Effects of KW-3902, a Novel Adenosine $A_1$-Receptor Antagonist, on Cephaloridine-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), a selective and potent adenosine $A_1$-receptor antagonist, against cephaloridine (CER)-induced acute renal failure (ARF) in rats. ARF was induced by intravenous injection of CER at a dose of 600 mg/kg body weight. KW-3902 at doses higher than 0.01 mg/kg (p.o.) dose-dependently attenuated the decrease of creatinine clearance and the increase of proteinuria in rats with CER-induced ARF. In contrast, furosemide and trichlormethiazide (TCM) increased urinary protein and aggravated the serum parameters. These results suggest that KW-3902 has some advantages over furosemide and TCM when used in combination with CER. In the diuretic study in the rats with established ARF induced by CER, KW-3902, furosemide and TCM caused a significant increase in sodium excretion, whereas acetazolamide was ineffective. These results suggest that the proximal tubule is functionally damaged in rats with CER-induced ARF, in accord with the histological observation demonstrating the degeneration of the proximal tubule. From the fact that KW-3902 induces diuretic action even in CER-induced ARF, it is suggested that KW-3902 acts, directly or indirectly, on the proximal tubule or other tubular sites in the kidney, resulting in the diuretic effect.

Keywords: KW-3902, Adenosine $A_1$-receptor antagonist, Diuretic effect, Renal protective effect

Cephaloridine (CER), one of the cephalosporins, has been successfully used for the treatment of a wide variety of bacterial infections (1). However, use of an excessive dosage of CER produces renal injury in humans as well as in laboratory animals (2–4). The main toxic effect of CER has been ascribed to the action of this drug on the proximal tubule (5, 6). Suppression of the renal organic anion transport system, reduced glomerular filtration rates, glucosuria, proteinuria, and other effects were observed following the administration of CER (7–9). Histopathological studies (5, 6, 10) demonstrated nuclear pyknosis, cytoplasmic disintegration, hydropic changes and necrosis in the proximal tubular cells and patchy loss of the brushborder membranes in the proximal tubule. Several mechanisms for the CER-induced nephrotoxicity have been proposed. For example, lipid peroxidation ascribable to oxygen free radicals produced in the kidney has been proposed to be the cause of CER-induced nephrotoxicity (4, 11–14).

The newly synthesized compound KW-3902 (8-(noradamantan-3-yl)-1,3-dipropylxanthine) is selective and the most potent adenosine $A_1$-receptor antagonist reported to date (15). In the receptor-binding study, the dissociation constant values of KW-3902 for adenosine $A_1$-receptor and $A_2$-receptor are 1.1 nM and 330 nM, respectively (15, 16). In anesthetized rats, KW-3902 antagonizes the 5'-N-ethylcarboxamidoadenosine (NECA)-induced bradycardiac response mediated via adenosine $A_1$-receptors, with little influence on the NECA-induced hypotensive response mediated via adenosine $A_2$-receptors (17). Blockade of the adenosine $A_1$-receptor with KW-3902 induces significant increases of urine volume and sodium excretion with little change of potassium excretion in saline-loaded rats (17, 18). From the results of the Li clearance study and stop-flow method, KW-3902 is thought to act mainly on the proximal tubule, resulting in diuresis and natriuresis (18). Additionally, we previously reported that KW-3902 possesses renal protective effects against glycerol- or cisplatin-induced acute renal failure (ARF) (17, 19). Thus, it is assumed that the diuretic and the renal protective effects of KW-3902 are due to the blockade of adenosine $A_1$-receptors.
In the present study, we studied possible renal protective effects of KW-3902 against CER-induced ARF in comparison with those of furosemide and trichlormethiazide (TCM). Moreover, the effect of KW-3902 on urinary volume and electrolyte excretion were determined in rats with established CER-induced ARF.

MATERIALS AND METHODS

Experimental animals

Male Wistar rats, weighing 200–250 g (Japan Shizuoka Laboratory Animal Center, Inc., Hamamatsu), were used in the present study. The animals were kept at 22°C under a 12 hr light-dark cycle. They had free access to tap water and commercial chow.

