Renal Tubular Site of Action of KW-3902, a Novel Adenosine A_1-Receptor Antagonist, in Anesthetized Rats

Hideaki Mizumoto and Akira Karasawa

Department of Pharmacology, Pharmaceutical Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., 1188 Shimotogari, Nagaizumi-cho, Sunto-gun, Shizuoka 411, Japan

Received September 3, 1992 Accepted January 18, 1993

ABSTRACT—The mechanism of the diuretic action of KW-3902 (8-(noradamantan-3-yl)-1,3-dipropylxanthine), an adenosine A_1-receptor antagonist, was investigated by a lithium clearance study and stop-flow method in anesthetized rats. KW-3902 increased urine volume (UV), sodium excretion and renal clearances of sodium (C_{Na}) and lithium (C_{Li}), when UV and C_{Na} increased more than C_{Li}. KW-3902 did not affect the stop-flow pattern, whereas trichlormethiazide inhibited the reabsorption of water and sodium at the distal nephron. These results suggest that the adenosine A_1-receptor blockade exhibits diuretic effects via the inhibition of reabsorption of water and sodium mainly at the proximal tubule. The additional small contribution of the distal action can not be ruled out.

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

Although the diuretic effects of alkylxanthines have been known for many years (1), it was only recently found that adenosine receptor antagonism is the basis for this effect. Several years ago, it was demonstrated that 8-phenyltheophylline, a non-selective adenosine receptor antagonist, exhibits a diuretic effect (2). KW-3902 (8-(noradamantan-3-yl)-1,3-dipropylxanthine) is a selective and the most potent adenosine A_1-receptor antagonist reported to date (3). KW-3902 significantly increases urine volume and sodium excretion with little change in the potassium excretion of saline-loaded rats (4). Therefore, it seems that diuretic effects of adenosine antagonists are due to the blockade of adenosine A_1-receptors (5). In the previous study, we found that KW-3902 causes significant diuresis and natriuresis with no change in renal plasma flow and creatinine clearance in anesthetized rats (4). These results suggest that the adenosine A_1-receptor antagonist causes diuretic effects by inhibiting reabsorption of water and sodium at tubular sites rather than by change in the renal hemodynamics. In the present study, the tubular site of the diuretic action of KW-3902 was investigated by a lithium clearance study and stop-flow experiment.

Male Wistar rats (weighing 258-305 g, Japan Shizuoka Laboratory animal Center, Inc., Hamamatsu) were used for the present study, and they were kept at 22℃ with a 12-hr light-dark cycle. Commercial chow and tap water were available ad libitum before the experiment. Prior to the experiment, rats were anesthetized with urethan (1.3 g/kg, s.c.).

In the lithium clearance study, polyethylene catheters were cannulated into left carotid artery, right femoral vein and urinary bladder for blood collection, infusion and urine collection, respectively. After the surgery was completed, saline containing 3 mg/ml lithium carbonate (Wako Pure Chemical Industries, Ltd., Osaka) and 5 μg/ml creatinine (Wako Pure Chemical Industries, Ltd.) was infused with a constant flow infusion pump (Pump 22, Harvard Apparatus Inc., South Natick, MA, USA) at a rate of 2 ml/hr/rat. After equilibration for 90 min, vehicle was administered to all rats, and urine was collected during a 1-hr control period. After the control period, KW-3902 or vehicle was administered to rats, and urine was collected during a 1-hr clearance period. KW-3902 was dissolved in saline containing 1% dimethylsulfoxide and 0.01 N NaOH (vehicle), and the solution was intravenously administered to rats at a volume of 1 ml/kg. Heparinized blood was collected at the midpoint of each urine collection period, and the plasma was separated. Urine volume (UV) was determined gravimetrically. Urine and plasma creatinine concentrations were measured by an autoanalyzer (AU510, Olympus, Tokyo), and

Table 1. Effects of KW-3902 on renal excretory responses in anesthetized rats

Group	UV (%)	Na Excretion (%)	C_{CRE} (%)	C_{Na} (%)	C_{Li} (%)
Control	77± 7	78± 8	94± 6	78± 7	90± 2
KW-3902, 0.001 mg/kg (i.v.)	98± 8	102± 8	105±13	101± 8	107± 2
KW-3902, 0.01 mg/kg (i.v.)	219± 4**	200±18*	112±10	201±19*	133± 3**
KW-3902, 0.1 mg/kg (i.v.)	366±46**	305±50**	102± 5	309±50**	121± 8**

