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Effects of the Acute and Subacute Administration of 1-(N-methyl) piperazinyl-3-phenyl-isoquinoline on Rat Kidney

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ABSTRACT *: To evaluate the renal toxicity of the antitumor agent, 1-(N-methyl)piperazinyl-3-phenyl-isoquinoline (CWJ-a-5), rats were treated with CWJ-a-5 (acute : 100 mg/kg, i.p., single and subacute : 10 mg/kg, i.p., daily for 7 days). The changes in the body weights, water consumption, kidney weights and urine volume after and during the treatment were observed. The concentrations of urinary creatinine, the activities of N-acetyl-β-D-glucosaminidase (NAG), alanine aminopeptidase (AAP), γ-glutamyl transpeptidase (γ-GT) and lactate dehydrogenase (LDH) in 24 hr urine were also determined. The body weight and water consumption were decreased after the acute and subacute administration. However, the excretion of urine was not changed except the 1 day after the acute treatment. The excretion of creatinine was significantly decreased from 1 day after acute administration and continuously decreased. Also the excretion of creatinine was decreased during subacute administration. However, the protein excretion did not changed in both treatment. Those indicate that CWJ-a-5 might decrease the metabolic rate of muscle. The urinary activities of NAG, AAP, γ-GT, and LDH were significantly affected by the drug treatment. The urinary activities of NAG, AAP and γ-GT were significantly increased 1 and 3 days after the acute administration and then returned to the control value. However, the urinary activities of LDH were increased 7 days after acute treatment. During subacute treatment, the urinary activities of γ-GT were not changed. However, the urinary activities of NAG, AAP and LDH were only significantly increased after the third administration. These results indicate that either the high acute dose or the subacute administration with low dose of the compound might induce a temporal damage in the kidney cells.*

Key Words *: 1-(N-methyl)piperazinyl-3-phenyl-isoquinoline, 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, chemotheraphy 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. To develop more valuable compound, it is important index to observe the kidney toxicity of the synthesized antitumor agent. These toxicities are usually related to the chemical structure of the agents. Also, their metabolites often have toxic effects.

1-(N-methyl)piperazinyl-3-phenyl-isoquinoline (CWJ-a-5) is an intermediate product during the synthesis of benzo[c]phenanthridine compounds, which are reported to have a narrow antitumor spectrum (Arisawa *et al.*, 1984; Sufness and Douros, 1979). It has also been reported that CJW-a-5 showed the antitumor activities against the various cell lines as well as the mouse leukemia P388 (Chun *et al.*, 1998).

It has been reported that in the damaged kidney, the glomerular filtration rates (GFR) are changed and total levels of some urinary enzymes are increased (Ohata *et al.*, 1987; Wachamuth *et al.*, 1982). Thus, the level of excreting creatinine and the activities of the enzymes in the urine can be used as an indicator of renal damage without injury to the examinee (Hofmeister *et al.*, 1986; Shin *et al.*, 1989).

Antitumor agents are usually administered for a

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The list of Abbreviation: 1-(N-methyl)piperazinyl-3-phenyl-isoquinoline, CWJ-a-5; N-acetyl-β-D-glucosaminidase, NAG; Alanine aminopeptidase, AAP; Gamma-glutamyl transpeptidase, γ-GT; Lactate dehydogenase, LDH.

long period. Although the in vitro antitumor activities of CWJ-a-5 in various cell types is more potent than the other derivatives (Chun *et al.*, 1998), the toxic effects of this agent are not sufficiently identified.

Therefore, the present study is designed to evaluate the renal cytotoxicity of CWJ-a-5.

II. MATERIALS AND METHODS

1. Animal and materials

Male Spraque-Dawley rats weighing 200~250 g, housed under 12 hour light/dark cycle. 23±1°C, 605% humidity, were used. All animals had free access to food and water. CWJ-a-5 was synthesized according to the method of Chun *et al.* (1998). MPS-1 (micropartition system-1) and membrane were purchsed from Amicon (Denver, U.S.A.). The diagnostic kit for γ-GT was purchased from Gilford (Cleveland, U.S.A.). The other chemicals were purchased from Sigma (St. Louise, U.S.A).