CER-induced ARF

CER-induced ARF was produced according to the previous method (20). In brief, the rats were divided into 6 groups: the normal group (5 rats) and 5 groups of CER-induced ARF (12 rats in each group). The rats in each CER-induced ARF group received a single injection of CER in saline solution (4 ml/kg body weight) through the tail vein, at doses of 200, 400, 500, 600 and 800 mg/kg body weight on day 0. The normal rat received an equivalent volume of saline. At the 7th day after the CER administration, body weight was measured. Soon after the measurement, blood was collected from the abdominal aorta under ether anesthesia, and the serum was obtained by centrifugation (3000 rpm, 10 min, 4°C) (18). As indexes of renal failure, serum creatinine (S-CRE) and urea nitrogen (S-UN) were measured with an autoanalyzer (AU510; Olympus, Tokyo). It is reported that both S-UN and urinary glucose (U-GLU) begin to rise at the 1st day, reaching the maximal level on the 3rd day, with the values being 10 times higher than the control level, and are still higher until the 7th day compared with the normal level (20). Based on this previous observation, the period for this experiment was set up.

Histological examination

At the end of the experiment, the left kidney was removed of adhering connective and fat tissues. Coronal slices of the kidney from the control and CER-treated rats were fixed in 10% buffered formalin (pH 7.25). Tranverse kidney slices were embedded in paraffin for light microscopy, sectioned at 4-μm thickness by a microtome and stained with hematoxylin/eosin.

Protective effects against CER-induced ARF

Rats were divided into the normal group, and the 7 groups of CER-induced ARF (7 or 8 rats in each group), i.e., one group of repeated oral administration of vehicle at 2 ml/kg (control group), the four groups of KW-3902 (0.01, 0.1, 1 and 10 mg/kg/day) and the two groups of furosemide (30 mg/kg/day) or TCM (1 mg/kg/day). The doses of furosemide and TCM used were equivalent to that of 1 mg/kg of KW-3902 in inducing the diuretic effect. Drugs were suspended in distilled water containing 5% arabic gum. Rats were fasted for 18 hr, and ARF was produced by injections of CER in saline solution (600 mg/kg body weight) through the tail vein at day 0. One hour before and 4 hr after the injection of CER, vehicle or drug suspension was orally administered to rats at a volume of 5 ml/kg body weight, followed by the oral administrations once a day from 1st day to 6th day.

Rats were fasted for 18 hr after the last administration. At the 7th day, rats were treated with saline at a volume of 20 ml/kg and individually placed in metabolic cages without food or water. Urine was collected for 4 hr and its volume was measured. Urinary excretion of creatinine (U-CRE), U-GLU and urinary protein (U-TP) were measured with the autoanalyzer. Soon after the urine collection, blood was collected from the abdominal aorta under ether anesthesia, and the serum was obtained by centrifugation (3000 rpm, 10 min, 4°C) to measure S-CRE and S-UN. As an index of glomerular filtration rate (GFR), creatinine clearance (CL.CRE) was calculated by the following standard formula:

$$\text{CL.CRE(l/kg/4 hr)} = \frac{\text{UV(l/kg/4 hr)} \times \text{U-CRE(mg/ml)}}{\text{S-CRE (mg/ml)}}$$

Tubular reabsorption rate of water (ABS.H2O) was calculated as an index of kidney function by the following formula:

$$\text{ABS.H2O(%) = } \frac{\text{CL.CRE(l/kg/4 hr)} - \text{UV(l/kg/4 hr)}}{\text{CL.CRE(l/kg/4 hr)}} \times 100$$

The value of U-TP (and U-GLU) was used as an index of proteinuria (and glucosuria). At the end of the experiment, the effects of KW-3902, furosemide and trichloromethiazide on the damage of the kidney were also determined by histopathology as described above.