Data are expressed as a percentage of each value during the clearance period to that during the control period. Values represent means±S.E. of 5 animals. UV=urine volume, C_{CRE}=renal clearance of creatinine, C_{Na}=renal clearance of sodium, C_{Li}=renal clearance of lithium. UV, Na excretion, C_{CRE}, C_{Na} and C_{Li} during the control period were 2.46±0.14 ml/kg/hr, 324.8±22.1 μEq/kg/hr, 275.0±8.8 ml/kg/hr, 2.40±0.17 ml/kg/hr and 106.1±4.1 ml/kg/hr, respectively. *P<0.05, **P<0.01, when compared with the control value by the Kruskal-Wallis's test followed by the Williams-Wilcoxon's test.

a standard formula was used to calculate the renal clearance of creatinine (C_{CRE}), an index of glomerular filtration rate (GFR). Sodium and lithium concentrations in the urine and plasma were measured by flame photometry (775-A, Hitachi Ltd., Tokyo), and the renal clearances of sodium (C_{Na}) and lithium (C_{Li}) were determined. C_{Li} is assumed to provide an index of the reabsorption of water and sodium at the proximal tubule, since lithium ions are filtered at the glomerulus into the renal tubular lumen and reabsorbed mainly at the renal proximal tubule in the same proportion as water and sodium (6).

Table 1 shows the results of the C_{Li} study. KW-3902 at doses higher than 0.01 mg/kg (i.v.) significantly increased UV and sodium excretion without any changes of C_{CRE}, indicating that the diuretic effects of KW-3902 in anesthetized rats were due to the inhibition of water and sodium reabsorption along the nephron segments. KW-3902 at doses that exhibited diuretic effects significantly increased C_{Na} and C_{Li}, suggesting that KW-3902 inhibited the reabsorption of water and sodium at the proximal tubule. However, the increases of UV and C_{Na} were greater than that of C_{Li}, suggesting a possibility that KW-3902 produces its diuretic effects also by inhibiting the reabsorption of water and sodium at the tubular sites beyond the proximal tubule (6, 7).

The stop-flow technique was used in an attempt to determine the effects of KW-3902 on the reabsorption of water and sodium at the distal nephron. After ligating the vascular pedicle of the right kidney, polyethylene catheters were cannulated into left carotid artery, right femoral vein and left ureter for blood collection, infusion and urine collection, respectively. After the surgery was completed, saline containing 0.15 g/ml mannitol (Wako Pure Chemical Industries, Ltd.) and 5 μg/ml creatinine was infused with a constant flow infusion pump at a rate of 30 ml/kg/hr. After equilibration for 30 min, the vehicle was administered to all rats. Five minutes after the administration of vehicle, the left ureter was clamped for 10 min. Upon release of occlusion, 15-urine samples of 3 drops of

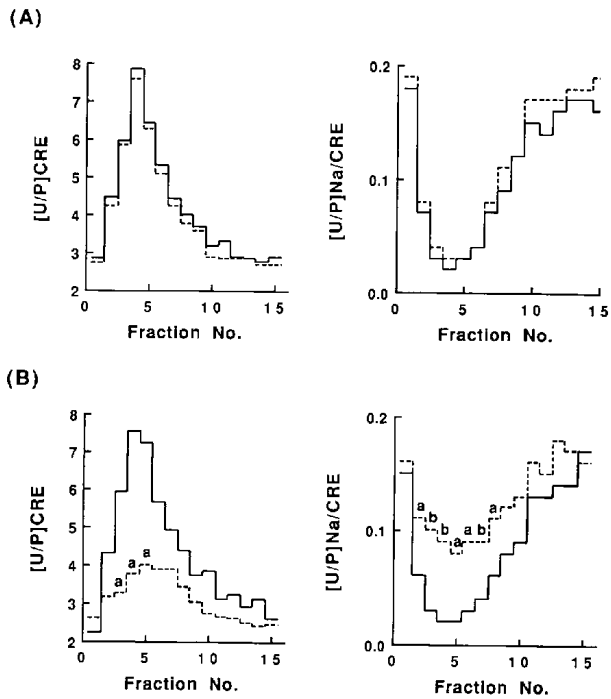

Fig. 1. Effects of intravenous administration of KW-3902 (0.1 mg/kg) (A) and TCM (1 mg/kg) (B) on the stop-flow patterns. Solid lines and dotted lines represent stop-flow patterns after the administration of vehicle and drugs, respectively. Values represent means of 5 animals. [U/P]CRE=the ratio of creatinine concentration in urine and plasma; [U/P]Na/CRE=the ratio of sodium concentration in urine and plasma divided by the ratio of creatinine concentration in urine and plasma. [a]: P<0.05, [b]: P<0.01, when compared with the control period value by the paired *t*-test.

urine (about 40 μl) were collected into microtubes. Heparinized blood was collected immediately after the urine collection, and the plasma was separated. After recovery, the same procedure was repeated 5 min after the administration of KW-3902 or trichlormethiazide (TCM; Sigma Chemical Co., St. Louise, MO, USA) to estimate the influence of drug on the stop-flow pattern. The solution of KW-3902 or TCM was intravenously administered to rats at a volume of 1 ml/kg. Creatinine and sodium concentrations in the urine and plasma were measured as described before. The following parameters were calculated: [U/P]CRE = [urinary creatinine concentration]/[plasma creatinine concentration], which estimates the reabsorption of water at the distal nephron (8, 9); [U/P]Na/CRE = [urinary sodium concentration]/[plasma sodium concentration]/[U/P]CRE, which estimates the reabsorption of sodium at the distal nephron (8, 9).

