Effect of *N*-Benzyl-D-glucamine Dithiocarbamate on Renal Toxicity of Inorganic Mercury in Rats

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The effect of *N*-benzyl-D-glucamine dithiocarbamate (BGD) on the renal toxicity of inorganic mercury in rats was studied. Rats were injected i.v. with saline or $HgCl_2$ (300 μg Hg/kg) and 30 min later they were injected i.p. with saline or BGD (2778 μmol/kg, a quarter of an LD_{50}). Urinary excretion of γ-glutamyl-transpeptidase (γ-GTP), which is a brush border enzyme, in rats after mercury treatment significantly increased compared to that of the control in the 12—24 h urine specimen and reached a maximum value within 24 h after the treatment. Urinary excretion of *N*-acetyl-β-D-glucosaminidase (NAG), which is a lysosomal enzyme, also significantly increased after mercury treatment compared to that of the control in the 12—24 h urine specimen and reached a maximum value within 48 h after the treatment. A change in urinary aspartate aminotransferase (AST) activity after mercury treatment followed a pattern similar to that observed with the urinary NAG. BGD treatment did not increase the urinary excretions of γ-GTP, NAG, and AST. The uptake of *p*-aminohippuric acid (PAH) by renal cortical slices significantly decreased 24 h after mercury treatment. BGD injection after mercury treatment did not decrease the uptake of PAH by cortical slices. In addition, the microscopic examination of renal tissue from mercury-treated rats revealed necrosis of the proximal tubular cells. However, a photomicrograph of rat renal cortex after BGD treatment showed little abnormality. These results indicated that the mercury-induced renal damage was protected by the injection of BGD 30 min after mercury treatment.

Keywords inorganic mercury; renal toxicity; protective effect; *N*-benzyl-D-glucamine dithiocarbamate

Mercury has been recognized as a metal highly toxic to man for many years. Inorganic mercury is predominantly accumulated in the renal cortex, and affects the morphology and function of the proximal tubules.[1-3] Mercury-induced renal damage is manifested by proteinuria, enzymuria, and glucosuria.[4,5] It is important to develop an effective chelation therapy for mercury to prevent the renal disease it induces. The most potent mercury detoxicating agents are SH-containing compounds, one of which, 2,3-dimercaptopropanol (BAL), is used in clinical medicine for acute mercury poisoning. Some studies showed, however, that the injection of BAL to mice pretreated with mercury caused the redistribution of the mercury to brain.[6-8] Gabard reported that BAL caused the redistribution of mercury to the kidney in rats.[9] In addition, we reported that the injection of BAL (400 μmol/kg) to rats pretreated with mercury caused the redistribution of mercury to lung, heart, and brain.[10] We also recently reported that the injection of the new dithiocarbamate derivative, sodium *N*-benzyl-D-glucamine dithiocarbamate (BGD), into rats treated with mercury was effective in decreasing the mercury content in the kidney without redistribution of this metal to tissues such as the brain, heart and lung, and that the effect of BGD in reducing kidney mercury content and its protective effect against mercury-induced renal damage, as shown by the examined indices of proteinuria, glucosuria, and enzymuria (aspartate aminotransferase, AST), were almost the same as those of BAL.[11]

The present study was undertaken to further evaluate the effect of BGD on $HgCl_2$-induced renal toxicity by determining the urinary excretion of three enzymes, γ-glutamyltranspeptidase (γ-GTP), *N*-acetyl-β-D-glucosaminidase (NAG), and AST, which were chosen as specific cellular markers of this toxicity,[5,12,13] and by light microscopic examination of renal tissues.

Experimental

Materials Mercuric chloride was obtained from Wako Pure Chemical Ind. (Osaka). BGD was synthesized according to the procedure reported in our previous paper.[14] All other chemicals were of reagent grade.

Animals and Treatments Male Wistar rats, weighing 190—200 g, were purchased from Kyudo Co., Ltd. (Kumamoto) and housed in individual metabolic cages with diet (Nosan Lab Chow) and drinking water *ad libitum*. The animals were maintained on a 12-h light/dark cycle and the temperature of the animals care facilities was 23 to 26 °C.

The rats were injected i.v. with physiological saline (1.0 ml/animal) or $HgCl_2$ (300 μg Hg/kg) in a volume of 1.0 ml saline. Thirty min later they were injected i.p. with saline (control) or BGD (2778 μmol/kg, a quarter of an LD_{50}) in 2.0 ml saline. Urine samples were collected from each rat at 6, 12, 24, 48, 72 and 96 h after injection of saline or mercury and subjected to the determination of γ-GTP, NAG, and AST.

