Effects of famotidine and vitamin C on low dose radiation-induced micronuclei in mice bone marrow cells

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ABSTRACT

  The radioprotective effects of vitamin C and famotidine were investigated using the micronucleus test for anticlastogenic and cell proliferation activity. Various doses of vitamin C and famotidine were administered intraperitoneally 2 h before 2Gy gamma irradiation to NMRI adult male mice. Frequency of micronuclei in 1000 PCEs (MnPCEs) were scored for each sample. Cell proliferation ratio (PCE/PCE+NCE) was also calculated. Data were statistically evaluated using one-way ANOVA and Tukey's HSD test. The results indicated that gamma irradiation alone caused a significant increase in the MnPCEs and reduced the cell proliferation ratio. Administration of various doses of famotidine and vitamin C before gamma irradiation reduced MnPCEs and therefore clastogenic effects of radiation. Famotidine didn't change cell proliferation compared to the irradiation group but vitamin C significantly improved and increased cell proliferation to the control group's level. The dose reduction factor (DRF) calculated, shows a DRF=2 for famotidine and a DRF=1.7 for vitamin C which is indicative of a high radioprotective property of these compounds. The way in which these compounds reduced the clastogenic effects of radiation might be via antioxidant property and free radical scavenging mechanism.

Keywords: Radioprotection; Famotidine; Vitamin C; Micronuclei; Gamma irradiation

INTRODUCTION

  Exposure to ionizing radiation results in a deposition of energy in tissues and production of free radicals. Free radicals are highly reactive chemicals and can initiate a chain of events in the cell which will eventually lead to injury and even cell death [1]. If free radicals being neutral and inactive before reaction to critical molecules, an effective radiation protection exerts in the cells [1, 2]. Due to water radiolysis, the most abundant intracellular compounds, various types of free radicals are generated such as hydroxyl radicals (OH°), hydrogen radicals (H°) and solvated electrons [e^- (aq)]. In the presence of oxygen, reactive oxygen species (ROS) such as superoxide anion (O_2) and hydrogen peroxide (H_2O_2) are also formed, leading to induction of more DNA damage and radiation cytotoxicity in the cells [1, 3, 4]. The OH° is generally considered the most

damaging of the oxygen-based free radicals and it is believed to account for an estimated 50% of the total damages induced by free radical mechanisms [5]. Famotidine is a histamine H2-receptor antagonist usually used for peptic ulcer treatment [6]. It is demonstrated that famotidine, in addition to being a good inhibitor of histamine stimulated gastric acid secretion, is a highly powerful hydroxyl-radical scavenger that can provide protection against the harmful effects of ionizing radiation [7].

Researchers have shown radioprotective effects of famotidine against radiation induced lipid peroxidation [8], DNA damage [9], apoptosis [10] and radiation induced lethality [11]. Vitamin C is the major water soluble antioxidant in the body and is an effective free radical and reactive oxygen species scavenger that can donate electrons to the chain reactions initiated by free

radicals and stop oxidative stress conditions in the body [12, 13]. These properties have persuaded many researchers to study the radioprotective effects of vitamin C leading to obtain acceptable results.

Administration of vitamin C before gamma irradiation prevented chromosomal damage and micronuclei formation in bone marrow cells, peripheral blood lymphocytes and leukocytes [14, 15], induction of apoptosis in peripheral blood leukocytes [10] and radiation-induced lethality [16]. Vitamin C led to repair of double strand break (DSB) and improved cell survival [17] and had a radioprotective effect against mutagenesis in cells [18]. In this study, we intend to test and compare radioprotective effects of famotidine and vitamin C, which are potent free radical scavengers, non toxic, abundant, accessible and affordable, in mice bone marrow cells using micronuclei test. Micronuclei are acentric chromosomal fragments or lagging chromosomes that can't migrate to daughter cells, so remain in the mother cell cytoplasm and are visible as a small nucleus. The micronucleus test developed by Schmid and his colleagues in 1975 [19], is a reliable, sensitive and effective alternative method for the evaluation of clastogenic and cytotoxic effects of physical and chemical agents in vitro and in vivo [20, 21].

MATERIALS AND METHODS
Animals
Six-week old male NMRI mice were purchased from the Pasteur Institute (Karaj, Iran). They were housed in cages for one week, provided with proper light, temperature and moisture, and fed with standard mouse pellet and water ad libitum. Seven-week old mice weighting 25 ± 5 gr were used to experiments.

