Restoration of Radiation Injury by Ginseng. III. Radioprotective Effect of Thermostable Fraction of Ginseng Extract on Mice, Rats and Guinea Pigs

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Radiation protection/Ginseng extract/Rescue of irradiated mice, rats and guinea pigs

Radiation protection by post-irradiation injection of a thermostable fraction of the ginseng extract in mice, rats and guinea pigs was studied. The thermostable fraction lost "by-effects" of decrease in body weight and splenic hyperplasia which were caused in injected mice by the original ginseng extract. The fraction protected mice (male) irradiated with 720 R of X-rays and rats (male) irradiated with 825 R with the dose about 6 mg per 100 g of body weight. The fraction also protected guinea pigs, both female and male, irradiated with 325 R with the dose about 80 mg per 300 g of body weight. The thermostable fraction stimulated recovery of thrombocyte and erythrocyte counts, but not leukocyte counts, in 550-R irradiated mice. Recovery of all the three blood cell counts was stimulated by the fraction in rats irradiated with 630 R and guinea pigs irradiated with 200 R. Comparison of stimulated recovery by the thermostable fraction of the ginseng extract among the three blood cell counts showed that restoring action was the most marked on thrombocyte counts, commonly in the three species of the animals.

INTRODUCTION

In previous papers1, 2), we reported that a ginseng extract injected after X-irradiation increased the 30-day survival ratio and accelerated recovery of thrombocyte and erythrocyte counts as well as splenic weight and splenic DNA in mice. But the extract induced a splenic hyperplasia in unirradiated animals. The splenic hyperplasia is caused by some of radioprotective substances, such as carbon particles3) and bacterial endotoxins4). We found that the by-effects of decreasing body weight and splenic hyperplasia by the extract were almost diminished by heating the extract solution retaining radioprotective activity.

In this paper the efficacy of ginseng on mice, rats and guinea pigs was examined by using the thermostable fraction of the extract.
MATERIALS AND METHODS

Ginseng Extract and Its Thermostable Fraction

Ginseng extract was prepared fundamentally by the method of Oura et al.\(^9\) described previously\(^0\). The extract was dissolved in physiological saline (46 mg/ml) and the insolubles were centrifuged off. The supernatant was neutralized with 0.5 N NaOH, heated in a boiling-water bath for 15 min, and then cooled. The resulting precipitate was centrifuged off, and supernatant or the thermostable fraction was obtained. Weight of the thermostable fraction was calculated by subtracting that of the two dried precipitates from that of the original ginseng extract. In some cases for guinea pigs, the fraction was prepared from two-fold concentrated ginseng extract solution.

Animals

Mice of ICR strain, 4 weeks old male, were purchased from Charles River Japan, Inc. They were housed ten in a cage at 24±1°C and 60±10% of relative humidity, and administered with nutritional chow (Oriental Yeast Co., Ltd., Japan) and water ad libitum. Acidic water (pH 2 with HC1) was given to mice to prevent contamination by Pseudomonas bacteria. Rats of Wister strain, 4 weeks old male, were purchased from Japan Clea Co., Ltd., housed 4-6 in a cage (CT-2 of Japan Clea), and administered with heat-sterilized water and the same nutritional chow as for mice. Guinea pigs of Hartley strain, 4 weeks old female and male, were purchased from Funahashi Farm Inc., housed 4 in a cage (CT-2) and administered with heat-sterilized water and nutritional chow (GM-3 of Funahashi Farm).

Irradiation and Administration

Irradiation with X-rays (200 kV, 20 mA, 0.3 mm Cu + 0.5 mm Al filter, 50 R/min) was carried out as mentioned previously\(^1\). Animals were whole-body exposed in a revolving-partitioned-plastic chamber at 6, 5 and 5 weeks of age for mice, rats and guinea pigs, respectively. Immediately (within 3 min) after exposure animals were intraperitoneally injected with about 2 mg in 0.2 ml for mice (average body weight 30 g) and with about 6 mg in 0.7 ml for rats (about 100 g). The dose applied to guinea pigs was about 20 mg in 2.0 ml or 80 mg in 4.0 ml per 300 g of body weight. Animals injected with only physiological saline were served as the control.

