Diuretic Effects of KW-3902, a Novel Adenosine A₁-Receptor Antagonist, in Anesthetized Dogs

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The effects of intravenous infusion of KW-3902 (8-(noradamantan-3-yl)-1,3-dipropylxanthine), a novel adenosine A₁-receptor antagonist, on urine volume, urinary excretion of electrolytes and renal hemodynamics were examined in anesthetized dogs. KW-3902 at 10 and 30 $\mu\text{g/kg/min}$ for 20 min inhibited the decline of renal blood flow induced by intrarenal arterial injection of adenosine (0.5–2.0 μg). KW-3902 at these doses produced significant increases in urine volume and sodium excretion with little change in potassium excretion. The diuretic effect of KW-3902 at 30 $\mu\text{g/kg/min}$ for 20 min continued for longer than 1 h even after discontinuation of the KW-3902 infusion. KW-3902 did not affect creatinine clearance, renal blood flow, arterial blood pressure or heart rate. Furosemide at 10 $\mu\text{g/kg/min}$ for 20 min brought about significant increases in urine volume and excretion of sodium and potassium. The diuresis and saliuresis induced by furosemide continued for only 40 min after discontinuation of the drug infusion. Trichlormethiazide at 3 $\mu\text{g/kg/min}$ for 20 min also provoked increases in urine volume and sodium excretion, but did not affect potassium excretion. The diuretic and natriuretic effect of trichlormethiazide gradually disappeared after discontinuation of the drug infusion. The present study in anesthetized dogs suggests that KW-3902, an adenosine A₁-receptor antagonist, produces diuresis and natriuresis but not kaliuresis and that the diuresis and natriuresis are caused in large part by the inhibition of sodium reabsorption at tubular sites.

Keywords KW-3902; adenosine A₁-receptor antagonist; diuretic effect; anesthetized dog

Previous studies indicated that the alkylxanthine with high affinity for adenosine receptors produces diuresis and natriuresis and that these effects are ascribable to the antagonism by the alkylxanthine of the effect of endogenous adenosine.^{1a,b)} It has recently been assumed that the diuretic effects of adenosine antagonists are due to the blockade of adenosine A₁-receptors.²⁾

KW-3902 (8-(noradamantan-3-yl)-1,3-dipropylxanthine) is a selective adenosine A₁-receptor antagonist and the most potent one reported to date.³⁾ KW-3902 produces significant increases in urine volume and sodium excretion with little change in potassium excretion in saline-loaded rats.^{2b,4)} In anesthetized rats, KW-3902 produces significant diuresis and natriuresis with no change in renal plasma flow or glomerular filtration rate (GFR).^{2b)} Furthermore, our recent studies have demonstrated that KW-3902 at the diuretic dose increases lithium clearance while it does not affect the distal dip of the stop-flow pattern in anesthetized rats.⁵⁾ Knight *et al.* demonstrated that 8-cyclopentyl-1,3-dipropylxanthine, another selective adenosine A₁-receptor antagonist, also increases lithium clearance in anesthetized rats.⁶⁾ Our results as well as the report by Knight *et al.* suggest that, in anesthetized rats, the adenosine A₁-receptor antagonist produces diuretic effects by inhibiting the reabsorption of water and sodium mainly at the proximal nephron segments.

Terai *et al.* reported that (+)-(R)-1-[(E)-3-(2-phenylpyrazolol[1,5-*a*]pyridin-3-yl)acryloyl]-2-piperidine ethanol (FK453), a non-xanthine adenosine A₁-receptor antagonist, produced diuretic effects with significant increases in renal blood flow (RBF) and GFR in anesthetized dogs.⁷⁾ Thus, effects of the adenosine A₁-receptor antagonist on renal hemodynamics may be different between rats and dogs. In the present study, we examined the diuretic effects of KW-3902 in anesthetized dogs in comparison with those of furosemide and trichlormethiazide (TCM). We also

sought to learn whether or not KW-3902 increases RBF and GFR at the diuretic doses in anesthetized dogs. The mechanism of the diuretic effect of KW-3902 is discussed based on the results.

