RESTORATION OF RADIATION INJURY BY GINSENG EXTRACT II.

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INTRODUCTION

At the 3rd International Ginseng Symposum held in Seoul, Korea in 1980 we, the authors, mentioned that there were no radioprotective substances practically useful to man through post-irradiation administration[1]. The present situation is almost the same, and we believe ginseng is still one of the most promising materials for this purpose.

This report consists of two parts. Part one is about the radioprotective effects of ginseng by using a thermostable fraction of the ginseng extract. Survival ratios, recovery of blood figures of irradiated mice, rats and guinea pigs, and recovery of blood forming pluripotent stem cells in bone marrow of irradiated mice are examined. Effect of ginseng on occult blood appearance in feces of irradiated mice is also mentioned. Part two is about the differences in radioprotective activity among different portions of raw ginseng roots harvested in several habitats in 1981 and 1982 in Japan.

MATERIALS AND METHODS

Ginseng extract was prepared by the method of Oura et al.[2] as noted previously[1]. In this study we used dialysis, instead of application to the Sephadex G-25 column, to eliminate ammonium sulfate and other impurities of low molecular weight. The ginseng extract was dissolved in physiological saline (46mg/ml) and the insolubles were centrifuged off. The supernatant was neutralized, heated in a boiling-water bath for 15min, and cooled. The resulting precipitate was centrifuged off, and the supernatant or thermostable fraction was obtained. The weight of the thermostable fraction was calculated by subtracting that of the two dried precipitates from that of the original ginseng extract. For guinea pigs the fraction was prepared from two-fold concentrated ginseng extract solution.

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 fed with nutritional chow (Oriental Yeast Co., Ltd., Japan) and water *ad libitum*. Acidic water (pH 3 with HCl) 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 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 fed with nutritional chow (GM-3 of Funahashi Farm) and heatsterilized water.

Animals were whole-body exposed to X-rays (200 kV, 20 mA 0.3mm Cu + 0.5mm Al filter,

50 R/min) in a revolving–partitioned-plastic chamber at 6, 5 and 5 weeks of age for mice, rats and guinea pigs, respectively. Immediately after irradiation animals were intraperitoneally injected with about 2mg in 0.2ml for mice (average body weight of 30g) and with about 6mg in 0.7ml for rats (about 100g). The dose adopted for guinea pigs was 80mg in 4.0ml per 300g of body weight. Animals injected with only physiological saline served as th control. Survival ratios 30 days after irradiation were statistically examined by Chi-square test applying Yates' correction. For the measurement of blood figure the blood was sampled on days 1, 2, 4, 6, 8, 10, 14, 18, 22 and 30 after irradiation. Thrombocyte counts were counted automatically with a Toa PL-110 thrombocyte counter (Toa Electric Co., Ltd., Japan), erythrocytes and leukocytes with a Toa blood cell counter. The samples were 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 were repeatedly. Sampling from the same animals within 4 days was avoided in guinea pigs. Five to ten animals were used per each point.

Pluripotent blood forming stem cells were measured as CFUs (colony forming unit in spleen) by the method of Till and McCulloch[3]. Bone marrow cells of mice (donor) irradiated with 525 R were intravenously injected into mice (recipient) previously irradiated with 1050 R. Five recipient mice were prepared for each donor mouse, 5-10 donor mice used per point. Ten days after the injection mice were killed and the spleens were fixed in Bouin's solution. Colonies on the surface of the spleen were counted.

Determination of occult blood in feces of irradiated mice was carried out by the method of Wahba[4] modified by us to a quantitative one. Daily feces of mouse irradiated with 650 R and bred separately was dried at 115°C until the weight became constant, and was powdered in a mill. To 40mg of fecal powder 30% actic acid (2.0ml) was added, stayed overnight, and extracted with ether (5.0ml) on the next day. The ether layer (1.0ml) was added to a mixture of 1.4ml of 36% acetic acid, 0.3ml of 3% hydrogen peroxide and 0.6ml of 0.5% ortho-dianisidine /ethanol, and mixed with a thermomixer. After 20 min absorbance at 460nm was photometrically observed. Hemoglobin content was determined by comparing the absorbance with the standard curve for purified hemoglobin. More than five animals were used for each point.

RESULTS AND DISCUSSION

Table 1 shows the radioprotective activity of the thermostable fraction in mice compared with that of the original ginseng extract. More than 65% of the constituents of the ginseng extract was removed as precipitate after the heating, without changing radioprotective activity. Fig. 1 shows the splenic weight of mice injected with either ginseng extract or its themostable fraction. The heating procedure diminished "by-effect" of splenic hyperplasia induced by the original extract.

