# Concomitant Oral Ingestion of Germanium-132 and Curcumin Increased Mortality Rate by Aggravating Hepatic Dysfunction in Long-Evans Cinnamon Rats

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(Received May 16, 2006; Accepted August 27, 2006; Published online October 4, 2006)

To determine the effect of concomitant oral ingestion of germanium-132 (Ge-132) and curcumin on the onsets of hepatitis and hepatic cancer in Long-Evans Cinnamon (LEC) rats, 40 rats were administered sterile water (NT), corn oil (Vehicle), Ge-132 (Ge), curcumin (Cur), or Ge-132 plus curcumin (GeCur) for 50 weeks. Plasma enzyme levels of asparate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltrasferase (γGTP), and lactate dehydrogenase (LDH) were measured at *Pre* (6 weeks), *Early* (18–26 weeks), *Late* (30–38 weeks) and *End* (42–50 weeks) stages. Liver damage was assessed by the Histology Activity Index (HAI). In the group of Ge, LDH was significantly decreased at the *End* stage. In the group of Cur, LDH was remarkably decreased in the *Early* stage, whereas AST and LDH were significantly decreased in the *End* stage. In the GeCur group, AST, ALT, and γGTP were significantly increased in the *Early* stage, whereas LDH was significantly decreased in the *End* stage. The onset rate of icterus and the mortality rate were significantly increased in the GeCur groups (*vs*. Vehicle group, $p < 0.01$). There was no statistically significant difference in HAI among the groups. Thus, concomitant oral ingestion of Ge-132 and curcumin may aggravate hepatic dysfunction, thereby increasing the mortality rate in LEC rats.

**Key words** —— Long-Evans Cinnamon rats, hepatitis, hepatic cancer, germanium-132, curcumin

## INTRODUCTION

It has become widely recognized that the development of human hepatocellular carcinoma is predominantly due to chronic inflammation by virus, bacteria or chemical. Concerning chronic hepatic inflammation, the morbidity rates of hepatic steatosis and alcoholic steatohepatitis in Japan have increased due to a rise in food intake of a westernized diet and excessive drinking of alcohol, respectively.[1,2] Therefore, the number of people conventionally taking *Curcuma longa* seems to have increased as part of self-medication. *Curcuma longa* has been viewed by folk medicine as improving liver function. Curcumin is a medical ingredient of *Curcuma longa* and has been shown to improve liver function and to have antitumor and antioxidative actions.[3,4] For example, curcumin can effectively suppress diethylnitrosamine-induced liver inflammation and hyperplasia in rats.[5,6]

Germanium-132 (2-carboxyethyl germanium sesquioxide, Ge-132) is an organic germanium compound which is known to have immunostimulating effects including induction of interferon-γ through stimulating T-cells, enhancement of natural killer cell activity, and inhibition of tumor growth and its metastasis by stimulation of cytotoxic macrophages.[7,8] In addition, animal experiments have clarified that Ge-132 has tumor depression effects.[8–11] Therefore, the number of people taking nutritional supplementary food including germanium has increased since about the 1970's in hope of its having a beneficial effect on cancer.[12]

However, it is still controversial whether organic germanium compounds and/or curcumin ingestion inhibit hepatitis and hepatic cancer *in vivo*. Curcumin can cause cell damage by inactivating the Akt-related cell survival pathway and releasing cytochrome

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c.[13] In addition, some organic germanium compounds such as germanium dioxide, spirogermanium, germanium lactate citrate, propagermanium (3-oxygermylpropionic acid polymer), and Ge-132 affect the functions of liver, kidney, muscle, and neurons.[14–17]

The Long-Evans Cinnamon (LEC) rat is a spontaneous acute hepatitis model, a spontaneous hepatic cancer model, and Wilson's disease model rat,[18–20] which was discovered in a closed colony of the Long Evans strain.[21,22] LEC rats are characterized by the fulminant hepatitis occurring as a result of an abnormal hepatic deposition of copper (Cu) due to lack of the Cu-transporter P-type adenosine triphosphatase (Atp7b).[23,24] Spontaneous hepatitis associated with severe icterus occurred in 90% of adult LEC rats three to four months after birth.[21] Of this 90% with acute hepatitis, 50% died due to fulminant aggravation of hepatic dysfunction, and 40% of the LEC rats had remission.[21] Of this 40%, 10% died of recurrent acute or subacute hepatitis and 30% developed chronic hepatitis.[21] Furthermore, hepatic cancer appears in long surviving LEC rats after recovery from icterus as well as a few asymptomatic rats without icterus.[21]

In the present study, we investigated whether Ge-132 and/or curcumin ingestion depresses hepatitis and later hepatic cancer in LEC rats.

