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Potent radioprotective effect of therapeutic doses of ranitidine and famotidine against gamma-rays induced micronuclei *in vivo*

M. Shahidi [1] and H. Mozdarani [2*]

Department of [1]Medical physics, and [2]Radiology, School of Medical Sciences, Tarbiat Modarres University, Tehran, Iran

ABSTRACT

Background: Previous investigations have revealed, cimetidine, a histamine H2-receptor antagonist, show radioprotective effects against gamma- and neutron-induced micronuclei in bone marrow erythrocytes. In this study, the anticlastogenic effects of famotidine and ranitidine, which act similar to cimetidine as histamine H2-receptor antagonists, was investigated.

Materials and Methods: Balb/c male mice were injected i.p. with various doses of famotidine and ranitidine two hours before 2 Gy gamma irradiation. Frequency of micronuclei was determined in bone marrow erythrocytes following each treatment.

Results: The results indicated that gamma irradiation alone can cause a high frequency of micronuclei formation and decrease cell proliferation ratio. Pre-irradiation injection of famotidine and ranitidine, of various doses, effectively reduced the number of micronucleated polychromatic erythrocytes (MnPCEs), yet has no effect on cell proliferation ratio (PCEs/PCEs+NCEs). In fact, these two drugs reduce the clastogenic effects of gamma rays, while they are ineffective against the cytotoxic properties of gamma rays.

Conclusion: The dose reduction factor (DRF) calculated, shows a DRF=2 for famotidine and a DRF=1.8 for ranitidine which is indicative of a high radioprotective property of these drugs. The mechanism in which these drugs reduce clastogenic effect of gamma radiation is not fully understood. It might be due to their antioxidant and free radical-scavenging properties. *Iran. J. Radiat. Res.; 2003; 1(1): 29 - 35.*

Keywords: *Famotidine, ranitidine, gamma rays, micronuclei, mouse bone marrow erythrocytes.*

INTRODUCTION

Ionizing radiation can damage macromolecules such as DNA, which are of prime biological significance in the cell.

After the discovery of the radioprotective property of cysteine in 1949 (Patt *et al*. 1953), a vast number of compounds were studied with regard to their radioprotective potentials. Some of these newly discovered drugs, such as beta-mercaptoethylamine (MEA), cysteamine, amino-ethylisothiouronium (AET) and WR-2721, were declared as the most effective radioprotectors (Davidson *et al*. 1980). WR-2721, the most potent of this group, has the highest dose reduction factor (DRF = 2.7) against gamma rays (Durand 1983). Because of their toxic side effects that occur as vomiting, nausea, drowsiness, and hypotension the clinical application of radioprotective agents is limited (Davidson *et al*. 1980, Vardy *et al*. 2002). Studies to find radioprotective agents that would provide enough protection at concentrations more tolerable for patients are to be continued (Werner-Wasik 2001, Weiss 2000).

Our previous studies have shown that

*** Corresponding author:**
Dr. H. Mozdarani, Department of Radiology, School of Medical Sciences,Tarbiat Modarres University, P.O.Box: 14115-111, Tehran, Iran. E-mail: Mozdarah@modares.ac.ir

cimetidine, at a dose of 15 mg/kg , can protect the lymphohemopoietic tissues against gamma radiation of DRF>1.5 (Mozdarani and Vessal 1993). Also, it was shown that cimetidine is capable of reducing gamma and neutron-induced clastogenic effects (Mozdarani and Gharbali 1993, Mozdarani and Khoshbin-Khoshnazar 1998) as well as being effective in reducing benzene-induced micronuclei in bone marrow erythrocytes (Mozdarani and Kamali 1998).

The present study was conducted using the micronuclei assay in order to examine the effects of famotidine and ranitidine; which like cimetidine, are histamine H2-receptor antagonists and are used clinically for peptic ulcer treatment; on reducing gamma rays-induced clastogenic and cytotoxic effects.

The chemical structures of cimetidine, ranitidine and famotidine are shown in figure1.

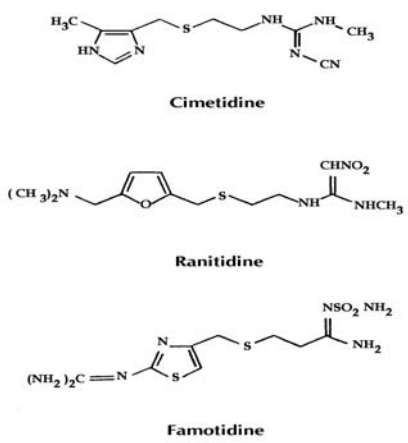

Figure 1. Chemical structures of cimetidine, ranitidine and famotidine.

