Radioprotective Properties of Histamine H₂ Receptor Antagonists: Present and Future Prospects

HOSSEIN MOZDARANI

H₂ receptor antagonist/Micronuclei/Chromosomal aberration/Radioprotection.

Various chemical agents were examined for their radioprotective capability to provide partial protection against radiation injury over the past 50 years. However, no suitable drug has yet been introduced for routine clinical use. In the present study, the radioprotective potential of H₂ receptor antagonists was examined in *in vivo* and *in vitro* conditions. For this purpose, an *in vivo* micronucleus assay and an *in vitro* metaphase analysis were used to test the effects of cimetidine, ranitidine, and famotidine on radiation-induced clastogenic effects. For micronuclei assay, BALB/c mice were irradiated in the presence or absence of drugs, and slides were prepared from bone marrow cells. The frequency of micronuclei was determined in bone marrow erythrocytes. For the *in vitro* assay, lymphocytes in whole peripheral blood were exposed to radiation in the presence or absence of drugs, and the frequency of chromosomal aberrations were determined. The results show that radiation produced a high number of micronuclei in polychromatic erythrocytes (PCE) and chromosomal aberrations in lymphocytes. All three drugs used in this study effectively reduced the frequency of radiation-induced micronuclei and chromosomal aberrations at various doses. Famotidine was found to be more effective than the other two drugs. From the results obtained, it can be concluded that H₂-receptor antagonists reduced the clastogenic effects of radiation with a dose reduction factor (DRF) of 1.5–2 *in vivo* and *in vitro*. The way in which these drugs reduce the clastogenic effects of radiation might be via a radical scavenging mechanism.

**INTRODUCTION**

Since the discovery of the radioprotective effect of cysteine in 1949¹, there has been an extensive search for additional compounds that provide radioprotection. Early research on the synthesis of radioprotectants and their testing concentrated on the analogues of cysteine; however, cysteine and its analogues was soon found to be too toxic for use on humans. In the early 1950s, the synthesis of amino-ethylisothiourea (AET) helped to understand that the structural features of sulphur compounds are important for radioprotection. At that time the most effective compounds found were those with a sulphydryl (-SH) group at one end of a 2 or 3 carbon chain and with a strong basic function, usually an amino group, at the end.

Among 4,000 chemicals with these features, tested for their radioprotective capability, aminothiols such as B-mercaptoethylamine (MEA), AET, and WR-2721 have been introduced as potent radioprotectors². These compounds not only are effective at high doses, but they also produce various side effects. WR-2721, with a generic name of Ethiofos in the United States and Gammaphos in Russia, is the best of them capable of producing DRF of about 2.7 for gamma irradiation in mice after an i.p. injection of doses from 100 to 800 mg/kg³. Mouse LD50/30 studies show DRF of up to 2.1 for AET at a dose of 400 mg/kg. Studies showed that AET penetrates easily into all tissues regardless of whether they are normal or malignant. AET afforded the same protection against radiation-induced damages to normal and cancer tissues⁴. On the other hand WR-2721 produced DRF of 2.4 and 2.7 against radiation for murine skin and bone marrow, respectively; but it did not change the response of solid tumors⁵. Also, the sizable systemic toxicity, such as hypotension, emesis, and vomiting, of WR-2721 in clinical trials limits an application of such a chemical protector in the treatment of cancer⁶.

For these reasons the search for more effective and less toxic radioprotectors has spurred interest in the development of different compounds. Some natural antioxidants (e.g., flavonoids), such as benzo-γ-pyrone derivatives, can suppress the formation of free radicals⁷,⁸. The radioprotective effects of structurally different flavonoids⁹,¹⁰ and 2-iminothiazolidine derivatives have been investigated recently against gamma irradiation in mice¹¹,¹²; these compounds, though toxic, did not produce a sig-

*Corresponding author: Fax: +98-21-800-6544,
E-mail: mozdarah@modares.ac.ir
School of Medical Sciences, Tarbiat Modarres University, P.O. Box 14115-111, Tehran, I.R Iran.
significant DRF (DRF < 1.5).

Since a wide variety of naturally occurring and synthetic immunomodulators appear as potential adjuncts to therapeutic and radioprotector regimens, we used cimetidine, which is a potent immunomodulator for clinical peptic ulcer treatment\(^\text{13}\) against lymphoid tissue injuries following whole body gamma irradiation. Cimetidine produced a DRF greater than 1.5 for a dose range of 1–8 Gray\(^\text{14}\). It was also effective against the clastogenic effects of gamma rays\(^\text{15}\) and low doses of neutron\(^\text{16}\).