Diuretic effects in normal rats

Diuretic effects of drugs in normal rats (6 rats in each group) were examined with a slight modification of the previous method (21). In brief, rats were fasted for 18 hr, and the drug suspension or vehicle was orally administered to the rat at a volume of 20 ml/kg. KW-3902 (1 mg/kg), furosemide (30 mg/kg), TCM (1 mg/kg), amiloride (25 mg/kg) and acetazolamide (6.3 mg/kg) were suspended in saline containing 0.5% arabic gum. After the administration, the rat was individually placed in a metabolic cage without food and water. Urine was collected for 4 hr and its volume was measured. Concentrations of sodium and potassium were measured by flame photometry (775-A; Hitachi Ltd., Tokyo), and sodium and
Diuretic effects in rats with CER-induced ARF

Rats were intravenously injected with 600 mg/kg of CER in saline solution through the tail vein at day 0. The normal rat received an equivalent volume of saline. At the 3rd day, the rats were fasted for 18 hr, and saline at a volume of 20 ml/kg was orally administered to the rat. After the administration, the rats were individually placed in metabolic cages without food or water. Urine was collected for 4 hr, and U-CRE was measured with the autoanalyzer. From the obtained value of U-CRE, the rats with CER-induced ARF were divided into 6 groups (6 or 7 rats in each group) that showed almost the same U-CRE values. At the 7th day, diuretic effects were determined by the same method as described above. Soon after the urine collection, blood was collected from the abdominal aorta under ether anesthesia, and S-UN was measured to confirm homogenous degree of ARF in each group.

Drugs

KW-3902 (8-(noradamantan-3-yl)-1,3-dipropylxanthine) was synthesized in our laboratories. Furosemide was purchased from Tokyo Kasei Co., Ltd. (Tokyo); trichloromethiazide (TCM) and amiloride, from Sigma Chemical Co. (St. Louis, MO, USA) and acetazolamide, from Aldrich Chemical Co., Ltd. (Milwaukee, WI, USA). Cephaloridine (Keflodin®) was purchased from Shionogi Co., Ltd. (Osaka). All other chemicals and solvents were used in their analytical pure form.

Statistical analyses

All the results are given as the mean±S.E. To define statistically significant differences among the groups for the protective effects, the data were subjected to Student’s t-test or the analysis of variance (ANOVA) followed by Steel’s test or Dunnett’s test (22). The data for the diuretic effect was analyzed using Student’s t-test. A P value of less than 0.05 was considered statistically significant.

RESULTS

CER-induced ARF

Table 1 shows the changes of body weight, S-CRE and S-UN at the 7th day following single intravenous injections of CER at doses from 200 to 800 mg/kg body weight. All the parameters in the 200 and 400 mg/kg groups showed no significantly different changes in comparison with those in the normal group. In the 500 and 600 mg/kg groups, the body weight decreased and S-CRE and S-UN significantly increased, with the values being 2–4 times higher than the normal level. In the 800 mg/kg group, most of the rats (11/12) died at the 7th day. Therefore, the single injection of CER at a dose of 600 mg/kg body weight was thought to be appropriate for studying the drug effect on CER-induced ARF, and this dose (600 mg/kg) was used for the further examinations.

In the histological examination, all the tubular structures, the cortex and the proximal tubule were evidently affected in the kidney of CER-induced ARF rats compared with that in normal rats. Especially, necrosis and regeneration of proximal tubular epithelium, tubular dilation, protein cast and delayed lumen in the cortical tubules were observed. Moreover, the nuclei seen in the lumen of proximal tubules disappeared. However, glomerulous medulla and papilla were histologically intact in the kidney of CER-induced ARF rats. Figure 1 (A and B) shows typical patterns of the kidney from normal and CER-induced ARF rats, respectively.

Protective effects against CER-induced ARF

Figure 1C shows a typical microphotograph of the kidney on the 7th day in CER-induced ARF rats treated with KW-3902 (1 mg/kg). KW-3902 attenuated the damage of

Table 1. Effects of cephaloridine on the change in body weight, serum creatinine (S-CRE) and serum urea nitrogen (S-UN) in rats

|               | Normal     | 200        | 400        | 500        | 600        | 800 (mg/kg) |
|---------------|------------|------------|------------|------------|------------|-------------|
| ΔBody weights (g) | 8.0±1.2    | 4.2±1.8    | 5.6±1.4    | -6.7±2.1*  | -5.4±1.6*  | -30         |
| S-CRE (mg/dl)   | 0.56±0.02  | 0.58±0.03  | 0.66±0.02  | 1.24±0.32* | 2.04±0.61* | 5.83        |
| S-UN (mg/dl)    | 20.5±1.3   | 19.6±2.4   | 21.8±2.8   | 42.6±15.2  | 78.0±26.9  | 252.4       |
| Mortality      | 0/12       | 0/12       | 0/12       | 0/5        | 0/5        | 11/12*      |