## MATERIALS AND METHODS

**Animals and Animal Care** —— All animals were cared for following the Standards Relating to the Care and Management of Experimental Animals (Notification No. 6, 27 March 1980, Prime Minister's Office, Tokyo, Japan) and the guide for animal experiments issued by University of the Ryukyus. All animal studies were reviewed and approved by the Animal Care Committee at University of the Ryukyus. Forty LEC rats 5 weeks of age were kindly donated by Japan Charles River Inc. (Tokyo, Japan), and were divided into five groups: Non treated (NT): ingestion of sterile water (solvent for Ge-132) from a drinking bottle *ad libitum*; Vehicle: ingestion of an equivolume of corn oil (solvent for curcumin) through a feeding syringe; Ge: ingestion of Ge-132; Cur: ingestion of curcumin; and GeCur: concomitant ingestion of Ge-132 plus curcumin. Each group was composed of 8 rats. All rats were bred on Rodent Diet CE2 solid food (CLEA Japan Inc., Tokyo) under a specific pathogen-free (SPF) condition. The breeding conditions of rats in the experimental period were: room temperature of 23 ± 1°C, humidity of 60 ± 5%, and 12 hr of the day-and-night cycle (daytime: 8:30–20:30).

**Preparation of Ge-132 and Curcumin** —— Curcumin (Sigma-Aldrich St. Louis, MO, U.S.A.) was dissolved in corn oil to make an oil emulsion containing 50 mg/ml according to the method of Kang *et al*.[4] Curcumin emulsion was daily administered at up to 100 mg/kg body weight using a disposable feeding syringe (Kenis Ltd., Osaka, Japan). Ge-132 [Orugano Germanium $(GeCH_2CH_2COOH)_2O_3$], Teichu Co., Hiroshima, Japan) was treated by electrostatic induction (1200 volts at 80–85°C) to increase its solubility in water and dissolved in sterile water to make a stock solution of 40 mg/ml. The stock solution was further diluted 200 times with sterile water (200 $\mu$g/ml) in a drinking bottle and was given *ad libitum*. Ingestion of these test solutions was started at 6 weeks of age.

**Measurement of Plasma Enzymes** —— After a rat was restricted by animal holding fabric, the tail vein was punctured with a 26-gauge needle, and then blood was sampled with heparinized capillary tubes (Drummond Scientific Co., Pennsylvania, U.S.A.), as previously reported.[25] Blood was sampled every month. After centrifugation, plasma was collected and stored at –80°C until assay. Plasma levels of asparate aminotransferase (AST), alanine aminotransferase (ALT), $\gamma$-glutamyltransferase ($\gamma$GTP), and lactate dehydrogenase (LDH) were measured with Hitachi-7170 automatic analysis equipment at Serotec Inc. (Hokkaido, Japan).

**Histopathological Study** —— When a LEC rat was found dead in the course of an experiment, an autopsy was performed. At the end of the experiment (62 weeks of age), all rats were euthanized by intravenous injection of Nembutal® (Pentobarbital sodium, Dainippon Sumitomo Pharma Co., Ltd., Osaka) and an autopsy was also performed. The liver was excised and preserved in 10% formalin solution at 4°C. The formalin-fixed liver of each rat was embedded in paraffin, and 5-$\mu$m thick sections were prepared. Sections were stained with hematoxylin and eosin for evaluation of histopathological grading and with Azan stain for evaluation of fibrosis. The histopathological grading for liver damage was assessed by the Histology Activity Index (HAI) according to the criteria in the previous paper.[26]

**Statistical Analysis** —— Statistical analysis was performed by a StatView version 5 (SAS Institute Inc., NC, U.S.A.) and a SuperANOVA version 10

for Macintosh (Abacus Concept, CA, U.S.A.). Data represent means ± standard error (SEM). Two-way repeated measure analysis of variance (ANOVA) was performed for comparison of time course of changes in the means. The contrast method was used for a multiple comparison of the means. The survival of the animals and the onset of icterus were computed according to Kaplan-Meier, and the difference between the groups was tested with the log-rank test.

## RESULTS

### Changes in Body Weight and Food Intake

Mean body weights of rats in all the groups were increased to about 300 g by 20 weeks of age, and thereafter gradually increased and saturated; however, at between 23 and 26 weeks body weight in all groups was transiently decreased by ca. 40 g (Fig. 1A). In the case of dead, the decrease in body weight started a couple of days prior to death. Since there were many deaths at 26 weeks of age, the mean body weight of each group transiently decreased from that time. We calculated the mean daily intake of solid food per rat from 14 to 41 weeks of age in order to determine the effect of Ge-132 and/or curcumin on their food intake, and found that the intake was slightly decreased in the Ge and Cur groups with statistical significance (Fig. 1B). Although there was no statistically significant difference of mean body weight among the groups, the mean in the groups of Ge, Cur and GeCur tended to be decreased as compared with those in the NT and Vehicle groups.

