ALTERATION OF RADIATION-INDUCED HEMATOTOXICITY BY BU-ZHONG-YI-QI-TANG IN MOUSE

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The purpose of this study reported here was to investigate the ability of Bu-Zhong-Yi-Qi-Tang (BZYQT), known to elevate hematopoietic functions, to protect mice undergoing treatment with whole body single gamma-irradiation. BZYQT was given (25 mg/kg B.W.) intraperitoneally at 36 and 12 hours before irradiation and 30 minute and 24 hours after irradiation. Recovery of neutrophil and lymphocyte counts was significantly stimulated by extract of BZYQT. Stimulated recovery by the extract from the BZYQT was also observed in thrombocyte. However, the anti-radiation effect of erythrocyte, hemoglobin and hematocrit was not as significant as that of leukocyte. Further studies are needed to better characterize the protective nature of BZYQT extract and its ingredients.

Keywords : Bu-Zhong-Yi-Qi-Tang, Radiation, Hematological value

1. INTRODUCTION

Despite the impressive accomplishments of the modern pharmaceutical industry, a large number of diseases remain for which treatment is of limited benefit. Furthermore, iatrogenic disorders have proved to be a consistent accompaniment of modern drug therapy. As a result, there is an increasing need for the development of so-called “alternative treatment by natural medicinal products” and health foods, for which predictable pharmacologic activity and therapeutic efficacy can be documented. A variety of such products have gained wide acceptance in east Asia and some European countries as mild nontoxic medications; however, for many products the active ingredients and mechanism of action remain to be determined. Traditional herbal medicines are widely used for treating many kinds of acute and chronic diseases. In Asia, drugs made of spray-dried powders of hot water extract obtained from mixtures of a number of plants used, as traditional Chinese medicines are available. Although the efficacy of herbal medicines has been acknowledged, their pharmacological activities as drugs have not yet been sufficiently evaluated experimentally or clinically.

As the diversity of radiation used in medicine, agriculture, industry, biochemical research and military operations increases, the risk from exposure including total body or local organs damage will be evaluated. Thus, the protection of individuals against severe radiation damage is an importance issue. Synthetic compounds have been studied for their ability to protect against the adverse effects of ionizing radiation since the original observation of radioprotection by Patt *et al.*[1]. Subsequently, many compounds have been tested for their ability to modify the effect of radiation[2]. Despite some toxic effects, newer compounds are currently under investigation as possible adjuvants in the radiation treatment of cancer[3]. Natural products such as herbal medicines have only recently begun to receive some attention as possible modifiers of the radiation response[4-9].

Bu-Zhong-Yi-Qi-Tang (BZYQT) consists of eight major herbs, *Ginseng radix, Angelicae radix, Cimicifugae rhizoma, Bupleuri radix, Aurantii nobilis pericarpium, Glycyrrhizae radix, Astragali radix and Atractylodis*

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rhizoma. Although clinical trials of BZYQT have been performed in Asia, no detailed hematological studies have reported. The present study was performed to determine the effect of BZYQT, a basic prescription as an energy tonic (Chinese medical concept : Bu-Qi) in irradiated mice.

2. MATERIALS AND METHODS

2.1 Animals

For measurement of hematograms and hematocrit, seven-week-old female ICR mice obtained from a specific pathogen-free colony at Oriental Inc. (Seoul, Korea) and allowed 1 week for quarantine and acclimatization. The Institutional Animal Care and Use Committee at Chonnam National University approved the protocols used in this study. The animals were cared for in accordance with the Guidelines for Animal Experiments. The animals were housed in a room maintained at 23 ± 2 °C, a relative humidity of 50 ± 5%, artificial lighting from 08:00 to 20:00 and 13-18 air changes per hour. The animals were housed four per stainless steel wire mesh cage, and were given tap water and commercial rodent chow (Samyang Feed Co, Korea) ad libitum.

2.2 Preparation and Treatment of Extract of BZYQT and Its Ingredients

Commercially obtained herbs were crushed and left in distilled water at 80 °C for 8 hours (100g of herb was extracted with 1000 ml of distilled water). The suspensions were spun at 1000g for 30minute and the supernatants were filtered and dried with a speed vacuum. An extract of BZYQT was prepared by mixing *Ginseng radix*(30g), *Angelicae radix*(15g), *Cimicifugae rhizoma*(9g), *Bupleuri radix*(9g), *Aurantii nobilis pericarpium*(15g), *Glycyrrhizae radix*(30g), *Astragali radix*(45g) and *Atractylodis rhizoma* (30g). BZYQT was given (25 mg/kg B.W.) intraperitoneally at 36 and 12 hours before irradiation and 30 minute and 24 hours after irradiation[10].

