively (9). In anesthetized rats, KW-3902 potently antagonizes the bradycardic response mediated via adenosine A1-receptors, with little influence on the hypotensive response, mediated via adenosine A2-receptors (10). KW-3902 exhibits diuretic effects in rats (10) and dogs (11, 12). Additionally, KW-3902 induces diuretic effects in various models of acute renal failure in rats (13). The purpose of this study was to determine whether the selective adenosine A1-receptor antagonist KW-3902 confers therapeutic effects during endotoxin-induced oliguria and the decrease in renal blood flow (RBF) in anesthetized dogs and to compare its effects with those of furosemide.
MATERIALS AND METHODS

The experiments were conducted in mongrel dogs of either sex, weighing 7.5 – 15.5 kg. All animals received humane care in compliance with the “Guiding Principles for the Care and Use of Laboratory Animals” formulated by the Japanese Pharmacological Society, and the protocol was approved by the Bioethical Committee of Pharmaceutical Research Institute, Kyowa Hakko Kogyo Co., Ltd.

Surgical preparation

The dog was anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and then artificially ventilated with room air using a constant volume respirator at a rate of 15 strokes /min. The femoral vein was cannulated for systemic infusion of saline containing 4% sodium pentobarbital at a rate of 3 ml/kg per hour. Arterial blood pressure was measured with a pressure transducer (AP-621G; Nihon Kohden, Tokyo) by a polyethylene catheter placed in the femoral artery. Heart rate (HR) was determined using a tachograph (AT-610G, Nihon Kohden). The left kidney was exposed, and all visible nerves were dissected away from the renal vessels and cut after ligation. A catheter for urine collection was inserted into the left ureter. An electromagnetic flow probe (MFV-3100, Nihon Kohden) was attached to the renal artery to measure the RBF. These mechanical parameters were recorded on a thermal array recorder (WS-681, Nihon Kohden). After completion of the surgical procedure, the animal was allowed to equilibrate for more than 1 h.

Experimental protocols

Following equilibration, the baseline values of urine volume (UV) were assessed in two consecutive 15-min control periods. Hereafter, LPS (0.5 mg/kg, E. coli 055B5; Difco, Detroit, MI, USA) was intravenously given as a bolus injection. When both arterial blood pressure and renal blood flow of the animal decreased to less than 70% of the baseline values within 5 min after the intravenous injection of LPS, the dogs were used for further experiments. KW-3902 (0.3, 1 mg/kg), furosemide (3.2 mg/kg) or their corresponding vehicle was given as a bolus injection. The mean arterial blood pressure (MBP), HR and RBF were recorded at 0, 5, 8, 15, 30, 60, 120, 180 and 240 min after the injection of LPS. Urine was collected every 15 min.

Drug

KW-3902 was synthesized in our laboratories. Sterile KW-3902 solutions were prepared as lipomulsion (vehicle). Furosemide (Lasix®) was purchased from Nippon Hoechst Marion Roussel, Ltd. (Tokyo). Saline was used as the vehicle for furosemide. The injected volume was: 0.67 ml/kg for KW-3902 at 0.3 mg/kg, 2 ml/kg for KW-3902 at 1 mg/kg, and 0.67 ml/kg for furosemide at 3.2 mg/kg. The doses of KW-3902 were selected so that this drug exhibits diuretic effects in anesthetized dogs. The dose of furosemide examined is reported to exhibit diuretic effects in anesthetized dogs (14). All other chemicals and solvents were used in their analytically pure form.

Statistical analyses

All data are expressed as means ± S.E.M. Statistical significance was estimated by using the Student’s t-test or the Aspin-Welch test for independence. P values of less than 0.05 were considered statistically significant.

RESULTS

Between the vehicle and drug-treated animals, no differences in the baseline values of HR, MBP, RBF and UV were observed for each treated group (Table 1).

Changes in hemodynamic parameters and UV after LPS injection

Changes in HR, MBP, RBF and UV after LPS injection are shown in Figs. 1 – 4. The LPS injection caused decreases in HR and UV. These parameters remained at lower levels than their baselines during the experimental period. The LPS induced immediate decreases in MBP and RBF, which reached peak levels at 5 min after LPS injection. These parameters also did not return to their baseline levels even 240 min after the LPS injection.

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<tr>
<th>Table 1. Basal heart rate, mean blood pressure, renal blood flow and urine volume of each group in anesthetized dogs</th>
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<td>Heart rate (beats/min)</td>
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<td>Renal blood flow (ml/kg per minute)</td>
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<td>Urine volume (ml/kg per 15 min)</td>
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Each value represents the mean ± S.E.M. of 4 – 5 animals.
**Effect of KW-3902 on the changes induced by LPS**

KW-3902 at 0.3 mg/kg had no effect on the decreases in HR, MBP and RBF, but tended to attenuate the decrease in UV, especially during the later period after LPS injection.

KW-3902 at 1 mg/kg had no effect on the decrease in HR, but tended to attenuate the decrease in MBP during the early period after LPS injection. The treatment with KW-3902 at 1 mg/kg significantly ameliorated the fall of RBF and UV induced by the LPS injection.

**Effect of furosemide on the changes induced by LPS**

Treatment with furosemide had no effect on the decreases in the HR, MBP and RBF induced by LPS. Furosemide tended to ameliorate the LPS-induced decline of UV during 30–90 min.