The weekly-averaged quantities of Ge-132 drunk in the Ge and GeCur groups were almost constant throughout the experiment period: the average daily intake being 43.3 ± 1.4 $\mu$g/kg body weight and 41.6 ± 1.6, respectively. There was no significant difference between the groups.

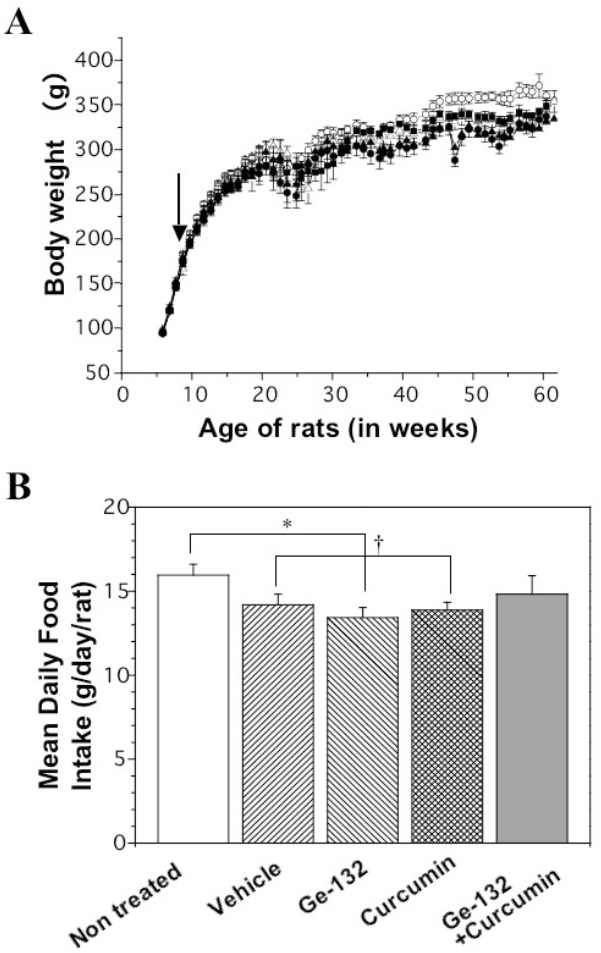


**Fig. 1.** Time Courses of Change in Body Weight and Overall Mean Daily Food Intake in Different Groups of LEC Rats

**A**: Line graphs showing time courses of changes in body weight of LEC rats. ○: Non treated group [ingestion of sterile water (solvent for Ge-132) in drinking bottle *ad libitum*], △: Vehicle group [ingestion of equivolume of corn oil (solvent for curcumin) by a feeding syringe], ●: Ge-132 group, ■: Curcumin group, and ▲: Ge-132 + Curcumin group. Although each group was composed of eight LEC rats at the start of the experiment, the number decreased due to animal deaths from sickness and from experimental procedures (see Table 1). **B**: Bar graphs showing averages of daily food intake of rats. Averages of daily food intake per rat from 14 to 41 weeks of age were calculated in groups. Data represent means ± SEM. A statistically significant difference of the mean value was tested by one way ANOVA, followed by multiple comparison by the PLSD method. *$p < 0.05$ *vs*. Non treated group. †$p < 0.05$ *vs*. Vehicle group.

### Changes in the Level of Plasma Enzymes

Data on the level of plasma enzymes were collected for four different stages: *Pre* (6 weeks of age), *Early* (from 18 to 26 weeks), *Late* (from 30 to 38 weeks) and *End* (from 42 to 50 weeks). Mean plasma enzyme levels were compared among the groups in each stage. The plasma levels of AST, ALT, $\gamma$GTP and LDH showed a rise with week of age in all groups. For AST, ALT and GTP, these plasma levels had two peaks at 22 and 34 weeks of age, whereas the plasma level of LDH had one peak at 30 weeks (data not shown).

In the *Early* stage, the plasma levels of AST, ALT and $\gamma$GTP of the GeCur group were increased significantly as compared with those of the Vehicle group ($p < 0.05$, Fig. 2); on the other hand, the plasma LDH level of the Cur group was remarkably decreased in comparison with the Vehicle group (Fig. 2,

