Promotive Effects of the Dietary Organic Germanium Poly-*trans*-[(2-carboxyethyl) germasesquioxane] (Ge-132) on the Secretion and Antioxidative Activity of Bile in Rodents

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Poly-*trans*-[(2-carboxyethyl) germasesquioxane] (Ge-132) is the most common organic germanium compound. This compound has many physiological effects, which are mediated via the modulation of immune-system activation. The intake of dietary Ge-132 causes fecal color changes in humans, and we studied the mechanism of this in a rodent model. Male Wistar rats were given a diet containing 0.05% Ge-132 for two weeks and were compared with rats given a germanium free diet. The color of their feces and cecal contents changed from grayish-green to yellow in rats fed with Ge-132. The concentrations of stercobilin, a major fecal pigment, and total bile acids in cecal contents, were significantly increased by dietary Ge-132. Stercobilin is a metabolite of the bile pigment bilirubin. These results were produced by increases in bile components, such as bilirubin and bile acids, and showed that dietary Ge-132 promotes bile secretion into the intestine. Next, we administered Ge-132 (per os) to male rats at 50 mg/kg body weight per day for four days to reveal its effect on bile (and particularly on bilirubin). Bile juice samples were collected, and their bilirubin content and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity were analyzed. The bilirubin level in the bile was significantly increased by the administration of Ge-132, and the DPPH radical scavenging activity of bile was also significantly increased. As the increases in these two factors were correlated, we supposed that the anti-oxidative properties of the bile of rats fed with Ge-132 were due to bilirubin glucuronides. Oral intake of Ge-132 also increased the mRNA expression of uridine diphosphate glucuronosyltransferase 1a1 (Ugt1a1) and bile acid coenzyme A: amino acid N-acyltransferase (Baat), which encode bile component conjugating enzymes for secretion. The acceleration of bile pigment secretion into the intestine as well as increases in the anti-oxidant activity of bilirubin was induced by oral intake of dietary Ge-132. It is suggested that the antioxidative effect of bile against oxidative stress occurs through radical trapping.

Key words —— poly-*trans*-[(2-carboxyethyl) germasesquioxane], bilirubin, bile acid, uridine diphosphate glucuronosyltransferase 1a1, organic germanium

INTRODUCTION

Poly-*trans*-[(2-carboxyethyl) germasesquioxane] (Ge-132) is the most common organic germanium compound and was first synthesized by Tsutsui *et al.*[1] Ge-132 is a water-soluble compound, and its safety has been confirmed.[2] The structures of Ge-132 and trihydroxy germyl propionic acid, a hydrolyzed compound of Ge-132, are shown in Fig. 1. It has many therapeutic effects such as anti-carcinogenic and anti-influenza virus infection effects and also aids the alleviation of rheumatism and prevents osteoporosis.[3–7] Ge-132 also induces γ-interferon production and activates both natural killer (NK) cells and macrophages.[8] Therefore, the activation of immune systems by dietary Ge-132 must contribute to disease protec-

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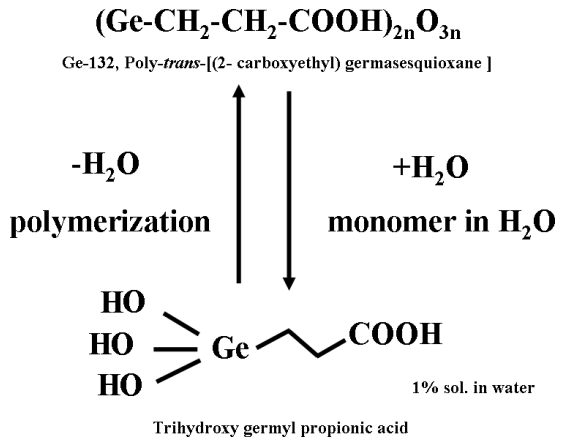

Fig. 1. The Structure of Ge-132 and Its Hydrolysis to Trihydroxy Germyl Propionic Acid

tion via the modulation of immune function or viral attack. In contrast, the toxicity of germanium dioxide (GeO_2), an inorganic germanium, has been elucidated. Significant oral intake of the compound results in renal insufficiency due to its accumulation in the kidney,[9] and the damage it causes is often fatal. We have already verified that dietary Ge-132 does not accumulate in a similar manner and only remains within the mammalian body for a few days.[10] Approximately 20% of Ge-132 is absorbed from the intestine, and it is almost totally excreted during the first 24 hr.