Each parameter was measured at the 7th day after administration of cephaloridine. Each value is the mean±S.E. of 5–12 animals. ΔBody weight means: (body weight at day 7) minus (body weight at day 0). The initial body weight was 28±1.4 g, and the value in each group was not statistically different among each other. Mortality means: number of deaths/number of treated. *: Significantly different from the value in normal rats at P<0.05 (Steel’s test). #: Significantly different from the value in normal rats (χ²-test).
Fig. 1. Light microphotographs of the kidney from normal (A), control (B) and KW-3902 (1 mg/kg, p.o.) (C) treated rats with CER-induced ARF on the 7th day. The rats in the ARF group received a single injection of CER at 600 mg/kg through the tail vein at day 0. The normal rat received a saline solution (4 ml/kg body weight). A – C: magnification (× 100).
the proximal tubule.

Figure 2 shows the effect of CER on the glomerular (CL.CRE) and tubular function (ABS.H2O) and the effects of the drugs on CER-induced ARF. After the injection of CER, CL.CRE in the CER-induced ARF group markedly decreased to 0.58 l/kg/4 hr, from 1.17 l/kg/4 hr in the normal group. KW-3902 at 1 and 10 mg/kg (p.o.) significantly improved the depressed CL.CRE. On the other hand, furosemide and TCM aggravated the decreases of CL.CRE and ABS.H2O.

The effects of KW-3902, furosemide and TCM, on the serum parameters in rats with CER-induced ARF are shown in Fig. 3. S-CRE and S-UN (1.06±0.14 and 32.2±5.18 mg/dl, respectively) in the control group were significantly higher in comparison with those (0.54±0.02 and 19.0±0.48 mg/dl) in normal rats. KW-3902 tended to prevent the increases of S-CRE and S-UN, whereas furosemide and TCM markedly aggravated the increases of these serum parameters (furosemide: 3.74±1.22 and 180.6±54.9 mg/dl; TCM: 6.12±2.18 and 254.4±88.7 mg/dl), the values being significantly different as compared with those in the control group.

Effects of the drugs on the proteinuria and glucosuria occurring in rats with CER-induced ARF are shown in Fig. 4. Proteinuria (U-TP, 0.036±0.006 g/kg/4 hr) in the control group was significantly higher in comparison with that (0.021±0.002 g/kg/4 hr) in normal rats. KW-3902 at doses higher than 0.01 mg/kg (p.o.) induced dose-dependent decreases of proteinuria. In contrast, furosemide and TCM induced marked increases of U-TP (furosemide: 0.039±0.05 g/kg/4 hr, TCM: 0.021±0.005 g/kg/4 hr). The drug effects on the glucosuria (U-GLU) were similar to those on the proteinuria.
Diuretic effects in normal rats

Furosemide (30 mg/kg, p.o.), TCM (1 mg/kg, p.o.) and acetazolamide (6.3 mg/kg, p.o.) caused significant increases of urine volume and sodium excretion. Amiloride, a diuretic acting on the distal tubule, caused a decrease in potassium excretion in addition to the increases of urine volume and sodium excretion. KW-3902 at the dose of 1 mg/kg (p.o.) caused diuresis and natriuresis without affecting potassium excretion (Fig. 5). The urine volume and sodium excretion in each drug-treated group were significantly and almost equally increased in comparison with those in the control group.