The thermostable fraction rescued the irradiated rats and guinea pigs from bone marrow death (death occuring 10-20 days after irradiation) as shown in Table 2. The fraction also enhanced recovery of blood cell counts of erythrocytes, leukocytes and thrombocytes in irradiated

Table 1. Radioprotective effect of the thermostable fraction of ginseng extract on mice irradiated with 720 R of - rays

Injection	No. of animals	Dose (mg)	30-Day survival ratio (%)	Difference
Ginseng extract	40	6.2	77.5	$P < 0.001$
Thermostable fraction	42	2.0	71.4	$P < 0.001$
Physiological saline	40	0.0	20.0	

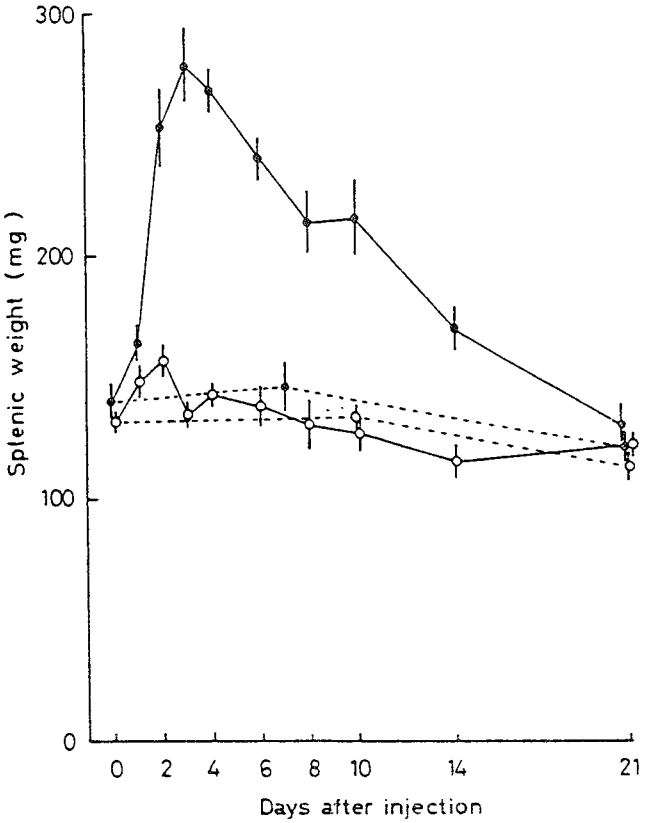


Fig. 1. Splenic weight of mice injected with ginseng extract (5.8mg) or its thermostable fraction (1.6mg). —●—, ginseng extract; - - ● - -, control for ginseng extract (saline); —○—, thermostable fraction; - - ○ - -, control for thermostable fraction (saline).

rats and guinea pigs. Fig. 2 shows comparison of stimulated recovery by ginseng among the three blood cell counts in mice, rats and guinea pigs after irradiation. Common to each of the three species of the experimental animals, the restoring action was the most marked on thrombocyte counts. The present result closely coincides with our previous one, in that the recovery of thrombocyte counts was one of the most important factors for survival of the irradiated mice[6].

Recovery of blood forming stem cells is the fundamental requirement for a radioprotective substance. Scheme 1 illustrates differentiation of blood forming stem cell. We examined recovery of pluripotent blood forming stem cells, which can be counted as CFUs (colony forming unit in spleen) by the method of Till and McCulloch[3]

Fig. 3 shows that the thermostable fraction of ginseng extract enhanced the recovery of the number of CFUs after exposure to 525 R of X-rays. The effect was significant on days 6-10.

Hemorrhage was found to be the primary symptom of high incidence in patients of Hiroshima and Nagasaki who had been exposed to atomic radiations. Radiation-induced hematopoietic death is thought to be caused by the cerebellomedulla oblongata hemorrhage[7]. Therefore, occult blood test is proposed as a good indicator for evaluating effectiveness of a test therapeutic treatment on animals exposed at midlethal or sublethal dose[8] Weimproved the assay method for occult blood in feces by converting to a quantitative one. Fig. 4 shows occult blood appearance in feces after 650 R of X-irradiation. Hemorrhage into daily feces was observed biphasically in the control group. Ginseng almost completely prevented the occult blood appearance. This would also mean that the tendency to hemorrhage after irradiation was prevented by ginseng.