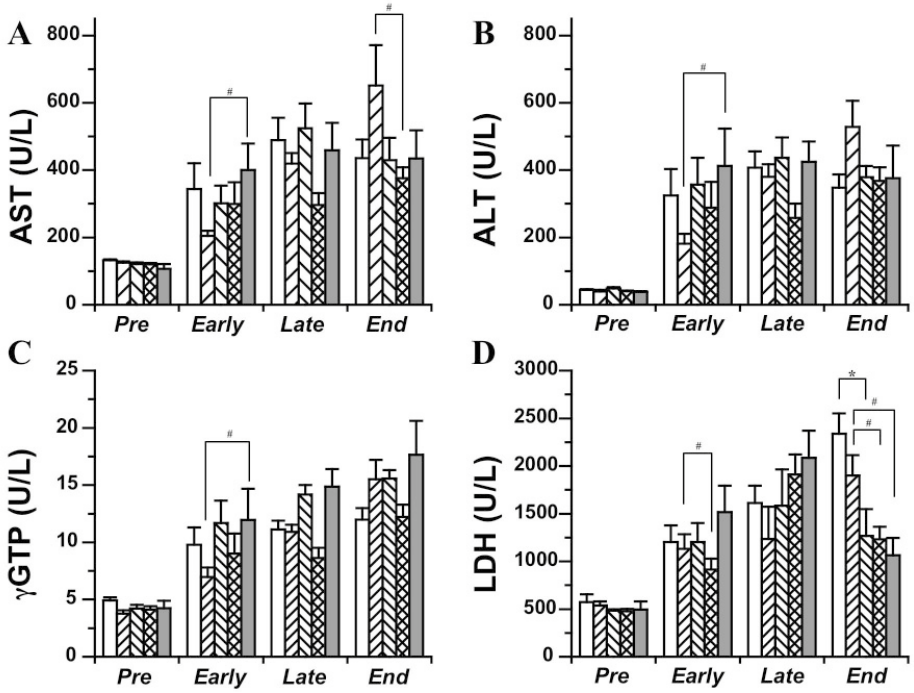


**Fig. 2.** Comparison of Time Courses of Changes in Levels of Plasma Enzymes in Different Groups of LEC Rats

Bar graphs showing the levels of plasma enzymes: AST (**A**), ALT (**B**), γGTP (**C**), and LDH (**D**) in different groups of rats. The data on the level of plasma enzymes were collected for four different stages: *Pre* (6 weeks of age), *Early* (from 18 to 26 weeks of age), *Late* (from 30 to 38 weeks of age) and *End* (from 42 to 50 weeks of age). Mean plasma enzyme levels were compared among the groups in each stage. Open bars, Non treated group; shaded bars, Vehicle group; back shaded bars, Ge-132 group; diamond-shaped bars, Curcumin group, and gray bars, Ge-132 + Curcumin group. Data represent means ± SEM of 4–8 rats of Non treated group, 4–8 rats of Vehicle group, 3–8 rats of Ge-132 group, 4–8 rats of Curcumin group, and from 1–8 rats of Ge-132 + Curcumin group. A statistically significant difference of the mean value was tested by one way ANOVA, followed by multiple comparison by the PLSD method. *$p < 0.05$ *vs*. Non treated group. #$p < 0.05$ *vs*. Vehicle group.

$p < 0.05$). In the *Late* stage, no significant difference was seen in any of the plasma enzyme levels. The levels of AST and LDH of the Cur group were decreased significantly in the *End* stage, as was that of LDH in the Ge and the GeCur groups.

## Comparison of Icteric Eruption and of Survival Curve

As shown in Table 1, the incidence rate of icterus was highest in the groups of Ge and GeCur (100%) and lowest in the Vehicle group (57.1%). In addition, the age at which icterus erupted in 50% of rats varied by group: GeCur, 16.7; NT, 17.1; Ge, 18.1; Vehicle, 20.1; Cur, 23.2 weeks of age (in the order of incidence rate). A cumulative incidence rate of icterus of each group was calculated by the Kaplan-Meier method (Fig. 3A). There was a statistically significant difference of cumulative incidence curve between the Ge and the Vehicle groups ($p = 0.004$) and between the GeCur and Vehicle groups ($p = 0.002$).

Survival rate of each group at the end of the experiment was: GeCur, 14.3%; Cur, 37.5%; Ge, 42.9%; and Vehicle, 57.1%. Survival analysis was also performed by the Kaplan-Meier method (Fig. 3B), and there was a statistically significant difference of cumulative survival curve between the GeCur and the Vehicle groups ($p = 0.014$).

## Histopathology of Liver

Although the icterus was observed with systemic skin, it was distinctly observed at appendicular palm sides and at auricular pars (Fig. 4A a); it was also present in subcutaneous tissue. Bilirubinuria and bilirubinemia were also noted with icterus. Autopsy on the rats that died in the *Early* stage demonstrated that the livers presented yellowish-brown, the sizes were enlarged (Fig. 4A b), and histologically bilirubin accumulation with a bile lake was observed. In some cases, multiple hemorrhagic lesions were present on the lung surface (Fig. 4A b). The hepatic histopathology section demonstrated central necrosis and microfatty degeneration of hepatic cells (Fig. 4B a and b). Oval cells were observed in some specimens (Fig. 4B c and d), and the tendency was stronger in the group of Cur. A few oval cells were

