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Oral Repeated-dose Toxicity Studies Especially in the Liver and Kidney of Rats Administered with Organic Germanium-fortified Yeasts

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Abstract

The object of this study was to examine whether the germanium fortified yeast administered to SD rat is accumulated in the liver and kidney. The administration doses were within 2,000 mg/kg which is the level of NOAEL (no observed adverse effect level) proved through the previous study of single/consecutive oral toxicity test. There were no significant clinical symptoms and mortality following the administration of organic germanium-fortified yeast (0, 500, 1,000, 2,000 mg/kg) during the whole test period, and also no difference in the consumed amount of feed and water for each group. No significant abnormalities of hematology and blood chemistry parameters were found in all groups of organic germanium-fortified yeast (0, 500, 1,000, 2,000 mg/kg). The amount of germanium accumulated in liver and kidney was 0 g/kg by ICP-AES method in the group of organic germanium-fortified yeast. In the positive control group of GeO_2 (150 mg/kg), the amount of accumulation was shown to 3135.0 and 4277.2 g/kg in each female and male kidney and 1044.3 and 2135.8 g/kg in each female and male liver, respectively. Organic germanium-fortified yeast, a biosynthetic product resulting from putting germanium into yeast, did not show any clinical symptoms, blood chemical significance, and residues in kidney and liver. It could be inferred that the non-toxic amount of organic germanium-fortified yeast was up to 2,000 mg/kg.

Key words: germanium-fortified yeast, repeated-dose toxicity, kidney, liver

INTRODUCTION

Germanium is a microelement naturally found in soil, water, plant, and animal body, and an adult generally takes about 0.367~1.5 mg daily (1). However, the symptoms for germanium has not been reported (2). The known functions of germanium are cell activation (3), strengthening the immunity, activation of oxygen provided by removing hydrogen ion of the waste material in blood (4,5), regulating immunity (6), antiviral effect (5-7), and secreting heavy metals by binding to cationic heavy metals, that is, excretion and detoxification of heavy metals such as mercury and cadmium (8,9). In spite of these various positive effects, there are many controversies over the safety of germanium. For the issue about the germanium poisoning, it was reported the 9 patients died (1) with clinical symptoms of kidney-toxic and anemia. Generally, this toxicity is a common symptom in inorganic germanium such as germanium dioxide (GeO_2) or germanium tetrachloride (10,11), whereas there are no reports for the toxicity in case of organic germanium of spirogermanium, germanium lactate, and carboxyethylgermanium sesquioxide (Ge-132), but its side effects are disrupted (1).

It was reported the yeast could convert inorganic germanium to organic one by proving that yeast could absorb the high density inorganic germanium and it was more than 95% of organic germanium which accumulated in the yeast (12). Klapcinska and Chmielowski (13) showed the accumulated germanium in *Ps. Putida* cell was mainly found in soluble fraction, and most of them was bound to nucleic acid and protein through the electron microscope analysis. In addition, the report that the yeast detoxifies the organic element (14) means the toxic elements are removed by biological anabolism of converting inorganic germanium to organic germanium. Van Dyke et al. (15) reported that *Saccharomyces cerevisiae* could be used in production of GeO_2 which showed the possibility for the production of organic germanium using this yeast, the full-scale research about it was started.

Herein we developed structurally safe organic germanium fortified yeast using the yeast with high industrial value (16,17), and examined its safety. The object

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of this study is to examine whether the orally administered germanium is accumulated in liver and kidney within the range of 2,000 mg/kg which is the scale of NOEL (no observed effect level) proved through single/consecutive oral administration of organic germanium fortified yeast.

MATERIALS AND METHODS

Test compound

Saccharomyces cervisiae (KCTC 1199) was used in this experiment. Organic germanium-fortified yeast (germanium: 3,210 ppm) manufactured by GERANTI PHARM Ltd. was used for the test material. The test material was stored in a refrigerator, and freshly prepared by different dosage for each group. The test materials for all groups were solved in the lukewarm water (distilled water) before administration.

Test animals

10 rats of each male and female 4-weeks old Sprague-Dawley (SD) rat were purchased from Hallym Laboratory Animal Science Co., and placed into random groups through 1-week acclimation. The environmental conditions for quarantine, acclimation and breeding were as follows, $23 \pm 2^{\circ}C$ of temperature, $55 \pm 10\%$ of relative humidity, $10 \sim 12$ times/hr of air-conditioning, 12 hours of illumination time and $15 \sim 200$ Lux of that intensity. Feed and water were given freely.