Diuretic effects in rats with CER-induced ARF

ARF was induced with a single injection of CER at a dose of 600 mg/kg body weight. To study the diuretic effect of each drug, rats were divided into 6 groups on the basis of the value of U-CRE at the 3rd day. In CER-induced ARF rats, U-CRE at the 3rd day decreased to 4.17±0.54 mg/kg/4 hr from 10.69±0.67 mg/kg/4 hr in the normal group. S-UN and S-CRE at the 7th day were significantly increased to 201.3±48.1 mg/dl and 6.83±1.64 mg/kg/4 hr, from 19.0±0.48 mg/dl and 0.55±0.02 mg/dl, respectively, in the normal group. The values of U-CRE at the 3rd day and S-UN at the 7th day were not different among all the groups for the diuretic study.

In the diuretic study in the ARF rat, only furosemide significantly induced the increase in urine volume as compared with the control group (Fig. 6). Whereas furosemide, TCM and KW-3902 caused significant increase of
sodium excretion, amiloride decreased the potassium excretion as compared with the control group. However, acetazolamide did not induce any diuretic effect at a dose of 6.3 mg/kg, which induced a diuretic effect in normal rats. In this series of experiments, KW-3902 did not affect endogenous creatinine clearance (0.37 ± 0.08 l/kg/4 hr as compared with 0.43 ± 0.10 l/kg/4 hr in the control group), suggesting that the diuretic effect of KW-3902 is ascribed to its tubular action. Furosemide and TCM also did not significantly change the creatinine clearance (0.40 ± 0.10 and 0.33 ± 0.10 l/kg/4 hr).

**DISCUSSION**

A variety of cephalosporins share common nephrotoxic properties if sufficiently high intracellular concentrations are achieved. The unique feature of CER is its unusual renal transport leading to the higher intracellular concentrations (23), which may be the cause of its greater degree of nephrotoxicity. Thus, CER has widely been used for the study of cephalosporin-induced ARF (24). In the present histological examination, intravenous injection of 600 mg/kg of CER severely damaged the proximal tubule in the cortex, but not the glomerulus. In the physiological study, however, proteinuria and the increases of S-CRE and S-UN occurred, suggesting damage to the glomerulus. It is known that CER generates superoxide anions in the renal cells (20). Thus, these oxygen radicals might have induced the peroxidation of the cellular membrane at the glomerulus, resulting in the functional damage of the glomerulus (25).

It has been reported that the LD50 value of CER by single intravenous administration in rats was 1.2 to 1.4 g/kg body weight and that the ND50 (nephrotoxic dose) value was 1.0 g/kg body weight (2, 4). The dose of CER used in the present study was lower as compared with that in the previous reports that referred to the CER-induced ARF. The reason for this difference in nephrotoxic doses is thought to be the difference of CER sensitivity between the strains of rats examined. In the present study, CER at a dose of 600 mg/kg induced glucosuria, proteinuria and reduction of the glomerular filtration rate (GFR). The reduction in the tubular reabsorption rate of water seems to be due to the reduction of GFR (7, 8).

In the present study, KW-3902, a potent adenosine A1-receptor antagonist, ameliorated the increases in S-CRE and S-UN, and it improved the depression of CL.CRE in CER-induced ARF. KW-3902 improved the damage of the kidney in the histological study. Moreover, KW-3902 inhibited the proteinuria accompanying the ARF. This is the first observation that the blockade of adenosine A1-receptors can inhibit the CER-induced ARF.

There are several possible mechanisms for the renal protective effect of KW-3902 against CER-induced ARF. The first plausible mechanism for the protective effect of KW-3902 may be the blockade of adenosine A1-receptors, which can possibly be involved in the pathogenesis of CER-induced ARF. Adenosine, acting on the adenosine A1-receptor, has been suggested to be a mediator of ARF in a variety of animal models, including the ARF's in-