These experimental results hitherto observed may indicate the promoting action of ginseng on recovery of thrombopoietic hematogenesis after irradiation at all following stages, *i. e.*

Blood forming stem cells (CFUs) ⟶ (Megakaryocytes) ⟶ Thrombocytes ⟹ Prevention of Hemorrhage

In preparing the extract from white ginseng we have met inactive ginseng materials. Therupon we started to examine difference in radioprotective activity of ginseng materials of different portions and different habitats by using raw ginseng roots harvested in Japan. Table 3 shows the yields of the extracts from 4-year roots harvested in 1981 and 1982 in Nagano, Fukushima and Shimane. Table 4 shows the radioprotective activity of each extract (30-31 mice were used for each group, and 40-43 for the control group). Among extracts from ginseng roots harvested in the three districts within the 2 years, those of Nagano had more activity than those of the other two districts (but, it was not always so from our experience in dried ginseng roots). The roots from Nagano grown in loamy soil were better than those grown in clay. The extract from xylem was

Table 2. Radioprotective effect of the themostable fraction of ginseng extract in rats and guinea pigs.

Animals (av. B. W.)	X-ray dose	Dose of the thermo-stable fraction	30-day survival ratio (exptl.: control)	Statistics
Rats (100g)	825 R	6.0mg	32/40 : 12/40	$P < 0.001$
Guinea pigs (male, 288g)	325 R	72mg	17/36 : 4/40	$P < 0.001$
(female, 294g)	325 R	76mg	13/20 : 2/20	$P < 0.01$

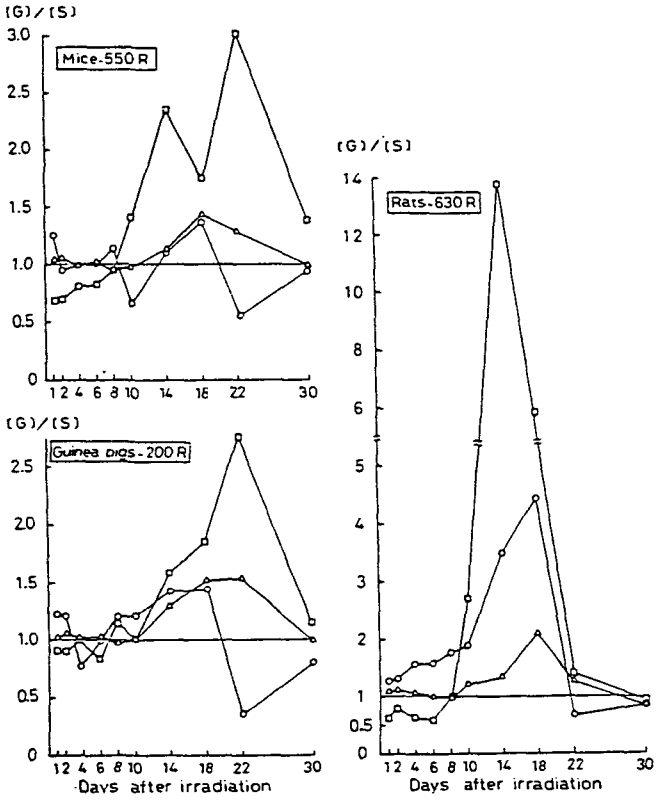


Fig. 2. Comparison of stimulated recovery by ginseng among the three cell counts in mice, rats and guinea pigs after irradiation.

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

—□—, thrombocyte counts; —△—, erythrocyte counts; —○—, leukocyte counts.

