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Chronic tubulointerstitial changes induced by germanium dioxide in comparison with carboxyethylgermanium sesquioxide

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Chronic tubulointerstitial changes induced by germanium dioxide in comparison with carboxyethylgermanium sesquioxide. Chronic nephrotoxicity was investigated in rats orally administered germanium dioxide (GeO_2) and carboxyethylgermanium sesquioxide (Ge-132) for 24 weeks. Increased BUN and serum phosphate as well as decreased creatinine clearance, weight loss, anemia and liver dysfunction were apparent at week 24 only in the GeO_2 treated group. Vacuolar degeneration and granular depositions were observed by light microscope in the degenerated renal distal tubules in the rats of this group, with the semiquantitative scores of tubular degeneration being 95 ± 9% in the GeO_2 group, 3 ± 1% in the Ge-132 group and 1 ± 1% in the control group, respectively. Electron microscopy revealed electron-dense inclusions in the swollen mitochondrial matrix of the distal tubular epithelium in the GeO_2 group. Although systemic toxicities were reduced after GeO_2 was discontinued at week 24, renal tubulointerstitial fibrosis became prominent even at week 40 (16 weeks after discontinuation). A Ge · Kα X-ray spectrum was clearly demonstrated in the mitochondrial matrix of the distal tubular epithelium in the GeO_2 group with the help of electron probe X-ray microanalysis. On the other hand, neither toxic effects nor renal histological abnormalities were manifested in either the Ge-132 or the control group. The renal tissue content of germanium was high at weeks 24 and 40 in the GeO_2 group. From these results, it is concluded that GeO_2 causes characteristic nephropathy while Ge-132 does not. In addition, it appears that residual GeO_2 remains for a considerably long time even after the cessation of GeO_2 intake.

Germanium (Ge; atomic number 32, atomic weight 72.59) belongs to the IV group of the periodic system and is a semiconductor that has been used mainly in the industrial field. It is an ubiquitous biomaterial, and is contained in almost all foods even if only in minute amounts [1]. Some biological activities such as erythropoietic action [2, 3] and antimicrobial activity [4] have been shown in either germanium dioxide (GeO_2) or organogermanium derivatives. Carboxyethylgermanium sesquioxide (Ge-132) was synthesized by Asai et al and has been well characterized in Japan [5]. This compound has also been reported to have an anti-tumor effect [6, 7], an inhibitory effect on amyloidosis [8], as well as possessing immunomodulative activity [9–11]. On the other hand, toxicities of some germanium compounds have been reported in spite of their medical utility [12–16]. However, there have been no reports describing toxicity of oral germanium compounds [17] except for Rosenfeld and Wallace's study [15].

In Japan, some people take Ge-containing compounds orally as a kind of elixir. We have experienced several cases of patients who took Ge-containing compounds and later went into progressive renal failure. These cases showed an uncommon clinical course and characteristic renal histology [18]. In particular, long-lasting renal dysfunction even after the cessation of Ge compound ingestion has been characteristic. Since these Ge compounds contained GeO_2, we reported the renal lesions as being GeO_2-induced nephropathy [19]. Recently, other similar cases have been reported in Japan [20–23]. Germanium has also recently gained in popularity in Germany, USA, and the United Kingdom, among other countries. According to Lancet [24], the popularity of germanium, which began in Japan in the 1970s, took off in Britain in 1987 with a claim (clearly directed at AIDS patients) that it would "rebuild your compromised immune system", and there are now 15 different brands of germanium compounds on the United Kingdom market.

Venugopal and Luckey [17] reported that organic derivatives of the metals of Group IV are more toxic than are the inorganic salts. However, we experienced two patients who developed renal failure due to the ingestion of GeO_2 after the cessation of Ge-132 or organic Ge compound (unpublished data). Furthermore, subacute nephrotoxicity was not induced by Ge-132 but by GeO_2 in our preliminary animal study [19].

In the present study, to examine the chronic nephrotoxicity of Ge compounds, we observed both renal function and histology for 16 weeks by administering two kinds of Ge compounds, GeO_2 and Ge-132, for 24 weeks. We tried to detect the localization of either Ge · Kα or Ge · Lα X-ray spectrum in the tubular epithelial cell organellae under X-ray microanalysis.