Measurement of Blood Figure

Unirradiated control groups, with and without the thermostable fraction, were prepared for the measurement of blood figure. The blood was sampled on days 1, 2, 4, 6, 8, 10, 14, 18, 22 and 30 after injection. Thrombocytes were counted automatically with a Toa PL-110 thrombocyte counter (Toa Electric Co., Ltd., Japan), erythrocytes and leukocytes with a Toa CC-110 blood cell
counter. The sample was obtained from eyelid for mice, from tail vein for rats, and from lower limb vein for guinea pigs. The same mice were never sampled again, but rats and guinea pigs repeatedly. Sampling from the same animals within 4 days was avoided in guinea pigs.

Statistics
Survival ratios 30 days after irradiation were statistically examined by Chi-square test applying Yates' correction. Blood cell counts were expressed as mean ± standard error.

RESULTS

Effect of Ginseng Extract and Thermostable Fraction of Survival and Weights of the Body and Spleen in Mice

Radioprotective effect of a thermostable fraction of the ginseng extract was examined in mice irradiated with 720 R. Table 1 shows that the thermostable fraction was as radioprotective as the original ginseng extract (P < 0.001). More than 65% of constituents of the extract was removed as precipitate after the heating procedure.

The weights of the body and the spleen were examined in mice injected with ginseng extract (5.8 mg) or the thermostable fraction (1.6 mg). The body weight was decreased by about 12% on day 1 (one day after injection, and so forth) by the ginseng extract. The growth recovered to the normal level on day 6 (Fig. 1A). On the other hand, administration of the thermostable fraction did not cause any decrease in the body weight (Fig. 1B). The ginseng extract resulted in a splenic hyperplasia; the splenic weight doubled on day 4, and more than 14 days were needed for recovery to the control level. But the thermostable

<table>
<thead>
<tr>
<th>Injection</th>
<th>Dose (mg)</th>
<th>30-Day survival ratio (%)</th>
<th>Difference from control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ginseng extract</td>
<td>6.0</td>
<td>77.5 (40)*2)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Thermostable fraction</td>
<td>2.0*3)</td>
<td>71.4 (42)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Physiological saline</td>
<td>0.0</td>
<td>20.0 (40)</td>
<td>—</td>
</tr>
</tbody>
</table>

*1) Injected in 0.2 ml of physiological saline.
*2) Numbers in parentheses show the number of animals.
*3) Two-thirds of the constituents of ginseng extract were removed as precipitate after heating procedure.
fraction caused only a slight increase in the splenic weight on days 1-2, showing recovery to the control level on day 3 (Fig. 2). The heating procedure diminished "by effects" of the ginseng extract, without changing radioprotective activity.

Fig. 1. Body weight of mice injected with ginseng extract (5.8 mg) or its thermostable fraction (1.6 mg). A, ginseng extract; B, thermostable fraction. –•–, experimental group; •••••, saline-injected control group.

Blood figure was measured on mice irradiated with 550 R. Thrombocyte, erythrocyte and leukocyte counts are shown in Figures 3, 4 and 5, respectively. In unirradiated mice the thermostable fraction resulted in a temporal decrease in thrombocyte counts on day 1 and the increase on days 4 and 18, and a temporal increase in leukocyte counts on day 6. Decrease in thrombocyte counts after irradiation preceded in the experimental group till day 6 (6 days after
irradiation, and so forth). The counts of both irradiated groups reached minimal values (about 7% of the normal) on day 10, and tended to recover after day 10. Recovery in thrombocyte counts was stimulated by the thermostable fraction and complete on day 22, while it was not complete in irradiated control group on day 30 (about 70% of unirradiated groups). Erythrocyte counts gradually decreased after irradiation, reaching minimal on day 14 and turned to increase thereafter. Recovery preceded in the experimental group, but the counts of both groups did not completely recover to those of unirradiated animals on day 30. Leukocyte counts decreased on day 1 in both irradiated groups, reaching minimal values on days 4-10. Recovery started on day 14 in both groups, but it was not complete in both groups on day 30 remaining about a half value of unirradiated animals. There seemed to be no effects of the thermostable fraction on recovery of leukocyte counts in mice.

Fig. 2. Splenic weight of mice injected with ginseng extract (5.8 mg) or its thermostable fraction (1.6 mg).
- ● --, ginseng extract;
- • --, control for ginseng extract (saline);
- ○ --, thermostable fraction;
- • - - -, control for thermostable fraction (saline).
Fig. 3. Thrombocyte counts of mice. —●—, 550 R-thermostable fraction; —○—, 550 R-saline; —●—, 0 R-thermostable fraction; —○—, 0 R-saline. Five animals for each point.
Fig. 4. Erythrocyte counts of mice. –○–, 550 R-thermo-stable fraction; –•–, 550 R-saline; —•—, 0 R-thermostable fraction; —○—, 0 R-saline.
Fig. 5. Leukocyte counts of mice. — ● —, 550 R-thermostable fraction; — ○ —, 550 R-saline; — ● ——, 0 R-thermostable fraction; —○—, 0 R-saline.
Radioprotective Effect of the Thermostable Fraction on Rats

Radiation protection by the thermostable fraction was examined on rats irradiated with 825 R. The 30-day survival ratio was significantly (P < 0.001) increased, from 30 to 80%, by injection of the fraction with the same ginseng-dose per body weight as for mice (6 mg per 100 g) as shown in Table 2.

