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Effects of the Administration of 5-(4'-Piperidinomethylphenyl)-2,3-dihydroimidazo[2,1-a] isoquinoline (SDZ-62-434) on Rat Kidney

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ABSTRACT : *To evaluate the renal toxicity of the antitumor agent, 5-(piperidonomethylphenyl)-2,3-dihydroimidazo[2,1-a]isoquinoline (SDZ-62-434), rats were treated with SDZ-62-434 of 50 mg/Kg, i.p., once and 10 mg/Kg, i.p., daily for 7 days. The kidney weights and urine volume after and during the treatment were observed. The concentrations of urinary creatinine, protein, and the activities of N-acetyl-β-D-glucosaminidase (NAG), alanine aminopeptidase (AAP), γ-glutamyl transpeptidase (GGT) and lactate dehydrogenase (LDH) in 24 hr urine were also determined. The kidney weights after acute and subacute administration was not affected. The urine excretions were increased 5 days after the acute administration and increased after the daily 3rd day-administration. The excretion of creatinine was similar as that of urine excretion. The excretion of creatinine was increased 5 days after the acute and subacute administration. However, the protein excretion didn't changed in both treatment. Those indicate that SDZ-62-434 might induce the diuresis and also suggest that diuresis might be due to the some metabolites rather than the compound itself. The urinary activities of NAG and LDH were not affected after the acute treatment. However, the urinary activities of AAP and GGT were slightly increased 3 days after the acute administration but, returned to the control value. In subacute treatment, the activities of GGT was not changed. And the activities of NAG were declined after the 7th day-administration. However, the activities of AAP were significantly increased after the 5th day-administration. Furthermore, the urinary activities of LDH were continuously increased during the subacute administration. These results indicate that the high and subacute administration might induce a weak damage on the kidney cells. Furthermore, the present results suggest that SDZ-62-434 might have relatively slow-emerging and mild toxicity to the kidney.*

Key Words : *SDZ-62-434, Creatinine, N-acetyl-β-D-glucosaminidase, Alanine aminopeptidase, γ-glutamyl transpeptidase, Lactate dehydrogenase*

I. INTRODUCTION

One of the most threatening diseases in recent years is cancer. Although various methods are applied in cancer treatment, chemotherapy with antitumor agents is getting more attentions. However, it is known that antitumor agent is toxic to various organs such as blood, liver, kidney and so on. These toxicities are usually related to the chemical structure of the agents. Also, their metabolites often have toxic effects.

It has been reported that 5-(4'-piperidinomethylphenyl)-2,3-dihydro imidazo[2,1-a]isoquinoline (SDZ-62-434) had the properties of an antagonist for platelet activating factor and showed antitumor effect during *in vitro* test and animal experiments (Houlihan *et al.*, 1995a).

It has been reported that in the damaged kidney, several enzymes are leaked into urine and total levels of urinary enzymes are changed. Thus, urinary activities of these enzymes can be used as an indicator of renal damage without injury to the examinee. (Hofmeister *et al.*, 1986; Ohata *et al.*, 1987; Wachsmuth, 1982)

Antitumor agents are usually administered for a long period. Although the survival rates of mice implanted with Meth A fibrosarcoma cells were increased by SDZ-62-434 (Houlihan *et al.*, 1995b), the

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toxic effects of this agent are not sufficiently identified. Therefore, the present study is designed to evaluate the renal cytotoxicity of SDZ-62-434.

II. METHODS

1. Animal and Materials

Male Sprague-Dawley rats weighing 200-250 g, housed under 12 hour light/dark cycle, 23±1°C , 60±5% humidity, were used. All animals had free access to food and water. SDZ-62-434 was synthesized according to the method of Houlihan *et al.* (1995a). MPS-1 (micropartition system-1) and membrane were purchased from Amicon (Denver, CO.). The diagnostic kit for GGT (γGT 14) was purchased from Gilford (Cleveland, OH.). The other chemicals were purchased from Sigma (St. Louis, MO.).