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Methods

Experimental design

Study I: Chronic administration and withdrawal study. Using Wistar female rats (Kyushu University Animal Center, Fukuoka, Japan), 140 to 190 g of body weight, three experimental groups were prepared: one group was treated daily with 75 mg/kg body wt of GeO_2 (molecular weight 104.59, Sumitomo Metal Mining Co., Tokyo, Japan) (GeO_2 group, $N = 20$ rats); another group was given 120 mg/kg of Ge-132 [$(GeCH_2CH_2COOH)_2O_3$, molecular weight 339.32, ASAI Germanium Institute, Tokyo, Japan] (Ge-132 group, $N = 19$); and a control group was administered no Ge compounds ($N = 16$). The dose of the two kinds of Ge compounds contained an equal amount of Ge. Each Ge compound was mixed into the powdered rat chow (CE-2, Clea Japan Inc., Tokyo, Japan). The constant dose of each Ge compound was administered in spite of the alteration of diet intake and body weight. To maintain an equal caloric and protein intake, all groups were pair-fed with the group which had minimal ingestion. All groups had free access to tap water. After week 24, Ge administration was discontinued and a Ge-free diet was provided to all the groups. To investigate both the tissue concentration of Ge and renal histology, rats were sacrificed at weeks 24, 26 and 40. The latter two periods were 2 and 16 weeks after the cessation of Ge. To determine the kidney concentration prior to a major fall in glomerular filtration rate, six rats from each group were sacrificed at week 6. Body weight, hematocrit, BUN, serum creatinine, and 24-hour urinary protein excretion were examined every four weeks. Systolic blood pressure was measured every eight weeks. The serum total protein, albumin, cholesterol, phosphate, GOT and GPT, and urinalysis were examined at weeks 24 and 40. Creatinine clearance (C_{Cr}), fractional excretion of sodium (FE_{Na}), urinary N-acetyl-beta-D-glucosaminidase (NAG) concentration and urinary Ge concentration were examined at week 24.

Study II: High dose study in GeO_2-treated rats for electron energy dispersive X-ray microanalysis (EDX). Five Wistar female rats (150 to 200 g body wt) were treated with 450 mg/kg body wt/day of GeO_2 for two weeks and sacrificed for light microscopic, electron microscopic study, and EDX analysis. The sections were observed and elementally analyzed with an electron probe X-ray microanalyzer.

Analytical methods

Hematocrit was determined by the microhematocrit method [25], serum and urinary creatinine by the Jaffe reaction [26], BUN by the reduced nicotinamide adenine dinucleotide-coupled reaction [27], serum total protein by the biuret reaction [28], albumin by the use of bromcresol green [29], cholesterol by an enzymatic method [30], and phosphate by the Fiske and Sabbarow's method [31]. GOT and GPT were determined by the method of Karmen, Wrobewski and Ladue [32], serum and urinary sodium by use of flame photometer, 24-hour urinary protein excretion by the sulfosalicylic acid method [33], NAG by the MCP-NAG method [34], and urinalysis by N-multistix (Ames-Sankyo Co., Tokyo, Japan). Systolic blood pressure was measured in the conscious state by the tail cuff method. Ge concentration of the tissue was determined by the flameless atomic absorption method (AA-670, Shimadzu, Kyoto, Japan), and urinary Ge concentration by a polarized Zeeman atomic absorption spectrometer (Z-6100, Hitachi Co., Tokyo, Japan). These determinations were performed by technicians who had no prior knowledge of the experimental groups.

Morphological analysis

The kidney and heart were fixed in 6% neutral buffered formalin and stained with hematoxylin and eosin (H&E) and periodic acid-Schiff reagent (PAS). The former was also stained with SUDAN III and the latter with Heidenhain's azocarmine (AZAN).

Histological evaluation was made independently by two investigators without prior knowledge of the experimental groups. A semiquantitative score was used to evaluate the degree of tubular changes according to the method of Risdon, Sloper and de Wardener [35]. The tubular change was expressed by the number of microscopic fields where unequivocal degeneration was found, observing a hundred consecutive microscopic fields in the cortex of each specimen with the aid of a 40× objective.

For the immunohistological studies, a 4 μm thick kidney section was exposed to fluorescein isothiocyanate conjugated anti-rat-IgG, IgA, IgM, or C_3 goat serum (Cappel Laboratories, Cochranville, Pennsylvania, USA).

For the electron microscopic studies, specimens were fixed in 3% glutaraldehyde buffered to pH 7.2 with 0.1 M cacodylate buffer at 4°C, postfixed in 1% osmium tetroxide, dehydrated and embedded in Spurr's low viscosity resin. Ultrathin sections were double stained with uranyl acetate and lead citrate at 50°C using the microwave system [36], and observed under the JEM-1200EX transmission electron microscope (Nihon-Denshi Co., Tokyo, Japan). Unstained ultrathin sections were analyzed with an EDX system (EDAX-PV-9800, Japan Philips, Ltd., Tokyo, Japan) at 40 to 60 kV accelerating voltage for 200 seconds. The obtained X-ray spectra were analyzed with a computer EDX system.

Statistical analysis

Data are expressed as mean ± SD. Statistical difference was calculated using the one way analysis of variance among groups and the unpaired *t*-test with Bonferroni's method, except where otherwise noted. A level of 0.05 was regarded as significant.

