Medical Journal of the
Islamic Republic of Iran

Volume 7
Number 2
Summer 1372
August 1993

Basic Science In Medicine

CIMETIDINE CAN MODIFY THE EFFECTS OF WHOLE BODY γ-IRRADIATION ON LYMPHOHEMATOPOIETIC SYSTEM

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ABSTRACT

The hematopoietic syndrome is anticipated when a dose of radiation greater than 100 cGy is received. The resulting clinical situation is life-threatening because of opportunistic infections and gradual decline in immune competency due to irradiation. Because of evidence of a possible immunomodulatory role for cimetidine, an antagonist of histamine H2 receptors, we studied the effects of this drug on radiation-induced lymphohematopoietic changes. The results obtained in this study indicate that cimetidine is effective in the reduction of radiation-induced injuries with a dose reduction factor of greater than 1.5. Therefore, it might be useful as a radioprotector for low doses of radiation usually used for radiation therapy.
MJIRI, Vol. 7, No.2, 95-99, 1993.

INTRODUCTION

Radiation-induced lymphohematopoietic syndrome is characterized by a depression in the peripheral blood levels of the white and red blood cells and platelets, as well as loss of weight and decrease in the size of lymphatic tissues such as spleen and thymus gland. The degree of depression and its duration is shown to be dose-dependent.[1] For reduction of radiation effect, certain drugs known as radioprotectors are used. Since the discovery in 1949 that cysteine has the ability to increase the survival of lethally-irradiated mice,[2] efforts for development of new compounds as suitable radioprotectors continued. Nowadays, aminethylisothrouronium (AET) and WR-2721 have been introduced as powerful radioprotectors.[3] Besides their various side effects, these drugs need to be administered at high doses. WR-2721, the best of these, is capable of producing a dose reduction factor (DRF) as high as 2.7 for gamma radiation in mice after intraperitoneal (I.P.) injection at high doses.[4] AET, at a dose of 400 mg/kg I.P. provides a DRF of up to 2.1.[5] In the present investigation, the effects of cimetidine on radiation-induced damage caused by various doses of Co-60 gamma rays was studied. Recently, it has bee shown that this drug is able to modulate the effects of mustard gas on the immune system.[6] Cimetidine is an antagonist of histamine type II receptors and is used clinically for treatment of peptic ulcer. This drug was chosen for two main reasons: first, it is not very toxic to T-lymphocyte activity; and secondly, it is available and does not posses major side effects.[7] Exposure to ionizing radiation causes skin erythema and release of histamine from arteries as well as production of free radicals due to indirect effects of γ-rays in the biological system.[8] It was shown that reaction of specific receptors with histamine can reduce cellular performance of multinucleated leukocytes and lymphocytes. Recently, it has been revealed that a group of T-cells having histamine receptors might have a suppression effect.[9] Therefore, an antagonist of histamine type II receptors

might play a role in the immune system and inhibit the function of suppressor T-cells. Thus, because cimetidine has an anti-suppressor cell activity,[10] it might prevent radiation-induced injuries in lymphatic tissues and lymphocyte production centers in bone marrow.

MATERIALS AND METHODS

Animals

Male mice of CD-1 strain and at the age of six weeks were purchased from Pasteur Institute, Tehran. Mice were housed in metal mesh cages in good condition and given food and water ad libitum for two weeks before being used for experiments. A total number of 200 mice were used in this study.

Irradiation

Irradiation was carried out using a therapy unit Co-60 gamma ray machine (ACEL Model 780, Canada). Animals were irradiated at various doses of radiation, 1, 2, 4, 6, and 8 Gy in a plastic box with a source sample distance (SSD) of 80 cm at room temperature (24 ± 2°C). Dose rate at this condition was 66.6 cGy/min.

Treatment

Cimetidine (200 mg/2 ml), commercially available, was diluted in physiologic serum and injected at a final concentration of 15 mg/kg body weight I.P. Mice were treated with cimetidine 2 hours prior to irradiation and its administration was continued for 10 days post-irradiation.

