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Orally Administered Ginseng Extract Stimulates Liver Regeneration in Partially Hepatectomized Rats

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Summary. **The effects of the oral administration of ginseng extract (GE, the root of *panax ginseng* C. A. Meyer) and its constituents (GRG, ginsenoside-Rg_1 and GRB, ginsenoside-Rb_1) on liver regeneration were examined in 66% hepatectmized rats. When liver regeneration was evaluated by the mitotic index of the hepatocyte (MI), a significant increase in the MI was observed 2 and 3 days after GE administration, and the MI response was dose-dependent. Food intake was not affected by GE administration, nor there was any change in the serum insulin concentration. It was also noted that the oral administration of GRG produced a MI response similar to that caused by GE administration. Serum parameters indicating liver and kidney function were unchanged after GE administration.**

These observations lead us to conclude that orally administered GE and its specific constituent are capable of stimulating liver regeneration without serious systemic effects.

Key words—saponin, liver resection, hepatocyte proliferation, rat.

INTRODUCTION

In the past few decades, it has been well demonstrated that ginseng enhances liver functions, namely biosynthesis of cholesterol,[1] synthesis of nucleic acid and protein in the rat liver.[2] Moreover, recent studies have revealed that a fraction of ginsenoside-Rg_1 present in the roots of *panax ginseng* C. A. Meyer induces a number of hepatocyte gene expressions[3] and nuclear RNA synthesis.[4-6] In *in vitro* experiments, it has been shown that ginsenoside-Rb_1 and -Rc lead to lactate dehydrogenase A- mRNA synthesis.[7] These findings imply that ginseng contains fractions modifying the transcriptional activity of the hepatocyte gene, but the effects of ginseng on liver regeneration have not yet been evaluated in freely moving animals. On the other hand, ginseng consists of many constituents: ginsenoside-Rb_1, -Rc, -Rd and -Rg_1 can be identified in the *panax ginseng* C. A. Meyer.[8-10]

This study was designed to investigate whether orally administered ginseng influences liver regeneration, taking into consideration the chemical structure of the constituent.

MATERIALS AND METHODS

Subjects

Eighty-four male Wistar rats (Charles River Japan Inc., Tsukuba, Japan) were used. They were housed in the laboratory animal facility of Niigata University. The animals were reared separately and allowed free access to laboratory chow (Oriental MF, Oriental Co., Tokyo, Japan) and tap water. The room temperature was controlled at 24±2℃ with 12 h/12 h light/dark cycles (lighting from 08:00-20:00 h). Estimates of individual body weight, food and water intake and surgery were made between 10:00 and 12:00 h.

Study design

The animals were divided into four groups: GE (ginseng extract from the roots of *panax ginseng* C. A. Meyer)-treated (n=30), GRG (ginsenoside -Rg_1)-treated (n=6), GRB (ginsenoside- Rb_1)-treated (n=6), and

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a control group for each of the three groups (n=42). The GE-treated group was further divided according to the dose: GEI, 125 mg/kg/day and GEII, 250 mg/kg/day. GEI was given from 3 days before hepatectomy, and continued until the rats were sacrificed.

Hepatectomy and liver regeneration score

When the animals attained a weight of about 180 g (5.6-5.8 weeks of age), hepatectomy was performed under ketamine hydrochloride (35 mg/kg, i.p.) anesthesia by the method previously described.[11] In brief, the median and left lateral lobes of the liver, constituting two-thirds of the total liver mass, were removed. The abdominal wall was closed in layers. After surgery, the animals were returned to their cages and allowed access to food and water. Liver regeneration was evaluated 0, 2, 3 and 5 days after hepatectomy, when the remainder of the liver was removed and specimens of the caudate lobe of the liver were prepared with hematoxylin and eosin, and the proportion of hepatocytes in mitosis per 1000 counts was expressed as the mitotic index (MI).[12]

Body fatty score

Body composition of fatty parameters, Lee-index [body weight $(g)^{0.33}$/nasoanal length (mm)$\times$100] and retro-peritoneal white adipose tissue weight (RPWT), were estimated.[13]