In this study we tested other histamine H\(_2\) receptor antagonists, ranitidine, and famotidine for their radioprotective capability by using an \textit{in vivo} micronucleus test and an \textit{in vitro} metaphase analysis. Figure 1 shows the chemical structures of these drugs. The micronucleus test developed by Schmid and his colleagues\(^\text{17}\) is a sensitive and reliable method for the assessment of genetic changes induced by chemical and physical agents \textit{in vivo}. A metaphase analysis on human peripheral blood lymphocytes was employed in \textit{vitro} to verify the need of drugs used in this study to be metabolized for their radioprotective capability.

**MATERIALS AND METHODS**

The \textit{micronucleus assay}

BALB/c male mice were purchased from the Razi institute, Karaj. They were housed under controlled living conditions (light/dark, 12:12 h; temperature, 22 ± 2°C) in the university animal house and given standard mouse pellets and tap water \textit{ad libitum}. All experiments were performed with 8-week-old mice. Cimetidine and famotidine (Guden Ritcher, Hungary) and ranitidine (Rambaspy, Spain), provided by the Chemidarou Co. in Tehran, were diluted in physiologic serum and injected i.p. at different concentrations 2 hours before irradiation to allow enough time for accumulation in bone marrow.

Irradiation was carried out with a Co-60 gamma ray machine (Teratron, Canada), at a dose rate of 99.77 cGy/min and SSD = 80 cm. The treated and control animals were irradiated with a dose of 2 Gy gamma rays. Twenty-four hours after treatment, the mice were sacrificed. Femoral bone marrow was flushed out, and cell suspension and slides were prepared. The slides were stained in May-Grunwald and Giemsa (Merck) to allow the identification of polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE). A total of 1,500 PCE was scored for the presence of micronuclei for each sample and for each sample mice in a group of five were treated. The ratio of PCE/ (PCE+NCE) was determined for each radiation dose to assess the influence of radiation and drugs on bone marrow proliferation.

**Metaphase analysis**

Heparinized blood samples were obtained from healthy male donors with no drug or radiation treatment in the last month before sampling. We cultured 0.4 ml of whole blood in 4.5 ml RPMI-1640 (Sigma) medium supplemented with 15% fetal calf serum (Sigma), antibiotics (penicillin and streptomycin), and L-glutamine. Cell cultures were initiated with the 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 treatments.

Lymphocytes were treated with 100 µmol/liter cimetidine, ranitidine, and famotidine 1 h before 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 h before the addition of PHA; i.e., lymphocytes of the whole blood were irradiated at the G\(_0\) phase of the cell cycle. The drug dose of 100 µmol/liter used in this study was similar to the dose used in two different studies\(^\text{18,19}\) to verify the radical scavenging properties of these drugs. Forty-eight hours after culture initiation, 0.2 µg/ml colchicine (Sigma) was added to the cultures for 2 h to arrest cells at metaphase. The cells were harvested and exposed to a hypotonic solution (KCl, 0.075 M) for 10 min, then fixed in Carnoy’s fixative (3:1 v/v methanol:Glacial acetic acid). The slides were prepared by using an air-drying technique and stained in 5% Giemsa solution (Merck). A total of 100 mitoses were analyzed for the presence or absence of chromosomal aberrations for each sample. Lesions were classified according to the international system of cytogenetic nomenclature for acquired chromosome aberrations (ISCN)\(^\text{20}\). Major chromosomal aberrations observed in this study were of chromosome types, including isochromatid gaps, isochromatid breaks, and chromosomal exchanges, mainly of a dicentric type. The frequency of isochromatid gaps were too low (about 1–1.5%) to be considered for statistical analysis.

**RESULTS**

The results obtained in experiments with micronuclei assay are summarized in Table 1, and the results of experiments with

![Chemical and molecular structures of H\(_2\) receptor antagonists used in this study.](image)

---

**Fig. 1.** Chemical and molecular structures of H\(_2\) receptor antagonists used in this study.
metaphase analysis are shown in Fig. 2. As seen, 2 Gy gamma rays effectively induced micronuclei in PCE (100.7 ± 5.5 vs. 5.5 in control) and reduced the ratio of PCE/(PCE+NCE) from 0.44 to 0.28. The pretreatment of mice with cimetidine led to a significant reduction of MNPCE, and it was more pronounced for a dose of 15 mg/kg cimetidine with a dose-dependent manner, which produced a DRF of 1.61 (Table 2). Doses of 2.5 and 5 mg/kg ranitidine and of 0.75 and 1.5 mg/kg famotidine more effectively reduced the number of MNPCE. These results show that both drugs are more effective than cimetidine at much lower concentrations. Because a dose of 1.5 mg/ml of famotidine produced an effect similar to ranitidine with a dose of 5 mg/ml, it seems that famotidine is more effective than ranitidine in reducing the number of MNPCE. However, cimetidine, despite ranitidine and famotidine, led to an increase in the ratio of PCE/(PCE+NCE), implying a reduction of radiation-induced cytotoxicity. DRF calculated for each dose of drugs, shown in Table 2, indicates a higher value of DRF for ranitidine and famotidine with much lower doses. A study of metaphases of irradiated lymphocytes in vitro with a dose of 3 Gy gamma rays in the presence of 100 μmol/liter drugs showed results similar to the in vivo study. All drugs effectively reduced the frequency of gamma ray induced chromosomal aberrations (Fig. 2) with DRF of 1.45–1.95. Famotidine was found more effective than the other two, producing DRF of 1.95 (Table 2).