Recently, Ge-132 has been used as an ingredient of health foods and supplements. We observed two typical changes in the characteristics of feces after the oral intake of Ge-132 in humans: fecal color changed from brown to yellow, and feces sometimes softened (unpublished results). The change in fecal color induced by dietary Ge-132 is drastic; therefore, we thought that Ge-132 might cause metabolic changes in the human body. Generally, the color of feces is caused by fecal pigments.[11] It is known that fecal pigments are metabolites of bilirubin, which is formed from the breakdown products of heme. Moreover, bilirubin has anti-oxidative effects.[12,13] The increasing levels of fecal pigment seen after Ge-132 ingestion suggest the enhancement of bilirubin excretion in the intestine. In this study, we first compared the color of the cecal pigment of rats. Simultaneously, we studied their bile components and other factors related to bile pigment and discussed the functions of Ge-132 as a food component that induces beneficial functional compounds. We secondly compared the concentration of bilirubin in bile juice and the radical trapping activities of bile juice among control and Ge-132 administered groups of rats using the stable radical reagent 1,1-diphenyl-2-picrylhydradyl (DPPH). Moreover, we evaluated the hepatic expression of genes involved in the conjugation of bile components in mice in a time course experiment after the forced administration of Ge-132. We report here an elevation of the anti-oxidative function of bile juice and its basis after oral intake of dietary Ge-132.

MATERIALS AND METHODS

Animals

Analysis of Fecal Pigment: Male Wistar rats (three weeks old, Japan SLC, Hamamatsu, Japan) were housed in a room under controlled temperature (23–25°C) and light (08:00–20:00) conditions. After the rats had been acclimated for 1 week, they were randomly divided into two groups (5 rats each) and maintained on one of the following diets: the control diet or the 0.05% Ge-132 diet for two weeks. The compositions of both diets are shown in Table 1. The Ge-132 content was chosen from the adult human daily intake (10 mg/kg body weight) that changed fecal color. In the Ge-132 diet, the Ge-132 replaced an equal amount of corn starch. The amount of Ge-132 consumed was approximately 50 mg/kg body weight per day. The rats were able to drink water freely. On the final day, the animals were anesthetized using pentobarbital and killed by bleeding from the abdominal aorta. Then, the cecum and major organs were excised. Blood samples were used for immediate hematocrit analysis.

Collection of Bile Juice: Male Sprague Dawley rats (six weeks old, Japan SLC) were maintained in the same conditions as described above. After acclimation, they were divided into two groups (6 rats each). The rats that belonged to one group received a forced administration of 0.5% Ge-132 solution at a dose of 50 mg/kg body weight per day for 4 days. The rats that belonged to the other group received a forced administration of the same volume of physiological saline solution. On the final day, the animals were anesthetized using pentobarbital. Under anesthetization, bile juice was collected through catheterization (cannula; Teflon tube 0.4 mm, inner diameter, 0.6 mm, outer diameter) of the bile duct. The rats were maintained on a hot sheet at 37°C throughout the gall collection.

Analysis of Gene Expression: Male ICR mice (ten weeks old, Clea Japan, Tokyo, Japan) were maintained in controlled temperature (22–23°C)

Table 1. Composition of Diets for Ge-132 Administration

	Control	Ge-132
	(%)	
Casein	23	23
Corn starch	61.5	61.45
Corn oil	5	5
DL-methionine	0.3	0.3
Vitamin mixture	1	1
Mineral mixture	4	4
Cellulose	5	5
Choline	0.2	0.2
Ge-132	—	0.05
Total	100	100