2. Animal treatment

Rats were adapted in metabolic cages for 5 days before the administration of SDZ-62-434. SDZ-62-434 was dissolved in saline and administered. Rats were injected intraperitonially in a dose of 50 mg/kg for the acute treatment. In the subacute treatment, rats were treated daily for 7 days with SDZ-62-434 in dose of 10 mg/kg, i.p.. Individual rats were kept in metabolic cages. After and during the administration of drug, 24 hour-urine was collected and measured at each rats. Also, the kidneys were removed and weighed after the acute and subacute administrations.

3. Pretreatment of urine for enzyme determinations

It has been reported that enzyme inhibitors are contained in urine. To remove the inhibitors micropartition systems were employed (Leathwood and Plummer, 1969; Ohata *et al.*, 1987); 24 hour-collected urine was centrifuged at 800×g, 4°C for 5 min. The diluted supernatant was added to MPS-1 and centrifuged at 1500×g, 5°C for 50 min. The filtrate in the lower tube of MPS-1 was used for creatinine quantification. To dissolve the enzymes attached to the membrane, phosphate buffered saline (PBS, 50 mM KH_2PO_4-K_2HPO_4 in saline, pH 7.4) was applied to the upper tube of MPS-1 and vortexed. After the same procedure was repeated, the washing solution was mixed with the first fluid. Then, it was used as the source for enzyme determinations. Enzyme activities were represented as creatinine ratios.

4. Creatinine measurement

It was determined by Jaffe reaction (Rock *et al.*, 1987); The diluted filtrate 1.5 m*l* and 0.36 M picric acid 0.5 m*l* were mixed. After 30 sec, 1.4 M NaOH was added to stop the reaction and 15 min stabilization followed. The detection wavelength for quantification was 500 nm.

5. Protein concentration

It was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard.

6. N-acetyl-β-D-glucosaminidase activity (NAG)

It was determined by Maruhn method (1976); 0.25 m*l* of substrate (5 mM p-nitrophenol-N-Acetyl-β-glucosaminide in 50 mM Citric acid-K_2HPO_4 -KOH buffer, pH 4.2) was added to 0.05 m*l* of enzyme sample. After 40 min incubation at 37°C, the reaction was stopped by adding 0.1 M borate buffer (H_3BO_3-KOH, pH 10.5) and the absorbance at 406 nm was determined.

7. Alanine aminopeptidase activity (AAP)

It was determined by Jung and Scholz method (1980); 0.08 m*l* of enzyme sample and 0.8 ml of substrate (2 mM L-alanine-4-nitroanilide) were incubated at 37°C for 20 min. The reaction was stopped by adding 20% sodium dodecyl sulfate (SDS) and the absorbance at 406 nm was determined.

8. Gamma-glutamyl transpeptidase activity (GGT)

It was determined by Szasz method (1974); The diagnostic kit for GGT was applied to the 0.02 m*l*

of enzyme sample. After 10 min incubaton at 25°C, the reaction was stopped by adding 20% SDS and the absorbance at 406 nm was determined.

9. Lactate dehydogenase activity (LDH)

It was determined by Bergmeyer and Bernt method (1974); Enzyme sample was incubated with NADH (1 mg/ml) and phosphate buffer (0.1 M K_2HPO_4-KH_2PO_4 containing 0.2% Triton X-100, pH 7.4) at 30°C for 5 min. The decreased optical density at 340 nm during the incubation was used to calculate the activity.

10. Statistics

Statistical significance was determined by Student's *t*-test.

III. RESULT

Changes in the kidney weight and urine volume after the acute and the subacute administration of SDZ-62-434 are shown at Table 1. Neither the acute nor the subacute administration of SDZ-62-434 did not change kidney weight. However, intestinal adhesion around the administration sites was observed in several animals treated with SDZ-62-434, 10 mg/Kg for 7 days. The excreted urine volume for 24hr was not changed 1 day after the acute administration. However, the 24hr urine volume was significantly increased on 5 days and 7 days after the acute administration of SDZ-62-434. Also, the subacutely treated animals excreted significantly high volume of urine after the 3rd day-administration.