MATERIALS AND METHODS

Drugs Used KW-3902³⁾ and furosemide were synthesized in our laboratories. Trichlormethiazide (TCM) was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Creatinine was purchased from Wako Pure Chemical Industries, Ltd. (Osaka). All other chemicals and solvents were used in their analytical pure form. KW-3902, furosemide and TCM were dissolved in saline containing 3% dimethylsulfoxide and 0.01N NaOH (vehicle).

Animal Preparation Mongrel dogs of either sex, weighing 8.5 to 18.5 kg, were anesthetized with sodium pentobarbital (25 mg/kg, i.v.), and then artificially ventilated with room air by a constant volume respirator at a rate of 15 strokes/min. Anesthesia was maintained by continuous intravenous infusion of sodium pentobarbital (5 mg/kg/h) throughout the experiment. The cephalic vein of the left forelimb and the medial saphenous vein of the right hindlimb were cannulated for infusion of saline and infusion of drugs, respectively. Two more catheters were placed in the medial saphenous artery of the right hindlimb and the left femoral artery for collection of arterial blood and measurement of arterial blood pressure, respectively. Arterial blood pressure was measured by a pressure transducer (MPU-05, Nihon Kohden, Tokyo) and heart rate was measured by a cardio-tachometer (RT-5, Nihon Kohden). The left kidney was exposed by a retroperitoneal flank incision as described.⁸⁾ All visible renal nerves were dissected away from the renal vessels and cut after ligation. An electromagnetic flow probe (Nihon Kohden) was

attached to the renal artery to measure the RBF with a square wave flowmeter (model MFV-3100, Nihon Kohden). A catheter for urine collection was inserted into the left ureter. Saline was infused into the cephalic vein of the left forelimb at a rate of 6 ml/kg/h. For measurement of creatinine clearance (C_{CRE}), a priming dose of creatinine (50 mg/kg) was intravenously administered, followed by continuous intravenous infusion of a maintenance dose (50 mg/kg/h). After completion of the surgical procedure, the animal was allowed to equilibrate for 60 to 90 min.

Experimental Protocol In a preliminary experiment, we determined the doses of KW-3902 which inhibit the RBF response induced by exogenous adenosine using 6 animals. Adenosine (0.5, 1.0 and 2.0 μ g) was injected into the renal artery at intervals of 7–8 min, and the RBF response was observed. Thereafter, KW-3902 at increasing doses of 3, 10 and 30 μ g/kg/min was intravenously infused at a flow rate of 1 ml/kg/min. During the infusion of KW-3902 at each dose, adenosine was repeatedly injected and the RBF response was determined.

In the experiment in which the effects of KW-3902, furosemide and TCM on renal functions were examined, 5 animals were used in each group. Urine was collected every 10 min and arterial blood was collected every 20 min until 200 min after the beginning of the experiment. Immediately after the blood collection, plasma was separated by centrifugation (3000 rpm, 10 min, 4 °C) for the analysis. At 20 min, vehicle was intravenously infused to all dogs for 20 min at a rate of 0.1 ml/kg/min. At 100 min, KW-3902 (3, 10 or 30 μ g/kg/min) was intravenously infused for 20 min at a flow rate of 0.1 ml/kg/min. In the furosemide and TCM groups, furosemide (1, 3 or 10 μ g/kg/min) or TCM (0.1, 0.3, 1 or 3 μ g/kg/min) was infused intravenously for 20 min instead of KW-3902. In the control group, vehicle was intravenously infused for 20 min again instead of the drug.

Analytical Procedures Concentrations of creatinine in urine (U-CRE) and plasma (P-CRE) were determined by an autoanalyzer (AU510, Olympus, Tokyo). Sodium and potassium concentrations in urine were measured by flame photometry (775-A, Hitachi Ltd., Tokyo). The standard formula was used to calculate C_{CRE} as an index of GFR as follows:

$$C_{CRE} \text{ (ml/g/min)} = \frac{\text{U-CRE (mg/dl)} \times \text{urine volume (ml/g/min)}}{\text{P-CRE (mg/dl)}}$$

Statistical Analyses Data are presented as means $\pm$ S.E. of 5 animals. Statistical significance was estimated using Tukey multiple comparison test, following the 2-way analysis of variance. Differences at p values of less than 0.05 were considered to be statistically significant.