Uptake of *p*-Aminohippuric Acid (PAH) by Slice of Rat Renal Cortex Rats were injected i.p. with saline (control) or BGD (2778 μmol/kg) 30 min after i.v. injection of saline or $HgCl_2$ (300 μg Hg/kg). Twenty-four h later the rats were killed by decapitation and kidneys were removed and chilled in ice-cold saline. The slice technique was carried out according to the method of Cross and Taggart with some modification.[15] The kidneys were sliced with a razor blade to obtain portions of the renal cortex (0.5—1.0 mm thick). The slices (about 140 mg wet wt.) were then placed in 5 ml of a medium containing 97 mM NaCl, 40 mM KCl, 0.74 mM $CaCl_2$, 7.41 mM sodium phosphate buffer (pH 7.4), and 0.074 mM PAH, and incubated in 95% O_2–5% CO_2 atmosphere for 1 h at 37 °C. The slices were lifted out as quickly as possible, blotted carefully on filter paper, weighed, and homogenized with a glass-Teflon homogenizer, to which were added 1.8 ml of 15% trichloroacetic acid and water to a final volume of 10 ml. A 2.0 ml aliquot of the slightly turbid suspending medium was treated in a similar manner. The homogenates were centrifuged at 2000 *g* for 10 min. The resulting supernatant fractions were filtered and 5 ml aliquots of the filtrates were used for the estimation of PAH by the method of Bratton and Marshall.[16] The uptake of PAH by the slices was calculated as the ratio of PAH concentrations in the slices and medium (S/M).

Histology The effect of BGD on the renal tissue of mercury-treated rats was determined. Rats were injected i.p. with saline (control) or BGD (2778 μmol/kg) 30 min after i.v. injection of saline or $HgCl_2$ (300 μ Hg/kg). Twenty-four h later the animals were killed by decapitation and kidneys were removed. The kidneys were fixed in neutral 10% formaline, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for examination by light microscopy.

Analytical Procedures AST and γ-GTP activities in the urine were determined using a commercially available kit, S.TA-Test Wako and γ-GTP C-Test Wako (Wako Pure Chemical Ind., Osaka), respectively. NAG activity in the urine was determined using another commercially available kit, NAG Test Shionogi (Shionogi Co., Osaka).

Statistical Analysis Data were compared by analysis of variance.

When the analysis indicated that a significant difference existed, the treated groups were compared to controls by Duncan's new multiple range test.

Results and Discussion

Figure 1 shows the urinary excretions of γ-GTP, NAG, and AST in rats injected with BGD 30 min after $HgCl_2$ pretreatment. Urinary excretion of γ-GTP, which is an enzyme associated with the renal tubular brush border,[12] after mercury treatment significantly increased compared to that of the control in the 12—24 h urine specimen and reached a maximum value within 24 h after the treatment. However, the enzyme level decreased thereafter and declined to almost the control level in 72 h after the treatment. Kyle *et al.* reported that urinary excretion of alkaline phosphatase (ALP), which is an enzyme associated with the tubular brush border, in rats after mercury treatment significantly increased compared to that of the control 9—24 h after mercury and the level decreased thereafter.[4] These results showed that the degeneration of the tubular brush border occurs during the 12—24 h after mercury treatment and that brush boder enzymes may be the most sensitive indicators of acute nephrotoxicity of $HgCl_2$. Urinary excretion of NAG, which is considered to be a lysosomal enzyme marker,[13] after mercury treatment significantly increased compared to that of the control in the 12—24 h urine specimen and reached a maximum value within 48 h after the treatment. NAG excretion decreased thereafter and declined to the control level 96 h after the treatment. A change in urinary AST activity in rats after mercury treatment followed a pattern similar to that observed with the urinary NAG. We reported that the urinary excretion of protein in these animals after mercury treatment significantly increased compared to that of the control 24 h after the treatment and a change in urinary protein showed a pattern similar to that observed with the urinary AST.[11] No changes in γ-GTP, NAG, and AST activities were observed in rats injected with saline or BGD alone. Increases in the urinary γ-GTP, NAG, and AST in rats after mercury treatment are considered to result from the necrosis of the proximal tubular cells, as shown by other studies.[4,5,17] In addition, the maximum excretion of γ-GTP, a brush border enzyme, occurred within 24 h after mercury treatment. In contrast, there was a delay in the urinary excretion of NAG, a lysosomal enzyme, and its maximum excretion was not observed until 48 h after the treatment. Thus, these results indicate that the damage to the brush border of the proximal tubular epithelium occurred before release of NAG into the urine or any significant lysosomal degradation. The present study also suggests lysosomal changes in mercury-induced renal toxicity but at a later time period, as indicated by the delayed urinary excretion of NAG.

