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Radioprotective effects of Chinese medicinal prescriptions in mice

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Abstract

Continuous oral administration of the Chinese medicinal prescriptions, Hotyû-ekki-tô (HET), Syô-saiko-tô (SST) and Zyûzen-taiho-tô (ZTT) since 3 weeks before irradiation with a dose of bone marrow death, increased the 30-day survival ratios of X-irradiated mice. The administration enhanced the recovery of blood cell counts, especially that of thrombocytes as well as blood forming stem cells (CFUs) in bone marrow. In the irradiated mice, occult blood appearance in feces which reflects hemorrhaging tendency in tissues, was diminished by the three prescriptions.

Kew words Chinese medicinal prescriptions, oral administration, mice, X-rays, hemopoiesis stimulation, prevention of hemorrhage, radiation protection

Abbreviations CFUs, colony forming unit in spleen ; HET, Hotyû-ekki-tô (Bu-Zhong-Yi-Qi-Tang), 補中益気湯 ; SST, Syô-saiko-tô (Xiao-Chai-Hu-Tang), 小柴胡湯 ; ZTT, Zyûzen-taiho-tô (Shi-Quan-Da-Bu-Tang), 十全大補湯

Introduction

Many substances which exert the effect of radiation protection in mice have been reported.[1,2] Among these substances, radical scavengers such as WR2721 including the thiol group in their molecular structure provide the most effective protection against exposure. However, most of these chemicals have to be administered by injection immediately before irradiation, as it provides little or no protection when given orally.[3] In addition, severe toxicity is unavoidable. Recently, some oral-type of thiol compounds were developed, but the doses used for radiation protection were about one third of their $LD_{50/30}$ for mice.[4] Therefore it is difficult to apply these chemicals to get a large number of people to keep vital resistance against exposure. Little is known about the practical application of non-thiol radioprotectors such as hypoxic agents or amins, too. Further, there are very few reports about physiological radioprotectors with very low side effects.

In order to find out physiologically efficient substances to protect mice from radiation injury, we examined several Chinese medicinal prescriptions which have been used for patients to accelerate recovery from severe diseases. In this paper, radiation protection by continuous oral administration of three prescriptions, Zyûzen-taiho-tô (Shi-Quan-Da-Bu-Tang), Hotyû-ekki-tô (Bu-Zhong-Yi-Qi-Tang) and Syô-saiko-tô (Xiao-Chai-Hu-Tang) to mice was investigated at the main stages of thrombopoietic hematogenesis after X-irradiation.

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Materials and Methods

Administration of Chinese medicinal prescriptions : Freeze-dried extracts of three prescriptions of herbs were supplied from Tsumura-Juntendo Co., Ltd., Japan. The extracts were dissolved in drinking water without elimination of a little volume of insolubles. Suspension was separately given to mice freely since 3 weeks before irradiation and throughout thereafter. The concentration was 0.4 or 1.2 g per liter, and daily dose per body weight was estimated to be equivalent to the human dose (about 2 g/day).

Animals and irradiation : Six week-old male mice of ICR strain were whole-body irradiated with X-rays (200 kV, 20 mA, 0.3 mm Cu + 0.5 mm Al filter, 50 R/min). Each of the ten mice were housed in a cage at 25±1℃ and 60±10% of relative humidity (all fresh air) and were administered with nutrient chow.

Determination of radioprotective activity : Survival ratios on day 30 after irradiation were statistically examined by Chi-square test applying Yates' correction.

Measurement of hemogram and 10-day CFUs : Blood was collected from retroorbital plexus of mice. The same mice were never sampled again. The blood cells were automatically counted with a thrombocyte or cell counter.

The number of blood forming stem cells (10-day CFUs) was measured by the method of Till and McCulloch.[5] 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. Ten days after the injection, the mice were sacrificed and the spleens were fixed with Bouin's solution. Colonies on the surface of the spleen were counted.