RESULTS

Effects on RBF Response Induced by Exogenous Adenosine Figure 1 shows the effect of KW-3902 on the RBF response induced by adenosine. Intrarenal arterial injection of adenosine (0.5, 1.0 and 2.0 μ g) induced dose-dependent biphasic RBF responses, *i.e.*, rapid decrease followed by an increase. KW-3902 dose-dependently inhibited the initial decrease in RBF at doses of

Fig. 1. Effects of KW-3902 on the Renal Blood Flow (RBF) Response Induced by Exogenous Adenosine in Anesthetized Dogs

Adenosine (0.5, 1.0 or 2.0 μ g) was injected into renal artery at intervals of 7–8 min. KW-3902 was intravenously infused at increasing doses of 3, 10 and 30 μ g/kg/min. Open column, control; dotted column, KW-3902 at 3 μ g/kg/min; hatched column, KW-3902 at 10 μ g/kg/min; closed column, KW-3902 at 30 μ g/kg/min. Each point represents the mean $\pm$ S.E. of 6 animals. * p < 0.05, ** p < 0.01 when compared with the control value.

3–30 μ g/kg/min. At doses of 1 and 10 μ g/kg/min it did not affect the latter increases in RBF, though at a dose of 30 μ g/kg/min KW-3902 significantly inhibited these increases.

Effects on Urine Volume and Electrolyte Excretion

Figures 2–4 show the effects of KW-3902, furosemide and TCM, respectively, on urine volume and electrolytes excretion. No significant difference was observed among the 0 time values for the KW-3902-, furosemide- and TCM-treated groups at each dose. KW-3902 at doses of 10 and 30 μ g/kg/min significantly increased urine volume and sodium excretion, but did not affect potassium excretion. The diuretic effect of KW-3902 at 30 μ g/kg/min persisted for longer than 1 h even after discontinuation of infusion (Fig. 2). Furosemide at 10 μ g/kg/min markedly increased urine volume and sodium excretion and also significantly increased potassium excretion. The diuretic effect of furosemide disappeared within 40 min after discontinuation of infusion (Fig. 3). TCM produced diuretic and natriuretic effects without significant change in potassium excretion at a dose of 3 μ g/kg/min. The diuretic effect of TCM gradually disappeared after discontinuation of infusion (Fig. 4).

Effects on Renal and Systemic Hemodynamics The effects of KW-3902 on C_{CRE} and RBF are shown in Fig. 5. C_{CRE} and RBF in the KW-3902 (10 and 30 μ g/kg/min)-treated group tended to increase during drug infusion. Though furosemide at 10 μ g/kg/min did not affect RBF or C_{CRE} during the infusion period, RBF tended to decrease (from 5.02 to 3.82 ml/g/min) and C_{CRE} decreased significantly (from 0.854 to 0.659 ml/g/min) after discontinuation of infusion. TCM (3 μ g/kg/min) did not

Fig. 2. Effects of KW-3902 on Urine Volume, Sodium and Potassium Excretion in Anesthetized Dogs

Closed circles, control group; open circles, KW-3902 3 µg/kg/min infusion group; open triangles, KW-3902 10 µg/kg/min infusion group; open squares, KW-3902 30 µg/kg/min infusion group. Each point represents the mean $\pm$ S.E. of 5 animals. * p < 0.05 when compared with the control value.

significantly affect RBF (data not shown); however, TCM showed a tendency to decrease C_{CRE} (from 0.933 to 0.848 ml/g/min) after discontinuation of infusion. KW-3902 did not affect mean arterial blood pressure or heart rate during the experiment (Fig. 6). No change in mean arterial blood pressure or heart rate was observed in either the furosemide- or the TCM-treated group (data not shown).