Results

Study I: Chronic and withdrawal study

Body weight, systolic blood pressure and hematocrit. The rats of the GeO_2 group developed a marked body weight loss after week 12 of treatment, and they became inactive and listless after week 20. One rat died from azotemia at week 23. As shown in Figure 1A, the body weight was significantly lower in the GeO_2 group than in either the Ge-132 or the control group after week 12 ($P < 0.05$), although there was no difference between the latter two groups throughout the experiment except week 24. At week 24 the body weight was 108 ± 7 g, 184 ± 12 and 170 ± 9 in the GeO_2, Ge-132 and control groups, respectively. Following the cessation of GeO_2 at week 24, the reduced body weight increased markedly but did not reach the level of other two groups by week 40.

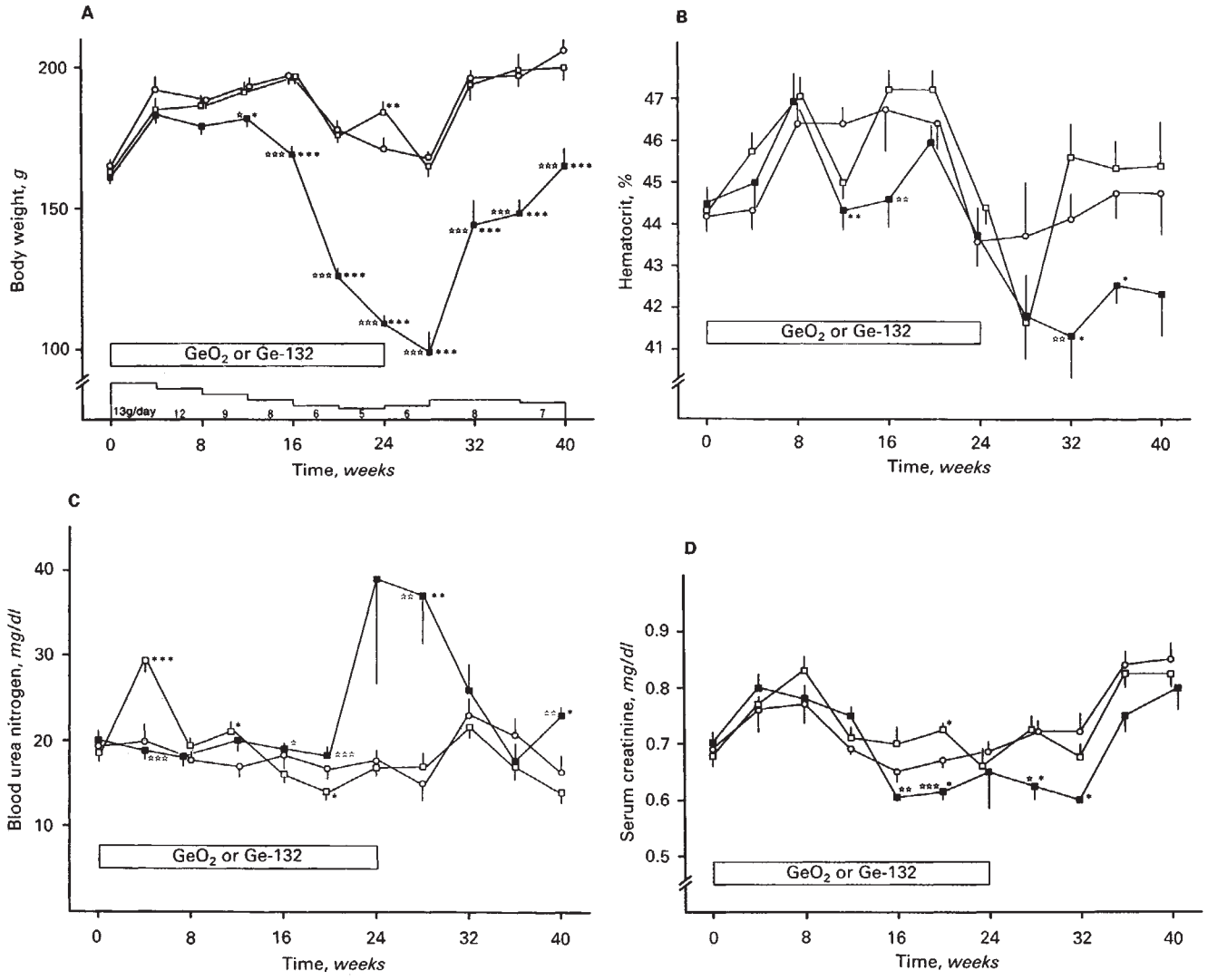

Fig. 1. *Time course of body weight, hematocrit, blood urea nitrogen, and serum creatinine.* **A** Body weight and amount of food taken (g). **B** Hematocrit (%). **C** Blood urea nitrogen (mg/dl). **D** Serum creatinine (mg/dl). Data are expressed as the mean ± SEM. Symbols are Group: GeO_2 (■), Ge-132 (□), and Control (○). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (vs. Control group), $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (vs. Ge-132 group).

Systolic blood pressure did not differ among the groups except week 24, when that in the GeO_2 group (109 ± 4 mm Hg) was significantly higher than that in the Ge-132 group (101 ± 6; $P < 0.01$). However, there was no significant difference between the GeO_2 or the Ge-132 group and the control group (105 ± 5).

