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In vitro radioprotective effects of histamine H_2 receptor antagonists against gamma-rays induced chromosomal aberrations in human lymphocytes

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ABSTRACT

Background: Radioprotective capability of histamine H_2 receptor antagonists have been shown in several *in vivo* studies mainly using animal models. However, to verify the effectiveness of these agents in clinical applications, studies should be performed on human cells. In the present study radioprotective properties of these agents was examined *in vitro* on human lymphocytes using metaphase analysis.

Materials and Methods: *In vitro* metaphase analysis technique was used to test the effects of cimetidine, ranitidine and famotidine on radiation induced clastogenic effects. Lymphocytes in whole peripheral blood were exposed to 3 Gy gamma-rays at a dose rate of 73.7 cGy/min in the presence or absence of various doses of the drugs used in this study. The frequency of chromosomal aberrations were determined after standard metaphase preparations and staining slides in 5% Giemsa.

Results: Results show that radiation produced a high number of chromosomal aberrations in lymphocytes compared to controls ($p<0.001$). All three drugs used in this study effectively reduced the frequency of chromosomal aberrations at all doses. Famotidine was found to be more effective than the other two drugs.

Conclusion: From the results obtained it can be concluded that H_2-receptor antagonists used in this study effectively reduced the clastogenic effects of radiation with a dose reduction factor (DRF) of 1.5-2 in human lymphocytes *in vitro*. The way in which these drugs reduce the clastogenic effects of radiation might be via radical scavenging mechanism. *Iran. J. Radiat. Res.; 2003; 1(2): 99 – 104.*

Keywords: *H_2 receptor antagonists, chromosomal aberration, radioprotection, human lymphocytes*

INTRODUCTION

The use of chemical agents to provide partial protection against radiation induced injuries has been a major field of study over 50 years. Since the discovery in 1949 that cysteine has the ability to increase the survival of lethality irradiated mice (Patt *et al*. 1949), a staggering number of compounds have been examined for their ability to function as radioprotector. The vast majority of radioprotective agents that have been developed and tested are the aminothiols. As a group, they are the most effective class of radioprotectors. WR-2721, the best of these, is capable of producing DRF about 2.7 for gamma irradiation in mice after i.p injection of doses between 100 - 800 mg/kg (Durand 1983). The substantial systemic toxicity, such as hypotension, emesis, vomiting, etc., of WR-2721 in clinical trials limits an application of such chemical protector in the

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treatment of cancer (Murray 1996). For these reasons the search for more effective and less toxic radioprotectors has spurred interest in the development of different compounds. The radioprotective effects of structurally different flavonoids (Castillo *et al.* 2000, Koleva *et al.* 2002) and 2-iminothiazolidine derivatives have been investigated recently against gamma irradiation in mice (Hosseinimehr *et al.* 2001, 2002). These compounds although toxic, did not produce a significant DRF (DRF <1.5).

Since a wide variety of immunomodulators both naturally occurring and synthetic show as potential adjuncts to therapeutic and radioprotector regimens, we used cimetidine which is a potent immunomodulator and used clinically for peptic ulcer treatment (Bardhan 1981) against mouse lymphoid tissue injuries following whole body gamma irradiation in our early studies (Mozdarani and Vessal 1993). This investigation was then extended to other histamine H_2 receptor antagonists, ranitidine and famotidine which similar to cimetidine are used clinically for peptic ulcer treatment. All these studies were performed using *in vivo* system. It was shown that cimetidine, ranitidine, and famotidine produced a DRF of 1.5 to about 2 against gamma rays induced micronuclei in mouse bone marrow erythrocytes (Mozdarani and Gharbali 1993, Shahidi and Mozdarani 2003).

The present study was conducted using metaphase analysis technique in order to examine the effects of histamine H_2 receptor antagonists on reducing gamma rays induced clastogenic effects *in vitro* on human lymphocytes. The main objectives of this study was to show the effect of *in vivo* metabolism of drugs in radioprotection and also to assess the effects seen in mouse cellular system in a human cell for future clinical trials.

MATERIALS AND METHODS

Heparinized blood samples were obtained from healthy male donors with no drug or radiation treatment last one month prior to sampling. 0.4 ml of whole blood was cultured in 4.5 ml RPMI-1640 (Sigma) medium supplemented with 15% foetal calf serum (Sigma), antibiotics (Penicillin, 100 iu/ml and Streptomycin, 100 µg/ml) and L-glutamine. Cell cultures were initiated with addition of 0.1 ml of phytohemaglutinin (PHA) (Gibco-BRL) at a final concentration of 5 µg/ml as mitogen to each culture vessel after drug and radiation treatment.

