Protective Effects of KW-3902, a Novel Adenosine A1-Receptor Antagonist, against Gentamicin-Induced Acute Renal Failure in Rats

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ABSTRACT—We investigated the possible renal protective effects of KW-3902 (8-(noradamantan-3-yl)-1,3-dipropylxanthine), an adenosine A1-receptor antagonist, against gentamicin (GM)-induced acute renal failure (ARF) in rats. ARF was induced by subcutaneous injection of GM at 80 mg/kg/day for 12 days. KW-3902 (0.001–0.1 mg/kg, p.o., twice daily) attenuated the increases of serum creatinine and urea nitrogen and the decrease of creatinine clearance in rats treated with GM. In contrast, furosemide and trichlormethiazide aggravated the GM-induced nephrotoxicity. These results suggest that KW-3902 can ameliorate the GM-induced ARF and that endogenous adenosine may be involved in GM-induced ARF via the adenosine A1-receptor.

Keywords: KW-3902, Adenosine A1-receptor antagonist, Acute renal failure

Gentamicin (GM) is an aminoglycoside antibiotic widely used for the treatment of Gram-negative infections. Unfortunately, the clinical usefulness of this drug is limited due to the development of GM-induced nephrotoxicity (1). The pathogenesis of aminoglycoside nephrotoxicity can be viewed as a two-step process (2). The first step involves the transport and accumulation of the antibiotic in high concentration by renal proximal tubular cells. The second step involves the adverse interaction between the polycationic drug with one or more intracellular processes. KW-3902 (8-(noradamantan-3-yl)-1,3-dipropylxanthine) is a newly synthesized and selective adenosine A1-receptor antagonist, which is the most potent one reported to date (3). In saline-loaded normal rats, blockade of the adenosine A1-receptor with KW-3902 induces significant increases of urine volume and sodium excretion with little change of potassium excretion (4). Additionally, we previously reported that KW-3902 possesses renal protective effects against glycerol-, cisplatin- or cephaloridine-induced acute renal failure (ARF) (4–6). In the present study, we studied possible renal protective effects of KW-3902 against GM-induced ARF in rats and compared them with those of furosemide and trichlormethiazide (TCM).

Male Wistar rats (Japan Shizuoka Laboratory Animal Center, Inc., Hamamatsu), weighing 170–200 g, were used in the present study. 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 Laboratories, Kyowa Hakko Kogyo Co., Ltd. The animals were kept at 22°C and in 12-hr light-dark cycle. They had free access to tap water and commercial chow. KW-3902 and furosemide were synthesized in our laboratories. TCM and gentamicin sulfate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and solvents were used in their analytical pure form. For oral administration, KW-3902 (0.001, 0.01 or 0.1 mg/kg), furosemide (30 mg/kg) and TCM (1 mg/kg) were suspended in saline containing 5% arabic gum. The doses of furosemide and TCM used were equivalent to 0.001–0.1 mg/kg of KW-3902 in inducing the diuretic effect in saline-loaded rats (4, 6).

The GM-induced ARF was produced according to the previous method (7). GM was subcutaneously injected to rats at 80 mg/kg/day for 12 days. Corresponding normal rats were injected with an equal volume of saline (1 ml/kg). In the separate groups of rats, the test drugs were administered twice daily (1 hr before and 4 hr after each GM injection). On the 13th day from the initial injection of GM, the rats, which had been starved overnight but allowed free access to water, were orally dosed with saline (25 ml/kg). After the administration, the rats were
housed individually in metabolic cages without food or water. Urine was collected for 4 hr and its volume was measured. Urinary excretions of creatinine (U-CRE), protein (U-TP) and glucose (U-GLU), and urinary lactate dehydrogenase activity (U-LDH) were measured with an autoanalyzer (AU510; Olympus, Tokyo). Soon after obtaining the urine, blood was collected from the abdominal descending aorta under ether anesthesia, and the serum was separated. Serum creatinine (S-CRE) and urea nitrogen (S-UN) were measured with the autoanalyzer. As an index of the glomerular filtration rate (GFR), creatinine clearance (C_{CRE}) was calculated by the standard formula as follows:

\[
C_{CRE} (\text{l/kg/4 hr}) = \frac{U-CRE (\text{mg/dl}) \times \text{Urinary volume (ml/kg/4 hr)}}{S-CRE (\text{mg/dl}) \times 1000}
\]