Repeated-dose toxicity study

The test materials were orally administered once a day. The dosage was 500, 1,000, 2,000 mg/kg for the organic germanium-fortified yeast group and GeO_2 150 mg/kg for positive control group (18) based on the results of previous study and administered repeatedly for 13 weeks. The negative control group (0 mg/kg) was fed with yeast without germanium.

The general symptoms were observed daily for each animal during the test, and weight change and feed and water consumptions were measured every week.

For hematology test, blood samples collected from saphenous vein were left for 30 minutes at room temperature to coagulate and centrifuged (3,000 rpm, 30 min), then it was tested for blood glucose, total cholesterol, blood urea nitrogen (BUN), total bilirubin, aspartate transaminase (AST), alanine transaminase (ALT), triglyceride (TG), creatinine, albumin, Ca, alkaline, phosphatase (ALP), and total protein. The accumulation of germanium was analyzed with sampling tissues from the liver and kidney (left, right) of all animals. Germanium standard solution (Sigma-Aldrich, Ge standard solution, USA) was used and ICP-MS (Inductively Coupled Plasma Mass Spectroscopy, ELAN 6100, Perkin-Elmer SCIEX) was used in resulting solution of second reaction of HNO_3 and H_2O_2 after HNO_3 reaction. During this time, each sample was measured 3 times.

Statistical analysis

Result was analyzed by SAS (Statistical Analysis System) PC package program. Mean value and standard deviation were calculated for each group. For comparison by dosage, the difference between each group was examined with Duncan's multiple range tests for items, which showed significant difference in ANOVA analysis.

RESULTS AND DISCUSSION

General symptoms

There were no significant clinical symptoms by dosage during the test period after administering organic germanium fortified yeast in dosages of 0, 500, 1,000, 2,000 mg/kg), and no animals died (Table 1). The weight change was showed in Fig. 1, which indicates that there was no significant difference among groups administered with organic germanium-fortified yeast (0, 500, 1,000,

Table 1. Mortality of rats on germanium-fortified yeast

Sex	Dose (mg/kg)	No. of animal	1	2	3	4	5	6	7	8	9	10	11	12	13	Mortality (dead/total)
Male	G1	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0% (0/10)
	G2	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0% (0/10)
	G3	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0% (0/10)
	G4	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0% (0/10)
	G5	10	0	0	0	0	0	0	0	0	1[1)]	0	1	0	1	30% (3/10)
Female	G1	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0% (0/10)
	G2	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0% (0/10)
	G3	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0% (0/10)
	G4	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0% (0/10)
	G5	10	0	0	0	0	0	0	0	1	0	1	0	1	1	40% (4/10)

G1, control (0 mg/kg); G2, germanium-fortified yeast 500 mg/kg; G3, germanium-fortified yeast 1,000 mg/kg; G4, germanium-fortified yeast 2,000 mg/kg; G5, GeO_2 150 mg/kg. [1)]Death No.