**Table 1.** Comparison of Onset Rate of Icterus and Mortality Rate among Five Different Groups of LEC Rats

| Group | Number of rats tested | Number of deaths from experimental procedures[a)] | Onset of icterus | | Deaths from sickness | |
|---|---|---|---|---|---|---|
| | | | (n) | (%) | (n) | (%) |
| Non treated | 8 | 0 | 5/8 | 62.5 | 3/8 | 37.5% |
| Vehicle | 8 | 1 | 4/7 | 57.1 | 3/7 | 42.8% |
| Ge-132 | 8 | 1 | 7/7 | 100 | 4/7 | 57.1% |
| Curcumin | 8 | 0 | 5/8 | 62.5 | 4/8 | 50% |
| Ge-132 + Curcumin | 8 | 1 | 7/7 | 100 | 6/7 | 85.7% |
| Total | 40 | 3 | 28/37 | 75.7 | 20/37 | 54.1% |

*a*) Data from rats that died of experimental procedures (ingestion of corn oil by feeding syringe, blood sampling) were not included in further statistical analysis.

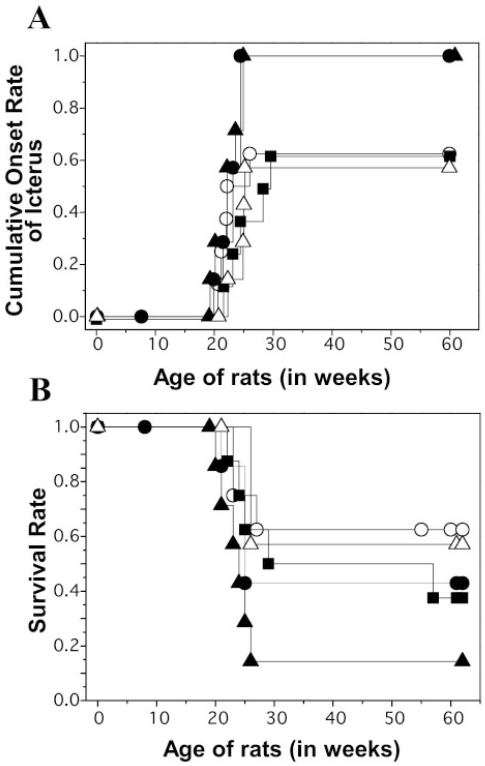


**Fig. 3.** Kaplan-Meier Analysis for Comparison of Cumulative Onset of Icterus and Survival Rate in Different Groups of LEC Rats

Line graphs showing cumulative curves for onset of icterus (**A**) and survival rate (**B**) of rats. ○: Non treated group, △: Vehicle group, ●: Ge-132 group, ■: Curcumin group, and ▲: Ge-132 + Curcumin group. Each group was composed of eight LEC rats at the start of the experiment.

also present in the group of GeCur. Liver damage was assessed by HAI, and there was no statistically significant difference in the grades of inflammation, degeneration and/or necrosis and fibrosis in the hepatic histopathology among the groups (Table 2). Among all the hepatic specimens from the rats that died in the *End* stage and from those euthanized at the end of the experiment, 88% were found to have hepatic cancer (Table 3). Abnormal bile ducts proliferated, and were surrounded by abundant fibroblasts and fibrosis (Fig. 5 a–d). A hepatocellular regenerating nodule was present in fibrotic lesions (Fig. 5 d).

## DISCUSSION

Our data demonstrated that 60% of LEC rats (NT and Vehicle groups) developed icterus, all of which died by 26 weeks of age. It has been reported that approximately 90% of LEC rats develop icterus at around 16 weeks of age and 60% die in the acute phase.[20–22)] Thus, in our experiment, the time of icterus was delayed and the rate of its onset was decreased as compared to previous reports.[20–22)] Since copper content has been known to be involved in the development of hepatitis in LEC rats,[27,28)] this discrepancy might be explained by the copper content in solid food and water. When cumulative curves of the onset rate of icterus were compared among the groups, curves of the Ge and GeCur groups were significantly different from that of the Vehicle group (Fig. 3A), and each rate of icterus onset was 100% (Table 1), suggesting that the development of this condition was enhanced by the ingestion of Ge-132. On the other hand, the cumulative curve of onset rate of icterus in the Cur group did not differ from that in the Vehicle group. These results suggested that the icterus onset was enhanced by ingestion of Ge-132. The levels of plasma AST, ALT and γGTP in the *Early* stage were significantly increased in the GeCur group, suggesting that liver damage was severe (Fig. 2). However, hepatic histopathological examination revealed no significant difference in HAI index between the GeCur and Vehicle groups