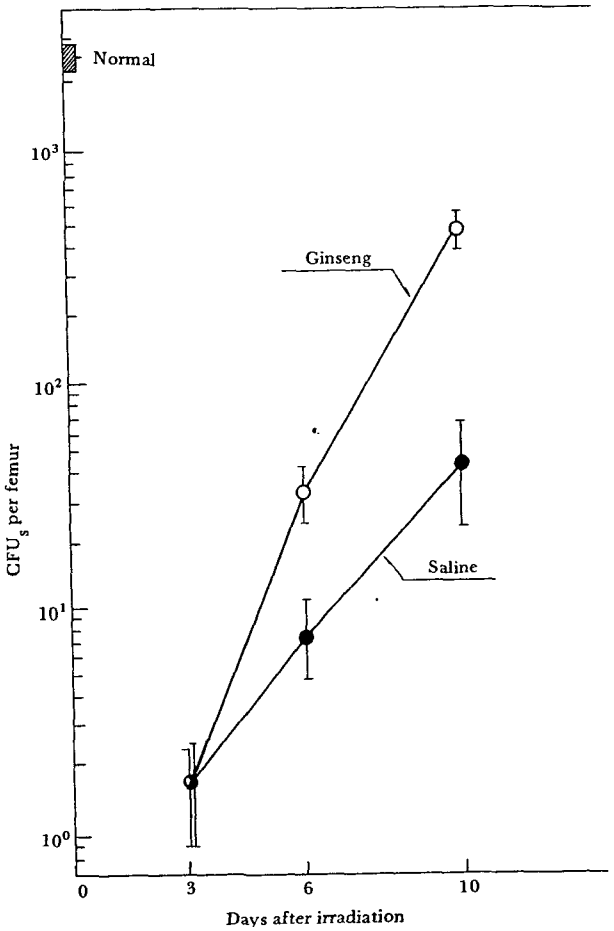


Fig. 3. Stimulated recovery of pluripotent blood forming stem cells by ginseng in bone marrow of mice irradiated with 525 R of X-rays.

more radioprotective than that from cortex and rootlet. This result agrees with our previous finding that the radioprotective principle of ginseng was not saponin[9].

Partially purified radioprotective fraction of the ginseng extract was composed of more than 90% protein and/or peptid and about 1.6% sugar moiety such as arabinose and galactose. It is interesting but complicated that under unirradiated conditions ginseng saponin is the active principle on bone marrow cells[10,11], but non-saponin fraction was the active component under irradiat-

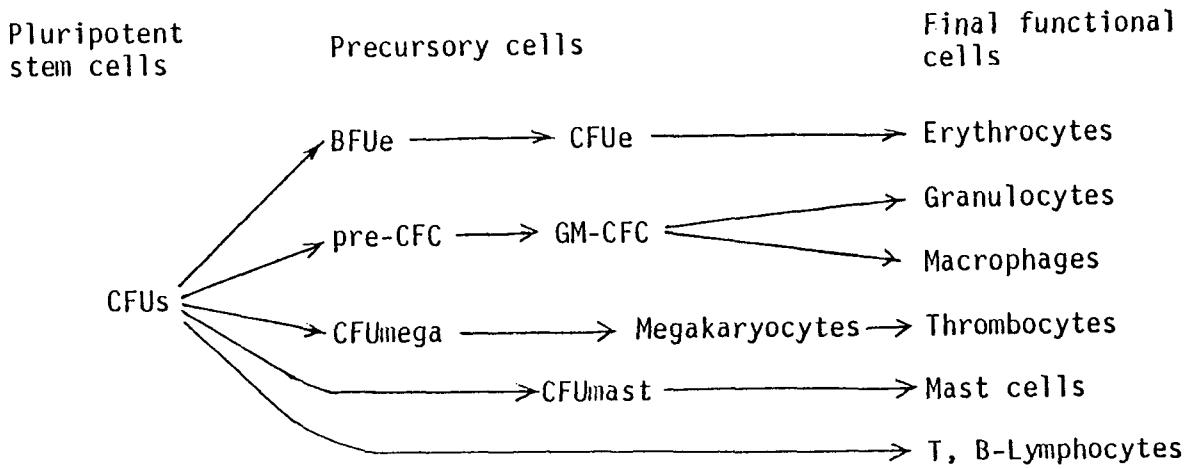
Table 3. Yield of the ginseng extract from various portions of raw ginseng roots harvested at various habitats in Japan

Harvested in 1981

Habitat	Soil	Portion	Wt. of roots(g)	Extract(g)	Yield(%)
Nagano	Loamy soil	whole	323	4.24	1.3
		Xylem	241	2.65	1.1
		Cortex & Rootlet	374	6.37	1.7
Nagano	Clay	Whole	322	3.87	1.2
		Xylem	296	2.40	0.8
		Cortex & Rootlet	326	5.10	1.6
Fukushima	—	Whole	408	5.02	1.2
		Xylem	278	2.85	1.0
		Cortex & Rootlet	459	8.30	1.8
Shimane	—	Whole	346	4.23	1.2
		Xylem	270	2.32	0.9
		Cortex & Rootlet	360	5.51	1.5

Harvested in 1982

Habitat	Soil	Portion	Wt. of roots(g)	Extract(g)	Yield(%)
Nagano	Loamy soil	Whole	319	2.63	0.8
		Xylem	264	1.86	0.3
		Cortex & Rootlet	497	5.48	1.1
Nagano	Clay	Whole	372	3.87	1.0
		Xylem	225	1.47	0.7
		Cortex & Rootlet	474	5.53	1.2
Fukushima	Loamy soil	Whole	369	4.93	1.4
		Xylem	247	2.35	1.0
		Cortex & Rootlet	451	6.43	1.4
Shimane	Loamy soil	Whole	296	3.60	1.2
		Xylem	226	2.69	1.2
		Cortex & Rootlet	359	5.10	1.4



Scheme 1. Differentiation of blood forming stem cells.