Chemical analysis

Blood for chemical analysis was obtained from the aorta, cooled immediately with iced water, and centrifuged at 2,200 rpm for 20 min. The separated serum was stored at -20℃ until measurement of the following parameters with an autoanalyzer Hitachi-736, Hitachi, Tokyo, Japan):[14] total protein (TP, biuret method), albumin (Alb, bromcrezol green method), glutamic pyruvic transaminase (GPT, ultraviolet method), alkaline phosphatase (Alb, besseylowry method), total bilirubin (TB, azobilirubin method), total cholesterol (TC, cholesterol oxidase colorimetric method), triglyceride (TG, glycerol oxidase colorimetric method) and blood urea nitrogen (BUN, urease ultraviolet method). Plasma concentrations of insulin and glucose were determined by double-antibody radioimmunoassay and the glucose oxidase method described previously.[15]

Test solutions

GE (Tsumura & Co., Tokyo, Japan), GRG (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and GRB (Extra synthese, Cedex, France) dissolved in saline were used. These agents were orally given with a feeding tube either once (10 : 00-12 : 00 h) or twice a day (10 : 00-12 : 00 h and 18 : 00-20 : 00 h). The volume of each time was 0.1 ml. Saline was given as the control. It was first observed that the test solution was entirely swallowed without spitting when it was dropped onto the posterior part of the tongue.

Statistical analysis

The statistical significance of differences among the values was evaluated by ANOVA and Duncan's multiple range test: $p<0.05$ is defined as significant.

RESULTS

Orally administered GE increased the number of mitosis (Fig. 1). GE 125 mg/kg/day administration increased the MI after hepatectomy, and the MI response reached its peak 2 days after hepatectomy, then returned to the control level in another 3 days. When the same dose of GE was given twice a day and the MI was checked 3 days after hepatectomy, there was a dose dependency (Fig. 2).

When GRG and GRB were given, different responses in the MI were produced (Fig. 3). GRG 2.5 mg/kg/day had enhanced the MI 2 days after hepatectomy, whereas no MI response was induced by GRB 2.5 mg/kg/day.

Food and water intake are shown in Table 1. GE administration did not change the volume of food intake during the 6 days before and after hepatectomy, but water intake was increased by GE administration, and the increase tended to be dose dependent.

Insulin concentrations in the blood are shown in Table 2. GE administration had not affected the insulin concentration 0 and 3 days after hepatectomy (Table 2). GRG and GRB also brought about no changes in the insulin concentration (data not shown). There was no difference in glucose concentrations in all groups of rats on GE.

Blood chemical parameters indicating liver and kidney functions are shown in Table 3. No noticeable change in the parameters was caused by GE administration except for cholesterol metabolic parameters. GE administration had evoked a reduction in TC and TG concentrations 3 days after hepatectomy, and the response disappeared in about 2 days (Fig. 4).

Body fatty accumulative scores were affected by GE administration. RPWT was decreased 3 days