and light (08:00–20:00) conditions. After the mice had been acclimated for a week, they were randomly divided into three groups (7 mice each) and maintained on a commercial diet (D10001, Research Diets, Inc., New Brunswick, NJ, U.S.A.) based on AIN76. The mice were fasted on the last day before sacrifice. The mice of each group underwent forced administration of 2 ml of 2 mg/ml Ge-132 aqueous solution at 0, 6, or 12 hr before sacrifice and then were anesthetized with diethyl ether and killed by bleeding of their whole blood from the abdominal aorta. Then, the liver was collected, dipped in RNA later (Applied Biosystems Japan, Tokyo, Japan), and kept at −20°C until the extraction of total RNA. Hepatic samples from the mice were maintained in RNA later at −20°C. Then, the tissue samples were homogenized using a SK mill (Tokken, Inc., Kashiwa, Japan), and total RNA was isolated with the SV total RNA isolation system (Promega, Madison, WI, U.S.A.), according to the manufacturer's instructions. Two μg extracted total RNA were used as a template for cDNA synthesis via reverse transcription with an oligo deoxythymidine primer and Super Script III (Invitrogen, Carlsbad, CA, U.S.A.). The synthesized cDNA was used for quantitative polymerase chain reaction (PCR) analysis. Quantitative real time PCR was performed for uridine diphosphate glucuronosyltransferase 1a1 (Ugt1a1), bile acid Coenzyme A: amino acid N-acyltransferase (Baat), and beta-actin using the following primer pairs: Ugt1a1: sense primer 5′-GCA CGA AGT TGT GGT CAT AGC A-3′ and anti-sense primer 5′-TCC GTC CAA GTT CCA CCA AA-3′; Baat: sense primer 5′-TGT CAG AGC CTT GGT TTG AGA-3′ and anti-sense primer 5′-CTT GGC CAT TTT TTG CAG AG-3′; beta-actin: sense primer 5′-AAG TAC CCC ATT GAA CAT GGC A-3′ and anti-sense primer 5′-CTG GAT GGC TAC GTA CAT GGC T-3′. The cDNA was amplified using SYBR Premix Ex Taq II (Takara Bio, Ohtsu, Japan) on an Opticon 2 (Bio-Rad laboratories, Hercules, CA, U.S.A.), which was programmed for 95°C for 30 sec, followed by 40 cycles of denaturation (95°C for 5 sec), annealing, and extension (60°C for 30 sec). Each expression value was calculated according to the threshold cycle value, and the data were displayed as the expression ratio of each gene to beta-actin.

All animal experiments were conducted at Asai Germanium Research Institute Co., Ltd. according to the guidelines provided by the ethical committee of experimental care, which are based on public guidelines set by the Japanese Ministry of Education, Culture, Sports, Science and Technology.

Analysis of Fecal Pigments —— The fecal pigments and lipids of the rats were extracted from the cecal contents or the feces with chloroform/methanol (2 : 1, vol/vol) by the method of Folch *et al.*[14] First, the extracted pigments were separated by thin layer chromatography (TLC) using a silica gel TLC plate (Silicagel 60, Merck, Darmstadt, Germany). TLC spotted aliquots of the extracted pigments were then developed in chloroform/methanol/water (65 : 25 : 4, vol/vol). The standard samples used as references were L-stercobilin (Porphyrin Products Inc., Logan, UT, U.S.A.), biliverdin (Frontier Scientific Inc., Logan, UT, U.S.A.), bilirubin (Wako Pure Chemical Industries, Ltd., Osaka, Japan), and protoporphyrin IX (Sigma-Aldrich Inc., St. Louis, MO, U.S.A.). Then, the extracted pigments were analyzed by a HPLC system with a UV/Visible detector after filtration. The analytical column was a Shim-pac TMS column (4.6 × 150 mm, Shimadzu, Kyoto, Japan). The mobile phase used was methanol-phosphate buffer (pH 7.4) 6 : 4 (vol/vol), the flow rate was 0.5 ml/min, and the pigments were detected at 490 nm.

Analysis of Bile Acid Concentration in Cecal Content —— The bile acids in the cecal content were extracted by the method of Folch *et al.*[14] The concentration of the bile acids was measured enzymatically according to the method of Marion *et al.*[15]

Assay of β-glucuronidase Activity in Cecal Content —— The activity of β-glucuronidase was measured by the method of Ishikawa *et al.*[16] An eighty-fold dilution of the cecal content was used as an enzyme solution. A substrate solution (180 μl) consisting of 0.1 M phosphate buffer (pH 7.4) containing 4 mM p-β-D-glucuronide (Sigma-Aldrich Inc.)

and 0.133 mM Na-EDTA was added to the enzyme solution (60 μl). The reaction mixture was incubated for 20 min at 37°C, before being stopped by the addition of 240 μl Na_2CO_3 and centrifuged. Finally, the absorption of p-nitrophenol in the supernatant was measured at 415 nm.