Table 4. Radioprotective activity of the thermostable fraction of ginseng extract from various portions of raw ginseng roots harvested at various habitats in Japan

Harvested in 1981

Habitat	Soil	Portion	Inject. dose (mg/animal)*1)	30-Day survival ratio (%)*2)	Statistics
Nagano	Loamy soil	Whole	4.3	48.4	$P < 0.001$
		Xylem	1.5	67.7	$P < 0.001$
		Cortex & Rootlet	6.2	61.3	$P < 0.001$
Nagano	Clay	Whole	4.8	19.4	$P < 0.05$
		Xylem	1.6	29.0	$P < 0.01$
		Cortex & Rootlet	7.0	12.9	$P > 0.05$
Fukushima	—	Whloe	5.0	3.2	$P > 0.05$
		Xylem	1.2	16.0	$P > 0.05$
		Cortex & Rootlet	6.7	12.9	$P > 0.05$
Shimane	—	Whole	4.1	6.5	$P > 0.05$
		Xylem	1.1	6.5	$P > 0.05$
		Cortex & Rootlet	5.7	13.3	$P > 0.05$

Harvested in 1982

Habitat	Soil	Portion	Inject. dose (mg/animal)*1)	30-Day survival ratio (%)*3)	Statistics
Nagano	Loamy soil	whole	5.0	33.3	$P < 0.05$
		Xylem	2.0	66.7	$P < 0.001$
		Cortex & Rootlet	6.1	3.3	$P > 0.05$
Nagano	clay	Whole	5.2	43.3	$P < 0.01$
		Xylem	2.0	43.3	$P < 0.01$
		Cortex & Rootlet	6.2	6.7	$P > 0.05$
Fukushima	Loamy soil	Whole	4.7	10.0	$P > 0.05$
		Xylem	1.4	26.7	$P < 0.01$
		Cortex & Rootlet	6.1	6.7	$P > 0.05$
Shimane	Loamy soil	Whole	4.4	16.7	$P > 0.05$
		Xylem	1.2	16.7	$P > 0.05$
		Cortex & Rootlet	6.3	6.7	$P > 0.05$

*1) 9.2 mg of ginseng extract was used per animal.
*2) Survival ratio of saline-injected control group was 2.5 - 7.5%.
*3) Survival ratio of saline-injected control group was 5.0 - 2.5%.

ed conditions. In our preliminary study a single *per os* administration failed to rescue the irradiated mice (adminsitration of red ginseng in chow pellets protected irradiated mice, but the effect was rather small compared with injection of the ginseng extract).

CONCLUSION

1. The thermostable fraction of ginseng extract increased survival ratios of X-iradiated mice, rats and guinea pigs.
2. The fraction accerelated recovery of blood cell counts, especially that of thrombocyte counts of irradiated mice, rats and guinea pigs.
3. The fraction enhanced recovery of pluripotent blood forming stem cells in bone marrow of irradiated mice.
4. The fraction prevented occult blood appear-

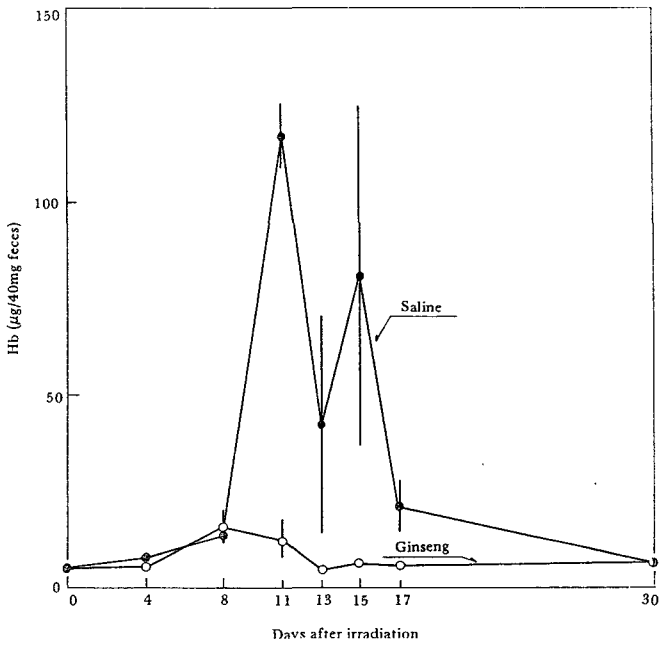


Fig. 4. Prevention of occult blood appearance by ginseng in daily feces of mice irradiated with 650 R of X-rays.

ance in feces of irradiated mice.