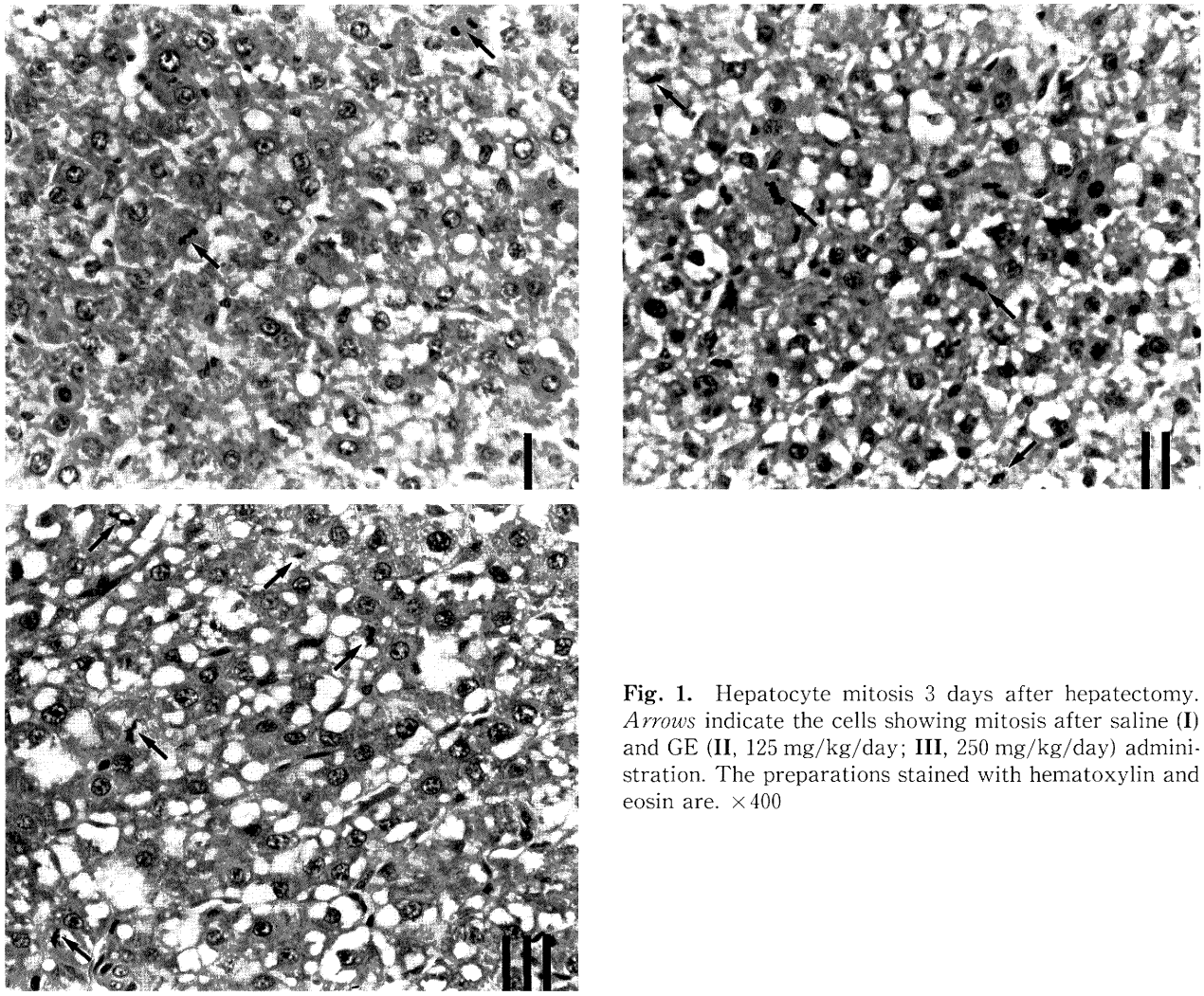

Fig. 1. Hepatocyte mitosis 3 days after hepatectomy. *Arrows* indicate the cells showing mitosis after saline (**I**) and GE (**II**, 125 mg/kg/day; **III**, 250 mg/kg/day) administration. The preparations stained with hematoxylin and eosin are. ×400

after hepatectomy, and the decrease tended to be dose dependent. On the other hand, there was no change in the Lee-index (Table 4).

DISCUSSION

We found that the oral administration of GE is effective in stimulating liver regeneration: GE produced an increase in MI after hepatectomy. The MI response due to GE might be peculiar to GE, because the response was dose-dependent. This is partially in keeping with the view that ginseng powder increases the rate of incorporation of labelled precursors into nuclear and cytoplasmic RNA in the rat liver.[4-6]

When a major constituent of GE, GRG, was administered, the MI was increased. This coincides with the finding that GRG induces a number of hepatocyte gene expressions and nuclear RNA synthesis.[4-6] Because the yield of GRG from the GE was estimated to be nearly 2-5%,[16] and the dose of GRG used in this study was almost equal to this rate, it is possible that GRG is one of the principle constituents stimulating liver regeneration. Concerning GRB, it has been shown that parenteral application of GRB 1.0 mg/100 g B.W. causes a noticeable increase in LDH A-mRNA in the regenerating rat liver.[17] However, in the present experiment, orally given GRB 0.25 mg/100 g B. W. failed to change the MI. This could mean that GRG is inactivated in the alimentary canal.