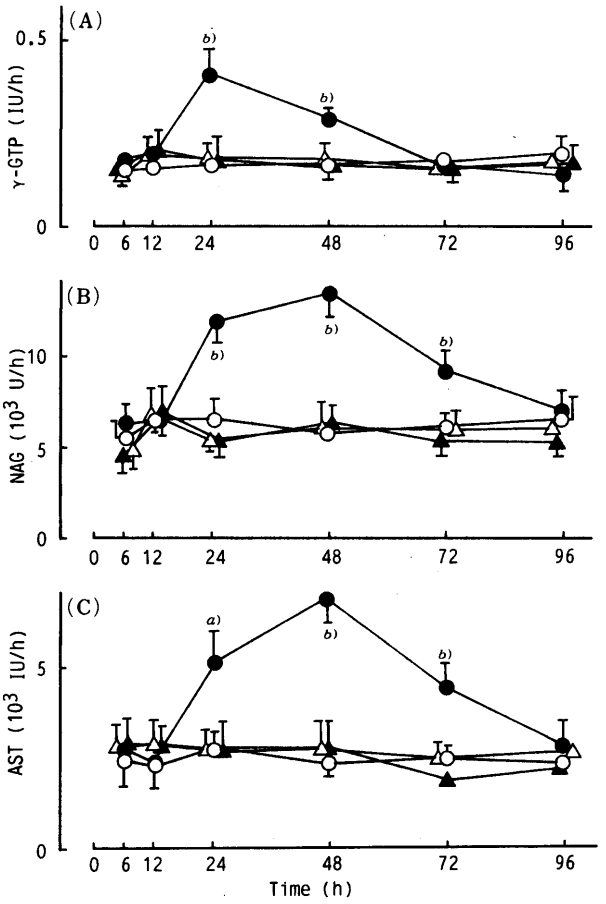

Fig. 1. Effect of BGD on Urinary Excretions of γ-GTP (A), NAG (B), and AST (C) in Rats Pretreated with $HgCl_2$, 30 min Earlier

The values represent the mean ± S.D. for 4 animals. ○, control; ●, Hg; △, BGD; ▲ Hg+BGD. Significantly different from control, *a*) $p<0.05$; *b*) $p<0.01$.

The injection of BGD at 30 min after mercury treatment did not increase the urinary excretions of γ-GTP, NAG, and AST, indicating the protective effect of BGD against mercury-induced renal damage. Such an effect of BGD is suggested to be due to the efficient removal of mercury from the body mainly through the bile, as shown in our previous paper.[11]

We further investigated the protective effect of BGD on the renal dysfunction after mercury treatment. The uptake of PAH by renal cortical slices of rats injected with BGD 30 min after $HgCl_2$ treatment is shown in Table I. This uptake significantly decreased 24 h after mercury treatment and was about 75% of the control, being approximately consistent with the report by Kyle *et al.*,[4] in which $HgCl_2$ (250 μg Hg/kg) was injected to rats. However, the uptake of PAH by cortical slices did not decrease in those rats injected with BGD after mercury treatment, indicating that BGD protected against mercury-induced renal dysfunction.

Light microscopic examination was made of renal tissue from mercury- and/or BGD-treated rats (Fig. 2). Partial proximal tubular damage was evident in animals treated with mercury, and some of the renal tubular cells showed necrosis. Kyle *et al.* reported that morphological alterations in proximal tubular cells were observed at 24 h after the injection of $HgCl_2$ (250 μg/kg) to rats.[4] The photomicro-

Table I. Effect of BGD on PAH Uptake by Renal Cortical Slices of $HgCl_2$-Pretreated Rats

Treatment	PAH uptake[a] (S/M)
Control	6.48 ± 0.38
Hg	4.86 ± 0.07[b]
BGD	6.58 ± 0.11
Hg+BGD	6.25 ± 0.14

a) Expressed as the ratio of PAH concentrations in the slices and medium (S/M). Significantly different from control: *b*) $p<0.01$.

