RESTORATION OF RADIATION INJURY BY GINSENG EXTRACT

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Introduction

There have been many attempts to obtain a radioprotective substance stimulating recovery for radiation injury by post-irradiation administration. Neverthless, none were practically useful for men because of the lack of reproducibility, toxicity, difficulty of exclusion, or other reasons. Bone marrow transplantation gives marked rescurative effect in experimental animals. But, in this case, forcoming immune response is serious. On the other hand, death by ionizing radiation occuring 10 to 20 days after irradiation is known to be primarily produced by the damage of blood forming tissues, and is called bone marrow death[1].

Recently, stimulating actions of ginseng on rat bone marrow and other tissues have been investigated[2-4]. Oral administration of a ginseng extract resulted in an increase of the number of mitotic cells both in myeloid and erythroid cells[3], and intraperitoneal injection of the extract also increased the rate of synthesis of serum albumin and gamma-globulin as well as DNA, RNA, protein and lipid synthesis in bone marrow cells[2].

We studied the restorative effect of the ginseng extract in mice given an acute dose of X-rays, expecting that it might prevent death of the irradiated animals by stimulating mitosis of the surviving bone marrow cells. In this paper we described the restorative effect of a single post-irradiation injection of partially purified ginseng extract in mice whole-body exposed to X-rays. Stimulation of the recovery of blood cell counts of the irradiated animals and that of the splenic weight were also briefly mentioned. In addition, a partial purification of the active components were reported.

Materials and Methods

Ginseng extract was prepared by the method of Oura *et al.*[4] of Research Institute of Oriental Medicines, Toyama Medical and Pharmaceutical University. Ginseng from Korea, which was dried and cut into round slices, was powdered in a ball mill. The powder was extracted with 0.05 *M* Tris-HCl buffer (pH 7.6) in a refrigerator for 2 days. The filtrate was centrifuged, and the clear supernatant was concentrated in cellophane tubes by blowing with an electric fan until the volume became about half the original. The concentrate was brought to 0.7 saturation with ammonium sulfate and centrifuged. The precipitate was dissolved in deionized water and the insolubles were discarded after centrifugation. The supernatant was applied on a Sephadex G-25 column, and the preceding fractions that showed negative

Nessler's test were collected. After lyophilization a slightly ocherous flake was obtained. This preparation corresponds to fraction 3 of the purification procedure of ginseng saponin described by Oura *et al.* Before injection the extract was dissolved in physiological saline and the insolubles were elimainated by centrifugation or ultrafiltration.

SPF mice of ICR strain, 4 weeks old male, were purchased and housed ten in a cage at 25 ± 1°C and about 60% of relative humidity, supplied with nutritional chow and with tap water *at libitum*. At 6 weeks of age they were whole-body irradiated with X-rays using a therapeutic X-ray generator(200 kV, 20 mA, 0.3 mm Cu + 0.5 mm Al filter, 50 R/min). The mice were immediately (within 5 min after exposure) injected intraperitoneally with the ginseng extract in 0.2 ml of physiological saline. Mice injected without the extract were served as the control. The control was set up at every examination. Difference between survival ratios 30 days after exposure was statistically examined by Chi-square test applying Yates' correction.

For the measurement of blood cell counts the blood was obtained from the eyelid for erythrocyte and leukocyte, and from the tail vein for thrombocyte. Erythrocyte and leukocyte was counted automatically, thrombocyte under microscope. The blood was sampled between 10:00 to 11:00 a.m. to avoid the diurnal change of the cell counts. The mice were killed by decapitation and the spleen and thymus were weighed.

Increased Survival of X-Irradiated Mice by the Extract

Difference of the survival ratio of mice irradiated with 720 R of X-rays between saline-injected control and the extract-injected group was statistically significant ($P < 0.001$) even with the dose of 1.8 mg per animal (about 30 g of body weight). The survival ratio increased with increasing dose of the extract. It increased from 5% (saline group) to 82.5% with 6.8 mg of the extract (Table 1).

Table 1. Effect of ginseng extract on the 30-day survival ratio of mice irradiated with 720 R of X-rays.