Food intake has been shown to modify liver regeneration after hepatectomy,[18,19] and ginseng has been shown to increase food intake,[20] but the dose of GE used in this study produced no effect on food intake (Table 1). It is considered that the food factor was

Table 1. Food and water intake during GE administration

	Saline	GE I	GE II
Food intake (g)	115.8±3.6	119.9±3.1	113.3±3.5
Water intake(ml)	170.1±13.9	192.6±8.7	198.0±10.2[a]

These values were obtained during the 6 days before and after hepatectomy. Values are the mean ±SEM (n=6). [a]$p<0.01$ vs saline.

Table 2. Serum concentrations of insulin and glucose with GE administration

	Saline	GE I	GE II
Glucose (mg/dl)			
Day 0	140.1±2.6	143.5±3.5	145.3±1.5
Day 3	144.8±8.2	140.8±4.3	147.3±4.0
Insulin (pM)			
Day 0	151±3	152±3	154±5
Day 3	150±2	145±4	153±5

These values were obtained 0 and 3 days after hepatectomy. Values are the mean ±SEM (n=6).

Table 3. Serum chemical scores after GE administration

		Saline	GE I	GE II
TP	(g/dl)	4.1±0.1	3.9±0.1	4.0±0.2
Alb	(g/dl)	1.7±0.1	1.7±0.0	1.7±0.1
TB	(mg/dl)	0.1±0.0	0.1±0.0	0.1±0.0
GTP	(U/L)	46.6±6.9	44.6±3.3	40.1±3.2
Alp	(U/L)	1117±120	1149±138	939±58
BUN	(mg/dl)	13.3±0.4	12.7±0.7	14.8±1.1

These values were obtained 3 days after hepatectomy. Values are the mean ±SEM (n=6).

Table 4. Body composition of fatty tissue following GE administration

	Saline	GE I	GE II
RPWT (mg)	621±28	483±28[a]	414±46[a]
Lee-index	301.2±2.8	301.6±1.6	300.0±1.9

These values were obtained 3 days after hepatectomy. Values are the mean ±SEM (n=6). [a]$p<0.01$ vs saline.

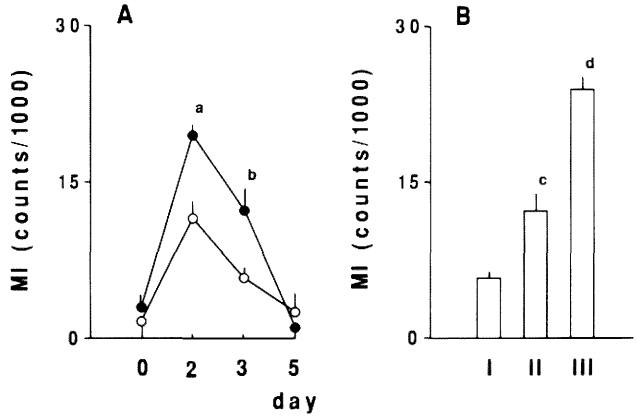

Fig. 2. **A.** Time courses for the MI after GE administration. GE (●, 125 mg/kg/day) or saline (○) was orally given. Day 0 is the day of hepatectomy. Values are the mean ±SEM (n=6). [a]$p<0.05$ vs saline. [b]$p<0.01$ vs saline. **B.** Responses in MI after different doses of GE. Three doses of GE (II, 125 mg/kg/day; III, 250 mg/kg/day) and saline (I) were administered, and the MI 3 days after hepatectomy was obtained. Values are the mean ±SEM (n= 6). [c]$p<0.01$ vs I. [d]$p<0.01$ vs II.