Cimetidine and famotidine (Guden Ritcher, Hungary) and ranitidine (Rambaspy, Spain), provided by the Chemidarou Co. in Tehran were diluted in physiologic serum. Lymphocytes were treated with various doses (10-200 µmol/L) of drugs 1 h prior to irradiation with 3 Gy gamma rays generated by a Co-60 source (Teratron, Canada) at a dose rate of 73.7 cGy/min. All blood samples were irradiated in the presence or absence of drugs 1 hour prior to the addition of PHA, i.e. lymphocytes of the whole blood were irradiated at G0 phase of the cell cycle. Drug dose of 100 µmol/L used in this study was similar to the dose used in two different studies (Ching *et al*. 1994, Lappena *et al.* 1994) to verify the radical scavenging properties of these drugs. Forty-eight hours after culture initiation 0.2 µ g/ml colchicine (Sigma) was added to the cultures for 2 hours to arrest cells at metaphase. Cells were harvested and exposed to hypotonic solution (KCl, 0.075 M) for 10 minutes, then fixed in Carnoy's fixative (3:1 v/v methanol: Glacial acetic acid). Slides were prepared using air drying technique and stained in 5% Giemsa solution (Merck). 200 mitoses were analysed for the presence or absence of chromosomal aberrations for each treatment. Lesions were classified according to the international system of cytogenetic nomenclature for acquired chromosome aberrations (ISCN 1985). Major chromosomal aberrations observed in this study were of chromosome types including isochromatid gaps, isochromatid breaks and chromosomal exchanges, mainly of dicentric type. The frequency of isochromatid gaps were too low (about 1-1.5%) to be considered for statistical analysis. The significance of any inter-group differences in the number of chromosomal aberrations was statistically evaluated by one-way analysis of variance and student's *t-test*.

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RESULTS

Results obtained in this study are summarized in table 1 and shown in figure 1. As seen, 3 Gy gamma rays effectively induced chromosomal aberrations mainly of exchange (dicentric) type (64.4 versus 0 in control) ($p<0.001$). Treatment of lymphocytes with cimetidine, ranitidine and famotidine alone did not produce chromosomal aberrations. Pre-treatment of lymphocytes with cimetidine reduced the frequency of chromosomal aberrations and it was more pronounce for a dose of 100 µmol/L with a DRF of 1.43 (figure 1) ($p<0.05$). Similar effect was observed for cells pre-treated with ranitidine and famotidine. Dose reduction factor calculated for ranitidine range from 1.37 to 1.5. The overall effect of ranitidine at all dose ranges used in this study was slightly more than cimetidine but statistically not significant (table 1, figure 1). There was not a significant difference between the DRF produced by different doses (10- 200 µmol/L) of ranitidine. Famotidine was found more effective than the other two, producing DRF of 1.95 (table 1). All drugs effectively reduced the frequency of gamma ray induced chromosomal aberrations with DRF of 1.45 – 1.95 (figure 1).

Table 1: Percentage of chromosomal aberrations in human lymphocytes following gamma irradiation in the absence or presence of various doses of cimetidine (CIM), ranitidine (RAN) and famotidine (FAM). At least 200 cells were scored for each treatment.

Treatment	**% Chromosomal aberrations**			**% Total aberrations excluding isogaps**
	Isogaps	**Breaks**	**Exchanges**	
Control	1	0	0	0
Solvent control	0	0	0	0
Cimetidine alone	0	0	0	0
Ranitidine alone	0	0	0	0
Famotidine alone	0	0	0	0
Gamma-rays 3 Gy	1.5	24	40.5	64.5
γ + CIM 10 µM	1.5	14.5	39.5	54
γ + CIM 28 µM	0	15.5	35	50.5
γ + CIM 100 µM	0.5	11.5	33	45
γ + CIM 200 µM	0	13.5	38	51.5
γ + RAN 10 µM	4	10	35	45
γ + RAN 28 µM	0	7.5	39.5	47
γ + RAN 100 µM	0	6	37	43
γ + RAN 200 µM	0	15.5	29	44.5
γ + FAM 10 µM	0.5	11.5	37.5	49
γ + FAM 28 µM	0	13.5	29.5	43
γ + FAM 100 µM	1	12.5	20	32.5
γ + FAM 200 µM	1	11	26	37