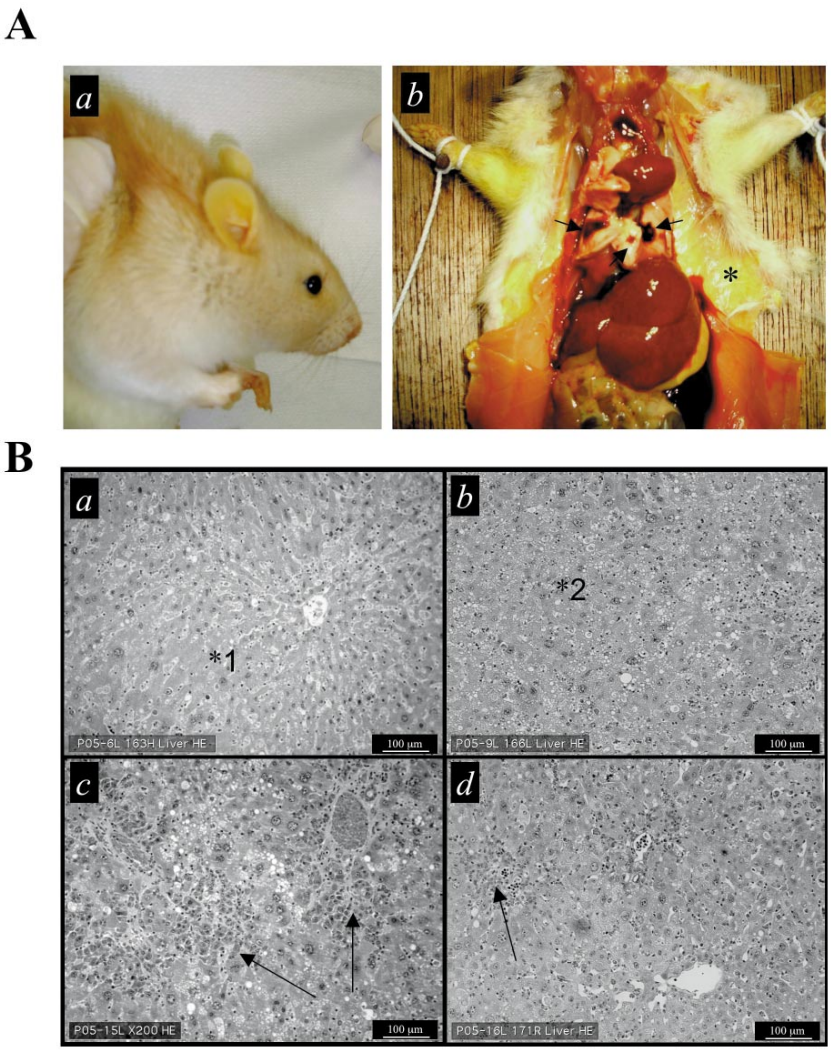


**Fig. 4.** Postmortem Pathological Examination of LEC Rats that Died of Fulminant Hepatitis

**A**: (a) Onset of icterus was confirmed by yellowish external skin color on any part of the body, especially at palms and auricles. (b) Gross pathology in male rats at 24 weeks of age. Arrows indicate multiple hemorrhage at lungs and an asterisk indicates icterus of subcutaneous tissue. **B**: Histopathology of liver from rats in Vehicle (a), Ge-132 (b), Curcumin (c), and Ge-132 + Curcumin (d) groups. The sections were stained with hematoxylin and eosin for evaluation of necroinflammatory grading. Asterisk (1) indicates central necrosis, asterisk (2) indicates microfatty degeneration, and arrows indicate the proliferation of oval cells. Scales indicate the length of 100 $\mu$m. Amplification × 200.

(Table 2). Therefore, the earlier of icterus onset might not be explained only by hepatic dysfunction. This was supported by the data that the icterus onset was earlier, although the plasma levels of AST, ALT and $\gamma$GTP and the HAI index in the *Early* stage did not rise in the Ge group.

With respect to mortality rate, most rats died in the *Early* stage and none died except in the Cur group in the *End* stage. Comparison of the mortality rate in the *Early* stage showed it to be high in the following order: GeCur (96.7%), Ge (57.1%), Cur (50%), Vehicle (42.9%) and NT group (37.5%). The rate in the GeCur group was thus significantly higher than that in the Vehicle group. These results suggest that ingestion of Ge-132 raised the icterus onset rate and mortality rate by acting on an organ besides the liver.

In the present study, the mortality rate was significantly higher in the group of GeCur. None of the plasma enzyme levels of the Ge and GeCur groups significantly increased in the *Late* stage. The hepatic histopathological examination of specimens from the rats that lived until the end of the experiment revealed that the ingestion of Ge-132 did not inhibit tumorigenesis (Table 3).