Dose (mg/animal)	Number of mice	Survival ratio (%)	Statistics (P)
0	40	5.0	—
1.8	40	45.0	<0.001
3.4	40	75.0	<0.001
6.8	40	82.5	<0.001

Effect of the Ginseng Extract on Haematological Recovery of the Irradiated Mice

Blood cell counts were measured of mice irradiated with 550 R of X-rays.

Erythrocyte counts decreased progressively and to the minimal (about 1/2 of the normal) on day 14, and increased thereafter in the saline-group. The counts decreased similarly till day 6 in ginseng-group, but started to increase on day 8.

The extract gave no marked effects on the recovery of leukocyte counts.

The thrombocyte counts decreased to about 1/13 the normal value on days 8–10 in saline-group. Recovery of the thrombocyte counts was significantly accerelated by the extract: the counts started to increase on day 10 in ginseng-group reaching to the normal level on day 22, while they started to increase on day 14 in saline-group and to the normal level on day 30. Blood figures on day 14 are illustrated in Fig. 1.

The splenic weight was reduced to about 1/3 on days 2–10 after irradiation. The decrease was lesser in ginseng-group. Recovery of the splenic weight was also accerelated with the extract. On day 10. the weight recovered to the normal level in mice injected with the extract while that of saline-group still remained in the low level and recovered to the normal on day 14–18.

There was little effect of ginseng on the recovery of thymic weight.

Partial Purification of the Active Components

As ginseng saponin are known to be the active components of the stimulation of bone marrow

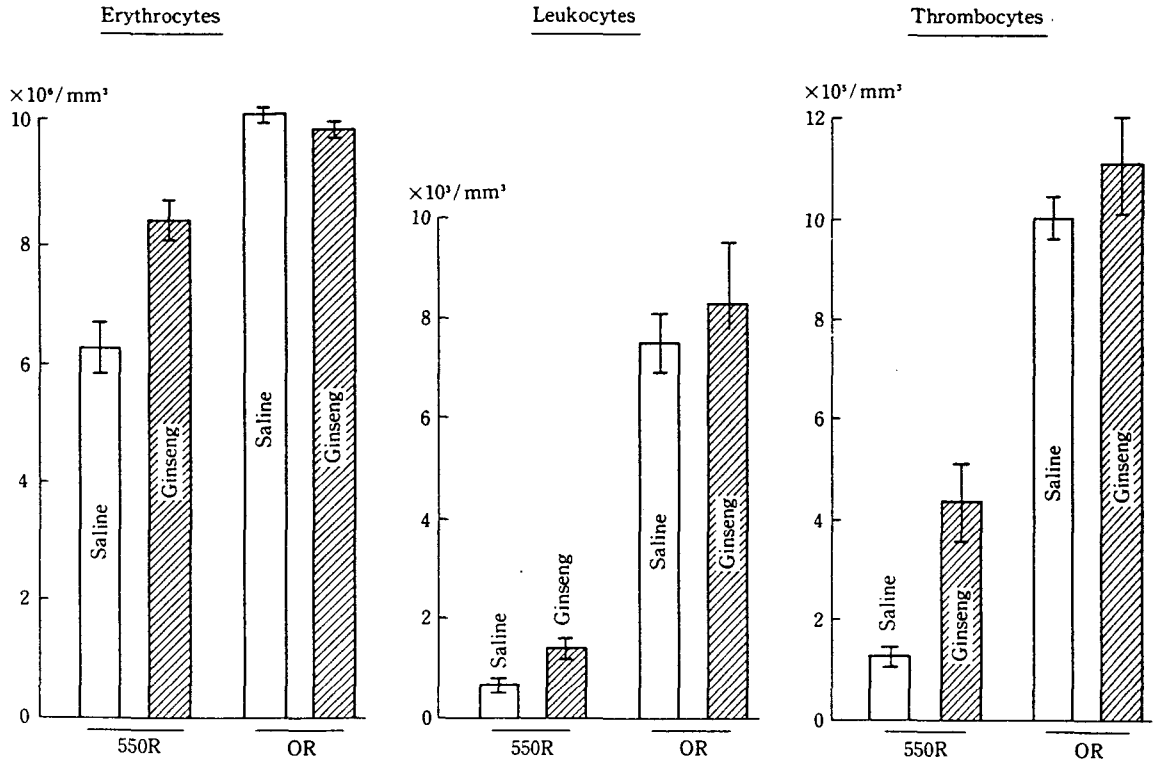

Fig. 1. Blood cell counts of X-irradiated mice on day 14.