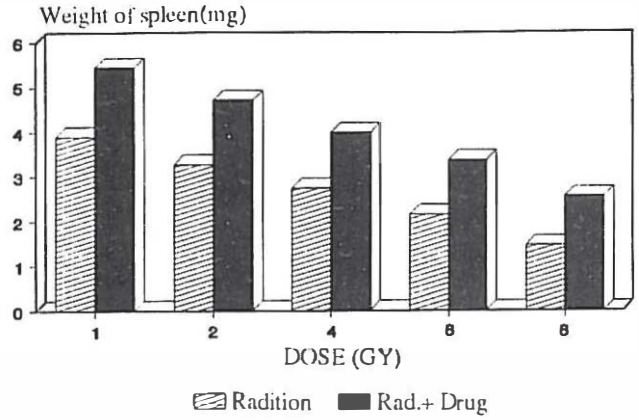

Fig. 1. Effect of cimetidine on radiation-induced changes in the weight of spleen.

Assay procedures

A group of mice were exposed to various doses of γ-rays and similar group were injected cimetidine I.P. 2 hours prior to irradiation. Mice were sacrificed on 1, 5, 10, 20, and 30 days post-irradiation. They were anesthetized with ether, and then weighed using a digital balance (Kern, Swiss) with precision of 0.01 g. Venous blood was collected in a heparinized tube; thymus gland and spleen were removed and immediately wighed using a digital balance with precision of 0.0001 g (Kern-S 2000, Swiss). Blood samples were counted using an automatic cell counter (cell counter system 8000, UK).

Table I. Effects of various doses of radiation on weight of spleen at different post-irradiation time intervals in the absence or presence of cimetidine. Data are mean values obtained from three mice ± SE of mean.

SPLEEN(weight, mg)

Time+ / Treatment	1 DAY	5 DAY	10 DAYS	20 DAYS	30 DAYS
Control	*3.1 ± 0.4	*4.8 ± 1.1	*6.1 ± 1.1	*6.9 ± 1.1	*9.3 ± 0.7
Drug	6.3 ± 1.6	8.2 ± 0.5	9.4 ± 2.0	9.6 ± 0.9	9.8 ± 0.1
Serum	6.6 ± 1.0	7.2 ± 0.3	7.3 ± 1.8	8.1 ± 0.0	
Rad. + 1 Gy #	4.8 ± 0.3	4.6 ± 1.1	5.4 ± 0.8	6.0 ± 0.5	6.4 ± 0.9
Rad. + 2 Gy	4.1 ± 0.7	3.9 ± 1.0	4.8 ± 0.2	5.3 ± 1.1	5.7 ± 1.3
Rad. + 4 Gy	3.5 ± 0.1	2.7 ± 0.7	3.9 ± 0.3	4.6 ± 0.8	5.5 ± 1.7
Rad. + 6 Gy	2.6 ± 0.4	2.5 ± 0.2	2.8 ± 0.2	4.1 ± 0.4	4.8 ± 1.2
Rad. + 8 Gy	2.5 ± 0.7	2.1 ± 0.2	2.5 ± 3.1	3.1 ± 0.5	
Rad. 1 Gy	3.6 ± 0.5	3.1 ± 0.1	3.8 ± 0.4	4.2 ± 0.1	4.8 ± 0.2
Rad. 2 Gy	3.0 ± 0.7	2.5 ± 0.3	3.2 ± 0.8	3.7 ± 0.2	3.9 ± 1.1
Rad. 4 Gy	2.5 ± 0.4	1.7 ± 0.6	2.9 ± 0.5	3.0 ± 0.1	3.6 + 0.2
Rad. 6 Gy	1.8 ± 0.4	1.5 ± 0.2	1.7 ± 1.3	2.6 ± 2.4	3.1 ± 0.0
Rad. 8 Gy	1.5 ± 0.2	1.1 ± 0.3	1.3 ± 0.5	1.9 ± 0.0	