Hematocrit in the GeO_2 group was 44.3 ± 1.6%, which was significantly lower than that of 46.4 ± 1.1% in the control group at week 12 ($P < 0.01$) as shown in Figure 1B. Even after GeO_2 was discontinued, hematocrit in this group remained significantly lowered, compared with that in the control group at weeks 32 and 36 ($P < 0.05$). In contrast, there was no significant difference in hematocrit between the Ge-132 and control groups throughout the experiment.

Blood and urine chemistries. BUN in the Ge-132 group increased at weeks 4 and 12, following by a significant decrease at week 20 compared with the control group ($P < 0.05$, Fig. 1C). In contrast, BUN in the GeO_2 group increased greatly at week 28 and slightly but significantly at week 40 (37 ± 11 and 23 ± 1 mg/dl, respectively, $P < 0.05$). BUN in the control group remained unchanged during the 40 weeks observation.

Serum creatinine was significantly lower in the GeO_2 group than in the Ge-132 or control group at weeks 16, 20, 28, and 32 ($P < 0.05$; Fig. 1D). BUN/serum creatinine ratio was significantly higher in the GeO_2 group than in the Ge-132 or control group from week 16 to 28 and at week 40 ($P < 0.05$).

C_{Cr} was significantly lower in the GeO_2 group (123 ± 51 ml/day/g kidney wt) than in the Ge-132 group (452 ± 151) or control (436 ± 243) at week 24 ($P < 0.05$). Both FE_{Na} and $NAG/U_{protein}$ levels were two to three times higher in the GeO_2 group (1.3 ± 0.4% and 39 ± 14 mU/mg) than in the Ge-132 group (0.3 ± 0.1 and 9 ± 2) or control (0.5 ± 0.2 and 18 ± 11) at week 24 ($P < 0.05$).

Urinary protein excretion was slight and did not differ among

Table 1. Serum cholesterol (Chol), GOT, GPT, and phosphate (P)

Group	No. of rats	Chol	GOT	GPT	P
		mg/dl	*U/liter*		*mg/dl*
Week 24					
GeO_2	13	103 ± 24[a,d]	419 ± 312[a,c]	42 ± 18[a,c]	5.6 ± 2.0[d]
Control	10	73 ± 12	83 ± 32	19 ± 4	4.2 ± 1.0
Ge-132	13	76 ± 9	79 ± 17	21 ± 6	3.8 ± 0.5
Week 40					
GeO_2	4	67 ± 4	73 ± 11	22 ± 2	4.1 ± 0.4[b,e]
Control	5	55 ± 9	86 ± 20	19 ± 4	3.1 ± 0.6
Ge-132	4	59 ± 4	76 ± 13	20 ± 2	2.9 ± 0.2

Abbreviations are: GeO_2, rats were administered with 75 mg/kg body wt/day of GeO_2; Ge-132, rats were administered with 120 mg/kg body wt/day of Ge-132; Control, rats were bred with a Ge free diet. Ge compounds were discontinued at week 24. Data are expressed as the mean ± SD.

[a] $P < 0.001$, [b] $P < 0.05$ (vs. Control group)

[c] $P < 0.001$, [d] $P < 0.01$, [e] $P < 0.05$ (vs. Ge-132 group)

Table 2. Organ weight

Group	No. of rats	Kidney	Heart	Liver
		g		
Week 24				
GeO_2	6	1.45 ± 0.14[a,e]	0.60 ± 0.06	3.55 ± 0.14[c,d]
Control	5	1.09 ± 0.08	0.56 ± 0.04	4.45 ± 0.68
Ge-132	5	1.24 ± 0.10	0.59 ± 0.04	5.44 ± 0.42[c]
Week 40				
GeO_2	4	1.15 ± 0.02	0.60 ± 0.05	4.43 ± 0.25
Control	5	1.22 ± 0.04	0.64 ± 0.04	5.04 ± 0.70
Ge-132	4	1.13 ± 0.09	0.60 ± 0.04	3.75 ± 0.25[b]

Ge compounds were discontinued at week 24. Data are expressed as the mean ± SD.

[a] $P < 0.001$, [b] $P < 0.01$, [c] $P < 0.05$ (vs. Control group)

[d] $P < 0.001$, [e] $P < 0.05$ (vs. Ge-132 group)

the three groups. Neither hematuria nor glucosuria was detected in any of the rats from the three groups throughout the experiment.

Serum cholesterol, GOT, GPT, and phosphate were all significantly more elevated in the GeO_2 group than in either the Ge-132 or control group at week 24 ($P < 0.01$). At week 40, or 16 weeks after treatment discontinued, the serum phosphate remained high in the GeO_2 group ($P < 0.05$) but other variables had returned to the control levels (Table 1). In contrast, neither serum total protein nor the albumin levels differed among the groups.