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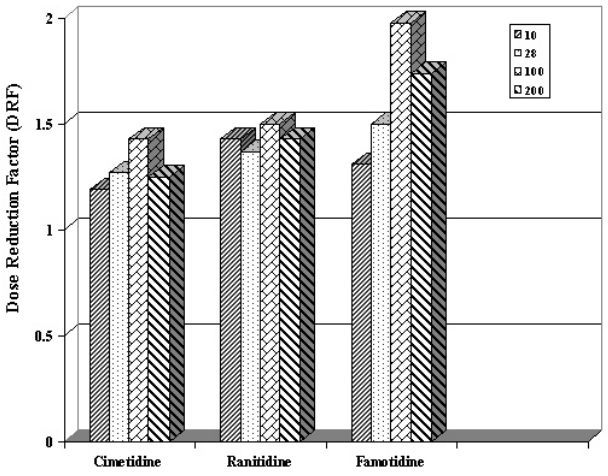

Figure 1. Dose reduction factors calculated for different doses of cimetidine, ranitidine and famotidine when used in combination with 3 Gy gamma rays. All doses indicated are in μmol/L.

DISCUSSION

Results of these experiments show clearly that histamine H_2 receptor antagonists are potent radioprotectors when used *in vitro*.

For clinical treatment of peptic ulcer, histamine H_2 receptor antagonists such as cimetidine, ranitidine and famotidine are used (Freston 1982, Parsons and Lewin 1991). Apart from their capability for gastric acid suppression and the pepsin secretion (Lipsy *et al*. 1990), most of them are potent hydroxyl radical scavengers (Ching *et al*. 1993). Ching *et al.* (1994) showed these compounds are good scavengers of HOCl produced by neutrophyls via the oxidation of Cl^- by neutrophil derived myeloperoxidase and H2O2 can rapidly attack and oxidize a wide range of biologically relevant molecules (Halliwell and Gutteridge 1989). Radiation chemical studies have shown that free radicals are primarily responsible for the indirect effects of radiation. These drugs when applied *in vivo* also showed radioprotective effect on mouse bone marrow erythrocytes. Cimetidine was found more effective when used *in vivo* (Mozdarani and Gharbali 1993). This effect might be due to the augmentation of the proliferative capacity of lymphocytic cells by cimetidine (Giffored *et al.* 1983). This and many other investigations indicate that administration of cimetidine before irradiation leads to the inhibition of T suppressor cells and increases the proliferation of $CD4^+$ lymphocytes. This process causes production of glutathione reductase and catalase enzymes which prevents DNA damage and eventually reduced the clastogenic effects of radiation (Mozdarani and Gharbali 1993, Mozdarani 1996, Mozdarani and Khoshbin-Khoshnazar 1998). Therefore cimetidine reduces clastogenic effects of radiation via a radical scavenging mechanism through enzyme catalysis. However nearly similar DRF was obtained both *in vitro* (~1.4) and *in vivo* (1.6) might be indicative of potential radioprotectve effects of cimetidine.

Ranitidine and famotidine do not have immunomodulatory role in the body, but it was shown that these drugs are potent radical scavengers of oxygen radicals (Lappena *et al.* 1994). *In vitro* effects of all drugs were similar to *in vivo*, implying that very low level of drugs reach to the bone marrow system, while the drugs are totally present in the cellular environment *in vitro* at the time of irradiation. Reduction of the frequency of chromosomal aberrations in lymphocytes indicate that all drugs might reduce the clastogenic effect of radiation via radical scavenging mechanism and famotidine is more effective than the other two histamine H_2 receptor antagonists studied in this research (figure 1). These findings are in accord with the report of Lappena *et al.* (1994), although nearly similar DRF is produced by cimetidine and ranitidine. They showed that these drugs scavenge hydroxyl radical (OH°) with a very high rate constant, which is about 10 fold higher than that of the specific scavenger "manitol", for famotidine (1.7×10^{10}/mol/s) and cimetidine (1.6×10^{10} mol/s); ranitidine displaying a rate constant of 7.5×10^{9}/ mol/s. These OH° scavenging effect was also found to be significant at 100 µmol/l concentration for famotidine, cimetidine and ranitidine respectively (Lappena *et al.* 1994). Based on their study on HOCl scavenging properties of these drugs, Ching *et al.* (1994) concluded that the

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presence of sulphur atom in the compound is important for their scavenging activity (figure 1).

A radioprotective drug or regimen that is to be used by radiotherapy patients, radiation workers or personnel in a nuclear battlefield should meet several criteria. It should provide significant protection to reduce the effects of radiation at least by a DRF>1.5 and the drug should be easily self administered. Relatively long term protection is necessary for a practical drug.

All histamine H_2 receptor antagonists studied in this investigation have routine and wide spread clinical use for peptic ulcer treatment with no apparent side effects at doses much higher than therapeutic levels and are administered orally. These drugs are widely available and chemically stable and also not too expensive. These features make these drugs suitable candidates for being used as chemical radioprotectors especially for radiotherapy patients who are at risk of bone marrow damage.

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