The micronuclei test which was developed and completed by Schmid *et al*. (1970) is used as an effective and reliable method to examine the mutagenic effects of chemical and physical agents (Matter *et al*. 1971, Ledebur *et al*. 1973, Schmid 1975, Heddle *et al*. 1991).

In this paper, data are presented for the *in vivo* effects of 2 Gy gamma rays and the effects of ranitidine and famotidine on the frequency of gamma-rays induced micronuclei and on the cell proliferation ratio.

MATERIALS AND METHODS

Animals

Four-week old male Balb/c mice were purchased from the Razi Institute (Karaj, Iran). They were housed in cages for 2 weeks, provided with proper light, temperature and moisture, and fed with standard ration, before the beginning of experiments at the university animal house. Six-week old mice were used for all experiments.

Drug treatment

Ranitidine (Impex Quimica, S.A., Spain) and famotidine (Globe Organics Limited, India) powders were provided as gifts from the Chemi Daru Co. (Tehran, Iran). Various doses of ranitidine (2.5, 5, 10, 20 and 40 mg/kg) and famotidine (0.625, 1.25, 2.5, 5, 10 and 20 mg/kg) were dissolved in normal saline solution, and injected to the mice i.p. The selected doses were multiplication of the conventional doses normally used for the treatment of duodenal ulcers.

Radiation

A 60-Co radiotherapy unit (ACEL, model 780, Canada) was used for gamma irradiation. The mice were grouped into 3, kept in a partitioned cardboard box, and irradiated with 2 Gy gamma rays at a dose rate of 95.19 cGy/min with irradiation condition of 85cm SSD and at room temperature (24 ± 2 °C). The mice were irradiated, some in the presence and some in the absence of different doses of famotidine or ranitidine.

Sampling, Mn preparation and staining

The mice were killed by cervical dislocation 24 hours after irradiation. Similarly, control groups were also sampled 24 hours after injection. Their femoral bone marrow was flushed out by means of fetal calf serum, and a cell suspension was duly prepared. The suspension was centrifuged for 6 minutes at 1000 rpm. After centrifuging, the supernatant was removed and cells were resuspended in the remaining serum and a smear was prepared,

fixed and stained by the May Grunwald-Giemsa method (Schmid 1975). In this method of staining, polychromatic erythrocytes (PCEs) are stained blue-violet, while normochromatic erythrocytes (NCEs) are stained yellow-orange.

Microscopic and statistical analysis

A Zeiss microscope with ×100 objective lens was used for scoring the cells. For each animal, 1500 PCEs were scored. At the same time, the NCEs/1500 PCEs as well as the PCEs and NCEs containing micronuclei were counted and recorded. In order to study the cytotoxic effects of gamma rays on the proliferation of the bone marrow cells-the ratio of PCEs/PCEs+NCEs was calculated. The significance of any intergroup differences in the number of micronucleated PCEs as well as the ratio of PCEs/PCEs+NCEs was statistically evaluated by the one-way analysis of variance and Student's *t-test*.

RESULTS

The results of the injection of various doses of ranitidine and famotidine alone or in combination with radiation are summarized in table 1 and shown in figures 2 and 3. Statistical analyses revealed that the groups that had received various doses of the two compounds showed no significant difference either in comparison with the control group or with each other ($P < 0.01$). Therefore, different selected doses of these drugs alone do not have any clastogenic or cytotoxic effects.

Table 1. The frequency of MnPCEs and the ratio of PCEs/PCEs+NCEs in bone marrow erythrocytes exposed to 2 Gy gamma rays in the presence or absence of various doses of ranitidine or famotidine.