HPLC Analysis of Bile —— Bile juice samples were filtered with an ultra-filtration unit (centrifree micropartition system, amicon, Millipore Co., Billerica, MA, U.S.A.) and a 0.45 μm membrane filter (DISMIC-25CS, ADVANTEC, Tokyo, Japan). A 30 μl aliquot was taken from each filtered sample for direct injection, and separation was performed on a reverse phased HPLC system equipped column, the ODS 80-Tm (Tosoh, Tokyo, Japan) at 30°C. Elution was performed at a flow rate of 0.6 ml/min with an HPLC pump (PU980, Jasco, Tokyo, Japan). The mobile phase was 0.05 M citrate buffer (pH 5.0)-methanol-acetonitrile 8 : 9 : 3 (vol/vol), the total analysis time was 40 min, and the pigments were detected with a UV/Vis detector (UV970, Jasco, Tokyo, Japan) at 450 nm.

DPPH Radical Scavenging Activity —— Bile juice samples were filtered with a 0.45 μm membrane filter (DISMIC-25CS, ADVANTEC), and 60 μM DPPH in methanol and 450 μl of distilled water were added to 50 μl samples. A 0.5 ml volume of the mixture was then mixed with a voltex mixer, and the reaction mixture was reacted for 1 hr in dark conditions. The absorption of the reacted mixture was measured at 517 nm (UV 2200, Shimadzu), and bile dilutions at the same concentration were used as references.

Data Analysis —— The values of the first experiment are presented as the mean ± S.D. of 5 rats. The values of the bile juice experiment are presented as the mean ± standard error of the Mean of 7 rats. Significant differences among two groups were analyzed using the Student's t test. On the other hand, the results of the gene expression experiment are presented as the mean ± S.E.M. of 7 mice. The experimental data concerning gene expression were analyzed using analysis of variance (ANOVA). The statistical significance of all experiments was defined as $p < 0.05$.

RESULTS

Effects of Ge-132 Intake on Fecal Characteristics and Cecal Changes

Fecal characteristics are changed by dietary Ge-132. The fecal color turned from grayish-brown to yellow a few days after the beginning of Ge-132 administration in rats. A similar change was confirmed in a human sample a day after Ge-132 intake. The fecal pigments from the feces of rats and humans were separated on silica gel TLC plate, and the TLC pattern is shown in Fig. 2. The fecal samples of rats were collected on the first day of the second week of Ge-132 administration. The fecal pigments of the rats were compared with those of the human fecal samples before and after Ge-132 intake. The two major pigments of rat feces (lanes 4 and 5) were separated on Rate of flow 0.38 and 0.56. The pigment at Rf 0.38 was identified as L-stercobilin using the commercial standard in lane 3. The same spots were detected in both groups; however, the amounts of the pigments were differed slightly between the two groups. The spot at Rf 0.56 in the Ge-132 intake group was higher than that of the controls. The pigment detected at Rf 0.56 was unknown but its Rf value was similar to that of protoporphyrin in lane 8, and they had the same specific red fluorescence in UV radiation (data

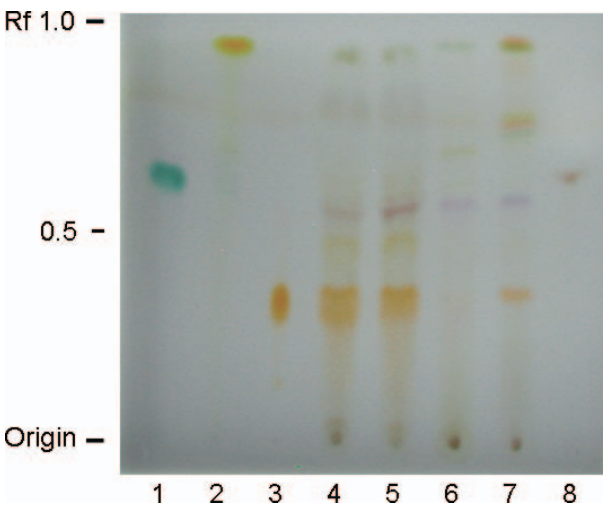

Fig. 2. TLC Pattern Changes in Fecal Pigments from Rat Feces Induced by Dietary Ge-132 Intake

