Effect of the Selective Adenosine A₁-Receptor Antagonist KW-3902 on Tubuloglomerular Feedback in Radiocontrast-Media-Induced Nephropathy in Rats With Chronic Nitric Oxide Deficiency

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ABSTRACT—To study the possible mechanism of renoprotective effects of adenosine A₁-receptor antagonist against radiocontrast media (RCM)-induced nephropathy, we investigated the effects of adenosine A₁-receptor antagonist on tubuloglomerular feedback (TGF) activity prior to and following application of RCM in chronic NO-depleted rats. TGF in NO-depleted rats was significantly enhanced compared with that in normal rats. After RCM application, the enhanced TGF was continued. A selective adenosine A₁-receptor antagonist, KW-3902 (8-(noradamanan-3-yl)-1,3-dipropylxanthine), inhibited the enhanced TGF. These results suggest that KW-3902 could inhibit TGF in chronic NO-depleted rats. Renoprotective effects by adenosine antagonists could be partly due to an inhibition of TGF via the blockade of the adenosine A₁-receptor.

Keywords: Contrast media, Adenosine, Tubuloglomerular feedback

The administration of radiocontrast media (RCM) has long been recognized to produce profound changes in renal hemodynamics and in some cases to lead to the initiation of acute renal failure, principally in patients presenting with one or more pre-existing risk factors (1). In such patients, the underlying mechanisms of RCM-induced nephropathy (RCIN) are unclear.

A role for adenosine in the pathogenesis of RCIN has been suggested by several studies. Theophylline, an adenosine receptor antagonist, has proven effective in the prevention of RCIN in both animals (2, 3) and humans (4, 5). Arakawa et al. (6) demonstrated an activation of adenosine A₁-receptor to be involved in the renal hemodynamic response to RCM. Pretreatment with the selective adenosine A₁-receptor antagonist, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), prevented the decline in glomerular filtration rate (GFR) and renal blood flow following RCM application in rats (2). These observations suggest that blockade of adenosine A₁-receptor activation may significantly attenuate the nephrototoxic effects of RCM.

It has been proposed that increase in tubular fluid flow is responsible for the reduction in renal blood flow (RBF) and GFR; that is, a tubuloglomerular feedback (TGF) signal is being generated to reduce filtration (7). Adenosine plays a role in signal transmission in TGF (8). It is known that the large increase in urine output after RCM administration is caused by an osmotic diuretic effect in the proximal tubule (2). The diuresis leads to an increase in solute at the macula densa and release of adenosine (3); afferent vasoconstriction follows, which reduces RBF and GFR. However, RCM induced deterioration in renal function not in normal rats, but in rats treated with the nitric oxide synthesis inhibitor, N-o-nitro-L-arginine methyl ester (L-NAME)(2). This tends to indicate that the effects of RCM on renal function are modulated by endothelium-derived nitric oxide, which could exert a protective role in physiologic conditions. The exact pathomechanisms of RCIN, however, are still incompletely understood.

KW-3902 (8-(noradamanan-3-yl)-1,3-dipropylxanthine) is a potent and selective adenosine A₁-receptor antagonist (9). In the receptor-binding study, the dissociation constant value of KW-3902 for adenosine A₁-receptor and A₂-receptor are 0.19 and 170 nM, respectively (10). In anesthetized rats, KW-3902 potently antagonizes the response mediated via adenosine A₁-receptors with little influence on that mediated via adenosine A₂-receptors (11). Recently, Kawabata et al. (12) reported that KW-3902 suppresses
TGF in rats. To study the possible mechanism of renal protective effects of adenosine A<sub>1</sub>-receptor antagonist against RCIN, we investigated the effects of KW-3902 on TGF activity prior to and following application of RCM in rats with chronic nitric oxide deficiency, established as a model for RCIN by Erley et al. (2).

Male Sprague-Dawley rats (Charles River Laboratories, Sulzfeld, Germany) were used after approval of the study protocol by the Institutional Board for Animal Use. All animals were given a standard chow (containing 0.9% calcium, 0.7% phosphorous, 0.2% magnesium, 0.2% sodium and 1.0% potassium). The initial weight of the rats was 180 to 220 g. Rats were given L-NAME, which was added to the drinking water (50 mg/l, approximating an intake of about 5 mg/kg per day) for 8 weeks.

KW-3902 was obtained from Kyowa Hakko Kogyo (Tokyo). KW-3902 was dissolved in saline containing 1% dimethylsulfoxide (DMSO) and 0.01 N NaOH (vehicle).

