Radioprotective Effects of a Preparation (HemoHIM) of a Herb Mixture

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1. Introduction

The proliferation of radioactive materials in industry, medicine, scientific and medical research, the military, and as a source of energy has increased the likelihood of an accidental exposure to ionizing radiation. Synthetic radioprotective agents have been studied for decades but their application was limited due to their toxicity [1]. Thus, the radioprotective agent to protect individuals against severe radiation damage is required.

A preparation (HemoHIM) of a mixture of 3 edible herbs was designed to protect the gastrointestine and hematopoietic organs and to promote recovery of the immune system against radiation damage. In this study, we evaluated its radioprotective effects with regards to reduction of DNA damage, immune cell repopulation, intestinal crypt survival, and 30-day survival rate.

2. Methods and Results

2.1 Preparation of HemoHIM and its fractions

The mixture of 3 edible medicinal herbs (Angelica Radix, Cnidium Rhizoma, Paeonia Radix) was decocted with boiling water to obtain the total extract (T.W). The total extract was fractionated into methanol-soluble (F.M), ethanol-soluble(F.E) and ethanol-insoluble polysaccharide (F.P) fractions. HemoHIM was prepared by adding an ethanol-insoluble fraction to the total extract.

2.2 Inhibitory effects on radiation-induced DNA damage

The DNA damage of the lymphocytes exposed to γ-ray (2Gy) was determined by tail movement in a single cell gel electrophoresis [1]. The preparation HemoHIM and the total water extract of the herb mixture, the polysaccharide and ethanol fractions showed significant reductions of the tail movement (Figure 1). The methanol fraction showed the tendency to reduce the tail moment. These results show that HemoHIM and its fractions were effective at protecting DNA from γ-ray.

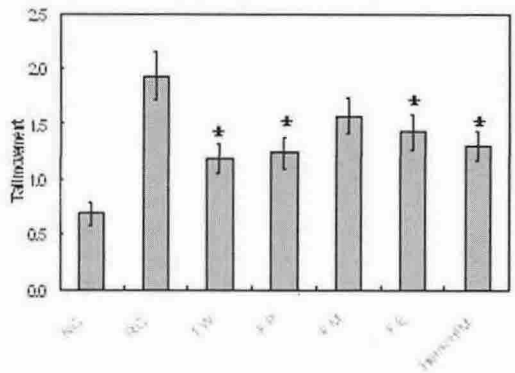

Figure 1. Inhibitory effect of HemoHIM and its fractions (400 μg/ml) against DNA damage induced by 2 Gy of gamma-ray (Mean±SE). *p<0.05.

2.3 Promotion of immune cell repopulation

Immune cells are greatly depleted after whole-body irradiation. The effects of HemoHIM on the regeneration of the white blood cells and lymphocytes in mice irradiated with a sublethal dose of γ-irradiation (4 Gy) are presented in Figure 2. The white blood cells and lymphocytes were depleted rapidly in the peripheral blood after irradiation and began to recover at day 3 after irradiation. In the HemoHIM administration group, blood cells were repopulated rapidly, and there was a significant recovery to the normal levels in the number of white blood cells and lymphocytes within 3 weeks after irradiation. In the untreated group, the recovery in the blood cells took place slowly, and continued steadily to completion within 7 weeks after irradiation.

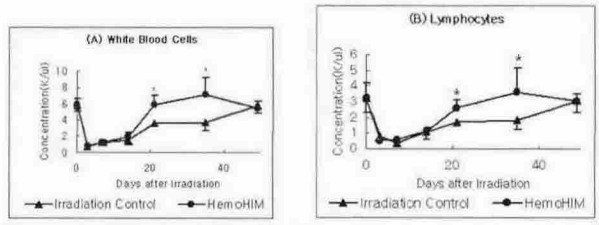

Figure 2. Effects of HemoHIM on the regeneration of white blood cells and lymphocytes after irradiation (4 Gy). Mice (6 mice/group) were i.p. injected with saline or HemoHIM (3mg/mouse) twice before and 7 times after irradiation. Mean ± SD. *p<0.05 as compared with the irradiation control group.

2.4 Enhancement of intestinal crypt survival

A high dose of irradiation causes damage to the intestinal crypt. The effects of HemoHIM and its fractsions on crypt survival were examined by the microcolony technique [2].