Each pair of fecal pigments from rat and human samples was extracted from the same weight of feces. Then, the extracts were dissolved in the same volume of chloroform/methanol (2 : 1, vol/vol). Forty microliter samples of fecal pigment solutions were spotted and developed. Development was carried in chloroform/methanol/water (65 : 25 : 4, vol/vol). Lanes are: 1: biliverdin, 2: bilirubin, 3: stercobilin, 4: the fecal pigment from the rats that ate the control diet, 5: the fecal pigment from the rats that ate the 0.05% Ge-132 diet, 6: the human fecal pigment before Ge-132 intake, 7: the human fecal pigment from the day after Ge-132 intake, 8: protoporphyrin. Human fecal samples were obtained before and after 60 mg/kg body weight of Ge-132 intake. The samples shown in lanes 6 and 7 were obtained on continuous two days from the same person.

not shown). Minor pigments were detected at Rf 0.46 and 0.92, both of which were unknown. On the other hand, the human fecal pigments were changed by Ge-132 intake. Little stercobilin was identified in either sample, and especially in the no intake group (lane 6). In human feces, the intake of Ge-132 increased the concentrations of every detected pigment. In addition, new spots were detected for rats, at Rf 0.96 and 0.75. Bilirubin was detected at Rf 0.96 (lane 2), and the spots of human fecal pigment of the same Rf value were different colors (orange and green) from that of bilirubin (yellow). No biliverdin spot was detected in any of the extracted fecal samples (referring to lane 1, commercial biliverdin).

Dietary Ge-132 had no effect on body weight or the weight of major tissues in the rats (data not shown). A picture of a rat cecum is shown in Fig. 3. The color of the rat cecum was grayish-green after the administration of the control diet for 2 weeks. However, the cecal color of the Ge-132-containing diet group became yellow, and its brightness was increased after 2 weeks administration.

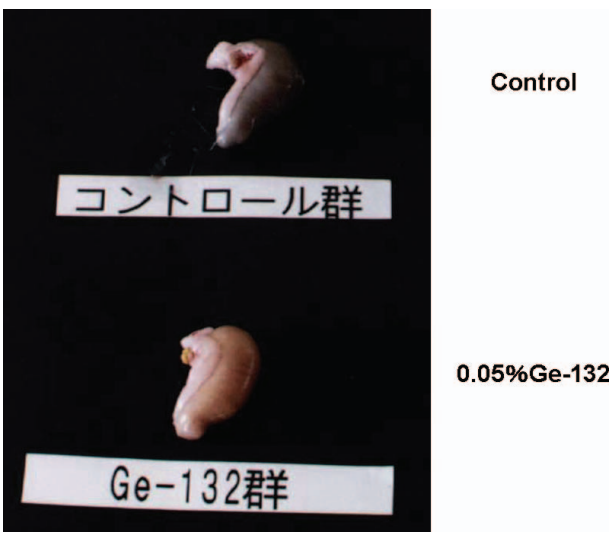

Fig. 3. Effects of Dietary Ge-132 on the Color and Form of Ceca Were Evaluated

Samples were obtained from each rat after two weeks administration of either diet. The scale is the same for both pictures. Dietary Ge-132 intake clearly changed the cecal color.

Analyses of Pigment and Factors Related to Cecal Content

The changes in cecal content and related factors are shown in Table 2. The concentration of cecal stercobilin was quantified by HPLC. A simple chromatogram with two peaks was obtained. The peaks detected at Retension Time 8.9 and 15.1 min were stercobilin and bilirubin, respectively (data not shown). The concentrations of stercobilin in the ceca of the control and Ge-132-fed rats were 14.4 and 24.6 μg/g cecal content, respectively. The stercobilin concentration of the Ge-132 group was significantly ($p < 0.05$) higher than that of the control group; *i.e.*, dietary Ge-132 intake induced fecal stercobilin secretion.

The total bile acid concentration increased significantly ($p < 0.05$) from 122 to 153 μg/g cecal content after dietary Ge-132 intake. The hematocrit value, which shows the ratio of the volume of red blood cells to whole blood, was not different between the two groups, at 46.7 and 45.5%, respectively.

β-glucuronidase is a deconjugating enzyme for compounds conjugated with glucuronic acid. The enzyme is produced from microorganisms present in the intestinal flora, and it also deconjugates bilirubin glucuronide. Dietary Ge-132 significantly ($p < 0.05$) enhanced β-glucuronidase activity from 1.6 to 2.6 unit/g cecal content.