Treatment	Mean number of MnPCEs/1500PCEs*	Mean ratio of PCEs/PCEs+NCEs
Control	5.00 ± 1.52	0.41 ± 0.03
Solvent control	5.00 ± 0.99	0.43 ± 0.05
FT(0.625)**	2.67 ± 0.33	0.37 ± 0.06
FT(1.25)	3.00 ± 0.58	0.48 ± 0.04
FT(2.5)	3.33 ± 1.45	0.37 ± 0.02
FT(5)	1.67 ± 0.33	0.54 ± 0.05
FT(10)	3.67 ± 0.33	0.49 ± 0.03
FT(20)	4.00 ± 0.08	0.49 ± 0.03
RN(2.5)	3.33 ± 1.45	0.46 ± 0.06
RN(5)	3.33 ± 0.67	0.42 ± 0.07
RN(10)	4.00 ± 1.53	0.49 ± 0.05
RN(20)	1.67 ± 0.67	0.43 ± 0.09
RN(40)	4.00 ± 0.58	0.43 ± 0.05
Gamma rays alone	106.40 ± 11.16	0.25 ± 0.03
Gamma+RN(2.5)	55.67 ± 7.72	0.16 ± 0.01
Gamma+RN(5)	53.33 ± 4.70	0.17 ± 0.02
Gamma+RN(10)	58.00 ± 5.51	0.20 ± 0.01
Gamma+RN(20)	57.00 ± 6.56	0.21 ± 0.01
Gamma+RN(40)	61.50 ± 13.50	0.18 ± 0.03
Gamma+FT(0.625)	47.67 ± 7.13	0.24 ± 0.05
Gamma+FT(1.25)	60.67 ± 9.61	0.18 ± 0.01
Gamma+FT(2.5)	58.67 ± 4.36	0.28 ± 0.05
Gamma+FT(5)	43.67 ± 2.33	0.23 ± 0.02
Gamma+FT(10)	45.00 ± 1.53	0.19 ± 0.02
Gamma+FT(20)	45.33 ± 7.31	0.14 ± 0.01

* Values indicate the mean values obtained from three mice. A total number of 4500 PCEs were scored for each sample. Errors are standard errors of mean values.

** Values in parentheses indicate the ranitidine and famotidine dose (mg/kg body weight) injected i.p. and the dose of gamma-radiation was 2 Gy.

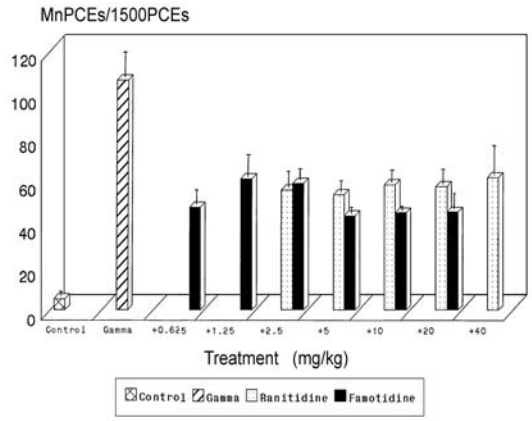

Figure 2. The frequency of MnPCEs in gamma-irradiated mice in the absence or presence of various doses of ranitidine and famotidine.

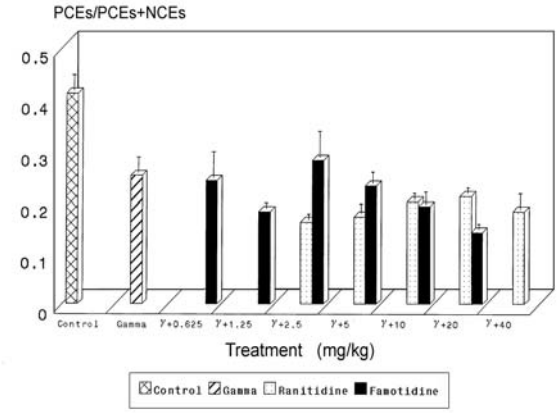

Figure 3. Changes in cell proliferation ratio in gamma-irradiated mice in the absence or presence of various doses of ranitidine and famotidine.

As shown in figure 2 and table 1, the gamma radiation caused significant increase in the frequency of MnPCEs in comparison with the control group that is statistically different with $P<0.001$. Any doses of famotidine or ranitidine injected before radiation exposure reduced the frequency of MnPCEs to almost 50% comparing to those that had received sheer gamma irradiation. This is also verified by statistical analyses ($P<0.01$). Comparing the frequency of MnPCEs between the groups having received injections of various doses of ranitidine or famotidine showed no significant difference.

Results obtained suggest that all doses of ranitidine or famotidine can effectively reduce the frequency of MnPCEs induced by gamma radiation, and such reduction is not a dose-dependent phenomenon ($P<0.01$) at the dose ranges used in these experiments. Table 2 illustrates the DRF calculated for various doses of famotidine and ranitidine.

The statistical analysis performed for the cell proliferation ratio (PCEs/PCEs+NCEs) showed a significant difference between the control and gamma-irradiated groups ($p<0.01$); but no such difference was found between the gamma-irradiated group and each of the groups having received either famotidine or ranitidine prior to irradiation (figure 3). These results indicate that none of the various doses of these two drugs produce any effect on radiation-induced cytotoxicity. Moreover, while comparing the effects of similar doses of these two compounds, statistical analysis using the Student's *t-test* showed no significant difference in reduction of Mn formation by gamma rays, as well as the ratio of PCEs/PCEs+NCEs.