Non-irradiated controls show the density of the crypts at the bases of the villi. As shown in Table 1, there is an average of about 160 crypts per complete circumference. Pre-injection with the total water extract of the herb mixture resulted in a significant increase in the number of surviving crypts compared to those in the irradiation control. This activity was due to the methanol fraction of the extract. The injection of HemoHIM before or after irradiation significantly increased the number of surviving crypts. The oral administration with HemoHIM increased the number, but not significantly.

Table 1. Effect of HemoHIM on the intestinal crypt survival in the irradiated mice

Group	Crypt per circumference (M±SD)
Untreated control	157.25 ± 6.05
Irradiation control (12 Gy)	21.29 ± 9.71
T.W[a] (50mg/kg B.W.) + irradiation	35.12 ± 11.00*
F.M[a] (33mg/kg B.W.) + irradiation	41.16 ± 10.01*
F.E[a] (8mg/kg B.W.) + irradiation	32.70 ± 9.18
F.P[a] (8.5mg/kg B.W.) + irradiation	26.04 ± 18.90
Untreated control	158.52 ± 9.26
Irradiation control (12 Gy)	10.75 ± 7.45
HemoHIM[a] (50mg/kg B.W.) + irradiation	26.17 ± 10.79*
HemoHIM[b] (2mg/ml of drinking water) + irradiation	18.27 ± 14.11
Irradiation + HemoHIM[c] (50mg/kg B.W.)	21.42 ± 7.05*
Irradiation + HemoHIM[d] (2mg/ml of drinking water)	14.83 ± 6.09
Diethyldithiocarbamate[e] (1000mg/kg B.W.) + irradiation	34.15 ± 13.01**

[a]Sample was given I.P. at 36 and 12 hours before irradiation. [b]Sample was given P.O. for 7 days before irradiation. [c]Sample was given I.P. at 30 min and 24 hours after irradiation. [d]Sample was given P.O. for 9 days after irradiation. [e]Diethyldithiocarbamate was given I.P. at 30 min before irradiation. *$p<0.05$, **$p<0.005$ as compared with irradiation control group.

2.5 Increase of survival rate of irradiated mice

To evaluate the comprehensive radioprotective effects, the survival rate at day 30 after irradiation was investigated (Figure 3). HemoHIM was administered with drinking water (1 mg/ml) from 7 days before irradiation to the day of death, or given (25 mg/kg B.W.) i.p. twice each before and after irradiation (8Gy).

In the irradiation control group, 10% of the mice survived 30 days after irradiation. However, the survival rate of the HemoHIM administered groups was 35% for the orally administered group and 40% for the intraperitoneally administered group.

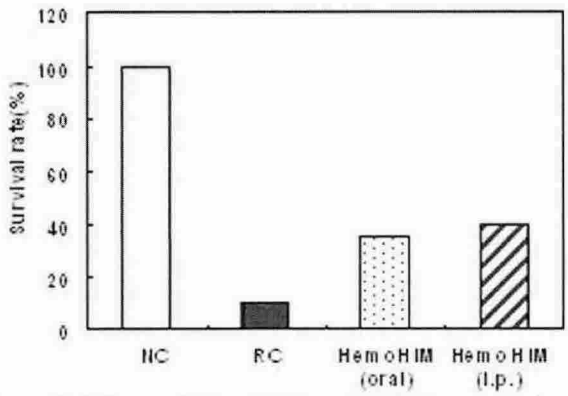

Figure 3. Effects of HemoHIM on the 30-day survival rate after irradiation (8 Gy). NC, normal control group; RC, irradiation control group; HemoHIM(oral), orally administered group; HemoHIM (i.p.), i.p. injected group. n=20.

3. Conclusion

The survival and recovery of the self-renewal tissues and hematopoietic organs are the fundamental requirements for radioprotection [4, 5]. In this study we have demonstrated that a herb mixture preparation (HemoHIM) effectively reduced radiation-induced DNA damage, enhanced the recovery of immune cells, increased the survival of intestinal self-renewal tissue, and finally increased the survival after lethal irradiation. This results suggest that HemoHIM is a good radioprotective agent, especially since it is a relatively nontoxic natural product. Further studies are being undertaken to investigate the active components and their mode of action.

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