2. Animal treatment

Rats were adapted in metabolic cages for 5 days before the administration of CWJ-a-5. CWJ-a-5 was dissolved in saline and administered. Rats were injected intraperitonially in a dose of 100 mg/kg for the acute treatment. In subacute treatment, rats were treated daily for 7 days in dose of 10 mg/kg, i.p. The doses were chosen according to our preliminary study. Individual rat was kept in metabolic cages. At 1, 3, 5 and 7 days after and during the administration of drug, 24 hour-urine was collected and volume was measured at each rat. Also, the kidneys were removed after the last collection of urine and weighed.

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 *et al.*, 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

Creatinine was determined by Jaffe reaction (Rock *et al.*, 1987); The diluted filtrate and 0.36 M picric acid 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

Protein 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)

The activity of NAG 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_3PO_4-KOH, pH 10.5) and the absorbance at 406 nm was determined.

7. Alanine aminopeptidase activity (AAP)

The activity of AAP was determined by Jung and Scholz method (1980): 0.08 m*l* of enzyme sample and 0.8 m*l* 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 (γ-GT)

The activity of γ-GT was determined by Szasz

method (1974); The diagnostic kit for γ-GT was applied to the 0.02 ml 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)

The activity of LDH 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 AND DISCUSSION

Changes in the body weight during and after the administration of CWJ-a-5 are shown in Fig. 1. In acute administration of CWJ-a-5, the body weight was significantly decreased. Also some rats were dead from 3 days and the mortality is 25% at 4 days after the acute administration. In subacute administration of CWJ-a-5, the change of body weight and the death were not observed, but the rate of growth was significantly decreased. Changes in water consumption are shown in Table 1. Water consumption was significantly decreased by both treatments, the decreases in water consumption were profound in the acute treatment, which showed maximum at 3 days after the treatment. The occurrence in the maximum decrease

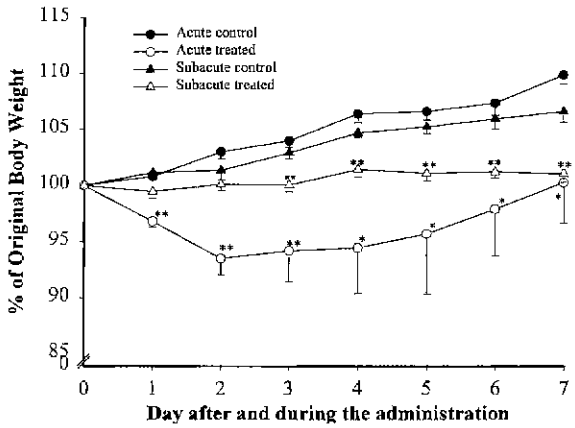

Fig. 1. Changes in the body weight after and during the administration of CWJ-a-5. Rats were treated either 100 mg/kg for acute or daily 10 mg/kg for 7 days, i.p., of CWJ-a-5. The values represent mean±S.E. for 5-7 animals The 100% value represents the body weight before the administration of CWJ-a-5. *P<0 05, **P<0.01 compared to the respective control value.

in water consumption after the acute treatment was superimposed with mortality. During the subacute administration, the decreased water consumption was gradually returned to the control level. However, there are no changes in the kidney weight per body weight after the acute and the subacute administrations (data not shown) and the appearances in kidneys were normal. These results indicate that CWJ-a-5 induces the reduction in water consumption and the decrease in growth rate. It is known that the water consumption is depend on the various body conditions, such as physical, psychological and neuronal states. Although the exact mechanisms are not clear, these results suggest that the compound might affect the consumatory behaviors and reduce the metabolic rate of the animals.

Table 2 shows the changes in the urine volume. The excreted urine volume for 24 hr was significantly decreased at just 1 day after the acute administration.