Table 2. DRF calculated for the frequency of MnPCEs induced by gamma rays in the presence of famotidine or ranitidine.

Drug Dose (mg/kg)	DRF (RN)	DRF (FT)
0.625		2.23
1.25		1.75
2.5	1.91	1.81
5	1.99	2.43
10	1.83	2.36
20	1.83	2.34
40	1.73	

DISCUSSION

The Mn Test is a reliable and effective alternative method for the *in vivo* evaluation of clastogenic effects of physical and chemical agents (Heddle *et al.* 1991, Jenssen *et al.* 1980). The present study shows a remarkable increase in the number of MnPCEs for a dose of 2 Gy gamma radiation compared to control groups ($p<0.01$). These findings are consistent with

previous findings of many other investigators who have shown that gamma ray at the dose range used in this study is a potent inducer of Mn in bone marrow erythrocytes (Cole *et al.* 1981, Jenssen *et al.* 1976, Uma Devi *et al.* 1990, Mozdarani and Gharbali, 1993).

When various doses of ranitidine or famotidine were injected 2 hours prior to irradiation, the frequency of MnPCEs was reduced at a DRF of above 1.8 for ranitidine and about 2 for famotidine (table 2). This is compatible with our previous observations with cimetidine which effectively reduced gamma rays and neutron induced micronuclei (Mozdarani and Gharbali 1993, Mozdarani and Khoshbin-Khoshnazar 1998). It is known that sparsely ionizing radiation such as X and gamma rays produce biological effects due to their indirect effects, i.e. free-radical formation. A major proportion of the single- and double strand breakage in DNA molecules is caused by the formation of hydroxyl radicals (Cole *et al.* 1981, Jenssen *et al.* 1976, Uma Devi *et al.* 1990. Skov, 1984, Siddiqi *et al.* 1987). It was observed that OH scavengers play the most effective role in the preservation of the DNA strands against breakage (Billen *et al.* 1984, Achey *et al*. 1974). Taking into consideration that in micronucleus test the micronuclei are the indicators of simple chromosomal breakage (Matter *et al.* 1971, Von Ledebur *et al.* 1973), it is probable that most of the micronuclei are formed through the action of hydroxyl radicals.

Ching *et al.* (1993) have demonstrated that histamine H2-receptor antagonists such as cimetidine, ranitidine and famotidine are, in addition to being good inhibitors of histamine-stimulated gastric acid secretion, also highly powerful hydroxyl-radical scavengers. Lapenna *et al.* (1994) have shown that ranitidine and famotidine are powerful scavengers for $OH^{\cdot}$, HOCl and NH2Cl. The reaction rate constant for famotidine is 1.7×10^{10} mol^{-1} s^{-1} and is 7.5×10^{9} mol^{-1} s^{-1} for ranitidine (Lapenna *et al.* 1994). These rate constants are much higher than for the well-known hydroxyl-radical scavenger, mannitol ($1.7\times10^{9}mol^{-1}s^{-1}$) and the physiological scavenger of $OH^{\cdot}$, glucose (1×10^{9} mol^{-1} s^{-1}) (Ching *et al.* 1993, Lapenna *et al.* 1994).

Comparisons of these values show that histamine H2-receptor antagonists, especially famotidine, have a relatively high power for scavenging hydroxyl radicals.

A study on the effects of cimetidine, ranitidine, and famotidine on human neutrophil functions has shown that cimetidine and famotidine inhibited superoxide (O2-) and H2O2 production of the neutrophils in a dose-dependent manner, although the inhibitory effects were minimal. In contrast, ranitidine failed to change O2- or H2O2 production of neutrophils (Mikawa *et al.* 1999).

In conclusion, the reduction of the incidence of micronuclei observed by the action of ranitidine and famotidine in the present study might be due to their antioxidant and radical-scavenging properties. However, these two drugs were unable to reduce radiation-induced cytotoxicity in bone marrow cells, although the drug dose ranges used in this study did not affect bone marrow cells proliferation. It has been previously shown that ranitidine is associated with hepathotoxicity of unknown mechanism (Pixley *et al.* 1989) and famotidine is the least immunomodulative drug in the histamine H2-receptor antagonist group (Hahm *et al.* 1994). This observation is also consistent with the findings of Du *et al.* (1989) who reported that histamine H2-receptor antagonists such as ranitidine could inhibit hemopoietic reconstruction in regenerating bone marrow after sublethal gamma ray irradiation. Therefore, it is probable that ranitidine and famotidine are unable to help the bone marrow cell reconstruction after gamma irradiation.

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