Determination of occult blood appearance : Hemoglobin content in feces of irradiated mice was determined by the method of Yonezawa[6] which modified that of Wahba in a quantitative one. Daily feces of mice irradiated with 650 R and bred separately were collected, dried at 115℃, and powdered in a mill. More than 5 mice were used for each group. Hemoglobin content was determined by comparing the absorbance with the standard curve for purified hemoglobin.

Statistics : Indices are expressed as mean ± standard error.

Results

Effects of Chinese medicinal prescriptions on sur-

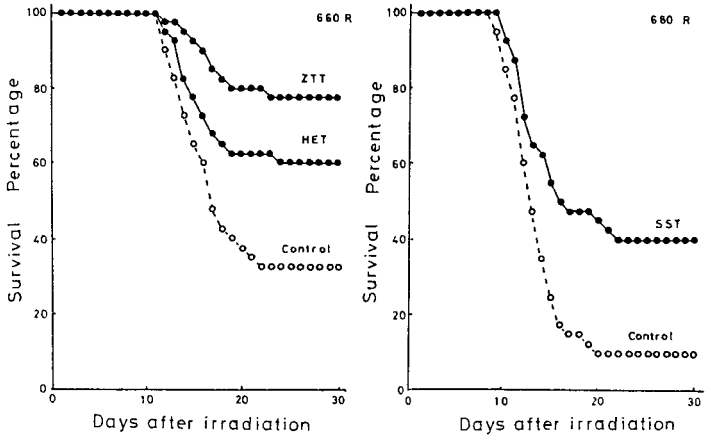

Fig. 1 Effect of ZTT, HET or SST on the survival of mice irradiated with 660 or 680 R of X-rays.
Forty animals were used for each group. The prescription was orally administered since 3 weeks before irradiation. ZTT : Zyûzen-taiho-tô, HET : Hotyû-ekki-tô, SST : Syô-saiko-tô.

vival in mice

Fig. 1 shows the time-survival relation of mice administered with three prescriptions, and irradiated with 660 R or 680 R. Protective effect of the drugs appeared at 10 to 20 days after exposure, that is, at the time of bone marrow death. Difference in 30-day survival ratios between the experimental and the control groups was significant (Table I).

Table I Effect of the Chinese medicinal prescriptions on the survival ratio and hemogram in mice.

Prescription	ZTT	HET	SST
Conc. (g/l)	0.4	0.4	1.2
Survival ratio			
Radiat. dose (R)	660	660	680
Significance	$p<0.001$	$p<0.4$	$p<0.005$
Hemogram			
Radiat. Dose (R)	550	550	550
Erythrocytes	+	+	±
Leukocytes	±	+	+
Thrombocytes	+	+	+

ZTT : Zyûzen-taiho-tô, HET : Hotyû-ekki-tô,
SST : Syô-saiko-tô

Prevention of occult blood appearance in feces of irradiated mice by Chinese medicinal prescriptions

When a positive occult blood reaction in feces of mice irradiated at the doses that produce bone marrow death, or even at a sublethal dose, was found, hemorrhage in various organs was also detected.[7] Therefore, the hemorrhaging tendency in various organs will be detected by the estimation of daily hemoglobin content in feces. In this experiment, the hemoglobin content in feces was estimated on days 11 and 15 after irradiation with 620 R, because the hemorrhage into daily feces was observed on those days with biphasical peaks in unadministered group.[6] Fig. 2 shows occult blood appearance in feces of irradiated mice. Among three prescriptions used, ZTT only diminished hemorrhage on day 11, and all the prescriptions did on day 15. Prevention of hemorrhage by the prescriptions is of use to avoid death in a critical condition of bone marrow injury.