Figure 1 shows [U/P]CRE and [U/P]Na/CRE as mean values of 5 animals before and after the administration of KW-3902 (0.1 mg/kg, i.v.) or TCM (1 mg/kg, i.v.). In the preliminary experiment, in which creatinine (50 mg/kg, i.v.) was administered just before releasing the clamp, the highest concentration of creatinine in the urine was observed in fraction No. 15. The highest concentration of creatinine indicates roughly the entry of new glomerular filtrate. In control experiments, when the same procedure was repeated twice with vehicle, sequential stop-flow patterns in all animals were found to be reproducible (data not shown). KW-3902 (0.1 mg/kg, i.v.) did not affect the stop-flow pattern obtained from the administration of vehicle. On the other hand, TCM (1 mg/kg, i.v.) significantly decreased [U/P]CRE, and increased [U/P]Na/CRE in specimens from the distal nephron, indicating that TCM inhibited the reabsorption of water and sodium at the distal nephron. These results suggest that the distal tubular action of KW-3902 was too small, if any, to detect by the conventional stop-flow technique.

In conclusion, the C_{Li} study demonstrated that KW-3902 increases C_{Na} and C_{Li} concomitant with its diuretic effects, when UV and C_{Na} increased more than C_{Li}. On the other hand, KW-3902 did not affect the distal dip of the stop-flow pattern. These results suggest that the adenosine A_1-blockade produces diuretic effects by inhibiting the reabsorption of water and sodium maily at the proximal nephron segments. Further studies are required to clarify the precise tubular site of the adenosine A_1-antagonist.

Acknowledgments

The excellent technical assistance of Ms. R. Yanagimoto is greatly appreciated. We are grateful to Drs. K. Miura of Osaka City University and T. Hirata of Kyowa Hakko Kogyo, Co., Ltd. for encouragement and support.

REFERENCES

1 Kleeman, C.R., Cutler, R., Maxwell, M.H., Bernstein, L. and Dowling, J.T.: Effects of various diuretic agents on maximal sustained water diuresis. J. Lab. Clin. Med. **60**, 224–244 (1962)

2 Collis, M.G., Baxter, J.R. and Keddie, J.R.: The adenosine receptor antagonist, 8-phenyltheophylline, causes diuresis and saliuresis in the rat. J. Pharm. Pharmacol. **38**, 850–852 (1986)

3 Shimada, J., Suzuki, F., Nonaka, H. and Ishii, A.: 8-Polycycloalkyl-1,3-dipropylxanthines as potent and selective antagonists for A_1-adenosine receptors. J. Med. Chem. **35**, 924–930 (1992)

4 Kobayashi, T., Mizumoto, H., Karasawa, A. and Kubo, K.: Diuretic and antihypertensive properties of KW-3902, a novel adenosine A_1 receptor antagonist. Japan. J. Pharmacol. **58**, Supp. **I**, 195P (1992)

5 Collis, M.G., Shaw, O. and Keddie, J.R.: Diuretic and saliuretic effects of 1,3-dipropyl-8-cyclopentylxanthine, a selective A_1-adenosine receptor antagonist. J. Pharm. Pharmacol. **43**, 138–139 (1991)

6 Thomsen, K.: Lithium clearance: A new method for determining proximal and distal tubular reabsorption of sodium and water. Nephron **37**, 217–223 (1984)

7 Johns, E.J.: A study of the renal actions of amlodipine in the normotensive and spontaneously hypertensive rat. Br. J. Pharmacol. **94**, 311–318 (1988)

8 Uchida, T., Hayashi, K., Suzuki, Y. and Matsumura, Y.: Effects of torasemide on renal haemodynamics and function in anaesthetized dogs. Clin. Exp. Pharmacol. Physiol. **18**, 497–504 (1991)

9 Yukimura, T., Ito, K., Takenaga, T., Yamamoto, K., Kangawa, K. and Matsuo, H.: Possible tubular site of action in anesthetized dogs of a synthetic α-human atrial natriuretic polypeptide. J. Pharmacol. Exp. Ther. **238**, 707–712 (1986)