On the day of the experiment, the rats were anesthetized with Inactin (Research Biochemicals International, Natick, MA, USA) at a dose of 120 mg/kg. The animal was placed on a heated-table, and the body temperature was maintained at 37°C via a servo-controlled rectal thermometer. After tracheotomy, the right jugular vein was cannulated with two polyethylene catheters for infusion saline (0.85 g/dl) and drugs. The total infusion rate was set at 0.8 – 1.4 ml/h per 100 g body wt. throughout the experiment. The right femoral artery was cannulated for continuous monitoring of mean arterial blood pressure (MAP) using a pressure transducer (PDA 23; Gould Inc., Cleveland, OH, USA). The left kidney was exposed by flank incision, carefully freed from perirenal fat, fixed with warm agar (3%) in a holder and covered with prewarmed (37°C) paraffin oil. The rats were allowed to stabilize for 60 min. A platinum pipette (O.D., 2 – 4 μm) filled with sodium chloride (1 M) and connected with a servo-nulling device (WPI, New Haven, CT, USA), was used to measure hydrostatic pressures in proximal tubules. Another pipette (O.D., 6 – 8 μm), filled with Ringer’s solution (136 mM NaCl, 4 mM KCl, 2 mM CaCl<sub>2</sub>, 4 mM NaHCO<sub>3</sub>, 7.5 mM urea, pH 7.4) stained with lissamine green (0.1%) and connected with a calibrated nanoliter perfusion pump (Hampel, Frankfurt, Germany), was used to identify the last accessible loop of proximal tubule. Two wax blocks (Paraffin 7159; Merck, Darmstadt, Germany) were injected into the proximal tubule, one upstream from the perfusion pipette inserted into the last accessible loop and the other into the first accessible loop of the proximal tubule. The servo-nulling pressure pipette was inserted upstream from the proximal wax block to monitor early proximal stop flow pressure (SFP). The activity of TGF was assessed as the difference of SFP (ΔSFP) in the early proximal tubule at 0 and 40 nl/min orthograde perfusion of Henle’s loop from endoprophinal tubule.

Experiment groups in the micropuncture study are shown in Fig. 1. The rats in group A (Vehicle + Saline, time control) received drinking water (normal tap water). ΔSFP was measured during a baseline period (period 1). The animals received an intravenous bolus injection of vehicle (1% DMSO, 0.01 N NaOH in saline) followed by saline (3 ml/kg) after 20 min. ΔSFP was measured again during 20 – 60 min after saline injection (period 2).

The rats in group B (KW-3902 + Saline) received drinking water (normal tap water). ΔTGF was measured during a baseline period (period 1). The animals received an intravenous bolus injection of KW-3902 (0.1 mg/kg body wt.) dissolved in saline containing 1% DMSO and 0.01 N NaOH followed by saline after 20 min. ΔSFP was measured again during 20 – 60 min after saline injection (period 2).

The rats in group C (Vehicle + RCM) received drinking water (normal tap water). ΔTGF was measured during a baseline period (period 1). The animals received an intravenous bolus injection of vehicle (1% DMSO, 0.01 N NaOH

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**Fig. 1.** Study protocol of micropuncture experiment. Group A: normal rat, vehicle + saline; group B: normal rat, KW-3902 + saline; group C: normal rat, vehicle + RCM; group D: L-NAME-treated rat, KW-3902 + saline; group E: L-NAME-treated rat, vehicle + RCM; group F: L-NAME-treated rat, KW-3902 + RCM. Vehicle, 1% DMSO, 0.01 N NaOH, in saline; KW-3902, 0.1 mg/kg; saline, 0.85% NaCl solution; RCM, radiocontrast media (Urografin 76%).
in saline) followed by RCM (3 ml/kg, sodium diatrizoate, Urografin®; Shering, Berlin, Germany) after 20 min. ΔSFP was measured again during 20–60 min after RCM injection (period 2).

The rats in group D (KW-3902 + Saline) received L-NAME (50 mg/l drinking water) for 8 weeks. ΔTGF was measured during a baseline period (period 1). The animals received an intravenous bolus injection of KW-3902 (0.1 mg/kg body wt.) dissolved in saline containing 1% DMSO and 0.01 N NaOH followed by saline after 20 min. ΔSFP was measured again during 20–60 min after saline injection (period 2).

The rats in group E (Vehicle + RCM) received L-NAME (50 mg/l drinking water) for 8 weeks. ΔSFP was measured during a baseline period (period 1). The animals received an intravenous bolus injection of vehicle (1% DMSO, 0.01 N NaOH in saline) followed by RCM after 20 min. ΔSFP was measured again during 20–60 min after saline injection (period 2).

The rats in group F (KW-3902 + RCM) received L-NAME (50 mg/l drinking water) for 8 weeks. ΔSFP was measured during a baseline period (period 1). The animals received an intravenous bolus injection of KW-3902 (0.1 mg/kg body wt.) dissolved in saline containing 1% DMSO and 0.01 N NaOH followed by RCM after 20 min. ΔSFP was measured again during 20–60 min after RCM injection (period 2).

All data are expressed as the mean ± S.E.M. Statistical analysis was performed using ANOVA and the t-test for paired and unpaired data. P values <0.05 were considered to be statistically significant.

