Diuretic Effects of KW-3902 (8-(Noradamanant-3-yl)-1,3-dipropylxanthine),
a Novel Adenosine A₁ Receptor Antagonist, in Conscious Dogs

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The diuretic effects of KW-3902 (8-(noradamanant-3-yl)-1,3-dipropylxanthine), a novel adenosine A₁ receptor antagonist, were determined and compared with those of trichlormethiazide (TCM) and furosemide in saline-loaded conscious dogs. KW-3902, at doses higher than 0.1 mg/kg (p.o.), produced dose-dependent increases of urine volume and sodium excretion and these effects were statistically significant at doses of 1 — 100 mg/kg. The increase in potassium excretion was lower than that of sodium, and the ratio of sodium to potassium excretion (Na/K) tended to be elevated. TCM (0.3 mg/kg) and furosemide (5 mg/kg) also induced increases in urine volume and sodium excretion. The diuretic effects of KW-3902 lasted for 4 h after administration, whereas TCM and furosemide caused significant natriuresis for 2 h after administration. Thus, KW-3902 exhibited a longer lasting natriuresis than TCM and furosemide. These results indicate that adenosine A₁ receptor blockade by KW-3902 causes consistent diuresis and natriuresis in dogs and suggest that adenosine A₁ receptor blockade is a promising approach to diuretic therapy.

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

It has been well-known for many years that caffeine, theophylline and other alkylxanthines provide diuretic effects.1) Recent studies have demonstrated that alkylxanthines with diuretic action have a high affinity for adenosine receptors,2) and suggested that the diuretic effect of alkylxanthines is based on the antagonism of adenosine at the receptor. More recently, the diuretic effect of 8-cyclopentyl-1,3-dipropylxanthine, a selective adenosine A₁ receptor antagonist, has been reported.3) Therefore, it seems that the diuretic effect of the adenosine receptor antagonist is due to blockade of the A₁ receptor.

KW-3902 (8-(noradamanant-3-yl)-1,3-dipropylxanthine), is a novel potent and selective adenosine A₁ receptor antagonist.4) In receptor binding studies, the dissociation constant values of KW-3902 for adenosine A₁ receptor and A₂ receptor are 1.1 and 330 nM, respectively.5) KW-3902 antagonized the 5'-N-ethylcarboxamidoadenosine (NECA)-induced bradycardic response which is thought to be mediated via the A₁ receptor, whereas the hypotensive response to NECA, which is predominantly mediated via the A₂ receptor, was not affected by KW-3902.4) KW-3902 causes significant increases in urine volume and sodium excretion with little change in potassium excretion in conscious rats.5) KW-3902 also causes significant diuresis and natriuresis without any changes in renal plasma flow and creatinine clearance in anesthetized rats,5) suggesting that the adenosine A₁ receptor antagonist causes diuretic effects not by the changes in renal hemodynamics but by the inhibition of water and sodium reabsorption at tubular sites. Moreover, KW-3902 at its diuretic doses induces less side-effects such as hypokalemia and hyperuricemia in rats, compared with established diuretics such as furosemide and trichlormethiazide (TCM).5) Thus, KW-3902 exhibits favorable diuretic action in rats. However, diuretic effects of KW-3902 in other species have not been examined prior to the present study.

In the present study, we determined the effects of KW-3902 on urinary volume and electrolyte excretion in conscious dogs following oral administration and compared these effects with those of the standard thiazide diuretic, TCM, and the loop diuretic, furosemide.

Materials and Methods

Experimental Animals. Experiments were carried out using 20 adult female beagle dogs weighing 7.5—12.0 kg, maintained on standard laboratory diet.

Materials. KW-3902 and furosemide were synthesized in our laboratories. TCM was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). KW-3902, furosemide and TCM were suspended in saline containing 0.05% w/v Tween 80 (vehicle).

General Procedure. On the day before the experiment, the dogs were anesthetized with sodium thiopental (25 mg/kg, i.v.). A Foley catheter (8—10 Fr.; Nihon Sherwood Medical Ind. Inc., Tokyo, Japan) was inserted into the bladder for urine sampling and a nasonesophageal catheter was put in place for the administration of saline (10 ml/kg·2 h) and drugs. The Elizabeth collar (Saf T shield; EJAY Int. Inc., Glendora, CA, U.S.A.), which is for protecting the catheters from the dog, was placed around the neck of the animal. The dogs were returned to individual cages and were allowed to recover from anesthesia. The dogs were deprived of food from the time of anesthesia to the beginning of the experiment, but were allowed free access to water.