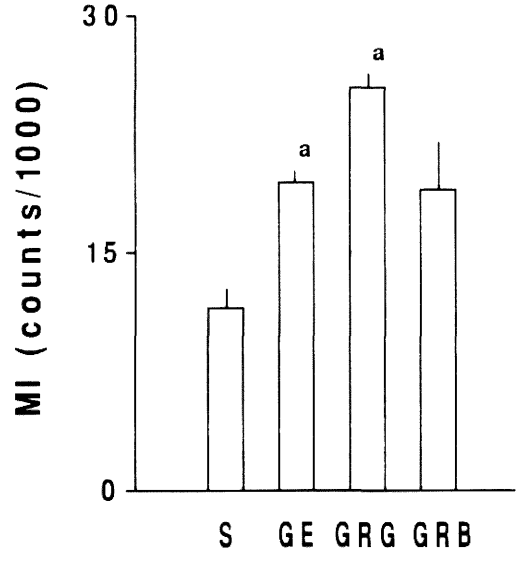

Fig. 3. Responses in the MI after GE and its fractions. GE (125 mg/kg/day), GRG (2.5 mg/kg/day), GRB (2.5 mg/kg/day) and saline (S) were orally administered, and MI 2 days after hepatectomy was obtained. Values are the mean ±SEM (n=6). [a]$p<0.01$ vs saline.

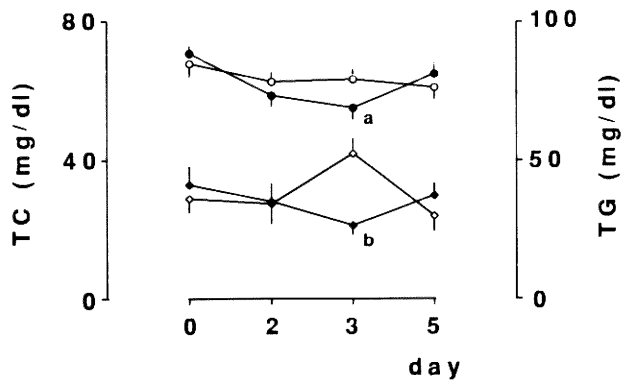

Fig. 4. Time courses for serum cholesterol parameters after GE administration. GE (●——◆, 125 mg/kg/day) or saline (○——△) was given, and TC (●——○) and TG (◆——◇) concentrations were estimated. Day 0 is the day of hepatectomy. Values are the mean ±SEM (n=6). [a]$p<0.05$ vs saline. [b]$p<0.01$ vs saline.

not involved in the regenerative response. Contrary to this, GE administration increased water intake, but the water factor could be eliminated from the observed response because changes in water intake have no influence on regeneration.[12,14]

Insulin is regarded as one of the hepatotrophic factors, because it exerts direct effects on organelles and stimulates DNA and protein synthesis.[21,22] In this study, GE did not cause any significant change in the serum insulin concentration (Table 2). It appears that the regenerative response caused by GE was not mediated by insulin secretion.

The finding that GE had no influence on TP or Alb (Table 3) implies that the nutrient condition of the animals was maintained normally during the experiment. Although ginseng has been shown to improve liver functional parameters GPT and Alp,[24] these parameters were unchanged. It is likely that the dose of GE is important in moving these parameters.

The visceral fatty accumulation, RPWT, was decreased by GE even when GE did not diminish food intake (Tables 1 and 4). Considering these findings together with the fact that the body fatty accumulation score, Lee-index, was unchanged, it is feasible that the regenerating rat liver primarily consumed the visceral fat store as an energy source.

Serum TC and TG concentrations were reduced by GE. It has been reported that partial hepatectomy increases hepatic and serum TC and TG concentrations.[24,25] It has also been found that ginseng suppresses the fatty parameters in animals with hyperlipid diets.[26,27] Our findings are in accordance with such reports,[24,26,27] and suggest that GE corrects the cholesterol metabolism in the liver after hepatic resection.

As mentioned above, GE consists of many different constituents,[16] but only a constituent enhancing liver regeneration was identified in this study. Further study on the remaining constituents of GE is necessary.

From these observations, we conclude that orally administered GE is capable of stimulating liver regeneration, and that GRG represents one of the active fractions of GE.

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