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[Fig. 6. Effects of KW-3902, furosemide, trichlormethiazide, amiloride and acetazolamide on urinary volume (UV) and electrolyte excretion in rats with cephaloridine-induced ARF. The rats in the ARF group received a single injection of CER at 600 mg/kg through the tail vein. The normal rat received a saline solution (4 ml/kg body weight). Seven days after the CER administration, the rats were used for this experiment. Each value is the mean ± S.E. of 6-7 animals. NOR: cephaloridine non-treated; CON: control (saline, 20 ml/kg, p.o.); KW: KW-3902 (1 mg/kg, p.o.); FUR: furosemide (30 mg/kg, p.o.); TCM: trichlormethiazide (1 mg/kg, p.o.); AML: amiloride (25 mg/kg, p.o.); ACT: acetazolamide (6.3 mg/kg, p.o.) *: Significantly different from the value in control rats at P<0.05 (Student's t-test).]
duced by glycerol (26) and cisplatin (27). In fact, plasma adenosine was shown to be increased in the ARF induced by glycerol (26). The increased adenosine could contribute to the aggravation of ARF, since adenosine decreases GFR, resulting from the constriction of afferent arterioles (28). Thus, KW-3902 might have ameliorated the CER-induced ARF by antagonizing adenosine A1-receptors. However, further investigation is necessary to clarify this point by measuring adenosine in the kidney of CER-induced ARF rats.

Second, the diuretic action of KW-3902 might contribute to the amelioration of CER-induced ARF (17). The decrement by KW-3902 of CER-induced toxicity might be ascribed to a dilution of CER in the proximal tubule due to the diuretic effect of KW-3902, which acts mainly on proximal tubules (18). The decreased concentration of CER in the proximal tubule could result in the attenuated nephrotoxicity of CER, which mainly injures the proximal tubule (8).

Finally, the effect on the cortical transport might have contributed the beneficial effect of KW-3902. In fact, it is known that CER-induced renal failure can also be prevented by probenecid and other organic anions (2, 3, 8). Both the renal cortical transport and the toxicity are prevented by the inhibitors of organic acid secretion, suggesting that the nephrotoxicity of CER is related to the high intracellular concentration that results from its active transport. Thus, KW-3902 might have reduced the CER-induced ARF by inhibiting this transport. Further investigation is required to clarify this possibility.

Furosemide and TCM tended to increase proteinuria and the serum parameters such as S-UN and S-CRE, resulting in the aggravation of the CER-induced ARF. It is well known that administration of furosemide to CER-treated rats increases urea nitrogen and creatinine in plasma (29) or sera (30, 31). Moreover, the enhancement by furosemide of CER-induced renal tubular necrosis has been demonstrated in numerous occasions in rats (29). Thus, furosemide treatment always increases the incidence and severity of renal dysfunction in CER-treated animals. The present results demonstrated that TCM, in addition to furosemide, aggravated the renal dysfunction occurring in CER-induced ARF. In fact, the increased renal damage by furosemide or TCM has also been observed in rats treated with the nephrotoxic agents such as gentamicin (K. Yao et al., unpublished data) and glycerol (32). At present, the mechanism underlying the enhancement of nephrotoxic action by these drugs is not fully understood, although there are many reports that the enhancement by furosemide of the renal necrosis occurs in animals and man (33, 34). Thus, KW-3902 may have some advantages over furosemide and thiazides when it is necessary to use a diuretic in combination with CER.

In the diuretic study in CER-induced ARF rats, furosemide and TCM caused significant increases of sodium excretion. Amiloride, a diuretic also acting mainly on the distal tubule, decreased potassium excretion as compared with the control group. The diuretic effects of these drugs in CER-induced ARF rats were qualitatively similar to those in normal rats. However, the diuretic effect of acetazolamide, an inhibitor of carbonic anhydrase, was abolished in the rats with CER-induced ARF at the dose which induces diuretic effects in normal rats. The inability of acetazolamide to induce diuresis in the rats with CER-induced ARF is likely to be due to the damage of carbonic anhydrase, which mainly exists in the proximal tubules of the kidney and activates Na/H transport. This assumption seems to be supported by the present histological observation that the proximal tubule was degenerated in the rats treated with CER.

KW-3902 significantly increased sodium excretion and tended to increase the urinary volume with little change of potassium excretion even in rats with CER-induced ARF at the dose that induces diuretic effects in normal rats. Since KW-3902 did not affect endogenous creatinine clearance, the diuretic effect cannot be ascribed to the change in glomerular filtration rate. From these results, it is suggested that KW-3902 acts, directly or indirectly, on the proximal tubule or other tubular sites in the kidney in CER-induced ARF rats. This implication is in accordance with the previous study (18) that examined the effects of KW-3902 on Li clearance and stop-flow patterns.