**DISCUSSION**

For the clinical treatment of peptic ulcer, histamine H2 receptor antagonists such as cimetidine, ranitidine, and famotidine are used. Besides their capability for gastric acid suppression and pepsin secretion, most are potent hydroxyl radical scavengers. Ching et al. showed that these compounds are good scavengers of HOCl produced by neutrophils via the oxidation of Cl− by neutrophil-derived myeloperoxidase, and H2O2 can rapidly attack and oxidize a wide range of biologically relevant molecules. Radiation chemical studies have shown that free radicals are primarily responsible for the indirect effects of radiation. The results obtained for cimetidine is similar to the results of Garriot and Grove, who found a reduction in micronuclei production in mouse bone marrow cells for AET at a dose of 0–2.5 Gy gamma rays. However, these authors used much higher doses of AET (300 mg/kg) for producing radioprotection. In the normal bone marrow cells, kidney, and liver of mammals, some enzymes such as glutathione reductase and catalase, which
have a role in hydrogen peroxide catalysis, are synthesized normally. It was shown that the inhibition of the activity of these enzymes leads to an increase in micronuclei production. It seems that CD4+ lymphocytes (effector T cells) have a role in the induction of glutathione reductase and catalyse enzymes. Gifford and his colleagues have shown that cimetidine augments the proliferation capacity of lymphocytic cells. This and many other investigations indicate that an administration of cimetidine before irradiation leads to the inhibition of T suppressor cells and increases the proliferation of CD4+ lymphocytes. This process causes a production of glutathione reductase and catalase enzymes, which prevents DNA damage and eventually reduces the clastogenic effects of radiation. Therefore cimetidine is most effective in the radical scavenging mechanism through enzyme catalysis. As observed in Table 2, similar DRF obtained in vitro (-1.5) and in vivo (1.6) might be indicative of the potential radioprotective effects of cimetidine.

Ranitidine and famotidine have no immunomodulatory role in the body, but it was shown that these drugs are potent radical scavengers of oxygen radicals. The in vitro effects of all drugs were similar to those in vivo, implying that a very low level of drugs reach the bone marrow system, but the drugs are totally present in the cellular environment in vitro at the time of irradiation. A reduction of the frequency of chromosomal aberrations in lymphocytes indicates that all drugs might reduce the clastogenic effect of radiation via the radical scavenging mechanism, and famotidine is more effective than the other two histamine H2 receptor antagonists studied in this research (Fig. 2). These findings are in accord with the report of Lappena et al. (1994), though nearly similar DRF is produced by cimetidine and ranitidine. They showed that these drugs scaveng hydrogen peroxide and 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 × 1010 mol/s) and cimetidine (1.6 × 1010 mol/s); ranitidine displays a rate constant of 7.5 × 109/mol/s. These OH· scavenging effects were also found to be significant at 100 µmol/liter concentration for famotidine, cimetidine, and ranitidine. Based on their study of HOCl scavenging properties of these drugs, Ching et al. (1994) concluded that the presence of sulphur atoms in the compound is important for their scavenging activity (Fig. 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, including (i) offer a good protection against acute and chronic radiation damages (DRF > 1.5); (ii) be suitable for oral administration and have the ability to be rapidly absorbed and distributed throughout the body; (iii) have no significant toxicological effects; (iv) be widely available and not too expensive; (v) be chemically stable to permit easy handling and storage.

All histamine H2 receptor antagonists studied in this investigation are administered orally and have routine and widespread clinical use for peptic ulcer treatment with no apparent side effects at doses much higher than therapeutic levels. These features make them suitable candidates for use as chemical radioprotectors, especially for radiotherapy patients who are at the risk of bone marrow damage.

ACKNOWLEDGEMENTS

This work was supported in part by the Tarbiat Modarres University Research Council. I would like to thank Ms. Ghorbani, Ms. Shahidi, and Mr Ebrahimi for their technical assistance.

REFERENCES


Kline and French, Hertfordshire.