In addition, the kidney fixed with formalin, sectioned and stained with hematoxylin/eosin was observed under light microscopy, as described previously (6).

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

No rats in the normal, control or the KW-3902-treated groups died during the experimental period, whereas 5 of 7 rats in the furosemide-treated group and 3 of 7 rats in the TCM-treated group died from the 7th day to the 13th day of GM injections. In the survivors of these groups, S-UN increased to 227.0-572.3 mg/dl, indicating more severe ARF than that of the control group. Thus, on the 13th day, we collected urine from the normal, control and the KW-3902-treated groups and examined the effects of KW-3902 against GM-induced ARF.

Table 1 shows the effects of KW-3902 on GM-induced ARF. Subcutaneous consecutive administration of GM for 12 days significantly inhibited the gain of body weight and doubled urine volume as compared with that in normal rats. Moreover, S-CRE, S-UN, U-GLU, U-TP and U-LDH significantly increased and C_{CRE} decreased following the administration of GM. These data indicate that the injection of GM produced nephrotoxicity in rats. KW-3902 did not significantly influence the reduced body weight. However, KW-3902 significantly prevented the increases of S-CRE and S-UN. Moreover, KW-3902 at 0.01 and 0.1 mg/kg significantly improved the depressed C_{CRE}.

In the urinary parameters, KW-3902 significantly prevented the increase of U-GLU, although this drug did not attenuate the increased U-TP. KW-3902 at 0.1 mg/kg significantly prevented the increase of U-LDH.

Figure 1 shows the light micrographs of the kidney from the rat given GM alone or GM and KW-3902 (0.1 mg/kg) on the 13th day. As shown in Fig. 1A, the treatment with GM alone induced severe tubular cell necrosis, cast formation and exposure of the tubular basement membrane. On the other hand, the kidney from the KW-3902-treated rat showed a reduced degree of renal lesions (Fig. 1B). These results demonstrate that KW-3902 significantly attenuates the nephrotoxicity of GM in rats. However, the protective effects of KW-3902 (0.001–0.1 mg/kg) were not dose-dependent. This observation is in accordance with the previous finding that KW-3902 in a dose-range from 0.001 to 0.1 mg/kg induces similar diuretic and natriuretic effects in saline-loaded rats (4).

In the present study, furosemide and TCM increased the severity of renal dysfunction in GM-treated rats. Furosemide is reported to enhance GM nephrotoxicity because this drug directly accelerates GM accumulation in renal tissues (8). The increased renal damage by furosemide or TCM has also been observed in rats treated with nephrotoxic agents such as cephaloridine (6) and glycerol (9). However, the mechanism underlying the enhancement of nephrotoxic action by these drugs has not fully been understood. Recently, we found that KW-3902, but not furosemide or TCM, has a diuretic effect even in the established ARF induced by GM (10). It is therefore likely that KW-3902 has some advantages over furosemide or TCM when used under the treatment with