In conclusion, the present study demonstrated for the first time that adenosine A1-receptor blockade can ameliorate the ARF associated with the administration of CER. In addition, the present study demonstrated that the adenosine A1-blockade with KW-3902, in contrast to acetazolamide, causes natriuresis even in the rats with established CER-induced ARF, in which the proximal tubule is damaged. This observation suggests that the adenosine A1-blocker induces diuresis and natriuresis via acting, directly or indirectly, on the proximal tubule or other tubular sites in the kidney.

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**REFERENCES**

1. Lewis, A.A.G.: Cephaloridine. Postgrad. Med. J. 43, Aug. Supp. 125 (1967)
2. Child, K.J. and Dodds, M.G.: Nephron transport and renal tubular effects of cephaloridine in animals. Br. J. Pharmacol. Chemother. 30, 354–370 (1967)
3 Tune, B.M.: Effect of organic acid transport inhibitors on renal cortical uptake and proximal tubular toxicity of cephaloridine. J. Pharmacol. Exp. Ther. 181, 250–256 (1972)

4 Welles, J.S., Gibson, W.R., Harris, P.N., Small, R.M. and Anderson, R.C.: Toxicity, distribution, and excretion of cephaloridine in laboratory animals. Antimicrob. Agents Chemother. 1965, 863–869 (1966)

5 Atkinson, R.M., Currie, J.P., Davis, B., Pratt, D.A.H., Sharpe, H.M. and Tomich, E.G.: Acute toxicity of cephaloridine, an antibiotic derived from cephalosporine C. Toxicol. Appl. Pharmacol. 8, 398–406 (1966)

6 Silverblatt, F., Turck, M. and Bulger, R.: Nephrotoxicity due to cephaloridine: a light- and electron-microscopic study in rabbits. J. Infect. Dis. 122, 33–44 (1970)

7 Perkins, R.L., Apicella, M.A., Lee, I.S., Cuppage, F.E. and Saslow, S.: Cephaloridine and cephaloglycin: Comparative studies of potential nephrotoxicity. J. Lab. Clin. Med. 71, 75–84 (1968)

8 Tune, B.M.: Relationships between the transport and toxicity of cephalosporins in the kidney. J. Infect. Dis. 132, 189–194 (1975)

9 Boyd, J.F., Butcher, B.T. and Stewart, T.: The nephrotoxicity and histology of cephaloridine and its polymers in rats. Br. J. Exp. Pathol. 52, 503–516 (1971)

10 Tune, B.M. and Fravert, D.: Mechanisms of cephalosporin nephrotoxicity. Cephaloridine and cephaloglycin. Kidney Int. 18, 591–600 (1980)

11 Goldstein, R.S., Pasino, D.A., Hewitt, W.R. and Hook, J.B.: Biochemical mechanisms of cephaloridine nephrotoxicity: Time and concentration dependence of peroxidative injury. Toxicol. Appl. Pharmacol. 83, 261–270 (1986)

12 Goldstein, R.S., Contardi, L.R., Pasino, D.A. and Hook, J.B.: Mechanisms mediating cephaloridine inhibition of renal glucose-neogenesis. Toxicol. Appl. Pharmacol. 87, 297–305 (1987)

13 Tune, B.M., Wu, K.Y., Fravert, D. and Hortzman, D.: Effect of cephaloridine on respiration by renal cortical mitochondria. J. Pharmacol. Exp. Ther. 210, 98–100 (1979)

14 Suzuki, Y. and Sudo, J.: Lipid peroxidation and generations of oxygen radicals induced by cephaloridine in renal cortical microsomes of rats. Jpn. J. Pharmacol. 52, 233–243 (1990)

15 Suzuki, F., Shimada, J., Mizumoto, H., Karasawa, A., Kubo, K., Nonaka, H., Ishii, A. and Kawakita, T.: Adenosine A1 antagonist. 2. Structure-activity relationships on diuretic activities and protective effects against acute renal failure. J. Med. Chem. 35, 3066–3075 (1992)