Recovery of hemogram in irradiated mice

Hemogram was examined in mice irradiated with the lower doses to avoid death within 30 days after the exposure. Fig. 3 shows thrombocyte counts of mice irradiated with 550 R. On irradiation, they decreased rapidly at the 4th day, reached minimum at the 10th day and then gradually recovered at the 14th day. The rapid recovery in the administered groups was found to begin at the 18th day, but recovery in the control group delayed. Among the prescriptions employed, the effect of ZTT was somewhat better

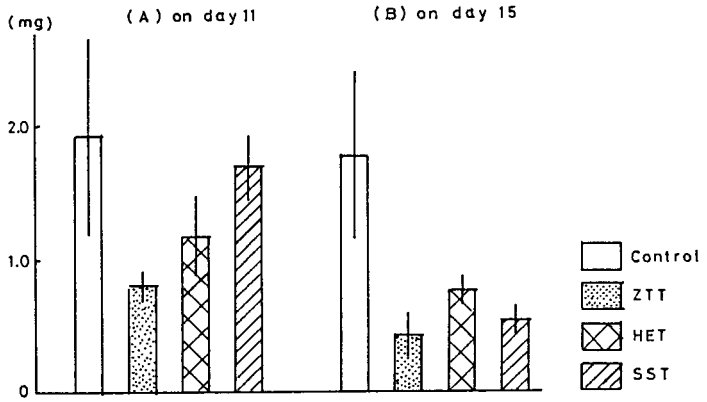

Fig. 2 Hemoglobin content in daily feces of mice irradiated with 620 R. Administered dose was 1.2 g/l.

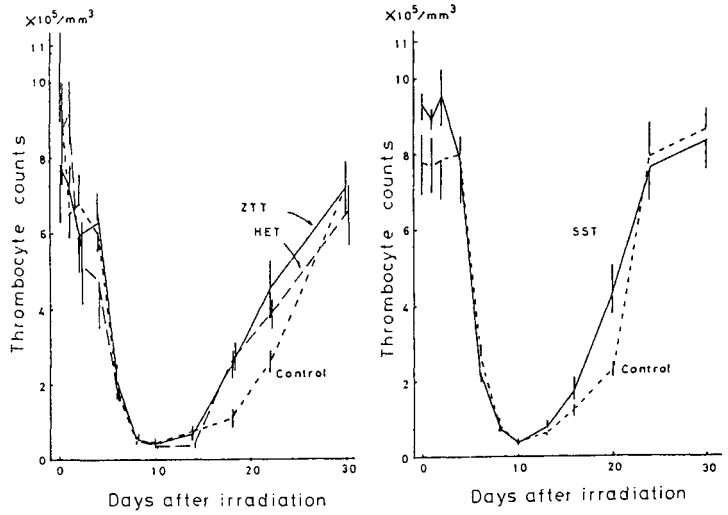

Fig. 3 Thrombocyte counts of mice administered with the prescriptions (1.2 g/l) and irradiated with 550 R. Five mice for each point.

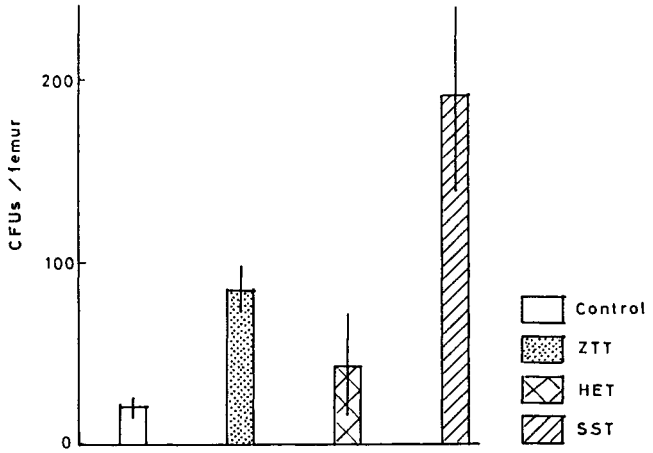

Fig. 4 Blood forming stem cells in mice irradiated with 525 R on day 10. Administered dose was 1.2 g/l.

than others. In the administered groups, the recovery of thrombocyte counts observed after the 14th day may be concerned in the prevention of hemorrhage on day 15 as in Fig.2.