Blood figure was observed in rats irradiated with 630 R. In unirradiated rats the thermostable fraction induced a temporal decrease (by about 70%) in thrombocyte counts on days 1-2 followed by an overshooting increase by about 90% on days 8-10. The observed values of thrombocyte counts fluctuated in unirradiated rats. After exposure the thrombocyte counts of 630-R saline group were decreased significantly, reaching minimal value (about 4% of the original) on days 10-14, and recovered to the original level on day 22. Decrease in thrombocyte counts preceded in ginseng group till day 8, but recovery was accelerated on and after day 10. The counts recovered to the original level on day 18 about 4 days earlier than in saline group (Fig. 6)

Erythrocyte counts of both irradiated groups decreased with time. The counts in saline group reached a minimal value (about 20% of the original) on day 18, and turned to recover thereafter. The thermostable fraction also accelerated recovery of erythrocyte counts which started to increase after day 10. But the recovery was still incomplete in both groups on day 30, with the counts of about 75% of those of individual unirradiated group (Fig. 7).

Leukocyte counts of both groups decreased significantly on day 1 after irradiation, reached minimal values (about 6% of the original in saline-group) on days 6-10. Decrease in the counts was less in ginseng group. The counts of saline group tended to increase on day 14, overshot to about 170% of the original on day 22, and recovered to unirradiated control level on day 30. Administration of the thermostable fraction accelerated recovery of leukocyte counts in irradiated rats; the counts recovered to a half of original on day 14 and recovered back to a normal level on day 22 including an overshooting multiplication to about 200% on day 18 (Fig. 8).

Table 2. Radioprotective effect of the thermostable fraction of ginseng extract in rats irradiated with 825 R of X-rays

<table>
<thead>
<tr>
<th>Injection</th>
<th>Dose (mg)</th>
<th>Dose ratio *1)</th>
<th>30-Day survival ratio (%)</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermostable fraction</td>
<td>6.0</td>
<td>1.0</td>
<td>80.0 (40) *2)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Physiological saline</td>
<td>0.0</td>
<td>—</td>
<td>30.0 (40)</td>
<td>—</td>
</tr>
</tbody>
</table>

*1) Ratio of dose to body weight compared with that for mice.
*2) Numbers in parentheses show the number of animals.
Fig. 6. Thrombocyte counts of rats. —●—, 630 R-thermostable fraction; —○—, 630 R-saline; —■—, 0 R-thermostable fraction; —□—, 0 R-saline. Ten (irradiated) or five (non-irradiated) animals for each point.
Radioprotective Effect of the Thermostable Fraction on Guinea Pigs

Effect of the thermostable fraction on survival ratio of guinea pigs exposed to 325 R was examined. Table 3 shows that the injection of dose-to-body weight ratio 4.1 (72 mg per 288 g) for male or 4.3 (76 mg per 294 g) for female was radioprotective, but injection with the same ratio as for mice (26 mg per 357 g) was not.

Blood figures were examined in male guinea pigs (average 293 g) irradiated with 200 R and injected with 105 mg of the thermostable fraction.

Thrombocyte counts were decreased two-stepwise by irradiation, firstly on day 1 and secondly on and after day 4. Decrease in the 1st step preceded in ginseng group. The counts of both groups were diminished to about 7% of the original on day 10 and then augmented thereafter. Recovery preceded in ginseng group, but the counts of both groups backed to the original level on day 30. In unirradiated animals the thermostable fraction brought about a decrease in the counts on days 1 and 2 (Fig. 9)
Fig. 8. Leukocyte counts of rats. —•—, 630 R-thermostable fraction; —○—, 630 R-saline; •—•—, 0 R-thermostable fraction; •—○—, 0 R-saline.
Fig. 9. Thrombocyte counts of guinea pigs. —•—, 200 R-thermostable fraction; —○—, 200 R-saline; ——•—, 0 R-thermostable fraction; ——○—, 0 R-saline. Five animals for each point.
Table 3. Radioprotective effect of the thermostable fraction of ginseng extract in guinea pigs irradiated with 325 R of X-rays