DISCUSSION

In receptor binding studies, the dissociation constant values of KW-3902 for adenosine A_1 -receptor and A_2 -receptor are 1.1 and 330 nM, respectively.^{3a)} In anesthetized rats, KW-3902 antagonizes the 5'-N-ethylcarboxamido-adenosine (NECA)-induced bradycardic response, which is thought to be mediated *via* adenosine A_1 -receptors, whereas this drug does not affect the hypotensive response to NECA which is predominantly due to adenosine A_2 -receptor activation.^{2b)} Thus, KW-3902 is a specific adenosine A_1 -receptor antagonist *in vivo* as well

Fig. 3. Effects of Furosemide on Urine Volume, Sodium and Potassium Excretion in Anesthetized Dogs

Closed circles, control group; open circles, furosemide 1 µg/kg/min infusion group; open triangles, furosemide 3 µg/kg/min infusion group; open squares, furosemide (Furo.) 10 µg/kg/min infusion group. Each point represents the mean $\pm$ S.E. of 5 animals. * p < 0.05, ** p < 0.01 when compared with the control value.

as *in vitro*. In the present study using anesthetized dogs, KW-3902 inhibited adenosine-induced vasoconstriction, mediated *via* adenosine A_1 -receptor, without affecting vasodilation, mediated *via* adenosine A_2 -receptor. These results indicate that KW-3902 is also a specific adenosine A_1 -receptor antagonist in dogs. Moreover, the present study revealed that KW-3902, presumably *via* the blockade of adenosine A_1 -receptor, produces diuresis and natriuresis in anesthetized dogs.

In general, the diuretic and natriuretic doses of furosemide and TCM are known to increase potassium excretion, whereas KW-3902 is reported to increase urine volume and sodium excretion without affecting potassium excretion.^{2,4,5)} In the present study, furosemide increased urine volume and sodium excretion with a significant increase in potassium excretion, whereas KW-3902 and TCM failed to increase potassium excretion significantly. Thus, the kaliuresis effects of KW-3902 and TCM were minimal under the present experimental conditions, as compared with that of furosemide. The present observa-

Fig. 4. Effects of TCM on Urine Volume, Sodium and Potassium Excretion in Anesthetized Dogs

Closed circles, control group; open circles, TCM 0.1 $\mu\text{g/kg/min}$ infusion group; open triangles, TCM 0.3 $\mu\text{g/kg/min}$ infusion group; open squares, TCM 1 $\mu\text{g/kg/min}$ infusion group; dotted triangles, TCM 3 $\mu\text{g/kg/min}$ infusion group. Each point represents the mean $\pm$ S.E. of 5 animals. * $p < 0.05$, ** $p < 0.01$ when compared with the control value.

tion is in accordance with our previous findings that KW-3902 produces diuresis and natriuresis without kaliuresis in conscious rats,^{2b)} anesthetized rats^{2b,5)} and conscious dogs.⁴⁾

In the kidney, adenosine A_1 -receptor and A_2 -receptor are located in the afferent renal arteriole and in the efferent renal arteriole, respectively.^{1b,9)} On the other hand, both these receptors exist in glomeruli¹⁰⁾ and in mesangial cells.¹¹⁾ These receptors are thought to regulate GFR and RBF in the kidney. In the present study, KW-3902 showed a tendency to increase C_{CRE} and RBF. It is thus presumed that the slight increase in these factor is due to an inhibition of the contraction of renal afferent arteriole mediated by adenosine A_1 -receptor. In fact, Terai *et al.* demonstrated that the adenosine A_1 -receptor antagonist FK453 could cause modest but statistically significant increases in GFR and RBF in anesthetized dogs.⁷⁾ However, despite the transient increase by FK453 of GFR and RBF, its diuretic and natriuretic effects lasted longer in association with the increased excretion of phosphate and HCO_3^- .¹²⁾ Likewise,

Fig. 5. Effects of KW-3902 on C_{CRE} and RBF in Anesthetized Dogs

Closed circles, control group; open circles, KW-3902 3 $\mu\text{g/kg/min}$ infusion group; open triangles, KW-3902 10 $\mu\text{g/kg/min}$ infusion group; open squares, KW-3902 30 $\mu\text{g/kg/min}$ infusion group. Each point represents the mean $\pm$ S.E. of 5 animals.