Drug treatment
Famotidine powder was provided from the Chemidarou Pharmaceutical Co, (Tehran, Iran) and vitamin C provided from the Osvah Pharmaceutical Co. (Tehran, Iran). Various doses of famotidine (5, 10 and 20 mg/kg) and vitamin C (50, 100 and 200 mg/kg) were dissolved in distilled water and injected to mice intraperitoneally, 2h before irradiation.

Irradiation
A 60-Co radiotherapy unit (Theratron II, 780 C, Canada) was used for gamma irradiation. The mice were grouped into 5, kept in a plexiglass box, and irradiated with 2Gy gamma rays. The source to sample distance (SSD) was 80 cm and the dose rate was 50 cGy/min. The mice were irradiated, some in the presence and some in the absence of different doses of famotidine or vitamin C.

The micronucleus assay
The mouse bone marrow micronucleus test was carried out according to the method described by Schmid [19]. 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 7 minutes at 1000 rpm. After centrifuging, the supernatant was removed and cells were resuspended in the remaining serum and a smear was prepared, fixed with methanol and stained by the May Grunwald-Giemsa method. In this method of staining, polychromatic erythrocytes (PCEs) are stained blue-violet, while normochromatic erythrocytes (NCEs) are stained yellow-orange.

Microscopic and statistical analysis
An Y100 Nikon microscope with $\times100$ objective lens was used for scoring the cells. A total of 1000 PCEs was scored for the presence of micronuclei for each sample. 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 ANOVA and Tukey's HSD test.

RESULTS
The results are shown in Table 1. Statistical analysis showed that the groups that had received maximum doses of famotidine (20 mg/kg) and vitamin C (200 mg/kg) alone, showed no significant differences in MnPCEs and cell proliferation ratio in comparison with the control group (P>0.05).

Therefore, lower doses of these compounds also do not have any clastogenic or cytotoxic effects. As shown in Table 1, the gamma radiation caused a significant increase in the frequency of MnPCEs in comparison with the control group (P<0.0001).

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 famotidine or vitamin C.

Treatment	MnPCEs/1000PCEs	PCEs/PCEs+NCEs
Control	4.8 ± 0.66*	0.44 ± 0.01
Fam 20**	4.8 ± 0.73	0.45 ± 0.005
VitC 200	3.2 ± 0.86	0.44 ± 0.004
Gamma rays (2 Gy)	97.2 ± 5.39	0.31± 0.004
Fam 5 + 2 Gy	46.4 ± 2.48	0.33 ± 0.007
Fam 10 + 2 Gy	57.2 ± 2.83	0.33 ± 0.004
Fam 20 + 2 Gy	43.4 ± 2.76	0.32 ± 0.004
VitC 50 + 2 Gy	69.2 ± 1.24	0.43 ± 0.003
VitC 100 + 2 Gy	59.6 ± 3.88	0.42 ± 0.001
VitC 2000 + 2 Gy	53.2 ± 3.00	0.42 ± 0.002

*Values indicate the mean values obtained from five mice. A total number of 5000 PCEs were scored for each sample. Errors are standard errors of mean values.
** Doses of drugs are in mg/kg body weight injected i.p. 2 h before irradiation. Fam and VitC are the abbreviations of famotidine and vitamin C, respectively.

Injection of various doses of famotidine and vitamin C, before radiation exposure showed a significant reduction in the MnPCEs in both famotidine and vitamin C groups (P<0.0001). In famotidine groups, there was no significant difference in the frequency of MnPCEs between 5 and 20 mg/kg (P>0.05), so, dose of 5 mg/kg could considered as optimal dose. In vitamin C groups, there was a significant difference in the frequency of MnPCEs between 50 and 200 mg/kg (P<0.05) but the dose of 100 mg/kg did not have any difference with 50 and 200 mg/kg, therefore the dose of 100 mg/kg could considered as optimal dose. Table 2 illustrates the DRF calculated for various doses of famotidine and vitamin C.
The statistical analysis performed for the cell proliferation ratio (PCEs/PCEs+NCEs) showed a significant difference between the control and gamma-irradiated group (p<0.0001) (Table 1).

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

Drug Dose (mg/kg)	DRF (Fam)	DRF (vitC)
5	2.09±0.24*	
10	1.7± 0.17	
20	2.23± 0.17	
50		1.4± 0.05
100		1.63±0.24
200		1.82± 0.2

*Values indicate the mean values of frequency of MnPCEs in treatment groups over the mean values of frequency of MnPCEs in gamma irradiated group. Errors are standard errors of mean values.