The mechanism by which the ingestion of Ge-132 increased the rate of icterus onset and the mortality rate remains to be identified. Since organic germanium compounds have immunostimulant and

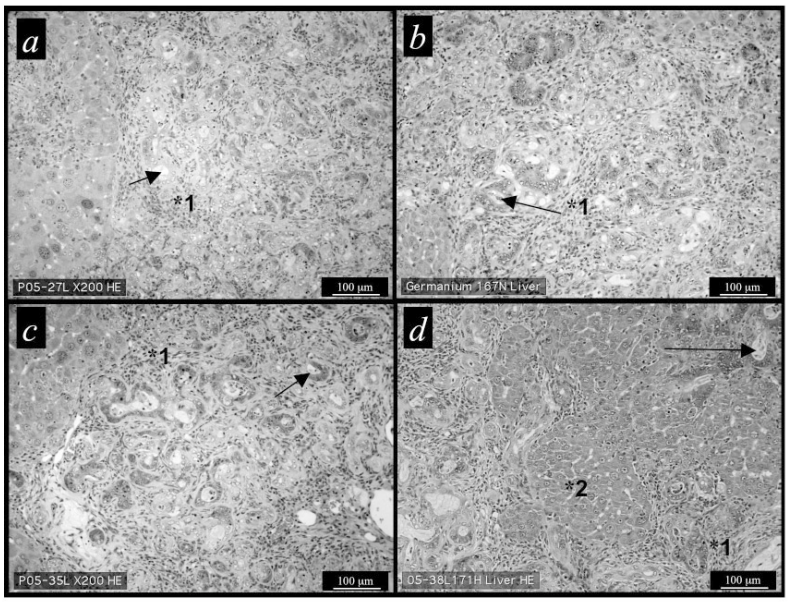


**Fig. 5.** Postmortem Pathological Examination of LEC Rats at 55–62 Weeks of Age

Histopathology of liver from rats in Vehicle (a), Ge-132 (b), Curcumin (c), and Ge-132 + Curcumin (d) groups. The sections were stained with hematoxylin and eosin for evaluation of necroinflammatory grading. Asterisks (1) indicate proliferation of fibroblast with fibrosis, an asterisk (2) indicates regeneration of hepatocyte, and the arrows indicate the proliferation of abnormal bile duct. Scales indicate the length of 100 $\mu$m. Amplification × 200.

**Table 2.** Histological Grading and Staging in Different Groups of Rats

| Group | HAI[a)] | | | | Tumor | Others |
|---|---|---|---|---|---|---|
| | Periportal Necrosis | Intralobular Degeneration and Focal Necrosis | Portal Inflammation | Fibrosis | | |
| Non treated | 0.33 ± 0.33 | 3.67 ± 0.33 | 0.67 ± 0.33 | 0.00 ± 0.00 | None | microfatty degeneration |
| Vehicle | 0.50 ± 0.29 | 2.75 ± 0.63 | 0.75 ± 0.25 | 0.25 ± 0.25 | None | |
| Ge-132 | 0.50 ± 0.29 | 3.75 ± 0.25 | 1.00 ± 0.00 | 0.00 ± 0.00 | None | |
| Curcumin | 0.00 ± 0.00 | 3.50 ± 0.29 | 1.50 ± 0.50 | 0.00 ± 0.00 | None | Oval cell (++) |
| Ge-132 + Curcumin | 0.33 ± 0.21 | 3.83 ± 0.17 | 1.00 ± 0.00 | 0.00 ± 0.00 | None | Oval cell (+) |
| Total | 0.33 ± 0.11 | 3.52 ± 0.16 | 1.00 ± 0.12 | 0.05 ± 0.05 | | |

*a*) HAI score was assessed based on the Knodell score as follows: Periportal Necrosis (none = 0, mild piecemeal necrosis = 1, moderate piecemeal necrosis = 3, marked piecemeal necrosis = 4, bridging necrosis = 5), Intralobular Degeneration and Focal Necrosis (none = 0, mild = 1, moderate = 3, marked = 4), Portal Inflammation (no portal inflammation = 0, mild = 1, moderate = 3, marked = 4), and Fibrosis (no fibrosis = 0, fibrous portal expansion = 1, bridging fibrosis = 3, cirrhosis = 4).

anti-viral infectious actions,[11)] the adjuvanticity by Ge-132, contrary to its antiinflammatory action, might have enhanced the inflammatory reaction and led to fulminant inflammation in our study. In addition, there are many reports demonstrating that germanium compounds caused renal disturbance in rat and human.[12,16,29–32)] Therefore, the mortality rate might have been increased by renal dysfunction by bilirubinemia accompanied with acute hepatitis, which might have been aggravated by Ge-132 *per se* in the present study. There is, however, a report that propagermanium inhibited lipopolysaccharide-induced hepatitis by inhibiting infiltration of inflammatory cells into a lesion.[33)] Therefore, it is assumed that Ge-132 has both stimulatory and inhibitory actions on an immune system.