Comparison of Bile Pigment Components

The bile pigments in the bile juice of rats were separated on HPLC. The concentrations of the bile pigments are shown in Fig. 4. Eight peaks were detected under these conditions. The peaks at RT 10.8 and 14.1 min were bilirubin diglucuronides (BDG),

Table 2. Effects of Ge-132 Administration on Stercobilin and Related Factors

	Control	Ge-132
Stercobilin (μg/g cecal content)	14.4 ± 5.7	24.6 ± 8.5*
Hematocrit Value (%)	46.7 ± 1.7	45.5 ± 2.2
Total Bile Acid (μg/g cecal content)	122 ± 22	153 ± 19*
β-glucuronidase activity (units/g cecal content)	1.6 ± 0.8	2.6 ± 0.6*

Data are presented as the mean ± S.D. for each group of five rats. Values marked with asterisks are significantly different from the control group ($p < 0.05$).

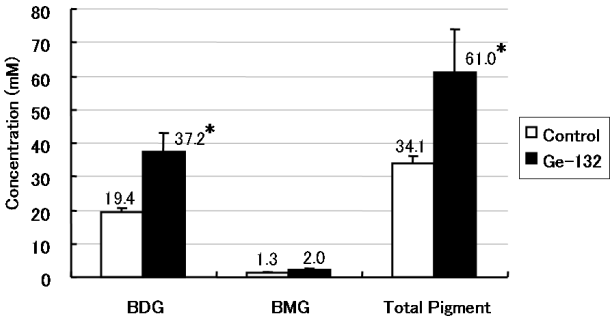

Fig. 4. Effect of Ge-132 Administration on the Bilirubin in Bile Juice

A sample of bile juice was obtained through a cannula and analyzed with reverse phased HPLC. BMG, BDG, and total pigment refers to bilirubin mono-glucuronide, bilirubin di-glucuronide, and the summation of other detected pigments peaks at 450 nm, respectively. Data are presented as the mean ± S.E.M. for each group of six rats. Values marked with asterisks are significantly different from the control group ($p < 0.05$).

and the peaks at RT 29 and 31 min were bilirubin monoglucuronide (BMG). The most major pigment was BDG. The BDG concentrations of the untreated rats (Control) and Ge-132 administered rats (Ge-132) were 19.4 and 37.2 mM, respectively. The BMG concentrations of the Control and Ge-132 groups were 1.3 and 2.0 mM, respectively. The ratios of the BDG and BMG values in the Ge-132 group to those in the control group were 1.92 and 1.53, respectively. The ratio of all detected peak areas of the administered rats to that of the untreated rats was 1.79 (data not shown). The administration of Ge-132 increased bilirubin conjugation and other pigment secretion from the liver.

DPPH Radical Scavenging Activity in Bile Juice

To evaluate the anti-oxidative activity of bile juice, DPPH, a stable radical reagent, was used. The absorption of DPPH at 517 nm was reduced in the reaction with the bile juice from rats, indicating that the bile juice possessed radical scavenging activity. The DPPH radical scavenging activity results are shown in Fig. 5. The ratios of DPPH radical scavenging in the control and Ge-132 groups were 46.0 and 79.9%, respectively; *i.e.*, the activity of the Ge-132 administered group was 1.74 fold higher. This suggests that the administration of Ge-132 enhanced the secretion of bile juice components possessing radical scavenging activity.

Expression of Genes Involved in the Metabolism of Bile Components in the Liver

A time course experiment was performed after the forced administration of Ge-132. The gene

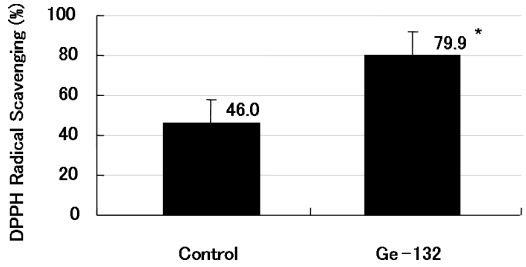

Fig. 5. DPPH Radical Scavenging Activity of Bile from Rats

A sample of bile juice was obtained through a cannula and was reacted with the stable radical reagent DPPH. Each scavenging percentage was calculated from the decrease in absorption at 517 nm. Data are presented as the mean ± S.E.M. for each group of six rats. Values with asterisks are significantly different from the control group ($p < 0.05$).