Such a difference was also seen between the gamma-irradiated group and various doses of vitamin C groups (p<0.0001); so that vitamin C increased cell proliferation ratio to control group's level. Comparing the different doses of vitamin C showed no significant difference between groups. In addition, there was no difference between the gamma-irradiated group and various doses of famotidine (P>0.05) indicating that famotidine could not reduce radiation-induced cytotoxicity. In this case, there is no difference between various doses of famotidine.

DISCUSSION

Ionizing radiation induces several damages to DNA as the most critical molecule in the cell. Among them, double strand break (DSB) is the most important damage and produces chromosomal aberrations and micronuclei [22]. The MN Test is a reliable and effective method for the evaluation of clastogenic effects of physical and chemical agents in vitro and in vivo [14,15].The present study showed a remarkable increase in the number of MnPCEs and decrease in the cell proliferation ratio for the dose of 2Gy gamma radiation compared to control groups. These findings are consistent with previous findings of many other investigators who have shown that gamma ray is a potent inducer of MN in bone marrow erythrocytes [23-25]. Selected doses of famotidine (without any clastogenic and

cytotoxic effects) reduced the frequency of MnPCEs. Calculated DRF was 2. These results are consistent with results obtained by other researchers that shown radioprotective effects of famotidine against induction of micronuclei in vitro and in vivo [26,27] and other family members of H2 histamine antagonists such as cimetidine [28]. In one study, non toxic dose of famotidine (2μg) was injected directly into mouse testis and DRF=2 was obtained [29]. It have demonstrated that histamine H2-receptor antagonists such as famotidine, in addition to inhibition of histamine stimulated gastric acid secretion, are also highly powerful OH˚, HOCl and NH2Cl scavengers [7,30]. Hydroxyl radical is the most destructive water radiolysis radical. Among histamine H2-receptor antagonists, famotidine has the highest reaction rate constant with OH˚ (1.7×10^{10} mol^{-1}s^{-1}).

This high value is attributed to 3 sulfur atoms in the structure of famotidine and is much higher than for the well-known hydroxyl-radical scavengers such as mannitol and glucose [30]. However, famotidine was unable to reduce radiation-induced cytotoxicity in bone marrow cells. This observation is consistent with finding of other researches. Du et al. reported that histamine H2-receptor antagonists inhibit hemopoietic reconstruction of bone marrow after sublethal gamma ray irradiation. Therefore, it is probable that famotidine is unable to help the bone marrow cell reconstruction after gamma irradiation [31]. Pre-treatment of mice with different doses of vitamin C, 2h before irradiation resulted in a significant reduction in the number of MnPCEs. Calculated DRF was 1.7. Harapanhaly and colleagues obtained DRF=1.7 with counting of nucleated cells of tibia after treatment of mice with vitamin C and whole body irradiation [32]. Injection of non-toxic dose (1.5μg) of vitamin C directly into mice testis, protected spermatogenesis process against internal irradiation of ^{125}I and ^{131}I with DRF= 2.63 [33,34].
The most important protection mechanism of vitamin C is free radical and reactive oxygen species scavenging before interacting with critical macromolecules such as DNA [14,15].

However, other mechanisms of protection have been proposed to vitamin C. Vitamin C can react directly with DNA molecule, leading to induction of more condensation in DNA structure and thus may protect against the incidence of DSB [35]. Vitamin C abrogates pro-apoptosis gene expression and increases antiapoptosis gene expression therefore, modulates apoptosis in bone marrow cells [36]. In addition, vitamin C has a direct role in the proliferation and differentiation of bone marrow cells [37,38].
The observed effect of vitamin C in increasing of cell proliferation ratio in the present study can be attributed to these mechanisms. In this study, famotidine was more effective in reducing clastogenic effects of gamma rays compared to vitamin C. This effect may be related to the higher reaction rate constant of famotidine (1.7×10^{10} mol^{-1} s^{-1}) with OH° than vitamin C (1×10^{9} mol^{-1}s^{-1}), yet vitamin C is effective than famotidine in reducing cytotoxic effect of gamma radiation.
Radioprotective agents used in radiotherapy patients, military personnel, radiation workers and people in nuclear accidents should meet several criteria such as: (i) offer a good protection against acute and chronic radiation damage (DRF>1.5). (ii) can be administrated easily (oral) and have the ability to be rapidly absorbed and distributed throughout of the body (iii) have no significant toxicity or side effects in the body (iv) be chemically stable (v) be widely available and cost-effective [27]. Famotidine and vitamin C show a good radiation protection.
This two compounds can be administrated orally. They are not toxic and have no side effects and are accessible and cost- effective. These features make them suitable for use as chemical radioprotectors for different exposure situations that there is a risk of damage to bone marrow.