Figure 1 shows the experimental schedule. The dogs were given 10 ml/ (kg·2 h) of saline via the nasonesophageal catheter. At time 0, the dogs received vehicle solution or drug suspension via the nasonesophageal catheter. Urine was collected from the bladder catheter every 2 h; 2—0 h for the control period and 0—2, 2—4, and 4—6 h after dosing. Sodium and potassium urine concentrations were measured by flame photometry (775-A, Hitachi Ltd., Tokyo, Japan). When the diuretic experiment ended, the catheters and the Elizabeth collar were removed from the dog, and it

![Fig. 1. Experimental Schedule](image)

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was returned to its own cage. Each dog underwent these separate assays at least one week apart.

**Experimental Protocols**

- **Experiment A**: KW-3902 (0.1, 0.3, 1 or 3 mg/kg) or vehicle was administered at time 0.
- **Experiment B**: KW-3902 (1, 10 or 100 mg/kg) or vehicle was administered at time 0.
- **Experiment C**: TCM (0.03 or 0.3 mg/kg), furosemide (0.3 or 3 mg/kg) or vehicle was administered at time 0.

**Statistical Analyses**

Data were presented as percentages of each value of the control period which was regarded as 100%. The ratio of sodium and potassium excretion (Na/K) during 0–2, 2–4 and 0–4 h was calculated and presented as percentages of each value of the control period, which was regarded as 100%. Each value represents the mean ± S.E. Statistical significance was examined using Steel’s multiple comparison test, and a p value of less than 0.05 was considered significant.

**Results**

- **Experiment A**: The urine volume during the control period in vehicle- and KW-3902 (0.1, 0.3, 1 and 3 mg/kg)-treated groups was 5.26 ± 0.6, 4.23 ± 0.98, 4.50 ± 0.36, 4.54 ± 1.02 and 3.68 ± 0.68 ml/(kg·2 h), respectively. The sodium excretion during the control period in each group was 0.98 ± 0.06, 0.81 ± 0.22, 0.79 ± 0.21, 0.67 ± 0.09 and 0.70 ± 0.12 meq/(kg·2 h), respectively. The potassium excretion during the control period in each group was 0.41 ± 0.14, 0.52 ± 0.19, 0.58 ± 0.21, 0.48 ± 0.13 and 0.52 ± 0.16 meq/(kg·2 h), respectively. There was no significant difference among these values in each group. KW-3902, at a dose of 0.1–3 mg/kg, caused dose-dependent increases in urine volume and sodium excretion. KW-3902, at doses higher than 1 mg/kg, significantly increased urine volume and sodium excretion, and the effects lasted for 4 h after administration (Fig. 2). On the other hand, the increase in potassium excretion was minimal, compared with sodium. The significant increase was observed at a dose of 3 mg/kg over the period 0–2 h after administration. KW-3902 caused dose-dependent increases in Na/K, although these were not statistically significant (Table I).

- **Experiment B**: The urine volume during the control period in vehicle- and KW-3902 (1, 10 and 100 mg/kg)-treated groups was 4.34 ± 0.50, 3.82 ± 0.71, 3.58 ± 0.30 and 5.06 ± 0.96 ml/(kg·2 h), respectively. The sodium excretion during the control period in each group was 1.00 ± 0.16, 0.72 ± 0.25, 0.68 ± 0.12 and 1.06 ± 0.31 meq/(kg·2 h), respectively. The potassium excretion during the control period in each group was 0.96 ± 0.07, 0.77 ± 0.10, 0.85 ± 0.10 and 0.84 ± 0.08 meq/(kg·2 h), respectively. There was no significant difference among these values in each group. KW-3902 produced stable diuretic and natriuretic effects and, at a dose of 10 mg/kg, produced significant diuresis for 4 h after administration, although at doses of 1 and 100 mg/kg significant diuresis only lasted for 2 h after administration (Fig. 3). KW-3902 caused stable increases in Na/K at doses from 1 to 100 mg/kg, but these did not reach statistical significance (Table II).