Effects of each prescription on both erythrocyte and leucocyte counts in mice irradiated with 550 R are shown in Table I.

Stimulated recovery of pluripotent blood forming stem cells (CFUs) by Chinese medicinal prescriptions

10-day CFUs in mice irradiated with 525 R of X-rays were rarely found until the 3rd day after exposure, regardless of administration or non-administration of the prescriptions. An experiment carried out by Yonezawa *et al.*[6] introduced the result that the stimulated recovery

of CFUs by ginseng was marked on and after the 6th day. According to the procedure, we compared stimulating effect of CFUs by the prescriptions on the 10th day after irradiation with 525 R. As shown in Fig. 4, the three prescriptions stimulated recovery of blood forming stem cells (CFUs). The result means that the recovery of thrombocyte counts is not only due to the stimulation of a cell differentiation promoting factor, but the activation of all over the reticuloendothelial system.

Discussion

It is well known that exposure with a sublethal or midlethal dose of ionizing radiation produces bone marrow death at 10 to 20 days post-irradiation. Nakamura *et al.* reported after determining occult blood appearance in feces and observing tissue hemorrhage that radiation-induced hematopoietic death in mice was closely related to thrombopenia and was caused by the cerebello-medulla oblongata hemorrhage.[7,8] Since the hemorrhage in various organs was reflected on hemoglobin content in feces, we also examined it. As in Fig. 2, hemorrhage into feces was significantly prevented in the drug-administered mice. This result would be related to the prevention of hemorrhage in tissues, and to the increase in survival ratio after exposure on the administered mice (Fig. 1). The prevention of hemorrhage is supported by the enhancement in recovery of thrombocyte counts (Fig.3). These results would show that the recovery of thrombocyte counts is essential for the maintenance of life.

We found that oral administration of the prescriptions, ZTT, SST, and HET, enhanced recovery of CFUs in mice after exposure (Fig. 4). Ito and Shimura reported that oral administration of the prescription such as ZTT or SST enhanced the function of the reticuloendothelial system (RES) in tumor-bearing mice, though the activity with administration of HET appeared only when it was injected.[9] Mori *et al.* showed that blockade of RES by injection of carbon particles promoted the recovery of pluripotent blood forming stem cells, and they pointed out that radiation-resistant stromal cells might control the multiplication of CFUs after exposure.[10,11] In considering these results, the radioprotective effect would appear through the stimulation of the RES in mice.

The prescriptions are not radioscavengers, so that the protective effect on radiation is observed below the dose of bone marrow death.

The three prescriptions commonly contain ginseng and licorice. Yonezawa *et al.* reported that a single injection of the partially purified extract of ginseng before or after whole-body X-irradiation protected mice from bone marrow death, and pointed out that the accelerating recovery of thrombocyte counts was one of the most important factors for restoration.[6,12] Likewise, licorice has been found to have protectivity against radiation injury, though acceleration of recovery of thrombocyte counts was not observed.[13] Shiki *et al.* suggested that glycyrrhizin, one of the components of licorice, may stabilize lisosome by inhibiting phospholipase A_2 activity, which was involved in the lipid metabolism of rat arterial wall.[14] These results show that the radioprotective effect observed within the 14 days after the irradiation may be caused by factors other than stimulated recovery of thrombocyte counts.

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和文抄録

補中益気湯，小柴胡湯または十全大補湯をICR系雄性マウスに3週齢から連続して経口投与し，3週間後にX線を骨髄死を起す線量で全身照射して，方剤の放射線防護効果を調べた。これらの方剤投与は照射30日後の生残率を増大させたが，その結果は被曝により障害を受けた造血幹細胞数および血小板数の回復促進，更に，糞便中への出血傾向で表わさ

れる組織内出血の抑制により支持された。照射マウスの赤血球数および白血球数にも方剤による回復促進効果がみられた。

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