Free Radical Research, 2002 VOL. 36 (3), pp. 255–263

Taylor & Francis
healthsciences

Protective Effects of Cimetidine on Radiation-induced Micronuclei and Apoptosis in Human Peripheral Blood Lymphocytes

YASUO KOJIMA[a,b], TAKASHI KONDO[a,*], QING-LI ZHAO[a], MIKI SHOJI[c] and RYUSUKE FUTATSUYA[d]

[a]*Department of Radiological Sciences, Faculty of Medicine, Toyama Medical and Pharmaceutical University, Sugitani 2630, Toyama 930-0194, Japan;*
[b]*Department of Obstetrics and Gynecology, Toyama Prefectural Central Hospital, Nishinagae, Toyama 930-8550, Japan;*
[c]*Radioisotope Research Center, Toyama Medical and Pharmaceutical University, Sugitani 2630 Toyama 930-0194, Japan;*
[d]*Department of Radiology, Toyama Saiseikai Hospital, Kusunoki, Toyama 931-8533, Japan*

Accepted by Professor N. Taniguchi

(Received 5 October 2001)

The radioprotective effects of cimetidine, which has been used clinically as an antagonist of H_2 receptor, on radiation-induced micronuclei and apoptosis in human peripheral blood lymphocytes (PBL) prepared from healthy donors were studied. Cells were treated with cimetidine before or after X-irradiation, and then cytokinesis-blocked micronucleus assay and flow cytometry for measurement of phosphatidylserine externalization were utilized to evaluate the radiation-induced cytogenetic damage and apoptosis. The protective effect of pre-irradiation treatment of cimetidine on radiation-induced micronuclei was dependent on the concentration. The maximum protection rates of cimetidine (1 mM) on frequencies of micronuclei were 38.8 and 30.2% for cells treated before and after X-irradiation (5 Gy), respectively. Protective effects of pre- and post-irradiation treatment with cimetidine on radiation-induced early apoptosis and decreased activity of caspase-3 were observed. A study of electron paramagnetic resonance-spin trapping with 5,5′-dimethyl-1-*N*-oxide revealed that the rate constant of cimetidine with radiation-induced OH radicals is about 4.5×10^9 l/mol/s. Cimetidine did not significantly increase the intracellular concentration of glutathione. These results suggest that cimetidine suppresses radiation-induced micronuclei and apoptosis via OH radical scavenging and an intracellular antioxidation mechanism. Cimetidine appears to be a useful candidate for the future development of post-irradiation radioprotectors.

Keywords: Cimetidine; Radioprotection; Micronucleus; Apoptosis; Lymphocytes

INTRODUCTION

Many chemicals which can protect cells against ionizing radiation have been found since the discovery of radioprotective effects of sulfhydryl compounds such as cyanide and cystein in 1949, and thiourea in 1950.[1] Among the numerous compounds tested, some drugs such as 2-mercaptoethylamine (MEA), *S*-(aminoethyl)isothiuronium bromide (AET) and *S*-2-(3-aminopropylamino)ethylphosphorothioic acid (amifostine, WR-2721) showed potent radioprotective effects. MEA, which proved to be a strong protector in mice, is still regarded as the most potent whole-body radioprotective agent. AET and mercaptoethylguanidine, which is considered the active structure of AET, have been noted for their lack of chronic toxicity and strong protective effects against intestinal damage in animals. In a large research program for synthesis of radioprotective agents at the Walter Reed Army Institute of Research in the US, many substances containing sulfhydryl groups were tested, but only amifostine was found to be acceptable with regard to the toxicity. Preclinical work suggested that amifostine and its active thiol, 2-[(aminopropyl)amino]ethanethiol (WR-1065) are clinically useful radioprotectors which protect normal cells effectively against the adverse effects of irradiation without affecting tumor cells. A recent phase III randomized trial in head and neck cancer[2]

*Corresponding author. Tel.: +81-76-434-7265. Fax: +81-76-434-5190. E-mail: kondot@ms.toyama-mpu.ac.jp

ISSN 1071-5762 print/ISSN 1029-2470 online © 2002 Taylor & Francis Ltd
DOI: 10.1080/10715760290019264

demonstrated that amifostine reduced acute and chronic xerostomia while preserving the efficacy of antitumor treatment.