- **Experiment C**: The urine volume during the control period in vehicle-, TCM (0.03 and 0.3 mg/kg)- and furosemide (0.3 and 3 mg/kg)-treated groups was 4.53 ± 1.18, 3.53 ± 0.27, 3.57 ± 0.19, 3.94 ± 0.24 and 3.56 ± 0.39 ml/(kg·2 h), respectively. The sodium excretion during the control period in each group was 1.01 ± 0.09, 0.91 ± 0.17, 0.88 ± 0.09, 1.09 ± 0.05 and 0.89 ± 0.15 meq/(kg·2 h), respectively. The potassium excretion during the control period in each group was 0.68 ± 0.06, 0.73 ± 0.05, 0.77 ± 0.07, 0.64 ± 0.05 and 0.69 ± 0.04 meq/(kg·2 h), respectively. These was no significant difference among these values in each group. TCM (0.3 mg/kg) and furosemide (3 mg/kg) caused significant increases in urine volume and sodium excretion during 0–2 h after administration (Fig. 4). Potassium excretion was significantly increased by
Fig. 3. Diuretic Effects of KW-3902 in Conscious Dogs
Vehicle or KW-3902 (1, 10 or 100 mg/kg) was orally administered at time 0. □, vehicle; □, KW-3902 1 mg/kg; ○, KW-3902 10 mg/kg; ■, KW-3902 100 mg/kg. Each value represents the mean ± S.E. (n = 6). a) Significant difference from the control value (p < 0.05).

Table II. Effects of KW-3902 on the Ratio of Sodium to Potassium Excretion (Na/K)
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Vehicle or KW-3902 (1, 10 or 100 mg/kg) was orally administered at time 0. Each value represents the mean ± S.E. (n = 6).

furosemide at a dose of 3 mg/kg (Fig. 4). TCM (0.03 and 0.3 mg/kg) and furosemide (3 mg/kg) caused increases in Na/K, and this reached statistical significance for the dose of 0.3 mg/kg of TCM (Table III).

Discussion
Adenosine receptors have been classified into two main subtypes (A1 and A2), both of which are located in a large number of tissue and cell types, including the kidney.6) Exogenous adenosine produces very intense antidiuretic and antinatriuretic effects in rats7) and dogs.8) These effects are receptor-mediated, since they are competitively antagonized by theophylline8) and mimicked by several adenosine...
anals.\textsuperscript{9} Recently, 8-cyclopentyl-1,3-dipropylxanthine, a highly selective adenosine A\textsubscript{1} receptor antagonist, was shown to elicit diuretic and natriuretic effects,\textsuperscript{9} suggesting that these effects are mediated \textit{via} blockade of endogenous adenosine at the A\textsubscript{1} rather than the A\textsubscript{2} receptors.

KW-3902 is a selective adenosine A\textsubscript{1} receptor antagonist and the most potent reported to date.\textsuperscript{43} In saline-loaded rats, KW-3902 causes significant diuresis and natriuresis with little change in potassium excretion at doses higher than 0.001 mg/kg (p.o.).\textsuperscript{34} Creatinine and p-aminohippuric acid clearances were not affected by the diuretic doses of KW-3902 in anesthetized rats.\textsuperscript{9} Moreover, KW-3902 was shown to antagonize the antidiuretic action of (−)-N\textsuperscript{6}(2-phenylisopropyl)adenosine, an adenosine A\textsubscript{1} receptor agonist, in anesthetized rats (unpublished observation). Thus, it seems that the diuretic effect of KW-3902 is mediated \textit{via} the blockade of A\textsubscript{1} receptors and is caused, not by a change in renal hemodynamics, but by the inhibition of water and sodium reabsorption at tubular sites. Our recent studies using lithium clearance and the stop-flow technique in anesthetized rats has demonstrated that KW-3902 produces diuretic effects by inhibiting the reabsorption of water and sodium mainly at the proximal nephron segments.\textsuperscript{10} Moreover, other recent study indicates that FK-453, another adenosine A\textsubscript{1} receptor antagonist, inhibits basolateral Na\textsuperscript{+}/HCO\textsubscript{3}\textsuperscript{−} cotransport in rabbit proximal convoluted tubules, leading to natriuresis and increased HCO\textsubscript{3}\textsuperscript{−} excretion.\textsuperscript{11} The renal tubular action of adenosine receptor blockade is in agreement with previous findings that the adenosine analog stimulates active sodium transport in toad kidney cells,\textsuperscript{12} and adenosine and adenosine analogs increase water permeability, mimicking the effect of vasopressin, in isolated rabbit collecting-ducts.\textsuperscript{13} Prior to the present study, the diuretic action of KW-3902 in animal species other than the rat had not been investigated. In the present study, the possible diuretic effects of KW-3902 were examined in conscious dogs and compared with those of TCM and furosemide.