Table 2 shows the changes in the creatinine and protein excretion after and during the acute and subacute administration of SDZ-62-434. The creatinine excretion was significantly increased from 5 days after the acute administration of SDZ-62-434. Also, animals excreted significantly high amount of creatinine after the 5th day-injection of subacute administration. The protein excretion after acute administration was continuously increased although it didn't show any statistical significance. However, the subacute administration of SDZ-62-434 didn't alter the urinary protein excretion so much.

Changes in the activities of various urinary en-

Table 1. Changes in the excretion of urine after and during the administration of SDZ-62-434

Treatment	1 day	3 day	5 day	7day
Acute 50 mg/kg	104.9±22.2	73.4±23.2	175.9±21.6*	160.8±19.9*
Subacute 10 mg/kg	92.3±32.6	147.5±20.0*	209.6±35.8**	219.0±31.9**

Rats were treated either 50 mg/kg for once or daily 10 mg/kg for 7 days, i.p.. of SDZ-62-434. The excreted urine for 24 hr were collected and measured. Each value was expressed as the percentage of 24 hr urine volume before the treatment, which values are 9.44±2.54 and 9.15±0.88 ml. Each value represents mean±S.E. for 4 or 6 animals. *, ** means significance at $p<0.05$ and $p<0.01$, respectively.

Table 2. Changes in the excretion of creatinine and protein after and during the administration of SDZ-62-434

Treatment	1 day	3 day	5 day	7 day
Creatinine[a]				
Acute 50 mg/kg	9.38±1.51	7.39±1.24	12.57±1.24**	15.52±1.91**
Subacute 10 mg/kg for 7 days	6.83±1.15*	9.61±1.01	12.30±1.03**	12.78±0.50**
Proteina				
Acute 50 mg/kg	1.18±0.48	1.25±0.42	1.91±0.53	2.07±1.17
Subacute 10 mg/kg for 7 days	1.68±0.28	1.48±0.40	1.64±0.21	1.16±0.36

Rats were treated with either 50 mg/kg, once or daily 10 mg/kg for 7 days, i.p., of SDZ-62-434. The excreted urine for 24 hr were collected and concentration of creatinine and protein were measured. a The control values of creatinine and protein are 9.37±0.45 mg and 1.76±0.24 mg/mg creatinine, respectively. Each value represents mean S.E. for 4 or 6 animals. *, ** means significance at $p<0.05$ and $p<0.01$, respectively.

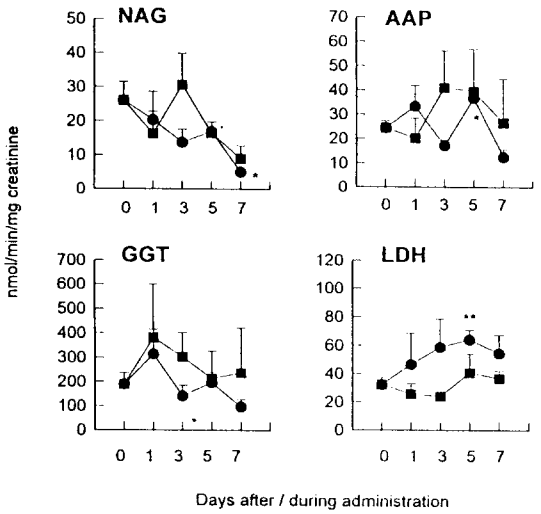

Fig. 1 Changes in various urinary enzyme activities after and during the administration of SDZ-62-434. Rats were treated with either 50 mg/kg for once (the closed square) or daily 10 mg/kg for 7 days (the closed circle), i. p., of SDZ-62-434. The excreted urine for 24 hr were collected and the various enzyme activities were measured. Each value represents mean ± S.E. for 4 or 6 determinations. * and ** means significance at $p<0.05$ and $p<0.01$, respectively.

