Effects of KW-3902, an Adenosine A₁-Receptor Antagonist, on Ascites Volume in Puromycin Aminonucleoside (PAN)-Induced Nephrotic Rats

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ABSTRACT—We investigated the effects of KW-3902 (8-(noradamantan-3-yl)-1,3-dipropylxanthine), a potent adenosine A₁-receptor antagonist, on the nephrotic edema induced by puromycin aminonucleoside (PAN; 100 mg/kg, i.v.) in rats. The treatment with PAN decreased urine volume and urinary excretions of sodium and potassium, resulting in the ascites formation in 7 days. In rats with the nephrosis, KW-3902 (0.01–1 mg/kg/day for 3 days, p.o.) showed diuretic effects and reduced the volume of ascites, as was the case with furosemide (30 mg/kg/day) and trichlormethiazide (1 mg/kg/day). These results suggest that even in the nephrotic state, the adenosine A₁-receptor antagonist can be an effective diuretic to ameliorate edema.

Keywords: KW-3902, Adenosine A₁-receptor antagonist, Diuretic effect

Edema and ascites are frequently associated with severe disturbances of systemic and renal hemodynamics (1). On the other hand, nephrotic syndrome manifests itself with massive proteinuria leading to edema and ascites (2). The administration of puromycin aminonucleoside (PAN) to rats results in the symptoms of proteinuria, hypoproteinemia, hypercholesterolemia, edema and ascites, resembling the changes observed in the nephrotic syndrome of humans. Thus, PAN-induced nephrosis can serve as a model of nephrotic edema (3).

KW-3902 (8-(noradamantan-3-yl)-1,3-dipropylxanthine), a potent adenosine A₁-receptor antagonist, shows diuretic and natriuretic effects in normal rats (4). Furthermore, KW-3902 induces diuretic and natriuretic effects even in rats with various types of acute renal failure (5, 6). The latter observations led us to the question of whether KW-3902 can also induce diuresis in the nephrotic state, resulting in the amelioration of edema. To address this issue, we employed a rat model of PAN-induced nephrosis and investigated whether or not KW-3902 shows the diuretic effect and ameliorates the formation of ascites.

Male Wistar rats (Shizuoka Laboratory Animal Center, Inc., Hamamatsu), weighing 210–260 g, were used in the present study. The animals were kept at 22°C and on a 12-hr light-dark cycle. They had free access to tap water and commercial chow. KW-3902 was synthesized at Sakai

Research Laboratories, Kyowa Hakko Kogyo Co., Ltd. PAN and trichlormethiazide (TCM) were purchased from Sigma Chemical Co., Ltd. (St. Louis, MO, USA). Furosemide was purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo). All other chemicals and solvents were used in their analytical pure form.

In order to elicit nephrotic edema in rats, each animal was injected with PAN (100 mg/kg, i.v.). Rats injected with saline (1 ml/kg, i.v.) served as the normal rats. Seven days after the injection of PAN, blood was collected from the tail vein, and the concentration of serum albumin was determined with an autoanalyzer (AU-510; Olympus, Tokyo). PAN-treated rats were divided into 7 groups, each consisting of 7 rats, so as to make the mean concentration of serum albumin almost equal in each group. KW-3902 (0.001, 0.01, 0.1 and 1 mg/kg), furosemide (30 mg/kg), TCM (1 mg/kg) or saline (5 ml/kg) was orally administered once a day to PAN-treated rats for 3 days. Saline (5 ml/kg) was also administered to the normal rats (without PAN treatment). The doses of furosemide and TCM examined are the ones comparable to those of KW-3902 (0.01–1 mg/kg) that induce diuresis and natriuresis following a single oral administration in normal rats (4, 6). At day 4, saline containing a drug was orally administered to PAN-treated rats in a volume 25 ml/kg, and urine was collected for 6 hr. Saline in the same volume was administered to the normal rats and the PAN-treated rats. After the collection of urine, ascites

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Table 1. Diuretic effects of KW-3902, furosemide and trichlormethiazide in rats with puromycin aminonucleoside-induced nephrosis