Organ weight. Kidney weight increased in the GeO_2 group at week 24, being significantly higher than in the Ge-132 or control group ($P < 0.05$). In contrast, liver weight significantly decreased in the GeO_2 group but increased in the Ge-132 group at week 24 ($P < 0.05$). At week 40, liver weight decreased in the Ge-132 group, compared with the control group ($P < 0.05$; Table 2).

Germanium concentration in tissue and urine. At week 6, kidney concentration of Ge was higher in the GeO_2 group (11.2 ± 2.7 μg/g wet wt) compared to the Ge-132 group (0.8 ± 0.2; $P < 0.001$, Student's *t*-test) or control (not detected).

As shown in Table 3, Ge concentrations in the kidney, heart, liver, muscle, and hair were higher in the GeO_2 group than in the other groups at week 24. Tissue Ge concentrations in these organs were reduced but remained higher in the GeO_2 group than in the Ge-132 or control group at week 26 or two weeks after the discontinuation of Ge compounds. Particularly in the kidney and hair, tissue Ge concentration was still significantly higher in the GeO_2 group than in the Ge-132 or control group even 16 weeks after Ge was discontinued (at week 40; $P < 0.05$ for kidney or hair, respectively).

The urinary excretion of Ge at week 24 was significantly higher in the GeO_2 (768.9 ± 376.9 μg/day) and Ge-132 (539.5 ± 228.3) groups than in the control group (8.1 ± 8.7; $P < 0.001$). However, there was no difference between the former two groups.

Morphological findings. At week 24, vacuolar degeneration was observed in the tubular epithelium in the GeO_2 group by light microscopic examination. Granular particles stained in purple with PAS and large vacuoles stained with SUDAN III were predominantly detected in the epithelium of dilatated distal tubules and collecting tubules. Tubular atrophy, epithelial cell necrosis and desquamation were partly evident in the GeO_2 group. Although proximal tubular injury also occurred such as vacuolar alteration of epithelial cells, the above mentioned histological changes localized mainly in distal segment of the tubules. A slightly sclerotic change was also partly observed in the GeO_2 group, although the glomeruli were mostly intact (Fig. 2A, B). By electron microscopic observation, the number of mitochondria increased and electron-dense granules were characteristically detected in the swollen mitochondria in distal and collecting tubule epithelium, in which both the cristae and matrix were often seen to have disappeared in the GeO_2 group (Fig. 2C). The same granules were also detected in the lysosomes. Sulfur, zinc, and a trace of Ge were found in these

Table 3. Tissue concentration of germanium (Ge)

Group	No. of rats	Kidney	Heart	Liver	Muscle	Hair
		μg/g wet wt				
Week 24						
GeO_2	6	34.0 ± 24.6[b,e]	7.9 ± 3.7[e]	27.7 ± 16.6	8.7 ± 4.3	171.2 ± 96.6 (13)[a,d]
Control	5	0.4 ± 0.4	ND	ND	ND	2.8 ± 1.5 (10)
Ge-132	5	0.8 ± 0.2	0.3 ± 0.2	ND	ND	7.2 ± 3.6 (13)
Week 26						
GeO_2	4	2.3 ± 0.6	0.5 ± 0.6	1.2 ± 0.2[f]	0.8 ± 0.3	107.8 ± 18.2 (8)[a,d]
Control	5	—	—	—	—	2.5 ± 0.9
Ge-132	4	1.6 ± 1.7	0.2 ± 0.1	0.6 ± 0.5	ND	5.2 ± 1.7 (8)
Week 40						
GeO_2	4	1.1 ± 0.1[b,e]	ND	0.3 ± 0.2	ND	14.2 ± 7.9[c,f]
Control	5	0.2 ± 0.1	ND	ND	ND	4.7 ± 1.3
Ge-132	4	0.4 ± 0.4	0.2 ± 0.3	0.3 ± 0.3	ND	3.8 ± 2.9

Tissue concentration of Ge in GeO_2 or Ge-132 group at week 26 was compared with that in the control group at week 24. The differences between GeO_2 and Ge-132 groups are calculated using Student's *t*-test in the heart at weeks 24 and 26, or in the liver at weeks 26 and 40. Ge compounds were discontinued at week 24. ND; not detected. The number of rats is in parentheses. Data are expressed as the mean ± SD.
[a] $P < 0.001$, [b] $P < 0.01$, [c] $P < 0.05$ (vs. Control group), [d] $P < 0.001$, [e] $P < 0.01$, [f] $P < 0.05$ (vs. Ge-132 group).

inclusions by EDX analysis. These changes were not evident in the Ge-132 and control groups. No deposition of immunoglobulin or complement such as IgG, IgA, IgM or C_3 was observed in any of the rats among the groups. No significant pathological changes were observed in the myocardial cells in any group.

At week 40 severe tubular atrophy, interstitial fibrosis and cellular infiltration manifested in the GeO_2 group, although PAS-positive granules in distal tubules almost disappeared (Fig. 2D). These changes were not detected in either the Ge-132 or the control group. As depicted in Table 4, tubular changes were more marked in the GeO_2 group than those in the Ge-132 or control group ($P < 0.001$).