2.3 Irradiation

The animals were irradiated with 4Gy of ^{60}Co gamma-rays (Gamma-cell Elan 3000, Nordion International, Canada) at a dose-rate of 10.0Gy per minute.

2.4 Measurement of Hematograms and Hematocrits

Whole blood was collected from the ophthalmic venous plexus of mice on different days after treatments and the fluctuation of hematograms, including leukocytes, erythrocytes, thrombocytes, hemoglobin and hematocrit were automatically counted by a hematology analyzer (HEMAVET 850+, CDC Technologies, Inc. U.S.A.). A average values for each group were obtained from six mice per group.

2.5 Statistical Analysis

Data collected from the results are presented by means and standard deviations. Statistical analysis was performed by Student's *t*-test to express the difference between two groups.

3. RESULTS

The prescription elevated the total leukocyte, neutrophil and lymphocyte counts from the 3th day after administration (Table 1). A significant difference from the irradiated controls was seen at day 14 of the group treated with BZYQT and substantial differences from the irradiated controls at day 3, 7 and 21 were detected (Table 1 and 2). The significant elevations of erythrocytes and thrombocytes by BZYQT were found at days 21 (Table 2). The recovery of erythrocytes after irradiation was found earlier in the groups treated BZYQT and radiation than in the irradiated control group. The thrombocytes count was also protected by BZYQT administration. The effect of BZYQT administration on day 21 and 28 was significantly superior to the protection in other periods after irradiation (Table 2 and 3).

4. DISCUSSION AND CONCLUSION

Many chemical compounds that exert the effect of radiation protection in mice have been investigated. Washburn *et al*.[11] and Cairnie[12] suggested that these compounds such as WR2721, which include the thiol group in their molecular structure, have some effects on radiation protection. However, most of these chemicals have to be administered by injection immediately before irradiation, as they provide little or no protection when given orally or after irradiation. In addition, they produce severe toxicity to normal cells. As a result, it is difficult to use these chemicals in patients who receive high doses of radiation without additional side effects.

Traditional Chinese medicine, including simple herbs and compounded prescriptions, are known to be effective in accelerating recovery from disease or injury and do not appear to have obvious side effects. Therefore, it may be practical and valuable to develop Chinese medicines for eliminating radiation damage. It has been reported that many kinds of crude drugs such as ginseng[8, 9, 13], *Cnidium officinale*[14], *Angelica sinensis*[15], *Acanthopanax senticosus*[16], *Astragalus menbranaceous*[17], and

Table 1. Hematological values in mice administered with the Bu-Zhong-Yi-Qi-Tang (BZYQT) on day 3 and 7 after irradiation with 4 Gy (mean ± S.D.).

Hematological markers	Days after irradiation			
	3		7	
	Irradiation control	BZYQT[a]	Irradiation control	BZYQT
Erythrocyte (M/ul)	8.87 ± 0.72	8.97 ± 1.30	7.45 ± 0.79	7.45 ± 1.03
Hemoglobin (g/dl)	13.50 ± 0.53	13.70 ± 0.59	11.64 ± 0.57	11.38 ± 0.98
Hematocrit (%)	47.24 ± 2.92	48.44 ± 7.32	45.14 ± 4.77	48.50 ± 3.79
Thrombocyte (K/ul)	880 ± 145	1013 ± 218	620 ± 81	576 ± 57
Leukocyte (K/ul)	0.83 ± 0.05	1.06 ± 0.32[b]	1.71 ± 0.66	2.66 ± 1.11[b]
Neutrophil (K/ul)	0.15 ± 0.04	0.30 ± 0.11[c]	0.36 ± 0.24	0.65 ± 0.32 [b]
Lymphocyte (K/ul)	0.53 ± 0.08	0.60 ± 0.21	1.14 ± 0.51	1.66 ± 0.71

[a] Extract of Bu-Zhong-Yi-Qi-Tang was given (25 mg/kg B.W.) i.p. at 36 and 12 hours before irradiation and 30 minute and 24 hours after irradiation.
[b]$p<0.05$, [c]$p<0.001$ as compared with the irradiation control group.

Table 2. Hematological values in mice administered with the Bu-Zhong-Yi-Qi-Tang (BZYQT) on day 14 and 21 after irradiation with 4 Gy (mean ± S.D.).