In this study, we used the RCIN model with a slight modification of the previous method (2). We had confirmed that GFR did not change in groups A, B, C and D (K. Yao et al., unpublished data). In addition, RCM application induced a significant decline in GFR in L-NAME-treated rats (Group E, −42.2 ± 11.5% of preinjection value). KW-3902 (0.1 mg/kg, i.v.) prevented the fall in GFR in response to RCM in L-NAME-treated rats (Group F, −3.1 ± 2.9% of preinjection value, P<0.05 vs group E).

Figure 2 shows the effects of RCM on TGF activity in normal and L-NAME rats. TGF activity remained unchanged by saline (group A) or RCM (group C) application in normal rats. KW-3902 treatment significantly inhibited the TGF activity in normal rats (group B). An enhancement in TGF activity was found in L-NAME-treated rats before RCM application (period 1 in groups D, E and F). After RCM application, the enhanced TGF activity was continued in group E. KW-3902 treatment significantly inhibited the enhanced TGF activity near the normal level with or without RCM application (period 2 in groups D and F).

Assessing the activity of the TGF by measuring stop flow pressure in the early proximal tubule while perfusing the loop of Henle from the endproximal tubule with modified Ringer solution, we showed that TGF activity was enhanced in L-NAME-treated rats as compared to that in normal rats. Several studies have shown that inhibition of nitric oxide synthesis (NOS) enhances the TGF response (13, 14), which implies that endogenous nitric oxide attenuates the TGF system. RCM induced deterioration in

![Fig. 2. Effects of contrast media and KW-3902 on tubuloglomerular feedback in normal and L-NAME-treated rats. Values are represented as the mean ± S.E.M. (n = 5 or 6). KW-3902 (0.1 mg/kg, i.v.) was administered 20 min before contrast media application (Group F, Fig. 1). Period 1: TGF before contrast media application, Period 2: TGF after contrast media application (Fig. 1). 1) Q: group A, vehicle + saline; Δ: group B, KW-3902 + saline; ○: group C, vehicle + RCM. 2) Δ: group D, KW-3902 + saline; ●: Group E, vehicle + RCM; •: Group F, KW-3902 + RCM. a: P<0.05, b: P<0.001 versus zero perfusion in each period; c: P<0.01, d: P<0.001 versus corresponding value in group A; e: P<0.05, f: P<0.01, g: P<0.001 versus corresponding value in period 1 in each group.]
renal function not in normal rats, but in NO-depleted rats (2). These results suggest that the enhanced TGF activity may accentuate the nephrotoxicity of RCM in rats with chronic NO blockade. The decrease in GFR may be caused by the hyperosmolarity of RCM and its effect on the TGF activity.

Several studies have suggested that endogenous intrarenal adenosine contributes to the renal response to RCM (3, 6). Our hypothesis was that RCM might potentiate TGF activity through the increased adenosine induced by RCM injection. However, in the present study, TGF activity was not changed after RCM application in L-NAME treated rats. RCM might have no effect on TGF activity. This does not support the hypothesis that increased renal adenosine mediates the RCM-induced hemodynamic change. In this study, we measured TGF activity during 20–60 min after RCM injection. Application of RCM induced a transient increase in urine flow. However, the urine flow returned to baseline level 20 min after RCM injection (K. Yao et al., unpublished data). The increase in intrarenal adenosine after injection of RCM might not last for more than 20 min. This may be the reason why we could not detect the more enhanced TGF after RCM application in L-NAME treated rats. Alternatively, nephrotoxicity also is documented for low-osmolarity RCM (1). A direct toxic effect ofionic contrast media on renal epithelial cells was shown by in vitro studies (15). Further studies are necessary to determine the mechanism of RCIN.

Infusion of adenosine is reported to elicit marked decreases in renal blood flow (8). Recent investigations have clarified that a sustained and profound vasoconstriction induced by the application of RCM was mediated by adenosine A<sub>2</sub>-receptor (6). Furthermore, the administration of adenosine A<sub>2</sub>-receptor antagonist DPCPX prevented the renal deterioration after application of RCM in rats (2). These observations suggest that inhibition of the adenosine A<sub>2</sub>-receptor has an important role in the nephroprotection. Recently, it was reported that the specific adenosine A<sub>2</sub>-receptor antagonist KW-3902 suppresses TGF in rats (12). The present study demonstrated KW-3902 attenuated TGF activity after RCM application in L-NAME treated rats. This effect may partially contribute to the protective effect of adenosine antagonists against RCIN. However, the precise mechanism for the nephroprotective effect by adenosine A<sub>2</sub>-receptor antagonist remains undetermined, and further studies are necessary.

In summary, TGF activity was significantly enhanced in rats with chronic NO deficiency. The adenosine A<sub>2</sub>-receptor antagonist KW-3902 could inhibit the enhanced TGF activity. The renoprotective effect by adenosine antagonists could be partly due an inhibition of TGF activity via the blockade of adenosine A<sub>2</sub>-receptor.

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