zymes after and during the acute and subacute administration of SDZ-62-434 are shown at Fig. 1. In the acute treatment, the urinary activities of all examined enzymes were not significantly changed. However, the activities of AAP and GGT were 1.5 times increased on three day and one day after the treatment, repectively. In the subacute treatment, the urinary activities of NAG were significantly decreased after the 7th day-administration. The activities of urinary AAP were significantly increased after the 5th day administration, but decreased after the last administration. The subacute administration of SDZ-62434 increased the GGT activity after the first injection and thereafter kept low level. However, the excretion of LDH was continuously elevated during subacute administration and the urinary activities of LDH were significantly increased after the 5th day-administraion.

IV. DISCUSSION

The present results demonstrate that the administration of SDZ-62-434 alters the renal function. The urine and creatinine excretions were increased. Although the urinary enzyme activities were not altered in acute treatment, the activities of LDH and AAP were increased and those of NAG were decreased after the subacute administration.

It has been reported that SDZ-62-434 has the antitumor activity as well as the antagonistic effect of platelet activating factor (Houlihan *et al.*, 1995a). Also, the compound shows the direct and macrophage-induced cytotoxic effect (Houlihan *et al.*, 1995b). The present results indicate that acute and subacute treatment with SDZ-62-434 does not affect kidney weight. However, the intestinal adhesion during subacute administration suggest that the compound might be toxic to organs or at least alter the actions of the organs.

It has been reported that many diuretics increase the urine excretion by increasing the glomerular filtration or by decreasing the tubular reabsortion (Rang and Dale, 1991). Also, the glomerular filtration of creatinine can be used for estimating GFR (Kee, 1991). The present results revealed that the urinary excretion was increased during subacute administration. Furthermore, the high acute administration showed a delayed diuretic effect. Thus, the present results suggest that some metabolites of SDZ-62-434 rather than the parent compound itself may induce diuresis. It has been reported that plasma creatinine concentration can be influenced by the size of muscle or diet (Hoffmann and Hardelander, 1981; Pfeifer *et al.*, 1975; Shin *et al.*, 1989). The present results indicate that the excretion of creatinine is similar as that of urine excretion. Although the consumatory effects of SDZ-62-434 are needed to further illustrate, the increased urine excretion might be due to the increased glomerular filtration.

It has been reported that most renal toxic substances induce necrosis in proximal tubular cells and thus, the lysosomal and cytoplasmic enzymes, such as NAG, AAP, GGT and LDH, would leak into the urine (Ohata *et al.*, 1987; Shin *et al.*, 1990). Since urinary activities of NAG and LDH were not affected in acutely treated animals, the acute cytotoxicity of SDZ-62-434 may not be severe. However, the high single dose of SDZ-62-434 induced slight increase in the activities of AAP and GGT, rich in brush border. Thus, the present results indicate that the acute high dose of SDZ-62-434 ad-

ministration might induce a weak damage to the brush border of the kidney. The present results also revealed that the activities of NAG after and during acute and subacute administration were decreasing trends. Harauchi and Yoshizaki (1990) reported that the urinary enzyme activities represented as creatinine ratio were more uniform than the total activities. Thus, the decreased urinary activities might be due to the relative reduction of activity/creatinine ratio followed by the increased creatinine excretion rather than the absolute reduction of excretion. The repetitive administration of SDZ-62-434 with low dose increase excretion of LDH, a cytosolic enzyme, while the single administration with high dose don't. Thus, the present results suggest that a repetitive subacute administration induce the increase in membrane permeability. In summary, the present results suggest that SDZ-62-434 might have relatively slow-emerging and mild toxicity to the kidney. However, the toxic effects of chronic administration are needed to be further investigated.

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