### Table 1. Effects of KW-3902 on gentamicin-induced acute renal failure in rats

| Drugs  | Dose (mg/kg, p.o.) | n  | Body weight (g)  | Urine volume (ml/kg/4 hr) | Serum creatinine (mg/dl) | Serum urea nitrogen (mg/dl) | Creatinine clearance (l/kg/4 hr) | Urinary glucose (mg/kg/4 hr) | Urinary protein (mg/kg/4 hr) | Urinary LDH (mg/kg/4 hr) |
|--------|-------------------|----|-----------------|--------------------------|------------------------|---------------------------|---------------------------------|-----------------------------|-----------------------------|--------------------------|
| Normal |                   | 7  | 226±3**         | 19.7±1.1**               | 0.52±0.01**            | 15.74±0.51**              | 1.00±0.04**                     | 0.18±0.07**                  | 28.6±3.8**                  | 0.24±0.04**              |
| Control|                   | 8  | 195±3           | 40.3±1.4                 | 1.19±0.09              | 36.13±3.25                | 0.66±0.06                      | 231.62±34.87                | 80.8±13.1                   | 8.06±0.65                |
| KW-3902| 0.001             | 8  | 202±2           | 31.1±1.9*                | 0.72±0.04**            | 20.14±1.85**              | 0.91±0.07                      | 43.77±14.07                | 59.7±2.9                   | 5.56±0.64                |
|       | 0.01              | 8  | 202±4           | 27.8±2.0**               | 0.71±0.04**            | 19.94±1.36**              | 1.06±0.12**                    | 35.04±10.35                | 74.6±9.4                    | 5.70±0.90                |
|       | 0.1               | 8  | 196±3           | 33.8±2.0                 | 0.72±0.08**            | 20.48±1.80**              | 0.98±0.07                      | 22.94±7.62                  | 72.6±6.1                    | 4.98±0.68*                |

Each value is the mean±S.E. Urinary LDH=urinary lactate dehydrogenase activity. **:*: Significantly different from the control at P<0.05 and 0.01, respectively.
Recently, adenosine, acting on the adenosine $A_1$-receptor, has been suggested to be a mediator in a variety of ARFs (4–6, 11, 12). In the glycerol-induced ARF, adenosine is assumed to constrict the afferent arterioles, leading to the depressed $C_{\text{CRE}}$, since the outflow of adenosine from the renal vein is increased after the glycerol-injection (12). In contrast, it was reported that the nonselective adenosine antagonist 8-phenyltheophylline produced little improvement in GM-induced ARF rats, suggesting that adenosine plays little, if any, pathophysiological role in this type of ARF (13). In the present study, we demonstrated that the selective $A_1$-receptor antagonist KW-3902 was effective in ameliorating some of the biochemical,
functional and morphological correlates of GM-induced ARF in rats. The protective effect of KW-3902 against GM-induced ARF is likely to be due to selective blockade of the adenosine A<sub>1</sub>-receptor. Thus, KW-3902 might have improved the C<sub>CRE</sub> via the blockade of the adenosine A<sub>1</sub>-receptor, the stimulation of which is known to constrict the afferent arterioles (14). The ineffectiveness of the non-selective adenosine antagonist (13) might be due to the inhibition of the adenosine A<sub>2</sub>-receptor mediated vasodilating effect of adenosine (14).

The diuretic action of KW-3902 might also contribute to the amelioration of GM-induced ARF. KW-3902 is known to induce diuretic action via the inhibition of sodium and water mainly at the proximal tubule (15) and, therefore, is expected to dilute the secreted GM in the proximal tubule. The decreased concentration of GM in the proximal tubule could result in the attenuated nephrotoxicity of GM, which is known to mainly injure the proximal tubule (1). In the present study, however, KW-3902 did not completely prevent GM-induced ARF (e.g., the increase of U-TP), suggesting that a factor other than adenosine is also involved in the pathogenesis of GM-induced nephrotoxicity. The exact mechanism of the protective effect of KW-3902 and the precise role of adenosine in GM-induced ARF remain unclear, and further investigations are necessary.

In conclusion, the present study demonstrates that the adenosine A<sub>1</sub> blockade by KW-3902 can ameliorate the GM-induced ARF in rats. Endogenous adenosine may be involved in GM-induced ARF via adenosine A<sub>1</sub>-receptors.

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