16 Shimada, J., Suzuki, F., Nonaka, H. and Ishii, A.: 8-Poly-cycloalkyl-1,3-dipropylxanthines as potent and selective antagonist for A1-adenosine receptors. J. Med. Chem. 35, 924–930 (1992)

17 Mizumoto, H., Karasawa, A. and Kubo, K.: Diuretic and renal protective effects of KW-3902, a novel adenosine A1-receptor antagonist, via pertussis toxin insensitive mechanism. J. Pharmacol. Exp. Ther. 266, 200–206 (1993)

18 Mizumoto, H. and Karasawa, A.: Renal tubular site of action of KW-3902, a novel adenosine A1-receptor antagonist, in anesthetized rats. Jpn. J. Pharmacol. 61, 251–253 (1993)

19 Mizumoto, H., Kobayashi, T., Karasawa, A., Nonaka, H., Ishii, A., Kubo, K., Shimada, J. and Suzuki, F.: Renal protective effects of a novel adenosine A1-receptor antagonist, KW-3902. Jpn. J. Pharmacol. 58, Suppl. 1, 194P (1992)

20 Suzuki, Y. and Sudo, J.: Changes in lipid peroxidation and activities of xanthine oxidase, superoxide dismutase and catalase in kidneys of cephaloridine-administered rats. Jpn. J. Pharmacol. 49, 43–51 (1989)

21 Karasawa, A., Kubo, K., Shuto, K., Oka, T. and Nakamizo, N.: Antihypertensive effects of the new calcium antagonist benidipine hydrochloride in rats. Arzneimittelforschung 38, 1684–1690 (1988)

22 Hochberg, Y. and Tamhane, A.C.: Multiple Comparison Procedures. John Wiley & Sons, New York (1987)

23 Tune, B.M., Fernholt, M. and Schwartz, A.: Mechanism of cephaloridine transport in the kidney. J. Pharmacol. Exp. Ther. 191, 311–317 (1974)

24 Child, K.J. and Dodd, M.G.: Mechanism of urinary excretion of cephaloridine and its effects on renal function in animals. Br. J. Pharmacol. Chemother. 26, 108–119 (1967)

25 Sugihara, K., Nakano, S. and Gemb, M.: Effect of cispłatlon on in vitro production of lipid peroxides in rat kidney cortex. Jpn. J. Pharmacol. 44, 71–76 (1987)

26 Thié, G., Wilson, D.R., Arce, M.L. and Oken, D.E.: Glycercol induced hemoglobinuric acute renal failure in the rats. II. The experimental model, predisposing factors and pathophysiological features. Nephron 4, 276–297 (1967)

27 Knight, R.J., Collis, M.G., Yates, M.S. and Bowner, C.J.: Amelioration of cispłatlon-induced acute renal failure with 8-cyclopentyl-1,3-dipropylxanthine. Br. J. Pharmacol. 104, 1062–1068 (1991)

28 Osswald, H.: The role of adenosine in the regulation of glomerular filtration rate and renin secretion. Trends Pharmacol. Sci. 5, 94–97 (1984)

29 Dodds, M.G. and Foord, R.D.: Enhancement by potent diuretics of renal tubular necrosis induced by cephaloridine. Br. J. Pharmacol. 40, 227–236 (1970)

30 Foord, R.D.: Cephaloridine and the kidney. In Progress in Pharmacology, p. 597, University Tokyo Press, Tokyo (1970)

31 Lawson, D.H.: Effect of furosemide on antibiotic-induced renal damage in rats. J. Infect. Dis. 126, 593–600 (1972)

32 Bidani, A.K., Churchill, P.C. and Packer, W.: Theophylline-induced protection in myoglobinuric acute renal failure: further characterization. Can. J. Physiol. Pharmacol. 65, 42–45 (1986)

33 Brown, J.J., Gleadle, R.I., Lawson, D.H., Lever, A.F., Linton, A.L., Macadam, R.F., Prentice, E., Robertson, J.I.S. and Tree, M.: Renin and acute renal failure: studies in man. Br. Med. J. 1, 253–258 (1970)

34 Barza, M.: The nephrotoxicity of cephalosporins. An overview. J. Infect. Dis. 137, Suppl. 60–73 (1978)