Hematological markers	Days after irradiation			
	14		21	
	Irradiation control	BZYQT[a]	Irradiation control	BZYQT
Erythrocyte (M/ul)	6.89 ± 1.36	7.85 ± 0.83	8.29 ± 0.72	9.31 ± 0.35[b]
Hemoglobin (g/dl)	12.86 ± 3.04	14.40 ± 0.81	14.96 ± 0.40	13.92 ± 2.54
Hematocrit (%)	40.96 ± 8.14	44.04 ± 2.05	50.22 ± 4.77	46.62 ± 3.71
Thrombocyte (K/ul)	630 ± 160	641 ± 89	919 ± 120	1143 ± 157[c]
Leukocyte (K/ul)	2.30 ± 0.96	3.18 ± 0.81[d]	3.91 ± 0.86	6.01 ± 1.67[c]
Neutrophil (K/ul)	0.35 ± 0.25	0.93 ± 0.41[c]	1.17 ± 0.64	1.67 ± 0.67
Lymphocyte (K/ul)	1.65 ± 0.93	2.66 ± 0.95[d]	2.29 ± 0.70	3.86 ± 1.39[e]

[a] Extract of Bu-Zhong-Yi-Qi-Tang was given (25 mg/kg B.W.) i.p. at 36 and 12 hours before irradiation and 30 minute and 24 hours after irradiation.
[b] $p<0.001$, [c] $p<0.005$, [d] $p<0.05$, [e] $p<0.01$ as compared with the irradiation control group.

Ganoderma lucidium[5] have some effects on promoting recovery of radiation damage. Recently, many investigations focused on the radioprotective effect of medicinal herb prescription. Xiao-Chai-Hu-Tang, Shi-Quan-Da-Bu-Tang [18], Jen-Sheng-Yang-Yung-Tang[19], Si-Wu-Tang[20, 21], Dang-Gui-Shao-Yao-San[22] and Kuei-Pi-Tang[23] are examples of effective prescriptions investigated.

BZYQT has been reported to possess antitumor, antibacterial, antinociceptive and antidepressive activities and to have some effects on impairment of hematopoietic organs, stress incontinence and male infertility[24-29]. Administration of BZYQT significantly ameliorated not only leukopenia but also thrombocytopenia and decrease of erythrocyte induced by radiation. According to the conclusions of Floershiem *et al*.[30], it would appear that BZYQT affords hematological protection by both preventing the destruction of blood cells and enhancing hematopoietic recovery. This means that not only the circulating blood cells but also the progenitor cells are protected from irradiation by BZYQT administration.

Table 3. Hematological values in mice administered with the Bu-Zhong-Yi-Qi-Tang (BZYQT) on day 28 and 42 after irradiation with 4 Gy (mean ± S.D.).

Hematological markers	Days after irradiation			
	28		42	
	Irradiation control	BZYQT[a]	Irradiation control	BZYQT
Erythrocyte (M/ul)	8.88 ± 0.39	9.20 ± 0.66	8.54 ± 0.75	9.18 ± 0.78
Hemoglobin (g/dl)	14.60 ± 0.55	15.76 ± 1.04	15.48 ± 1.11	15.80 ± 0.84
Hematocrit (%)	50.44 ± 0.55	50.80 ± 7.65	49.00 ± 4.34	50.78 ± 4.04
Thrombocyte (K/ul)	1020 ± 297	1279 ± 124[b]	1179 ± 197	1223 ± 88
Leukocyte (K/ul)	4.33 ± 1.13	4.80 ± 0.92	5.38 ± 1.34	5.39 ± 0.98
Neutrophil (K/ul)	1.24 ± 0.69	1.28 ± 0.42	1.37 ± 0.35	1.46 ± 0.33
Lymphocyte (K/ul)	2.75 ± 0.67	3.22 ± 0.61	3.42 ± 1.30	3.93 ± 0.62

[a] Extract of Bu-Zhong-Yi-Qi-Tang was given (25 mg/kg B.W.) i.p. at 36 and 12 hours before irradiation and 30 minute and 24 hours after irradiation.
[b] $p<0.05$ as compared to the irradiation control group.

The present study indicates that repeated administration of BZYQT in a single dose irradiation regime enhances post-irradiation hematopoietic recovery in sublethally irradiated mice. These findings suggest that BZYQT is potentially valuable in protecting against hematopoietic injury induced by high dose irradiation. Although our preliminary investigations might provide an experimental basis for the use of BZYQT as a selective radioprotector of hematopoietic cells, further studies are needed to better characterize the protective nature of BZYQT extract and its ingredients.

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