Study II: High dose study of GeO_2 for EDX analysis

All rats became cachectic after a one week administration of 450 mg/kg/day GeO_2. Vacuolation and purple granules were also observed in the renal tubular epithelium. The Ge · K line was clearly detected from the electron-opaque inclusion body of swollen mitochondrion in the distal tubular epithelium in the unstained ultrathin sections by EDX analysis with other elements, which included P · K (phosphorus), S · K (sulfur), Cl · K (chloride), Ca · K (calcium), Fe · K (iron) and Zn · K (zinc). A very large amount of iron and sulfur seemed to be included in the tissue, as shown in Figure 3 and Table 5.

Discussion

We have previously reported four patients who developed characteristic renal deterioration after the long-term ingestion of the Ge-containing compounds, as Ge-induced nephropathy [18]. Using the X-ray diffraction method, we observed GeO_2 in the Ge compounds which these patients had ingested [19]. The present study confirmed that GeO_2 induced chronic nephrotoxicity, while an organic germanium compound Ge-132 did not have any toxic effects. Furthermore, liver dysfunction as well as systemic toxicities (weight loss, anemia, and central nervous disorders) developed only in the GeO_2 group of the present vivo study, and GeO_2 proved to be more cytotoxic than Ge-132 in the macrophages of the in vitro study (unpublished data). These results indicate the clear difference in toxicity among the different Ge-compounds. Rosenfeld and Wallace [15] reported that germanium itself is pharmacologically inert, and toxicity of any compound seems due to the toxic effects of the nongermanium inorganic, aliphatic, or aromatic portion of the molecules. Although Venugopal and Luckey [17] reported that organic Ge compounds are more toxic than inorganic compounds, the present study revealed evidence to the opposite. Toxicological and pharmacokinetic studies previously revealed that Ge-132 has an extremely low toxicity [9, 37, 38]. Likewise, our present study demonstrated that Ge-132 had an extremely low toxicity. There have been some reports [9–11] describing that Ge-132 activates macrophage, possesses anti-oxidant activity [39], and has a protective effect on the warm ischemic injury in renal transplantation [40].

The precise mechanism of GeO_2-induced renal toxicity is still unclear. In the present study, the creatinine clearance significantly decreased in the GeO_2 group compared to the Ge-132 and the control groups. The decrease in body weight and the elevation of the BUN/serum creatinine ratio suggest that the renal functional deterioration might be induced by the reduction in ECF associated with gastro-intestinal toxicity of GeO_2 [41]. However, pair-feeding was performed to maintain the same amount of food intake among the groups, and a decrease in FE_{Na} was not observed in the GeO_2 group. Furthermore, the blood pressure in the GeO_2 group was higher than that in the other groups at week 24. These results suggest that the weight loss and the elevation of BUN/serum creatinine ratio is due to catabolism induced by the systemic toxicity rather than dehydration. Serum creatinine did not increase regardless of the

Fig. 2. *Renal histology in GeO_2 group.* **A, B** Vacuolar degeneration is observed in the tubular epithelium, and tubular atrophy is also partly observed. Purple dense deposits, large vacuoles, and dilatation are detected in the distal segments. Glomeruli are slightly sclerotic but mostly intact. (week 24, PAS-stain, A. ×220, B. ×320. **C** Electron-dense inclusions exist in the rounded mitochondria of distal tubular epithelium. (week 24, ×45,000). **D** Tubular atrophy, interstitial fibrosis and cell infiltration are manifested. Glomeruli are mostly intact. (week 40, PAS-stain, ×220)