	Dose (mg/kg)	n	Urine volume (ml/kg/6 hr)	Na Excretion (mEq/kg/6 hr)	K Excretion (mEq/kg/6 hr)
Normal	—	4	24.5 ± 0.8**	3.43 ± 0.36***	2.34 ± 0.19***
Control	—	7	6.3 ± 1.1	0.37 ± 0.12	0.92 ± 0.15
KW-3902	0.001	7	7.5 ± 0.9	0.46 ± 0.07	0.97 ± 0.13
	0.01	7	19.3 ± 0.6**	1.22 ± 0.07**	0.43 ± 0.04*
	0.1	7	19.7 ± 0.6**	1.16 ± 0.05**	0.31 ± 0.03**
	1	7	17.4 ± 1.0**	0.99 ± 0.06**	0.35 ± 0.06*
	30	7	23.3 ± 1.6***	1.91 ± 0.28***	1.61 ± 0.16***
Furosemide	30	7	23.3 ± 1.6***	1.91 ± 0.28***	1.61 ± 0.16***
Trichlormethiazide	1	7	17.2 ± 2.2***	1.53 ± 0.22***	1.73 ± 0.20***

Values represent means ± S.E. n: Number of the animals examined. *P < 0.05, **P < 0.01, ***P < 0.001, when compared with the control value.

volume was determined with a slight modification of the previously reported method (3). The rat was anesthetized with ether, and following laparotomy, the ascites fluid was collected by using absorbent cotton. The ascites was weighed and presented as ml/kg body weight. Thereafter, blood was collected from the abdominal aorta and centrifuged (3000 rpm, 10 min, 4°C) to obtain the serum. Concentrations of sodium and potassium in the urine and serum were determined by flame photometry (775-A; Hitachi, Ltd., Tokyo), and excretions of sodium and potassium were calculated.

All results are given as means ± S.E. To define statistically significant differences among the groups, the data were subjected to the Dunnett's test or to Student's *t*-test or the Aspin-Welch test following the *F*-test. A *P*-value of less than 0.05 was considered to be statistically significant.

Table 1 shows the effects of KW-3902, furosemide and TCM on urine volume, and excretions of sodium and

potassium in rats with PAN-induced nephrosis. In the PAN-treated rats, urine volume and excretions of sodium and potassium were markedly decreased as compared with those in normal rats. The depressed diuresis seems to be due to the renal glomerular lesion and proteinuria induced by PAN (3). KW-3902 at doses from 0.01 to 1 mg/kg/day (p.o.) significantly increased urine volume and sodium excretion in rats with PAN-induced nephrosis. Furosemide and TCM significantly increased the urine volume, sodium excretion and potassium excretion.

Table 2 shows the effects of KW-3902, furosemide and TCM on ascites volume, and serum concentrations of sodium and potassium in rats with PAN-induced nephrosis. Compared with normal rats, the rats with PAN-induced nephrosis exhibited increased ascites volume and high serum concentrations of sodium. KW-3902 at doses from 0.01 to 1 mg/kg/day (p.o.) significantly ameliorated the ascites, as was the case with furosemide (30 mg/kg/day) and TCM (1 mg/kg/day). None of the compounds had

Table 2. Effects of KW-3902, furosemide and trichlormethiazide on ascites volume, serum electrolytes in rats with puromycin aminonucleoside-induced nephrosis

	Dose (mg/kg)	n	Ascites volume (ml/kg)	Serum Na (mEq/l)	Serum K (mEq/l)
Normal	—	4	1.2 ± 0.05***	145.1 ± 0.20***	4.79 ± 0.06
Control	—	7	15.0 ± 1.35	148.5 ± 0.23	5.10 ± 0.21
KW-3902	0.001	7	12.8 ± 0.87	148.2 ± 0.39	5.23 ± 0.28
	0.01	7	3.9 ± 0.90**	148.0 ± 0.39	4.89 ± 0.29
	0.1	7	3.2 ± 0.42**	148.0 ± 0.31	4.97 ± 0.25
	1	7	4.3 ± 0.60**	148.0 ± 0.23	4.99 ± 0.26
	30	7	3.8 ± 0.65***	147.5 ± 0.23	4.99 ± 0.25
Furosemide	30	7	3.8 ± 0.65***	147.5 ± 0.23	4.99 ± 0.25
Trichlormethiazide	1	7	2.8 ± 0.84***	147.4 ± 0.58	5.11 ± 0.41