In clinical situations, the existing diuretics thiazides and furosemide sometimes cause hypokalemia following consecutive administration.\textsuperscript{14} Thus, exploitation of the new type of diuretic with little effect on potassium excretion was expected. In rats, KW-3902 caused significant diuresis and natriuresis without causing increases in potassium excretion,\textsuperscript{51} suggesting that KW-3902 may be a useful diuretic with very little potassium excretion. In the present study in dogs, KW-3902 caused significant kaliuresis at the higher dose of 3 mg/kg (p.o.) in experiment A and significant kaliuresis from 1 mg/kg in experiment B. The reason for the difference in the kaliuretic action of KW-3902 between experiments A and B was uncertain. The increase in potassium excretion was mild compared with that in sodium excretion, since stable increases of Na/K were observed at doses from 1 to 100 mg/kg. These data suggest that KW-3902 has a sodium-selective diuretic action in dogs. The mild kaliuretic action of KW-3902 might be due to the increase in renal cyclic AMP, which could be induced \textit{via} suppression of the inhibitory effect of adenosine A\textsubscript{1} stimulation on adenylate cyclase. In fact, epinephrine is known to induce renal potassium excretion through increase in cyclic AMP.\textsuperscript{15}

In rats, TCM causes significant kaliuresis and hypokalemia following repeated administration, which is not the case with KW-3902.\textsuperscript{51} In the present study, however, TCM failed to exhibit kaliuretic action in dog. The reason for this difference is not clear at present.

The diuretic and natriuretic effects of KW-3902 at 100 mg/kg (p.o.) were less prominent than those at 10 mg/kg (p.o.). In rats, KW-3902 caused stable diuresis in the dose range from 0.01 to 10 mg/kg (p.o.), but this effect declined above 10 mg/kg (unpublished data). Thus, the diuretic pattern of KW-3902 in dogs is similar to that in rats. In conscious dogs, oral administration of KW-3902 at 100 mg/kg did not affect blood pressure (unpublished data) indicating that the reason for the decrease in diuresis at 100 mg/kg (p.o.) cannot be ascribed to the decrease in renal perfusion pressure due to hypotension. At its high dose, KW-3902 might have induced antidiuretic action by blockade of the adenosine A\textsubscript{2} receptor. Further study is needed to clarify the bell-shaped diuretic action of KW-3902.

The present study demonstrated that KW-3902 produces significant increases in urine volume and sodium excretion at doses greater than 1 mg/kg (p.o.) in saline-loaded dogs. Thus, the diuretic activity of KW-3902 in dogs was estimated to be 1/100 to 1/1000 that in rats\textsuperscript{49} when it was orally administered. On the other hand, the diuretic activity of KW-3902 in mice and guinea-pigs was estimated to be 1/10—1/100 that in rats (unpublished data). Therefore, it seems that the diuretic activity of KW-3902 differs among the species, the activity in dogs being the weakest in the species tested so far.

In fact, our unpublished observations indicate that the plasma level of KW-3902 in dogs is about 1/10 that in rats when comparing administration of the same doses per body weight. Although differences in pharmacokinetics can contribute to species differences in potency, this difference cannot entirely be ascribed to pharmacokinetics. The difference in the diuretic effects of KW-3902 among animal species may be due to the difference in the distribution of adenosine receptors in the kidney among the species. In dogs, the distribution of adenosine receptor on tubular sites, which are involved in the reabsorption of water and sodium, may be lower than in rats. Alternatively, the kidney in dogs may be less sensitive to adenosine than in rats. Further investigation is required to clarify the mechanism for the species differences exhibited by KW-3902.

Our recent studies have demonstrated that administration of KW-3902 ameliorates the severity of glycerol- or cisplatin-induced acute renal failure in rats.\textsuperscript{49} KW-3902 promotes uricosuric action in oxonic acid treated rats and, moreover, it exhibits antihypertensive effects in spontaneously hypertensive rats.\textsuperscript{51} These results in previous studies as well as the present one suggest that the adenosine A\textsubscript{1} receptor antagonist, KW-3902, possesses favorable properties for a diuretic agent.

In conclusion, the present study demonstrated that KW-3902, an adenosine A\textsubscript{1} receptor antagonist, causes significant diuresis and natriuresis with only a mild increase in potassium excretion in conscious dogs. The effects of KW-3902 lasted longer than those of TCM and furosemide at equipotent doses. Adenosine A\textsubscript{1} blockade offers a promising new approach to diuretic therapy.
References