cells[2,4], we first examined methanol-soluble fraction of the extract. The methanol-soluble fraction (5.0 mg per animal) was injected to the irradiated mice. But, it did not increase the survival ratio. In additon, a crude extract prepared by the method of Shibata *et al.*[5] (*i.e.*, ginseng powder was extracted with hot methanol and the extract was precipitated with ether) also failed to protect the irradiated animals up to the dose of 10 mg. These suggest that the active components of the extract may not be saponin. Since the extract gave a positive Biuret reaction and was inactivated with acid or alkali, some proteins were assumed to be the active components.

The extract was chromatographed on CM-cellulose column equilibrated with 0.02 *M* KH_2PO_4, and eluted with a linear gradient of 0.05 *M* KH_2PO_4 and 0.05 *M* phosphate buffer (pH 7.6). The extract was separated into two fractions: non-adsorbing fraction containing saponin and the other being adsorbed-and-eluted not containing saponin. The former fraction was significantly efficacious to rescue the irradiated mice at 5% level and the latter at 0.1% level with doses proportional to their yields, 2.5 and 3.3 mg, respectively. We interpreted that the main active components were contained in the latter fraction. This fraction, dissolved in saline and neutralized, was then heated in a boiling-water bath for 15 min, and the resulting precipitate was removed by centrifugation. The supernatant was still radioprotective at 0.1% level with dose of 0.8 mg per animal. This supernatant was gel-chromatographed with a Sephadex G-75 column and three fractions were obtained. Two of them (fraction 1 and 3) were efficacious with doses of 0.44 and 0.84 mg, respectively, but one (fraction 2) was not. The two active fraction showed UV spectra like protein.

Discussion

Radiation protection by a single post-irradiation injection of a ginseng extract in ICR strain mice was confirmed. This result agrees with that in NIH-Swiss strain mice as reported previously[6]. The extract seemed to prevent bone marrow death because it prevented death of mice occuring 10 to 20 days after irradiation. This was

supported by the haematological observations; recovery of both erythrocyte and thrombocyte counts after irradiation was clearly accerelated by the extract. The extract also enhanced the recovery of weight of the spleen, a blood-forming tissue of mice.

Nakamura[7] reported that repeated transfusion of freshly prepared thrombocyte-rich plasma was beneficial on survival of irradiated mice, while thrombocyte-poor plasma which contained erythrocytes and leukocytes showed no effects. Our data agree with his result. Stimulation of the production of thrombocytes after irradiation might prevent bone marrow death of mice.

Column chromatography on CM-cellulose of the ginseng extract showed that the main active fraction did not contain saponin and was thermostable. There were more than two active components in gel-chromatography. It is very interesting for us to clarlify the active components.

It has-been reported by Brekhman and his coworkers[8] that a ginseng extract may improve survival of irradiated animals, although the increase in survival was rather slight. We suppose their injection (extracted ginseng with saline and injected) did not contain sufficient amount of the active components, which we have described above, for statistic examination.

Chairman: Now the time is open to discussion

Questioner (from Korea): We have also found very recently the radio-protective effect of crude saponin mixture of ginseng in Chinese hamster cells in culture. But the effect was different whether ginseng was administered before radiation or after radiation. I wonder if you have any comment to make on that difference whether you ever tried giving your preparation before in comparison to after radiation.

Yonezawa: Did you use it after radiation or before radiation? We compared before and after the radiation. I mean we have somewhat different result whether ginseng was administered before radiation or after radiation.

Questioner (from Korea): We do not use Chinese hamster and I am sorry I have no opinion in that. Well, you use white ginseng or red ginseng?

Yonezawa: I used white ginseng. I never used red ginseng.

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