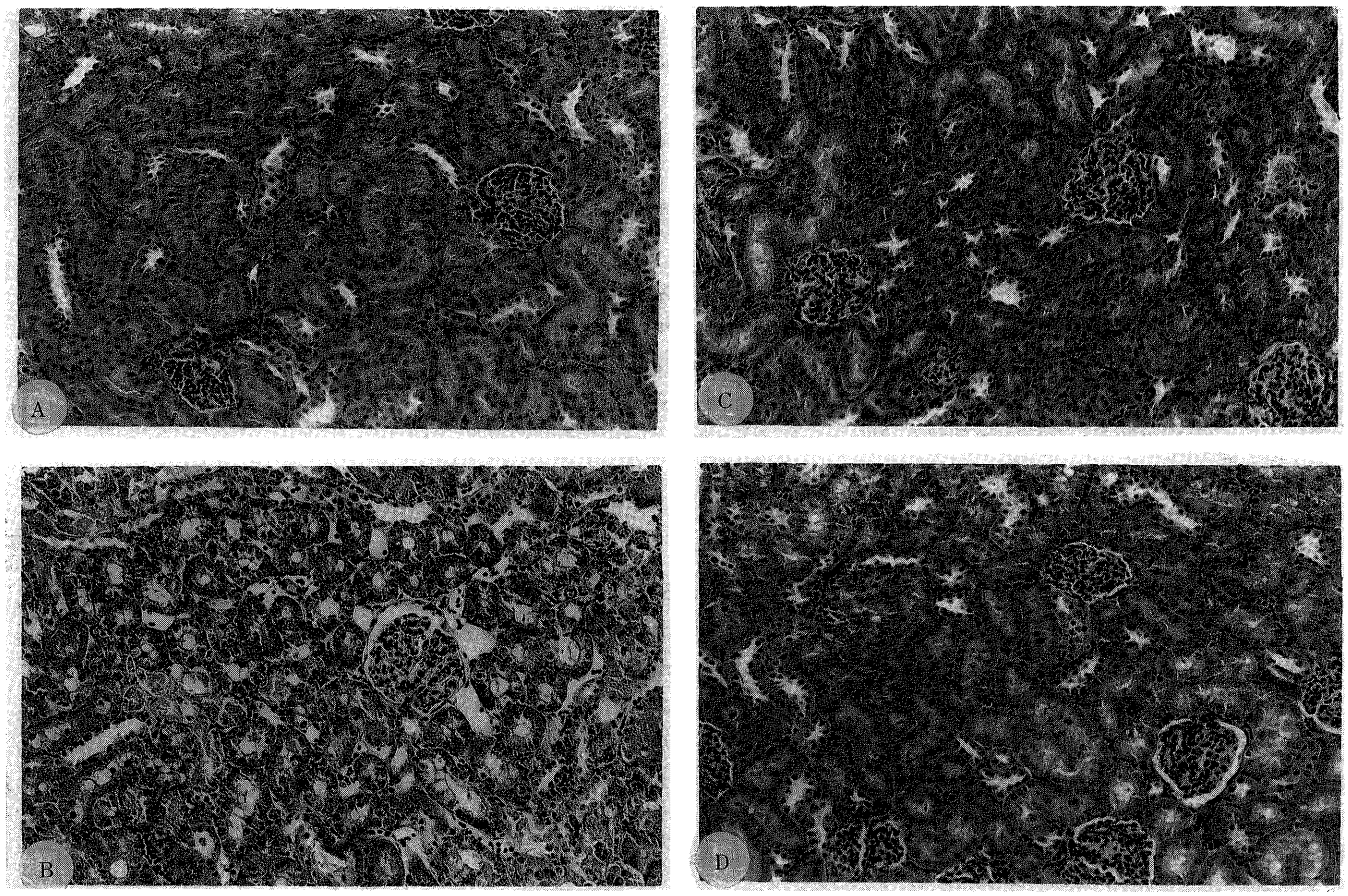

Fig. 2. Light Photomicrograph of Renal Tissue

A, Renal cortex from rat injected with saline 30 min after saline treatment showing proximal tubules of normal appearance. B, Renal cortex from rat injected with saline 30 min after mercury treatment showing proximal tubular cell necrosis. C, Renal cortex from rat injected with BGD 30 min after saline treatment showing proximal tubules of normal appearance. D, Renal cortex from rat injected with BGD 30 min after mercury treatment showing proximal tubules of normal appearance. Stain, hematoxylin and eosin; ×200.

graph of rat renal cortex after BGD treatment showed little such abnormality. This may be explained by the BGD effectively having removed the mercury, which induces a nephrotoxic effect, from the renal tissues.

The present study was carried out to evaluate the protective effect of BGD against the renal damage resulting from acute exposure to mercury in rats. The results indicated that mercury-induced renal damage was protected by the injection of BGD at 30 min after mercury treatment.

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