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REFERENCES

1.Steel G G. Basic clinical radiobiology.3th Edition. USA: CRC Press; 2002

2.Hosseinimehr SJ. Trends in the development of radioprotective agents. Drug Discovery Today 2007;12(19/20):794-805.

3.Hall E J. Radiobiology for radiobiologist.5thEdition. Philadelphia: Lippincott Williams & Wilkins; 2000.

4.Laverne JA. OH radicals and oxidizing products in the gamma radiolysis of water. Radiat Res 2000Feb;153(2):196-200.

5.Imlay JA, Linn S. DNA damage and oxygen radical toxicity. Science 1988 Jun 3;240(4857):1302.

6.Deakin M, Williams JG. Histamine H2-receptor antagonists in peptic ulcer disease. Efficacy in healing peptic ulcers. Drugs1992 Nov ;44 (5):709-19.

7.Ching TL, Haenen GR, Bast A. Cimetidine and other H2 receptor antagonists as powerful hydroxyl radical scavengers. Chem Biol Interact1993 Feb;86(2):119-27.

8.Della Rovere F, Broccio M, Granata A, Zirilli A, Brugnano L, Artemisia A, et al. Anti free radical action of calcium antagonists and H1 and H2 receptors antagonists in neoplastic disease. Anticancer Res1996 Jul-Aug;16(4A):1749-54.

9.Mozdarani H, Nasirian B, Haeri SA. In vivo gamma-rays induced initial DNA damage and the effect of famotidine in mouse leukocytes as assayed by the alkaline comet assay. J Radiat Res2007 Mar;48(2):129-34.

10.Mozdarani H, Ghoraeian P. Modulation of gamma-ray-induced apoptosis in human peripheral blood leukocytes by famotidine and vitamin C. Mutat Res 2008 Jan 8;649(1-2):71-8.

11.Mozdarani H, Salimi M, Froughizadeh M. Effect of cimetidine and famotidine on survival of lethally gamma irradiated mice. Iranian Journal of Radiation Research. 2008;5(4):187.

12.Rose RC. Ascorbic acid metabolism in protection against free radicals: A radiation model. Biochemical and Biophysical Research Communications 1990:169 (2): 430-6.

13.Wan XS, Ware JH, Zhou Z, Donahue JJ, Guan J, Kennedy AR. Protection against radiation-induced oxidative stress in cultured human epithelial cells by treatment with antioxidant agents. Int J Radiat Oncol Biol Phys 2006 Apr 1;64(5):1475-81.

14.Konopacka M, Widel M, Rzeszowska-Wolny J. Modifying effect of vitamins C, E and beta-carotene against gamma-ray-induced DNA damage in mouse cells. Mutat Res1998 Sep 11;417(2-3):85-94.

15.Konopacka M, Palyvoda O, Rzeszowska-Wolny J. Inhibitory effect of ascorbic acid post-treatment on radiation-induced chromosomal damage in human lymphocytes in vitro. Teratog Carcinog Mutagen 2002;22(6):443-50.

16.Jagetia GC, Rajanikant GK, Baliga MS, Rao KV, Kumar P. Augmentation of wound healing by ascorbic acid treatment in mice exposed to gamma-radiation. Int J Radiat Biol2004 May;80(5):347-54.

17.Fujii Y, Kato TA, Ueno A, Kubota N, Fujimori A, Okayasu R. Ascorbic acid gives different protective effects in human cells exposed to X-rays and heavy ions. Mutat Res Jun2010. 17;699(1-2):58-61.

18.Kinashi Y, Sakurai Y, Masunaga S, Suzuki M, Nagata K, Ono K. Ascorbic acid reduced mutagenicity at the HPRT locus in CHO cells against thermal neutron radiation. Applied Radiation and Isotopes 2004 ;61 (5) :929-32.

19.Schmid W. The micronucleus test. Mutat Res1975 Feb;31(1):9-15.