Table 1. Changes in water consumption after and during the administration of CWJ-a-5

	group	days of administration			
		1 day	3 day	5 day	7 day
Acute	control	49.2±4.2	39.7±2.2	40.2±3.4	41.3±1.8
	treated	14.0±3.1**	14.8±5.8**	30.3±9.4	23.0±9.4*
Subacute	control	38.8±2.4	33.0±1.3	39.6±2.4	34.4±2.6
	treated	24.3±3.2**	27.7±2.1	23.5±3.3**	28.0±3.3

Rats were treated either 100 mg/kg for acute or daily 10 mg/kg for 7 days, i.p., of CWJ-a-5. The consumed water for 24 hr were measured. The units is ml/100 g body weight. The values represent mean±S E. for 5-7 animals. *P<0.05, **P<0.01 compared to the respective control value.

Table 2. Changes in the excretion of urine after and during the administration of CWJ-a-5

	group	days of administration			
		1 day	3 day	5 day	7 day
Acute	control	16.70±1.67	16.32±1.58	16.50±1.11	14.92±1.53
	treated	7.78±1.36**	10.88±4.05	19.27±2.61	17.13±2.82
Subacute	control	13.83±0.48	13.17±0.48	11.36±0.14	9.56±0.92
	treated	11.37±1.11	14.37±0.88	10.67±0.93	10.24±1.30

Rats were treated either 100 mg/kg for acute or daily 10 mg/kg for 7 days, i.p., of CWJ-a-5. The excreted urine for 24 hr were collected and measured. The units is ml/100 g body weight. The values represent mean±S.E. for 5-7 animals. **P<0.01 compared to the respective control value.

Table 3. Changes in the excretion of total creatinine after and during the administration of CWJ-a-5

	group	days of administration			
		1 day	3 day	5 day	7 day
Acute	control	7.55±0.52	9.80±0.73	12.79±0.68	11.35±1.12
	treated	4.38±0.44**	5.73±1.44*	6.78±0.99**	7.26±0.99*
Subacute	control	9.44±0.24	9.01±0.19	10.20±0.31	8.86±1.23
	treated	7.14±0.59*	7.68±0.12**	7.41±0.62**	6.74±0.38

Rats were treated either 100 mg/kg for acute or daily 10 mg/kg for 7 days, i.p., of CWJ-a-5. The excreted urine for 24 hr were collected and the creatinine were measured. The units is mg. The values represent mean±S.E. for 5-7 animals. *p<0.05. **P<0.01 compared to the respective control value.

However, no changes in the urine volume were observed during the subacute treatment. The decreases in water consumption and the unaltered urinary excretion after acute and subacute treatment indicate that the metabolic rates of the animals do not rapidly decline.

Table 3 shows the changes in the creatinine after and during the acute and the subacute administration of CWJ-a-5. The excreted creatinine was decreased 42% at 1 day after the acute administration and continuously decreased. However, considering the urine volume, the amount of the excreted creatinine per urine volume was reduced at 5 days after the acute administration. In the subacute treatment, the excreted creatinine was decreased 24% at each administration. Also considering the urine volume, the amount of the excreted creatinine per urine volume was decreased from the third administration. However, the level of excreted protein was not changed (data not shown). It has been reported that the creatinine excretion is not changed in the damage of renal tubule and the level of creatinine was affected by the size of muscle and the food consumption (Hoffman *et al.*, 1981; Pfeifer *et al.*, 1975; Shin *et al.*, 1989). Although further study about the blood urea nitrogen and creatinine in blood is needed, the reduced amount of urinary creatinine was partly due to their reduced body weights. It has been reported that the glomerular filtration of creatinine can be used for estimating GFR (Kee, 1991) and the glomerular filtration activities are altered by hormonal or neuronal signals (Lee, 1986). The present results reveal that GFR might be decreased after both the acute and the late-stage of subacute treatment. Although the cytotoxic studies of the compound are needed, either high dose or long-term administration of CWJ-a-5 might affect the activities of kidney.