Most of the current radioprotective agents exhibit their protective effects against irradiation when they are administered before exposure, so the development of new drugs which are effective even when administered after exposure is desired for the amelioration of medical and accidental exposure to radiation. Cimetidine is a potent histamine H_2 receptor antagonist, which is clinically used in treating ulceration of the stomach and intestine. In *in vitro* studies, cimetidine reacted with copper ions to form copper/cimetidine complexes,[3] and was revealed to be a powerful hydroxyl radical scavenger.[4–7] Some authors have reported protective effects of cimetidine against γ-rays,[8] fast neutrons[9] and several chemicals[10–14] in mice or rats, however, the protective effects of cimetidine on radiation-induced micronuclei and apoptosis in human cells have not yet been reported.

The micronucleus assay has been used to assess chromosomal damage induced by ionizing radiation or chemicals. The cytokinesis-blocked technique[15–17] for analyzing micronuclei, which is based on the identification of binucleate cells inhibited when undergoing cytokinesis by cytochalasin B, is a common and reliable method for assessing the chromosomal damage due to irradiation in human peripheral blood lymphocytes (PBL).[18–22] Apoptosis is a physiologic mode of cell death that plays various critical roles in maintaining the normal cellular components, and evidence of apoptosis in PBL due to DNA damage or membrane peroxidation by ionizing radiation has been reported.[23–27]

Here, the radioprotective effects of cimetidine against the radiation-induced cytogenetic damage and apoptosis in PBL were investigated using a cytokinesis-blocked micronucleus assay and flow cytometry for the measurement of phosphatidylserine externalization. The results revealed that both pre- and post-irradiation treatment with cimetidine suppressed radiation-induced micronuclei and apoptosis effectively via OH radical scavenging and an intracellular antioxidation mechanism. To our knowledge, this is the first study to demonstrate the protective effects of cimetidine both before and after exposure on radiation-induced micronuclei and apoptosis in PBL.

MATERIALS AND METHODS

Blood Collection and Lymphocyte Preparation

Informed consent was obtained from four healthy, non-smoking human volunteers, three males and one female, aged between 27 and 40 years. Blood samples were collected in sterile vacutainer tubes (Vacutainer® CPT™, Becton Dickinson and Company, Franklin Lakes, NJ) containing sodium heparin anticoagulant, Ficoll–Hypaque solution and polyester gel. Lymphocytes were isolated from whole blood by density centrifugation and washed twice with PBS. They were cultured in RPMI 1640 medium (Gibco BRL, Paisley, Scotland, UK) containing 15% heat-inactivated fetal bovine serum (Gibco BRL) at a concentration of 5×10^5 cells/ml at 37°C in humidified air with 5% CO_2. When lymphocytes were treated with cimetidine, pure cimetidine powder (Wako Pure Chemical Industries, Osaka, Japan) was dissolved in the medium to each concentration.

X-irradiation

X-irradiation was carried out at room temperature by X-ray apparatus (MBR-1520R-3, Hitachi Medico Technology, Kashiwa, Japan) operating at 150 kVp and 20 mA at dose rate of 5 Gy/min determined by a Fricke dosimeter.