Many studies reported that curcumin induced apoptosis of various cultured cancer cells and inhibited oncogene expression.[3,34,35)] The icterus onset and mortality rate was not influenced by the ingestion of curcumin. Plasma levels of AST, ALT and $\gamma$GTP of the Cur group were slightly increased in

**Table 3.** Onset of Hepatic Cancer and Histological Grading and Staging in Different Groups of Rats

| Group | Number of tumor-positive cases | Histology | | | | |
|---|---|---|---|---|---|---|
| | | HAI[a)] | | | | Tumor |
| | | Periportal Necrosis | Intralobular Degeneration and Focal Necrosis | Portal Inflammation | Fibrosis | |
| Non treated | 3/4 (75%)[b)] | 1.00 ± 0.55 | 4.00 ± 0.00 | 1.00 ± 0.00 | 0.60 ± 0.25 | Hyperplasia Intrahepatic cholangiocarcinoma |
| Vehicle | 4/4 (100%) | 1.00 ± 0.00 | 3.00 ± 0.00 | 1.00 ± 0.00 | 1.25 ± 0.63 | Intrahepatic cholangiocarcinoma |
| Ge-132 | 4/4 (100%) | 0.33 ± 0.33 | 3.33 ± 0.33 | 1.00 ± 0.00 | 0.33 ± 0.33 | Intrahepatic cholangiocarcinoma |
| Curcumin | 3/4 (75%) | 0.50 ± 0.29 | 2.75 ± 0.63 | 1.00 ± 0.00 | 0.75 ± 0.25 | Intrahepatic cholangiocarcinoma |
| Ge-132 + Curcumin | 1/1 (100%) | 0.00 | 1.00 | 1.00 | 3.00 | Intrahepatic cholangiocarcinoma |
| Total | 15/17 (88%) | 0.71 ± 0.19 | 3.18 ± 0.23 | 1.00 ± 0.00 | 0.05 ± 0.05 | |

*a*) See the detail in footnote of Table 2. *b*) Histological test for one sample could not be conducted due to poor formalin-fixation.

comparison with the Vehicle group without statistical significance, whereas the plasma level of LDH was significantly decreased (Fig. 2). Hepatocellular regeneration in the Cur group was evidenced by the fact that oval cells were abundantly observed. A few oval cells also appeared in the GeCur group, although not in the Ge group. Taken together, the appearance of oval cells might be linked to decrease in the plasma level of LDH. Furthermore, the plasma levels of AST, ALT and γGTP in the Cur group tended to be decreased as compared with those in the Vehicle group. However, since postmortem hepatic histopathological examination in the *Late* stage was not performed, the relationship between the levels of plasma enzymes and liver damage was not pursued further. From the results that tumorigenesis was not restrained in the Cur group, the ingestion of curcumin did not seem to have an inhibitory effect on the development of hepatic cancer in LEC rats.

Since accumulated Cu in the liver of these rats reacts with tissue oxygen and produces free radicals, pathological development of hepatitis and hepatic cancer strongly depends on Cu homeostasis.[27,36–39)] Dietary curcumin does not increase the Cu level in LEC rat liver,[40)] so that curcumin *per se* may not aggravate hepatitis. Curcumin does, however, inhibit activator protein 1 activity that promotes gene transcription of metallothionein,[34,41)] implicating that the decreased degradation of free radicals due to downregulation of metallothionein might diminish the anti-tumorigenesis effect by curcumin in these rats. It was further reported that oral ingestion of zinc (Zn) compounds prevented the onset of icterus by inhibiting Cu absorption from the intestinal tract or increasing tissue metallothionein concentrations.[42)] If concomitant oral ingestion of Ge-132 and curcumin inhibits absorption of Zn and decreases tissue metallothionein concentrations, then accumulation of Cu in LEC rat liver would be increased. However, there are no reports indicating that Ge-132 and/or curcumin inhibit absorption of Zn from the intestinal tract.

In summary, concomitant oral ingestion of Ge-132 and curcumin remarkably increased icterus onset rate and mortality rate in the *Early* stage, which was likely caused by synergistic actions of liver and renal disturbances by Ge-132 and by curcumin-induced cytotoxicity. Neither Ge-132 nor curcumin has an inhibitory effect on tumorigenesis in LEC rats. Since both substances seem to have different pleiotropic pharmacological effects, however, those supplementary diets, in which Ge-132 and curcumin are concomitantly taken in the hope of its favorable effect on hepatitis and hepatic cancers, might be very toxic in some chemical-induced hepatic dysfunctions such as Wilson's disease. We would like to stress that nutritional supplementary food should be pharmacologically tested for any toxic side effects to support self-medication as a part of health promotion.

**Acknowledgements** The authors thank Prof. Naoki Yoshimi (Tumor Pathology, Faculty of Medicine, University of the Ryukyus) for his help in the histological study and critical reading of the manuscript.

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