<table>
<thead>
<tr>
<th>Sex</th>
<th>Average body weight (g)</th>
<th>Dose (mg)</th>
<th>Dose ratio</th>
<th>30-Day survival ratio (%) Experimental</th>
<th>Control</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>357</td>
<td>26</td>
<td>1.2</td>
<td>45.0 (20) *3)</td>
<td>40.0 (20)</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Male</td>
<td>288</td>
<td>72</td>
<td>4.1</td>
<td>47.2 (36)</td>
<td>10.0 (40)</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Female</td>
<td>294</td>
<td>76</td>
<td>4.3</td>
<td>65.0 (20)</td>
<td>10.0 (20)</td>
<td>P&lt;0.01</td>
</tr>
</tbody>
</table>

*1) Injected in 4.0 ml of physiological saline per 300 g of body weight.  
*2) Dose to body weight compared to that for mice.  
*3) Numbers in parentheses show the number of animals.

Fig. 10. Erythrocyte counts of guinea pigs. ---•---, 200 R-thermostable fraction; ---○---, 200 R-saline; ---•---•---, 0 R-thermostable fraction; ---○---○---, 0 R-saline.
Erythrocyte counts of irradiated groups decreased after day 8, and the counts of saline group reached a minimal value of about 30% of the original on day 18. The decrease was less in experimental group and stimulated recovery by the fraction was manifest on day 22. Recovery of erythrocyte counts was not complete on day 30 in both groups; the counts of the two groups were about 80% of unirradiated groups. There were no essential effects of the fraction on erythrocyte counts of unirradiated guinea pigs (Fig. 10).

Fig. 11. Leukocyte counts of guinea pigs. •, 200 R-thermostable fraction; -o-, 200 R-saline; •-•-, 0 R-thermostable fraction; -o-o-, 200 R-saline.
Leukocyte counts are shown in Fig. 11. The counts significantly decreased on day 1 after exposure, and they (about 30% of the original) continued at low values on days 4-10. Recovery preceded in experimental group; the counts increased to the normal level on day 22 including an overshoot on day 18, while the counts in control group reached the normal level on day 30 including an overshoot on day 22.

Fig. 12. Comparison of stimulated recovery by ginseng among the three cell counts in mice, rats and guinea pigs after irradiation.

\[
\frac{[G]}{[S]} = \frac{\text{cell counts of ginseng-group}}{\text{cell counts of saline group}}
\]

- ○, thrombocyte counts; - △-, erythrocyte counts; -○-, leukocyte counts.
DISCUSSION

Previously we reported that a ginseng extract, prepared by the method of Oura et al. increased 30-day survival ratio of X-irradiated mice, and stimulated recovery of thrombocyte and erythrocyte counts as well as splenic weight and splenic DNA contents. In addition, radioprotective substances in a fraction of the extract were thermostable at a neutral pH. On the other hand, the ginseng extract caused a temporal decrease in body weight and splenic hyperplasia in injected mice. In the present study we found that thermostable fraction of the ginseng extract lost those "by-effects", still maintaining radioprotective activity. Stimulated recovery of blood figures in mice by the thermostable fraction was observed so similar as by the original ginseng extract.

The thermostable fraction also increased 30-day survival ratios of both rats (male, irradiated with 825 R) and guinea pigs (female and male, irradiated with 325 R). Substances being radioprotective and efficacious administered even after irradiation for more than two species of mammals may be rare.

Examination of blood figures was carried out with the lower radiation doses than those for survival experiments to avoid death of the irradiated animals within 30 days after exposure. Recovery of thrombocyte, erythrocyte and leukocyte counts after irradiation were all enhanced by the thermostable fraction in rats and guinea pigs irradiated with 630 R and 200 R, respectively. Stimulation of recovery of the three blood cell counts expressed as the ratios of counts of experimental to control group was illustrated in Fig. 12. Restoring action was the most marked on thrombocyte counts, commonly in the three species of the experimental animals. Recovery of thrombocytosis may be one of the most important factors for survival from radiation-induced bone marrow death. This is quite similar to the result obtained previously by using splenectomized mice that recovery of only thrombocyte counts was significantly improved by ginseng extract.

In this respect, it is quite reasonable that several materials such as lipoprotein-nucleic acid complex and atabrine that stimulated leukopoiesis after irradiation failed to reduce radiation-lethality.

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REFERENCES