5. Ginseng seems to protect irradiated animals by enhancing thrombopoietic hematogenesis and deminishing hemorrhage.
6. The extract from xylem of ginseng roots was more radioprotective than that from cortex and rootlet.
7. Roots grown in loamy soil seems more radioprotective than those in clay.

ACKNOWLEDGMENT

A part of this work was supported by the Research Fund in Trust of Peaceful Use of Atomic Energy of Science and Technology Agency, Japan.

Fulder: We have done some studies on cell systems and found radio protection in cell culture. But we couldn't repeat this *in-vivo*. In fact, we found *in-vivo* that the animals were more sensitive to radiation. More animals died than the control when given ginseng, crude saponin mixture. So it is an opposite effect. You suggested this is possibly due to the route of the administration of the saponin. Have you got anything to add to this? Do you have any comments about why it should be that. If you give ginseng saponin, in one way you find radioprotection and in other way you find radiosensitivity?

Yonezawa: First point is that, I thin, in the *in-vitro* cell culture system, the type of the cell line you use is important. The second point is that whether your mice died within 10 days after radiation or not. When they survivied 10 days, the system, I think, might be clean. But when almost all the animals died within 10 days after radiation, the system might be infected with pseudomonas bacteria. If your system was clean, I have no idea because I don't know the actions of individual ginseng saponin on the irradiated animals. As far as the administration route was concerned, I think, intravenous injection would be better.

Cong: Let me ask you two questions. Did you measure the thrombocyte count? I just wonder whether you actually found any low red blood cell, white blood cell, and platelet counts in your study. The second point is that do you have any further information to substantiate that ginseng actually protects against hemorrhage?

Yonezawa: For the first question, initial decrease in thrombocyte count occured by injection of some stable fractions of ginseng extract. Though I didn't show you the individual cell counts, we have papers so we can show you the absolute value of each cell counts. And for the second question on the occult blood appearance in our experiment, the control group had eventually major occult blood appearence which maybe due to the diet that contains pea-powder. I think leucocyte count might be more important for man than mice.

인삼에 의한 방사선 손상 회복효과

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인체가 방사선에 의해 손상을 받게 되면, 실제적으로 치료, 회복시킬 수 있는 물질은 아직 발견되지 않았다. 이에 저자는 720R의 X-선을 조사시킨 mice에 Oura 등의 방법에 따라 부분 정제한 인삼 추출물을 투여하여 X-선 손상으로부터의 회복능을 검정하였다. 주사한 인삼 추출물의 용량에 의존적으로 30일간의 생존율이 증가하였다. Saline을 주사한 대조군과 인삼 추출물을 주사한 실험군 사이의 생존율의 차이는, 동물 한 마리당 1.8㎎을 투여한 실험에서 조차 통계학적으로 유의성을 보였다. ($P<0.001$)

550R의 X-선을 조사시킨 mice에 인삼 추출물을 투여하면 적혈구와 혈소판의 양적 회복이 촉진되었다. 또한 인삼 추출물중 열에 안정한 분획이 비장이 비대하여지는 것과 같은 부작용이 없어 방사선의 손상으로부터 보호 효과가 있음을 알았다. 이 분획은 mice뿐만 아니라, 반치사량의 X-선을 조사한 rat, guinea pig와 같은 실험동물에 있어서도 30일간의 생존율이 더 연장되므로서 현저한 효과를 보였다. 혈액상태 특히 혈소판의 양적 회복은 열에 안정한 이 분획에 의해서도 촉진되었다. 열에 안정한 분획을 투여한 mice에 있어서 X-선 조사에 의한 출혈이 방지되는데, 이를 매일 매일의 변에서 잠재혈액을 측정함으로써 정량적으로 관찰하였다.

결론적으로, 인삼 투여로 방사선에 의한 치사율이 감소되는데 이의 기전은 혈소판 생성을 촉진시키며, X-선에 의한 출혈을 감소시키기 때문이다.

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