* mean value ± SE + sampling time post-irradiation # radiation dose + drug

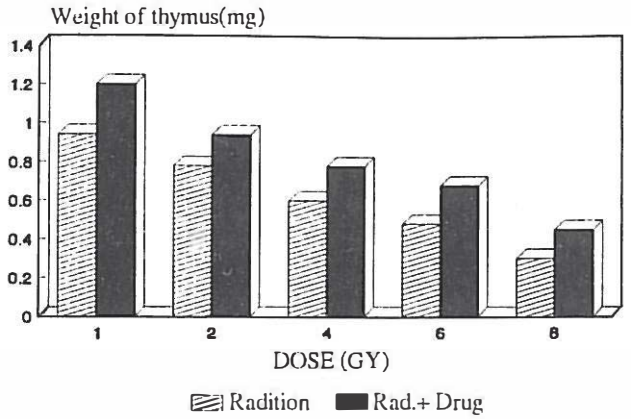

Fig. 2. Overall effects of various doses of radiation alone or in combination with cimetidine on the weight of thymus gland after five sampling times post-irradiation.

RESULTS

In this investigation, the effect of Co-60 γ-rays with a dose rate of 66.6 cGy/min on the lymphohemopoietic system of CD-1 mice, alone or in combination with cimetidine, was studied. Effects of radiation and drug were evaluated with determination of changes in WBC counts in peripheral blood and the weight of spleen and thymus gland. Data analysed using two-way analysis of variance. Results for WBC is expressed as mean number of total WBC reading of cell counter from three mice. Because of a small difference in the body weight, the weight of thymus and spleen are expressed as the ratio of weight of thymus and spleen to the total body weight of the related mouse.

Table I shows variation in spleen weight after irradiation alone or in the presence of cimetidine. Irradiation caused reduction in the weight and size of the spleen and this decrease was dose-dependent. Given time to animals after irradiation led to repair of injured tissues and after 30 days it rose up to 90% of initial value at day 1. In the group of mice treated with cimetidine 2 hours prior to irradiation, analysis of variance shows that there is an interaction effect between radiation and time with a $p<0.0001$. In cimetidine-treated mice $p<0.005$ for weight changes of spleen indicates that the difference between radiation effect alone and in combination with the drug is significcant. Weight decrease is dose-dependent and cimetidine-treated animals showed lesser decrease than those which received radiation alone. Mean values of data obtained for spleen for all sampling time in irradiated mice is compared with results obtained with cimetidine. (Fig. 1).

Similar results were obtained for variations in size and weight of the thymus gland as shown in Table II. Analysis of data shows that with 99.9% confidence ($p<0.001$) radiation induces significant changes in thymus weight. Time has a pronounced effect on repair of tissue after irradiation ($p<0.0001$). In cimetidine-treated mice a profound increase in thymus weight was observed (Fig. 2).

Whole-body irradiation of mice caused a dose-dependent remarkable decrease in the number of WBCs (Table III). In mice treated with cimetidine 2 hours before irradiation, the level of WBC in peripheral blood was higher with a ratio of more than 1.5 for all sampling times compared to'

Table II. Radition-induced changes in thymus weight (mg) at various sampling times and the effect of cimetidine. Data presented are mean values obtained for three mice ± SE of mean.