It has been reported that most renal toxic substances induce necrosis in proximal tubular cells and leak the lysosomal and cytoplasmic enzymes, such as NAG, AAP, γ-GT and LDH, into the urine (Ohata *et al*, 1987, Shin *et al*. 1990). Also Harauchi and Yoshizaki (1990) reported that the increase in the urinary enzyme activities represented the damage in the kidney and the activities ratio per creatinine were more uniform than the total activities. Figure 2 shows the changes in the activities of various urinary enzymes after and during the acute and the subacute administration of CWJ-a-5. A lysosomal enzyme, NAG, was increased at 1 and 3 days and then returned to the control value after the acute treatment. However, in the subacute administration, the urinary NAG activities were decreased after the first administration but significantly increased after the third administration. The results indicate that the high concentration of

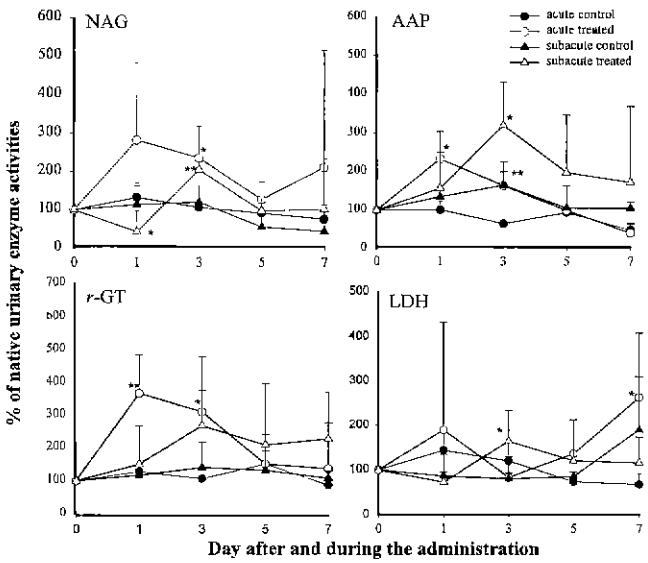

Fig. 2. Changes in various urinary enzyme activities after and during the administration of CWJ-a-5. Rats were treated either 100 mg/kg for acute or daily 10 mg/kg for 7 days. i.p., of CWJ-a-5. The excreted urine for 24 hr were collected and each enzyme activities were measured. The unit is expressed as the ratio to the enzyme activity of each untreated animal. The values represent mean±S.E. for 5-7 animals. *P<0.05, **P<0.01 compared to the respective control value.

CWJ-a-5 induces the damage in the kidney cell. The urinary activities of AAP and γ-GT, rich in brush border, show the similar effects. The activity of urinary AAP was increased at 1 and 3 days after the acute administration and then returned to the control value. Also in the subacute treatment, the urinary AAP activities were significantly increased only after the third administration of CWJ-a-5. The activity of urinary γ-GT, exist in the brush border, was also increased at 1 and 3 days after the acute administration and then returned to the control value. However, during the subacute administration, the urinary γ-GT activities were slightly increased but not significant. The results indicate that high dose of CWJ-a-5 induces the increases in the urinary AAP and γ-GT activities. The increases in these urinary enzymes suggest that the kidney brush border might be damaged by the compound, CWJ-a-5. Although it is needed to further elucidate the drug-induced changes in the rates of metabolism and the homeostasis of kidney cells, the urinary AAP and γ-GT activities after the fourth administration were not further increased. The results indicate that the brush border might be a little affected by the subacute administration with low dose. The activity of LDH, a cytoplasmic enzyme, was significantly increased at 7 days after the acute treatment. In the subacute treatment, the urinary activities of LDH were significantly increased only after the 3rd day treatment with CWJ-a-5. Although the analysis in the metabolism of CWJ-a-5 is needed, the results indicate that the cytotoxicity of the compound is appeared in the kidney cell membrane after the acute administration with high dose. However, the damage in the kidney cell membrane is not fully developed after the repetitive administration and suggest that the compound is relatively non-toxic. In summary, after the acute administration, the kidney cells in the renal tubule and brush border might be damaged by the compound and then recovered to the control condition. Also the acute administration with high dose induces the increase in membrane permeability. In the subacute administration with low dose, the kidney cells in the renal tubule and brush border were affected and then recovered to the control. However, the toxic effects of chronic administration are needed to be further investigated.

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