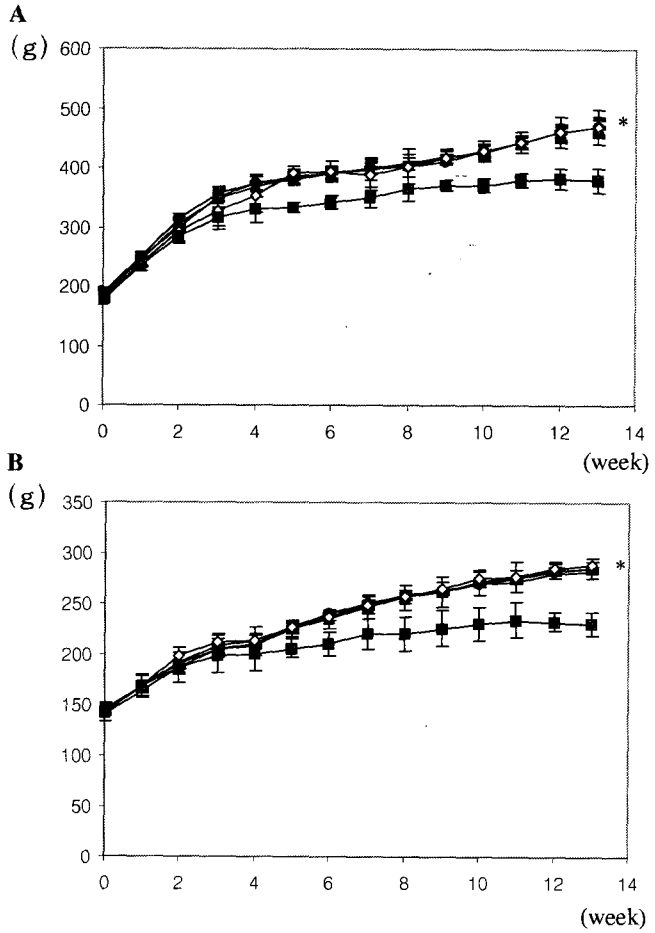

Fig. 1. Weight changes of rats by 13 weeks administration of organic germanium-fortified yeast.
A: male, B: female.
-▬-: control (0 mg/kg), -●-: germanium-fortified yeast 500 mg/kg, -▲-: germanium-fortified yeast 1,000 mg/kg, -◆-: germanium-fortified yeast 2,000 mg/kg, -■-: GeO_2 150 mg/kg.
*Significant difference between germanium-fortified yeast group and GeO_2 group at $p<0.05$.

2,000 mg/kg) but, there increased the weight for the organic germanium-fortified yeast (0, 500, 1,000, 2,000 mg/kg) increased more significantly than the GeO_2 (150 mg/kg) group ($p<0.05$). According to Sanai et al. (19), as like the result of weight change by 13-weeks administration of GeO_2 (150 mg/kg), this test showed that the weight for inorganic GeO_2 group did not increase after 3-weeks than that of organic germanium-fortified yeast. There was no difference in the consumption of feed and water in both female and male test group and control group (data not shown). It was proved that the safety of oral administration up to the concentration of 2,000 mg/kg in resulting from single/consecutive toxicity test (18,20) and genetic toxicity test (21) for organic germanium-fortified yeast, and Lee et al. (17) also proved the structure of organic germanium-fortified yeast was stable through the study about the binding condition for germanium. As we can see in this study, there was no significant change in weight, motility and clinical symptoms when oral administration of organic germanium-fortified yeast, which is considered this material, may be safe within the dosage of 2,000 mg/kg.

Hematology and blood chemistry change

For the group administered with organic germanium-fortified yeast (0, 500, 1,000, 2000 mg/kg), it did not show significant disorder in hematology and blood chemistry tests for all groups (Table 2), but the values of ALP which designated the liver function significantly increased ($p<0.05$). BUN and creatinine for kidney function decreased in the positive control group of GeO_2 (150 mg/kg) but those were not significantly different. So, it showed similar results with Matsusaka et al. (22) and Okada et al. (23), reported GeO_2 inducing kidney toxicity, and this may be considered inorganic GeO_2 influences on the function of liver and kidney. For the case of organic germanium-fortified yeast, its results were similar with 10-months consecutive administration (18) and 13-weeks repetitive toxicity test by Ahn et al. (24), and there was no blood chemistry change after oral administration. In particular, it may be considered the oral administration within 2,000 mg/kg dosage seeing from that there was no difference in blood chemistry values related to liver and kidney in organic germanium-fortified yeast group. Alkaline phosphatase (ALP) is a positive indicator for the serum concentration of parathyroid hormone or liver diseases, which resulted in the increase for GeO_2 administered group, particularly female with GeO_2 150 mg/kg.

In general, toxicity has been reported to come from inorganic compounds such as germanium dioxide (GeO_2) and germanium tetrachloride. On the other hand, organo germaniums such as spirogermanium, germanium lactate citrate and carboxylethylgermanium sesquioxide (Ge-132) have been reported to exert negligible adverse effects (1).

Germanium accumulation in liver and kidney

It was shown in Table 3 that the results of ICP-AES for measuring accumulated germanium in liver and kidney for the group administered organic germanium-fortified yeast (0, 500, 1,000, 2,000 mg/kg). There was no accumulated germanium in the group of organic germanium fortified yeast, but evidenced in positive control GeO_2 (150 mg/kg) that germanium was accumulated with 3135.09 (F) and 4277.2 (M) g/kg in the kidney, and 1044.3 (F) and 2135.8 (M) g/kg in the liver. This means inorganic GeO_2 was not secreted through the excretory organ by metabolizing, but accumulated on the liver and kidney, each of the storage organ and filter

Table 2. Serum biochemical values responded to dosages of germanium-fortified yeast in the male and female rat