THYMUS(weight, mg)

Time+ / Treatment	1 DAY	5 DAYS	10 DAYS	20 DAYS	30 DAYS
Control	*0.5 ± 0.0	*1.1 ± 0.6	*1.5 ± 0.3	*2.5 ± 0.0	*2.6 ± 0.6
Drug	1.8 ± 0.1	2.7 ± 1.4	3.2 ± 0.3	3.2 ± 0.7	3.3 ± 0.8
Serum	1.6 ± 0.6	2.0 ± 0.2	2.6 ± 1.5	2.9 ± 0.5	
Rad. + 1 Gy #	0.7 ± 0.3	0.8 ± 0.2	1.3 ± 0.4	1.5 ± 0.1	1.7 ± 0.2
Rad. + 2 Gy	0.6 ± 0.2	0.5 ± 0.0	1.1 ± 0.6	1.2 ± 0.2	1.3 ± 0.5
Rad. + 4 Gy	0.5 ± 0.0	0.4 ± 0.0	0.7 ± 0.5	1.1 ± 0.1	1.3 ± 0.1
Rad. + 6 Gy	0.4 ± 0.1	0.3 ± 0.2	0.6 ± 0.2	1.0 ± 0.2	1.1 ± 0.1
Rad. + 8 Gy	0.3 ± 0.2	0.2 ± 0.2	0.5 ± 0.1	0.8 ± 0.0	
Rad. 1 Gy	0.6 ± 0.2	0.5 ± 0.0	1.0 ± 0.1	1.2 ± 0.4	1.4 ± 0.1
Rad. 2 Gy	0.5 ± 0.0	0.4 ± 0.1	0.8 ± 0.1	1.0 ± 0.2	1.2 ± 0.2
Rad. 4 Gy	0.4 ± 0.1	0.3 ± 0.1	0.5 ± 0.1	0.8 ± 0.2	1.0 ± 0.1
Rad. 6 Gy	0.3 ± 0.2	0.2 ± 0.1	0.4 ± 0.1	0.7 ± 0.3	0.8 ± 0.0
Rad. 8 Gy	0.2 ± 0.2	0.1 ± 0.1	0.4 ± 0.3	0.5 ± 0.0	

* mean value ± SE + sampling time post-irradiation # radiation dose + drug

those which received radiation alone (Fig. 3). Similar results are obtained for RBC and platelets in peripheral blood which shows that cimetidine is effective in protection of the hemopoietic system from irradiation (results not shown). The results obtained with these experiments indicate that use of cimetidine at 15 mg/kg body weight two hours prior to irradiation causes a dose reduction factor (DRF) of more than 1.5 which is a very remarkable effect.

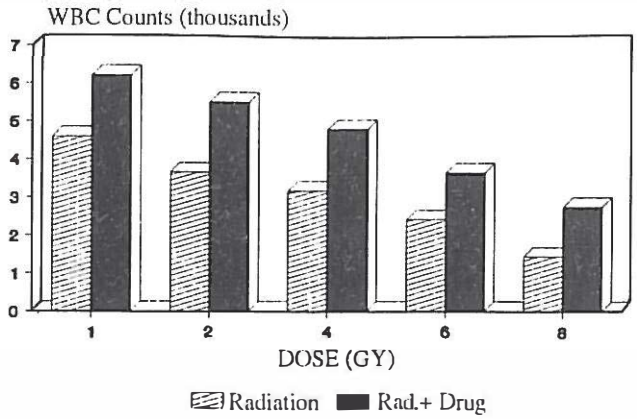

Fig. 3. Comparison of the effects of various doses of radiation alone or in combination with cimetidine on variations of WBC counts.

DISCUSSION

The mature elements of peripheral blood have a limited life span, and this places a cnstant demand on the hematopoietic elements of the hone marrow, a pluripotent stem cell (PPSC) population meets the demands of the system. Thus the hemopoietic and lymphoid systems are constantly dependent on the PPSC for the replacement of their functional cell types.[11] Therefore, if a specific agent such as radiation damages the PPSC, then all of these associated systems are compromized.

The mature blood cells (monocytes, platelets, RBCs and granulocytes) are functionally radioresistant to moderate doses of radiation. In contrast to other elements in blood, lymphocytes are extremely radiosensitive and have been suggested as a biological dosimeter.[12] In addition to the loss from the circulation (Table III), morphological changes rapidly appear in the lymphoid tissues (spleen and thymus) which quickly decrease in size (Tables I and II).[13] Therefore, changes seen in this investigation in WBC count, weight of spleen and thymus may be due to alterations induced in lymphocyte production centers by radiation (Table I-III).