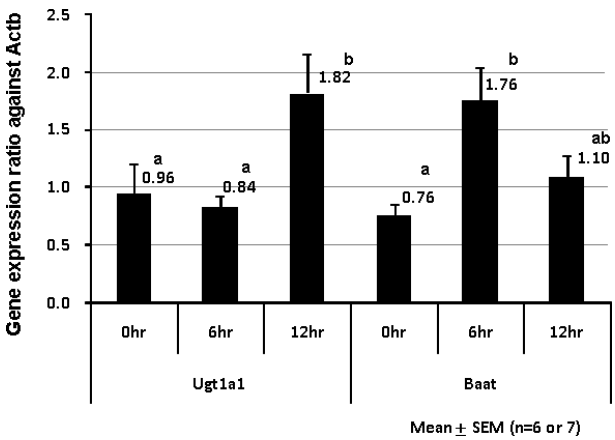

Fig. 6. Hepatic Gene Expression Related to Bile Component Conjugation Enzymes after Ge-132 Administration in Mice

Samples of total RNA were extracted from the livers of mice. The livers of the mice were collected after the forced administration of Ge-132. Each target gene value was obtained as a gene expression ratio against β-Actin (Actb) as a control gene. Values with different letters for each gene are significantly different between both groups ($p < 0.05$).

expression of Ugt1a1 and Baat, which are related to the conjugation of bile pigment and bile acid, respectively, were detected by quantitative reverse transcription (RT)-PCR. The ratios of the expression levels of the target genes to the expression of the control gene, beta-actin, in the liver after the forced administration of Ge-132 are shown in Fig. 6. The gene expression ratios of Ugt1a1 at 0, 6, and 12 hr after the administration of Ge-132 were 0.96, 0.84, and 1.82, respectively. The expression level of Ugt1a1 was significantly increased at 12 hr after Ge-132 administration compared with its expression at 0 and 6 hr ($p < 0.05$). On the other hand, the expression of Baat was temporarily increased at 6 hr and then decreased to its original level (0 hr). The gene

expression ratios of Baat at 0, 6, and 12 hr after Ge-132 administration were 0.76, 1.76, and 1.10, respectively. The administration of Ge-132 enhanced the expression of the bile component conjugation enzymes for both bile pigment and bile acids.

DISCUSSION

In this study, we first extracted the pigments from the feces of rats and humans (Fig. 2). The major fecal pigment of rats was identified as stercobilin. Moreover, the other major fecal pigment in rats was increased by the intake of dietary Ge-132. This pigment had a similar Rf value and response against UV radiation. Therefore, the spot may have been related to porphyrin. On the other hand, the fecal pigments of humans were increased after Ge-132 intake (lanes 6 and 7 shown in Fig. 2). The human feces had a high ratio of spots at Rf 0.96. The Rf values of these spots matched those of bilirubin. However, the colors of the spots in lane 7 were orange and green, respectively, rather than the yellow of bilirubin, but they may have been metabolites of bilirubin like mesobilirubin.[17] However, the stercobilin spot in the Ge-132 intake group was colored more intensely than that in the control. In this experiment, stercobilin was not different so much in Ge-132 diets on rat. Therefore, we examined cecal content samples in continuous studies to reveal the reasons for the fecal color change induced by dietary Ge-132. We used cecal samples rather than fecal samples in order to directly detect the condition of the intestine as some fecal pigments are easily oxidized by the oxygen in air, and the cecum is within the small intestine. The most common bile pigments were bilirubin and biliverdin. Bilirubin is secreted into the duodenum as a conjugate with glucuronic acid.[18] We speculate two hypotheses for the modulation of cecal color by dietary Ge-132: in the first case, only secretion of the pigment increases in the bile, and in the second case, the secretion of bile juice is also enhanced. In this study, we revealed that Ge-132 increased both bile pigment metabolites and total bile acids (Table 2) in the intestine. The enhancement of both bile pigment metabolites and bile acids supports the second hypothesis. Thus, we consider that the intake of Ge-132 may regulate bile secretion.

Shintani *et al.*[19] reported that there are receptors for food components such as fatty acids and amino acids on the surfaces of intestinal cells. The receptors trigger gastrointestinal hormone release after Ca influx into the cells when they recognize any signals from food components; and thereby, the secretion of digestive juices such as bile juice is accelerated. For this reaction, the importance of the carboxyl groups of these compounds is considerable. When Ge-132 is dissolved in water and hydrolyzed to a monomer, trihydroxy germyl propionic acid, the molecule formed is an organic acid bonding propionic acid (show in Fig. 1). The structure (carboxyl acid) and molecular weight of trihydroxy germyl propionic acid is similar to those of several major fatty acids and amino acids. Therefore, we speculate that, as a result, cells misrecognize them; and thus, bile secretion is increased in the intestine.