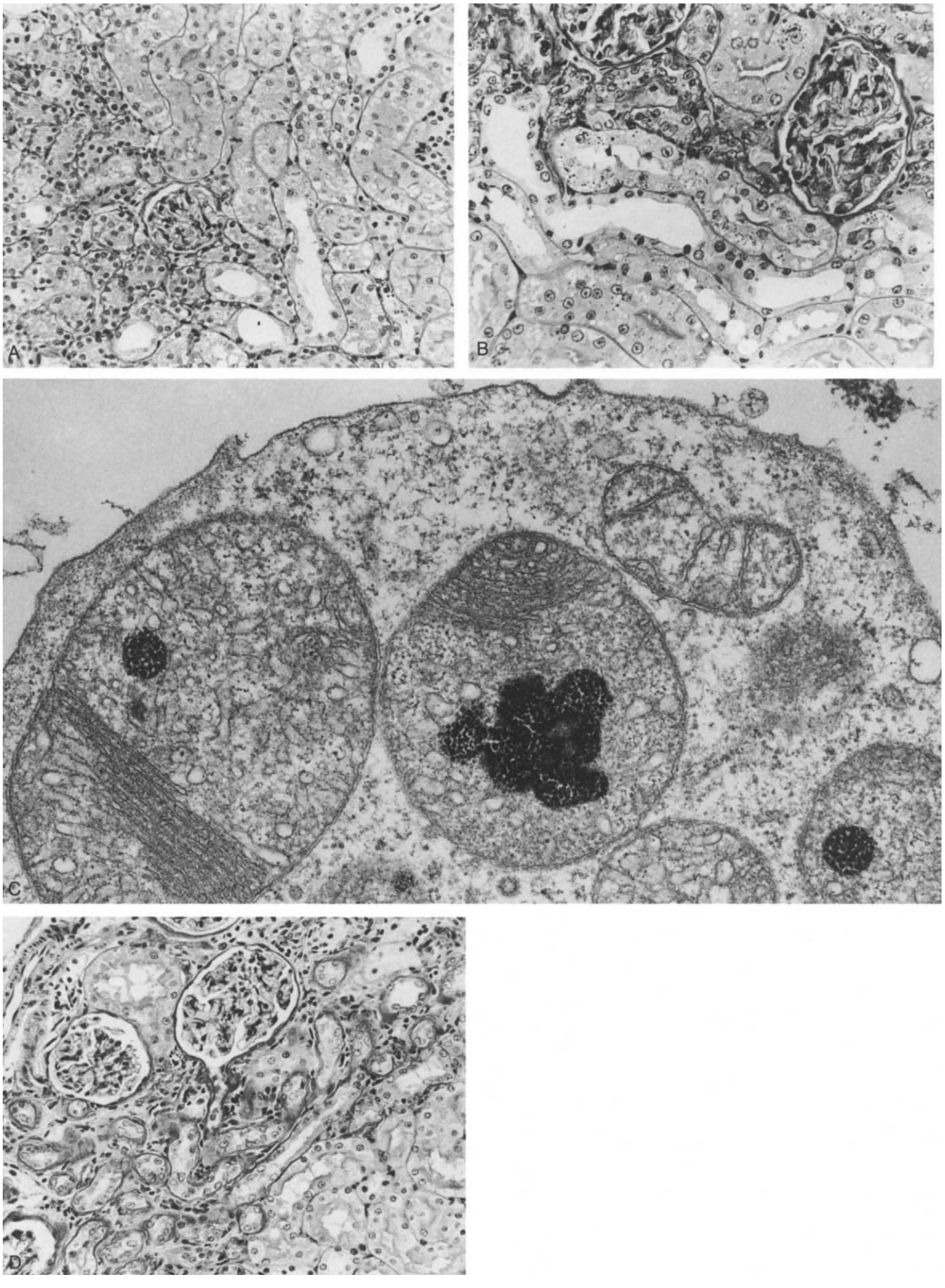
A
B
C
D

Table 4. Renal tubular degeneration (%)

Group	Week 24	Week 26	Week 40
GeO_2	95 ± 9 (6)[a,b]	85 ± 6 (4)[a,b]	77 ± 9 (4)[a,b]
Control	1 ± 1 (5)	—	2 ± 1 (5)
Ge-132	3 ± 1 (5)	4 ± 2 (4)	2 ± 2 (4)

Renal tubular degeneration in GeO_2 or Ge-132 group at week 26 is compared with that in the control group at week 24. Ge compounds were discontinued at week 24. The number of rats is in parentheses. Data are expressed as the means ± SD.

[a] $P < 0.001$ (vs. Control group)

[b] $P < 0.001$ (vs. Ge-132 group)

Table 5. Amounts of elements in the electron opaque inclusion body

Element	CPS	BKGD	CPS/BKGD	WT%
P · K	3.42	4.46	0.77	7.21
S · K	9.65	4.33	2.23	19.07
Cl · K	9.16	4.15	2.21	17.92
Ca · K	2.05	3.41	0.60	3.94
Fe · K	17.30	3.28	5.27	40.74
Zn · K	1.50	3.29	0.46	4.40
Ge · K	2.03	2.60	0.78	6.72

Abbreviations are: CPS, X-ray pulse counts per second; BKGD, background; WT%, atomic weight ratios %. Data are calculated from Figure 3.