Fig. 6. Effects of KW-3902 on Mean Arterial Pressure and Heart Rate in Anesthetized Dogs

Closed circles, control group; open circles, KW-3902 3 $\mu\text{g/kg/min}$ infusion group; open triangles, KW-3902 10 $\mu\text{g/kg/min}$ infusion group; open squares, KW-3902 30 $\mu\text{g/kg/min}$ infusion group. Each point represents the mean $\pm$ S.E. of 5 animals.

the slight and not significant increases by KW-3902 in C_{CRE} and RBF immediately disappeared after discontinuation of the drug infusion, whereas the diuretic and

natriuretic effects of KW-3902 continued for longer than 1 h even after discontinuation (Fig. 2). Our present results, as well as the previous reports,¹²⁾ suggest that the adenosine A₁-receptor antagonist produces diuretic and natriuretic effects mainly by inhibiting reabsorption of water and sodium at tubular sites, rather than by the increase in GFR and RBF in anesthetized dogs, as was the case with anesthetized rats.^{2b,4)} These results also suggest the possible existence of adenosine A₁-receptor in tubular cells.

In the present study, furosemide tended to decrease and TCM significantly decreased C_{CRE} at doses that produced diuretic effects. Such decrease was not observed in the KW-3902-treated groups. The decrease in GFR associated with diuretic action seems to be caused by the tubuloglomerular feedback (TGF) system.¹³⁾ It was reported that the adenosine A₁-receptor may play an important role in the transmission of TGF signals,¹⁴⁾ it is therefore likely that KW-3902, by inhibiting the TGF response, prevented the decrease in C_{CRE} associated with the diuretic action. In addition to the direct effect on the tubule, the inhibition of the TGF response, *via* antagonism of the adenosine A₁-receptor, may contribute to the diuretic effect of KW-3902.

In rats, KW-3902, treated either i.v. or *p.o.*, produces significant diuresis and natriuresis at doses of 0.001 or 0.01 mg/kg and above.^{2b,4)} However, a dose of 1 mg/kg or more of KW-3902 (*p.o.*) is necessary to induce significant diuresis and natriuresis in conscious dogs.¹⁵⁾ In the present study using anesthetized dogs, on intravenous KW-3902 dose of 10 µg/kg/min for 20 min (total 0.2 mg/kg) was necessary to cause significant diuresis and natriuresis. Thus, the diuretic and natriuretic activities of KW-3902 differ between rats and dogs, though the diuretic pattern of KW-3902 in dogs is quite similar to that in rats. In fact, the pharmacokinetics of KW-3902 following i.v. administration are similar in rats and dogs (unpublished data). Therefore, difference in the sensitivity to, or distribution of adenosine A₁-receptors, rather than a variation in mechanism of diuretic action, is likely to be responsible for the difference in the diuretic activity of KW-3902 between rats and dogs. This assumption is supported by the difference in the adenosine A₁-receptor antagonistic effect of KW-3902 between rats and dogs. In dogs, 0.2 mg/kg (i.v.) of KW-3902 was necessary to exhibit adenosine A₁-receptor antagonistic effect (Fig. 1), whereas in rats 0.01 mg/kg of KW-3902 antagonized the NECA-induced bradycardia.^{2b)}

Exogenous adenosine or the adenosine analogue decreases heart rate through the activation of adenosine A₁-receptors in the sinoatrial node and perhaps on adrenergic nerve terminals innervating this node.¹⁶⁾ If endogenous adenosine is important in the modulation of heart rate under the physiological condition, KW-3902 must increase heart rate. However, in the present study it did not affect heart rate or arterial blood pressure. This

suggests either that endogenous adenosine plays hardly any role in the modulation of heart rate, or that plasma concentration of endogenous adenosine may be quite low under the physiological condition. Moreover, the present results also suggest that, in the physiological condition, the adenosine A₁-receptor functions in the kidney, but not in the cardiovascular system. The kidney may be highly sensitive to adenosine, or this organ may itself have a high local production of this substance.

In summary, this study demonstrated that KW-3902 produces diuresis and natriuresis without significant change in potassium excretion in anesthetized dogs. The diuretic and natriuretic effects are probably caused not by changes in renal hemodynamics but by the inhibition of water and sodium reabsorption at tubular sites.

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