β-glucuronidase is an enzyme originating from microflora.[18] Dietary Ge-132 enhanced the activity of β-glucuronidase in the cecal content (Table 2). Generally, β-glucuronidase is considered to be a risk factor for colon cancer;[20] therefore, the enhancement of its activity is an unexpected result. However, the harmful effects of β-glucuronidase have not been definitively confirmed because some reports have suggested that there is no relationship between colon cancer and β-glucuronidase activity.[21]

Generally, bile pigments originate from the blood pigment heme;[22] hence, it is very important that the fecal color change induced by dietary Ge-132 originated from stercobilin, which is the final bile pigment metabolite of heme. The increase in the stercobilin concentration indicates the enhancement of heme degradation; nevertheless, there was no change in hematocrit values (Table 2). These results led us believe that the formation of new Blood Red Cells is involved in this process. Therefore, this phenomenon may be associated with erythropoietic effects of dietary Ge-132. Further study about this erythropoietic effect is necessary.

Incidentally, we previously reported the antioxidant activity of urobilinogen,[23] which is an intermediate metabolite of the reaction that produces stercobilin from bilirubin. Moreover, bilirubin is also a well-known antioxidant compound and shows strong antioxidant activity under physiological O_2 concentrations.[12, 13, 24] The enhancement of stercobilin must originate from its metabolism through bilirubin and urobilinogen. As a consequence, an anti-oxidative effect in the intestine is expected after the intake of dietary Ge-132 via the secretion of these heme metabolites. Bilirubin and/or urobilinogen, which are precursors of stercobilin, act

as anti-oxidants in the gastrointestinal tract; therefore, these compounds may exert protective functions such as anti-carcinogenic activity. The concentrations of the bilirubin glucuronides (BDG and BMG) were increased in bile by dietary Ge-132 (Fig. 4). In addition, the enhancement of bile pigment was confirmed, and this is the reason for the increases in the concentration of stercobilin in cecal content described above. Bilirubin and other bile pigments in bile juice were increased approximately 1.7 fold by dietary Ge-132, and the DPPH radical scavenging activity of bile (Fig. 5) was similarly increased. Therefore, these results suggest that the radical scavenging activity of bile juice mainly originates from bile pigments, and in particular, bilirubin. Ge-132 accelerates bilirubin release into the bile duct from liver tissue. The expression of Ugt1a1 was also upregulated by the intake of Ge-132, which indicates the enhancement of glucuronide conjugation (Fig. 6). Bilirubin is known to accumulate in jaundice patients. When hepatic damage occurs, bile secretion is stopped, and bile flows out into the blood. This suggests that the intake of Ge-132 results in the prevention or recovery from jaundice. It is reported that Ge-132 protects against the hepatic damage caused by glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) leakage.[25] Up-regulation of Ugt1a1 by Ge-132 may affect the detoxifying ability of the phase II detoxic system of the liver.[26, 27] Bile juice is constantly being produced, and bilirubin is contained in it; therefore, the radical scavenging action of this molecule must be considerably strong.

In conclusion, we observed that dietary Ge-132 increased the bilirubin level 1.7 fold with a similar elevation in the radical scavenging activity in bile. Dietary Ge-132 intake induces the expression of bilirubin and/or urobilinogen in the intestine and may regulate gastrointestinal condition. These compounds maintain a pre-digestion anti-oxidative state in the gastrointestinal tract.

The study of gene expression in mice suggests that the intake of Ge-132 up-regulates the expression of enzymes that catalyze the conjugation of bile pigments and bile acids in the liver, which then alters the secretion of these components into bile juice. The result for Ugt1a1 expression is in accord with the data for bilirubin glucuronide found by HPLC analysis of the bile juice of rats. Moreover, a detoxifying effect caused by the up-regulation of Ugt1a1 by Ge-132 intake is expected,[26, 27] and the elevation of detoxicity after Ugt1a1 induction by dietary Ge-132 is also predicted.

In this study, we revealed the effects of dietary Ge-132 intake on the acceleration of bile component excretion and the antioxidative effects of bile pigment. On the other hand, it is still unknown why these effects are induced by Ge-132, a simple structural organic germanium. A study of these functions using several analogous compounds of Ge-132 is required.

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