**DISCUSSION**

In this study, the administration of LPS to dogs caused marked decreases in UV and RBF with systemic hypotension. Several studies have linked the production of nitric
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Fig. 3. Effects of KW-3902 and furosemide on decrease in renal blood flow in anesthetized dogs with lipopolysaccharide (LPS)-induced shock. The drugs were intravenously administered at 5 min after LPS injection. Each point represents the mean ± S.E.M. of 4–5 dogs. ○: vehicle, ●: drug. *P<0.05, compared with the value in the vehicle-treated group.

Fig. 4. Effects of KW-3902 and furosemide on decrease in urine volume in anesthetized dogs with LPS-induced shock. The drugs were intravenously administered at 5 min after LPS injection. Each point represents the mean ± S.E.M. of 4–5 dogs. ○: vehicle, ●: drug. *P<0.05, **P<0.01, ***P<0.001 compared with the value in the vehicle-treated group.

Oxide (NO) to LPS-induced hypotension, vascular hyporesponsiveness and death, suggesting that the excess generation of NO plays an important role in the development of septic shock (15, 16). In addition, endotoxin is reported to induce the production of secondary mediators that cause renal vasoconstriction and ARF (2–5). It has been reported that adenosine acts as a potent renal vasoconstrictor (17). The infusion of adenosine elicits marked decreases in RBF (18, 19). In a clinical study, plasma concentrations of adenosine are known to be elevated 4- to 5-fold in patients with sepsis when compared with those in healthy volunteers (20). Moreover, Nishiyama et al. (7) have reported that the renal interstitial concentration of adenosine increases during endotoxin shock. Thus, the increase in endogenous adenosine has been suggested to play a pivotal role in the development of sepsis-associated ARF, possibly due to its potent renal vasoconstriction.

The present study demonstrated that KW-3902 ameliorated the LPS-induced renal vasoconstriction. It is reported that non-selective blockade of adenosine receptors with theophylline prevented renal failure in endotoxemic rats.
Theophylline, however, is a relatively non-selective adenosine antagonist, which also inhibits phosphodiesterase (21). Nishiyama et al. (7) reported that the pretreatment with the selective adenosine A1-receptor antagonist FK352 prevents the reductions in renal blood flow induced by administration of LPS. In the present study, we used KW-3902, which is a potent and selective adenosine A1-receptor antagonist (8). We demonstrated that treatment with KW-3902 ameliorated the decrease in RBF induced by LPS in dogs. Several investigations have clarified that adenosine A1-receptors, which cause preglomerular vasoconstriction, and adenosine A2-receptors, which cause postglomerular vasodilatation, have been identified in animal and human kidneys (22, 23). These observations suggest that the adenosine A1-receptor plays an important role in LPS-induced renal vasoconstriction.

In the present study, KW-3902 induced a remarkable increase in urine volume in the dogs treated with LPS. The effects of KW-3902 in dogs with endotoxemia are consistent with its diuretic effects reported in normal dogs (11, 12). We have previously demonstrated that KW-3902 induced diuretic effects in various models of acute renal failure, and the effect of KW-3902 was more prominent than that of furosemide (13). In the present study, KW-3902 had significant diuretic effects in the oliguric model, whereas furosemide showed transient diuretic effects. In addition, KW-3902, but not furosemide, significantly increased the RBF. KW-3902 might improve the RBF via the blockade of the adenosine A1-receptors, the stimulation of which is known to constrict the afferent arterioles. In fact, KW-3902 is assumed to act mainly on the proximal tubule, resulting in diuresis and natriuresis (24). However, the present results suggest that a renal hemodynamic effect may also be involved in the diuretic action of KW-3902 in the LPS-induced decrease in UV. A limitation of the present study is the lack of the data concerning the glomerular filtration rate. The exact mechanism of the diuretic effect of KW-3902 and the precise role of adenosine in LPS-induced oliguria remain unclear, and further studies are necessary.

In the present study, KW-3902 induced a remarkable increase in urine volume after LPS injection. It is possible that the diuretic actions of KW-3902 are responsible for the effects of treatment following endotoxemia. Patients with oliguric renal failures are usually treated with diuretics such as loop diuretics or mannitol for accelerating urination. In this regard, the large doses of furosemide (25) may improve the clinical course of acute renal failure. In the present study, the increasing effect of KW-3902 at 1 mg/kg on UV in the LPS-induced oliguric dogs was more potent than that of furosemide at 3.2 mg/kg, which was a sufficient dose to cause diuresis in normal dogs (26). Therefore, it is likely that KW-3902 is more useful as a diuretic than furosemide for the treatment of oliguria during endotoxemia.

In the present study, KW-3902 at 0.1 mg/kg tended to attenuate the decrease in MBP. Adenosine is known to attenuate the inotropic action of catecholamines in the ventricular myocardium solely via the A1-receptor interaction without direct (non-antiadrenergic) action on ventricular contractility (27-29). Such antiadrenergic action of endogenous adenosine may impair hemodynamic recovery in dogs with endotoxemia. In the dogs treated with LPS, KW-3902 might have enhanced the actions of the endogenous catecholamines and induced enhancement of the cardiac contractility, leading to the tendency of the increase in blood pressure. In the present study, however, the effects of KW-3902 on cardiovascular depression was not examined in detail. The precise role of adenosine in cardiac dysfunction during endotoxemia remains unclear, and further investigations are necessary.

In summary, the present study demonstrates that KW-3902 can ameliorate the decrease in UV and RBF during the early phase of LPS-induced shock. These results suggest that endogenous adenosine may be involved in the oliguria and the decrease in RBF induced by LPS via the adenosine A1-receptor. KW-3902 may be useful as a therapeutic agent for oliguria during endotoxemia.

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