20.Jenssen D, Ramel C. The micronucleus test as part of a short-term mutagenicity test program for the prediction of carcinogenicity evaluated by 143 agents tested. Mutation Research/Reviews in Genetic Toxicology 1980;75(2):191-202.

21.Hayashi M, Tice RR, MacGregor JT, Anderson D, Blakey DH, Kirsh-Volders M, et al. In vivo rodent erythrocyte micronucleus assay. Mutation Research /Environmental Mutagenesis and Related Subjects 1994;312(3):293-304.

22.Bender MA, Griggs HG, Bedford JS. Mechanisms of chromosomal aberration production III. Chemicals and ionizing radiation. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis1974;23(2):197-212.

23.Uma Devi P, Sharma AS. Mouse bone-marrow response to low doses of whole-body gamma irradiation: induction of micronuclei. Int J Radiat Biol1990 Jan;57(1):97-101.

24.Jagetia CG, Aruna R. The herbal preparation

abana protects against radiation-induced micronuclei in mouse bone marrow. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 1997; 393 (1/2):157-63.

25.Hosseinimehr SJ ,Tavakoli H, Pourheidari G, Sobhani A, Shafiee A. Radioprotective effects of citrus extract against gamma-irradiation in mouse bone marrow cells. J Radiat Res2003 Sep ;44 (3):237-41.

26.Ghorbani M, Mozdarani H. In vitro radioprotective effects of histamine H2 receptor antagonists against gamma-rays induced chromosomal aberrations in human lymphocytes. Iranian Journal of Radiation Research 2003;1(2):99-104.

27.Mozdarani H. Radioprotective properties of histamine H2 receptor antagonists: present and future prospects. J Radiat Res 2003 Jun ;44(2):145-9.

28.Mozdarani H, Gharbali A. Radioprotective effects of cimetidine in mouse bone marrow cells exposed to gamma-rays as assayed by the micronucleus test. Int J Radiat Biol1993 Aug;64(2):189-94

29.Mahdavi M, Mozdarani H. Protective effects of famotidine and vitamin C against radiation induced cellular damage in mouse spermatogenesis process. Iranian Journal of Radiation Research 2011 ;8(4):223-30.

30.Lapenna D, De Gioia S, Mezzetti A, Grossi L, Festi D, Marzio L, et al. H2-receptor antagonists are scavengers of oxygen radicals. Eur J Clin Invest1994 Jul ;24(7):476-81

31.Du XX, Zhou YJ, Xu YH. Effects of histamine H2-receptor antagonists on hemopoietic reconstruction in bone marrow. Sheng Li Xue Bao1989 Dec;41(6):597-601.

32.Harapanhalli RS, Yaghmai V, Giuliani D, Howell RW, Rao DV. Antioxidant effects of vitamin C in mice following X-irradiation. Res Commun Mol Pathol Pharmacol1996 Dec;94(3):271-87.

33.Narra VR, Howell RW, Sastry KS, Rao DV. Vitamin C as a radioprotector against iodine-131 in vivo. Journal of Nuclear Medicine: official publication, Society of Nuclear Medicine 1993; 34(4):637-40.

34.Narra VR, Harapanhalli RS, Howell RW, Rao DV. Vitamins as Radioprotectors In Vivo. I. Protection by Vitamin C against Internal Radionuclides in Mouse Testes: Implications to the Mechanism of Damage Caused by the Auger Effect. Radiation Research1994;137(3):394-9.

35.Yoshikawa Y, Suzuki M, Chen N, Zinchenko AA, Murata S, Kanbe T, et al. Ascorbic acid induces a marked conformational change in long duplex DNA. Eur J Biochem2003 Jul;270(14):3101.

36.Wambi C, Sanzari J, Wan XS, Nuth M, Davis J, Ko YH, et al. Dietary antioxidants protect hematopoietic cells and improve animal survival after total-body irradiation. Radiat Res2008 Apr;169 (4):384-96.

37.Choi KM, Seo YK, Yoon HH, Song KY, Kwon SY, Lee HS, et al. Effect of ascorbic acid on bone marrow-derived mesenchymal stem cell proliferation and differentiation. J Biosci Bioeng2008 Jun;105 (6):586-94.

38.Xian L, Lou M, Wu X. Pretreatment with antioxidants prevent bone injury by improving bone marrow microenvironment for stem cells. Stem Cell Discovery 20122(3):100-7.