Parameters		Dosage of germanium-fortified yeast (mg/kg)				GeO_2
		0	500	1,000	2,000	150 mg/kg
GLU (mg/dL)	Male	96.5±10.3[1)]	99.8±10.7	104.3±11.3	105.7±18.2	94.7±11.6
	Female	98.5±10.1	97.2±13.5	98.2±7.5	98.8±15.5	97.0±10.3
TC (mg/dL)	Male	51.5±5.85	50.8±5.54	49.01±6.83	48.02±4.85	45.3±4.43
	Female	59.6±10.8	58.7±13.7	58.0±10.3	57.5±12.9	56.8±9.8
TG (mg/dL)	Male	86.7±13.6	82.2±18.5	75.9±22.1	60.9±19.3	42.7±16.4
	Female	43.3±15.8	40.8±7.3	31.6±8.8	35.7±10.3	30.8±8.9
TP (g/dL)	Male	6.3±0.3	6.2±0.2	6.3±0.3	6.4±0.2	6.0±0.3
	Female	6.4±0.3	6.3±0.2	6.4±0.3	6.5±0.3	6.4±0.3
Albumin (g/dL)	Male	3.3±0.2	3.3±0.2	3.2±0.3	3.4±0.2	3.1±0.2
	Female	3.7±0.2	3.5±0.2	3.6±0.3	3.4±0.2	3.3±0.2
BUN (mg/dL)	Male	12.9±1.28	12.5±1.31	12.28±1.12	12.98±1.29	11.45±1.18
	Female	16.8±2.5	16.8±2.0	15.9±2.2	16.9±1.8	14.3±2.0
AST (IU/L)	Male	168.3±27.7	165.4±30.0	158.2±26.8	166.4±27.1	173.5±36.8
	Female	135.4±22.2	141.3±28.3	138.5±18.3	137.6±20.5	149.3±32.1
ALT (IU/L)	Male	32.3±3.0	32.8±2.3	32.0±3.7	31.6±2.8	43.1±4.3
	Female	22.2±3.3	22.8±5.3	22.6±3.4	24.8±4.8	38.3±2.6
ALP (IU/L)	Male	88.3±13.2	85.5±10.8	89.3±11.3	90.3±12.5	97.3±20.8
	Female	62.6±10.4[ab2)]	65.3±12.5[ab]	63.8±10.8[ab]	60.3±11.8[b]	74.1±13.6[a]
CRE (mg/dL)	Male	0.54±0.03	0.55±0.02	0.54±0.02	0.56±0.03	0.50±0.02
	Female	0.61±0.05	0.59±0.04	0.59±0.05	0.60±0.04	0.51±0.05

GLU, blood glucose; TC, total cholesterol; TG, triglyceride; BUN, blood urea nitrogen; AST, aspartate transaminase; ALT, alanine transaminase; CRE, creatinine; ALP, alkaline phosphatase; TP, total protein.
[1)]Values are mean±SD of 10 rats.
[2)]Values with different superscripts within a row indicate significant difference at $p<0.05$.

Table 3. Germanium concentrations responded to the dosage of germanium-fortified yeast in the kidney and liver of rat (μg/kg)

Parameters		Dosage of germanium-fortified yeast (mg/kg)				GeO_2
		0	500	1,000	2,000	150 mg/kg
Kidney	Male	0.0±0.0[1)]	0.0±0.0	0.0±0.0	0.0±0.0	4277.2±226.3
	Female	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	3135.0±163.1
Liver	Male	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	2135.8±121.5
	Female	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	1044.3±98.5

[1)]Values are mean±SD of 10 rats.

for inorganic materials in our body respectively. On the contrary, the organic germanium-fortified yeast, biosynthesized germanium, is considered not to be accumulated on the liver or kidney seeing from there was no evidence for accumulation.

In studies of four individuals, Schroeder and Balassa (25) reported a total of 1.4 mg germanium of the calculated average daily intake of 1.5 mg in urinary excretion. This suggests that dietary germanium is well absorbed from the intestinal tract and excreted largely through the kidneys. Also in Chen et al. studies (26), a single oral dose of 100 mg Ge-132, the urinary excretion rate of germanium peaked at around 3 hr. The rate of absorption of Ge-132 from intestine seems to be around 30%. The results of the excretion study indicate that the absorbed compound is completely excreted in urine within 72 hr (27). In addition, the excreted germanium in urine was isolated and proved to be identical to the germanium originally administered. These result indicate that germanium is not accumulated in the specific organs and any other metabolites were not detectable.

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