Values represent means ± S.E. n: Number of the animals examined. **P < 0.01, ***P < 0.001, when compared with the control value.

any significant influence on the concentrations of sodium and potassium in the serum, as compared with those in the PAN-treated rats.

The present study revealed that KW-3902, like furosemide and TCM, causes diuresis and natriuresis in the PAN-induced nephrotic rats. This observation is similar to the result of Ma et al. who showed the diuretic effect of the adenosine A_1 -receptor antagonist in the PAN-induced nephrotic rat (7). In fact, KW-3902 produces diuretic and natriuretic effects in various models of acute renal failure (ARF) more prominently than furosemide and TCM (5, 6). These observations suggest that KW-3902 can be an effective diuretic regimen not only in the normal state (4) but also in the renal disease state, including some types of acute renal failure (5, 6) and nephrosis (this study).

The PAN-induced ascites was prominently ameliorated by KW-3902 as well as by furosemide and TCM. As far as we know, this is the first demonstration that, employing the PAN-induced nephrotic rat, the diuretic agent can effectively ameliorate the ascites following nephrosis. The medical treatment of ascites with the nephrotic syndrome is aimed at creating a negative sodium balance to induce the translocation of body fluid from the peritoneal cavity to the intravascular space. Treatment of the ascites in nephrosis has thus been based on a low-sodium diet, water restriction and the treatment with loop diuretics (8, 9). The present result suggests that KW-3902 is comparable in efficacy to furosemide or TCM for the treatment of the patients with ascites following the nephrotic syndrome.

The treatment with KW-3902 significantly decreased potassium excretion in rats with PAN-induced nephrosis, although the serum concentration of potassium did not change. The reason for the decrease by KW-3902 of potassium excretion is unclear. In normal (4) and various acute renal failure rats (5, 6), KW-3902 causes diuresis and natriuresis with little change of potassium excretion. In contrast, furosemide and TCM sometimes cause urinary potassium excretion (5, 6, 10). It is thus probable that KW-3902 decreased the excretion of potassium at tubular sites in rats with PAN-induced nephrosis. The decrease of potassium excretion by KW-3902 might be due to the increase in tubular cAMP, which could be induced via suppression of the inhibitory effect of adenosine A_1 -receptor stimulation on adenylate cyclase (11). In fact, epinephrine is known to inhibit renal potassium secretion presumably via the elevated tubular cAMP (12). Further studies, however, are required to clarify the mechanism and the clinical implications of the decrease of urinary potassium excretion by KW-3902 in the PAN-induced nephrotic rat.

The present study demonstrated that the adenosine A_1 -receptor antagonist induces a natriuretic effect and

ameliorates ascites formation in rats with established PAN-induced nephrosis. On the other hand, the prophylactic treatment with dipyridamole, an inhibitor of facilitated carrier-mediated adenosine transport, was effective in preventing the development of proteinuria in PAN-induced nephrotic rats (13). This protective effect of dipyridamole seems to, at least partly, be due to the increased cAMP production in the glomerular cell, caused by the increased adenosine, via adenosine A_2 -receptor stimulation. Likewise, the adenosine A_1 -receptor antagonist can also increase cAMP production (14), possibly resulting in the prevention of PAN-induced nephrosis development. Further studies are required to determine whether the prophylactic treatment with KW-3902 protects against PAN-induced nephrosis.

In summary, the present study demonstrates that in saline-loaded rats with PAN-induced nephrosis, KW-3902, as well as furosemide and TCM, induces diuretic and natriuretic effects, resulting in the ameliorated ascites volume. These results suggest that the adenosine A_1 -receptor antagonist may be useful for the treatment of nephrotic patients with edema.

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