Results obtained with mice treated with cimetidine show that cimetidine has a profound effect in increasing the number of WBCs for doses from 1-8 Gy (Fig. 3). Weight of spleen and thymus in cimetidine-treated mice was at least 1.5 times more than those which received radiation alone (Fig. 1). Since irradiation causes breakage of hydrogen bonds and release of histamine from arteries,[8] interaction of histamine with leukocytes causes a reduction in some cellular action which can be prevented by cimetidine.[14] Cimetidine can augment the proliferative as

Table III. Variation in WBC count in peripheral blood after exposure of mice to various doses of radiation in the presence or absence of cimetidine. Data are mean values obtained for three mice ± SE of mean.

WBC(count)

Time+ / Treatment	1 DAY	5 DAYS	10 DAYS	20 DAYS	30 DAYS
Control	*2450 ± 0050	*2350± 2000	*5200 ± 2074	*5870 ± 1250	*7070 ± 0801
Drug	5900 + 3600	6300 ± 1200	6750 ± 0500	6900 ± 0524	9800 ± 1000
Serum	5700 ± 0100	6100 ± 1350	6130 ± 1900	6750 ± 0000	
Rad. + 1 Gy #	5850 ± 1650	4400 ± 1345	5530 ± 1136	6100 ± 1586	9200 ± 0500
Rad. + 2 Gy	4650 ± 2450	3800 ± 0176	5600 ± 2750	6467 ± 0906	7150 ± 0700
Rad. + 4 Gy	4550 ± 0300	3070 ± 0322	4933 ± 1345	5450 ± 0050	6100 ± 1100
Rad. + 6 Gy	3200 ± 0500	3030 ± 0985	3267 ± 0994	3800 ± 0950	4950 ± 1500
Rad. + 8 Gy	3100 ± 0350	2100 ± 2070	2750 ± 0000	3000 ± 0050	
Rad. 1 Gy	3770 ± 0650	2900 ± 0727	4800 ± 0400	5250 ± 0200	6300 ± 1750
Rad. 2 Gy	3000 ± 0200	2866 ± 0549	2950 ± 0318	4400 ± 0350	5170 ± 1250
Rad. 4 Gy	2700 ± 0100	1800 ± 0100	2950 ± 0900	3750 ± 0100	4750 ± 2000
Rad. 6 Gy	2300 ± 1700	1600 ± 0418	1670 ± 0900	3200 ± 0900	3300 ± 0000
Rad. 8 Gy	2100 ± 0100	0900 ± 0058	1333 ± 0240	1400 ± 0000	

* mean value ± SE + sampling time post-irradiation # radiation dose + drug

well as the cytotoxic responce of lymphocytes.[15] The manner in which cimetidine increases immune response is not completely clear. Activation of histamine receptors on T-cells has been shown to suppress both formation of antibody and cell-mediated toxicity.[16] It is also shown that an antagonist receptor of histamine type II may regulate action of T-cells.[14] However, as it is clearly shown in Figures 1-3, cimetidine at a low dosage (15 mg/kg) can reduce radiation effects effectively with a DRF of more than 1.5. The effect of this widely used drug compared to specially designed radioprotectors is very remarkable. In view of results reported here, it seems that cimetidine induces an augmentation in main centers for regulation of immune system after irradiation. It appears that cimetidine has the capability to promote a net increase in lymphocyte proliferation. The mechanism by which cimetidinereudces radiation effect on PPSC is not clearly understood.

ACKNOWLEDGMENTS

The authors express their gratitude to Mr. S. Faghihzadeh for his advice for statistical analysis. This work was supported by a grant from Tarbiat Modarres University Research Council.

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