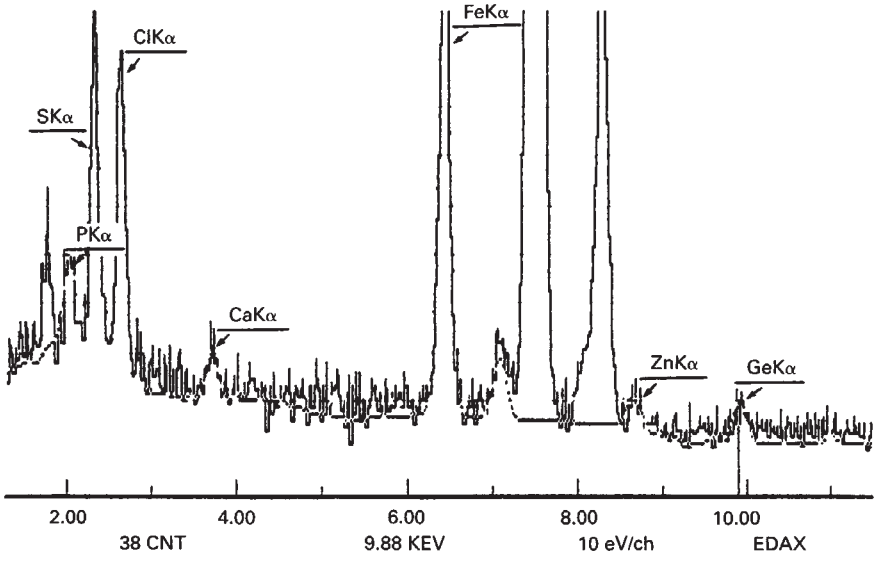

Fig. 3. *X-ray energy spectrum obtained from the electron-opaque inclusion body in the unstained 450 mg/kg GeO_2 administered rat renal distal tubular epithelial cell.* The X-ray energy peaks of P · K, S · K, Cl · K, Ca · K, Fe · K, Zn · K, and Ge · K were clearly demonstrated. A remarkable amount of S · K was noted with other elements. Nickel grid was used and the currsor is localized at the location of Ge · K X-ray energy peak, 9.88 keV. (60 kV, 200 seconds, calculated with "Super Quant" program of EDAX-PV-9800 analyzing system).

reduction in creatinine clearance. The discrepancy between serum creatinine and creatinine clearance may be explained by the reduction in lean body mass.

At week 6, prior to a major fall in GFR, kidney concentration of Ge was higher in the GeO_2 group than in the Ge-132 or control group, and Ge was also detected in the electron-dense granules in the present study. Therefore, the marked accumulation of the compound in the renal tissue seems to play an important role. In a previous death case, in which the patient had taken a long-term GeO_2 preparation, Ge accumulation into several organs including the kidney was observed [41]. Since neither immunoglobulin deposition nor significant interstitial cell infiltration was observed in the kidney at week 24 of GeO_2 treatment, the immunological mechanism does not seem to work in these renal changes. Tubular histological damage such as vacuolar degeneration, atrophy, necrosis and desquamation, and biochemical abnormalities such as the increase of FE_{Na} and NAG excretion, suggest that the direct cytotoxic effect of GeO_2 on the renal tubular epithelium contributes to renal damage in the GeO_2-treated rats.

Mitochondrion is the major source for the production of cellular high energy phosphate, ATP. In addition, enzymes of the Krebs cycle, such as oxidative phosphorylation and respiratory chain, are found to be largely confined to the mitochondrial fraction [42]. The ATP content and Na-K-ATPase activity in renal cortex of rats were reduced by cadmium administration, whereby the amount of cadmium increased in the mitochondrial fraction and mitochondria became swollen [43]. Cremer and Aldridge [16] reported that tri-n-butyl-germanium had some inhibitory effects on the respiration and oxidative phosphorylation processes of rat liver mitochondria, and a high concentration of this compound causes gross swelling of the mitochondria. Since cell degeneration and gross mitochondria swelling were evident in the present study, mitochondrial dysfunction might result in compromized cell's energy supply and structural disintegration in the GeO_2-induced nephrotoxicity.

Metallothionein, which contains sulfur, zinc, iron, and copper, is well known as a binding protein of cadmium [44, 45]. In the present study, these three elements were detected in the electron-opaque inclusion, and their distribution ratios in both CPS and WT% were high for sulfur, suggesting a possible role of metallothionein in GeO_2-induced nephrotoxicity. Further investigations may be needed to clarify the mechanism.

The histological damage in the GeO_2-treated rats at week 24 is mild compared with acute renal failure induced by heavy metals. In this model, vacuolar degeneration is prominent, but the general outline of the proximal tubule cells and nuclei are preserved. These histological characteristics were similarly

found in our human study [19], in which patients with deteriorated renal function had a well-preserved histology. The clinical feature of this nephropathy was that of subacute renal failure rather than that of acute renal failure. The renal dysfunction persisted for a long time even after GeO_2 administration was discontinued. However, proteinuria or hematuria was not evident, and other specific clinical symptoms or signs were lacking [19]. In the present study, neither interstitial fibrosis nor cellular infiltration was demonstrated at week 24 in the GeO_2 group, but these changes became manifest at week 40 or 16 weeks after discontinuation, when Ge concentration in the kidney remained high. These findings suggest that GeO_2-induced nephrotoxicity results in an irreversible chronic interstitial nephritis and lasts for a long-term period even after removing GeO_2 from food. This nephropathy may be in part related to the long-lasting GeO_2 accumulation in renal tissue.

In summary, we demonstrated the severe nephrotoxicity induced by GeO_2. Chronic GeO_2-induced nephropathy is characterized by progressive and long-lasting renal damages with a lack of any abnormal urinalysis. This nephropathy, which seems to be different from the previously reported renal diseases, might be a new and independent disease entity.

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