Micronucleus Assay

To assess the effects of cimetidine on radiation-induced micronuclei, pure cimetidine powder was dissolved in the medium to give a final concentration of 0.1, 1, 10, 100 μM or 1 mM, 2 h before irradiation or 1 mM immediately after irradiation. At the start of the culture, PHA (phytohemagglutinin; Difco Laboratories, Detroit, MI) at a final concentration of 50 μg/ml was added to each culture, and 44 h after PHA stimulation, cytochalasin-B (Sigma Chemical, St. Louis, MI) was added to the cultures to give final concentration of 3.0 μg/ml to block the dividing cells in cytokinesis. Twenty-eight hours after the addition of cytochalasin-B, cells were collected, decanted directly onto a glass slide, and stained with May-Grunwald Giemsa. All slides were evaluated under 1000-fold magnification, and the frequency of micronuclei in cytokinesis-blocked binucleate cells with well-preserved cytoplasm was recorded. Binucleate cells (1000 cells/culture) were counted to record the frequency of cells with one micronucleus, and two and three micronuclei. The criterion for identifying binucleated cytokinesis-blocked cells was that cells should have two nuclei of approximately equal size, which may be attached by a fine nucleoplasmic bridge or overlap slightly. A micronucleus was defined as a nucleus of diameter between 1/16 and 1/3 of that normal nuclei, non-refractile and not linked to normal nuclei via a nucleoplasmic bridge.[15–17]

Measurement of Intracellular Glutathione

Intracellular glutathione was analyzed using a total glutathione quantification kit (Dojindo Laboratories,

Kumamoto, Japan). The detection method of the kit used the reaction of 5,5′-dithiobis(2-nitrobenzoic acid) and glutathione to generate glutathione disulfide and 2-nitro-5-thiobenzoic acid, which is yellow in color (λ_{max}: 412 nm).[28–30] The assessment was performed according to the manufacturer's recommendations. Briefly, PBL were collected and washed after 24 h incubation, lysed with 10 mM HCl by freezing and thawing, and 5-sulfosalicylic acid was added to eliminate proteins and to prevent GSH oxidation and γ-glutamyl transpeptidase reaction. The samples were then centrifuged and the supernatant was transferred to 96 well microplates. After the reaction, the absorbance at 412 nm of each well was read using a microplate reader and the concentrations of GSH in the samples were determined from the calibration curve.

Assessment of Apoptosis and Caspase-3 Activity

Phosphatidylserine externalization of apoptosis was determined by two-color flow cytometric analysis of annexin-V/FITC binding propidium iodide (PI) uptake[24] using a Beckman-Coulter EPICS XL™ flow cytometer. The proportions of FITC+/PI− cells corresponding to early apoptosis and FITC+/PI+ cells corresponding to secondary necrosis were evaluated. A gate was put on annexin-V/FITC positive cells and back gating was performed on the scatter plot in order to distinguish platelets and debris from smaller apoptotic cells, with exclusion of monocytes. The cell permeable fluorogenic substance, PhiPhiLux-G1D2, was used to monitor the intracellular caspase-3 activity according to the manufacturer's recommendations (OncoImmunin, Geithersburg, MD). Briefly each sample containing approximately 10^6 cells/ml was gently centrifuged and the cell pellet was resuspended with 50 μl of 10 μM PhiPhiLux-G1D2 substrate solution in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum. After incubation for 1 h at 37°C in the dark, the sample was washed once and diluted with 0.5 ml of ice cold flow cytometry dilution buffer. Flow cytometric analysis was then performed.

ESR Measurement

Electron spin resonance (ESR)-spin trapping with 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) was utilized for the measurement of rate constants of cimetidine for reactions of OH radicals induced by X-irradiation. Aqueous DMPO solutions at a concentration of 10 mM saturated with air containing various concentrations of OH scavengers were exposed to X-rays (150 Gy). The ESR spectra of X-irradiated samples were measured in a quartz flat cell with an ESR spectrometer (RFR-30 Radical Analyzer System, Radical Research, Tokyo, Japan). First, the $C_{\frac{1}{2}}$ values, the scavenger concentrations at which the DMPO–OH adducts yield is decreased by 50% of the maximum yield, were obtained for potassium iodide, sodium formate, mannitol, glucose, sodium propionate and sodium acetate, all of which are known to be water soluble OH scavengers with different rate constants.[31] Second, the $C_{\frac{1}{2}}$ value of cimetidine was obtained and the rate constant was estimated from the relationships between the $C_{\frac{1}{2}}$ values and the rate constants of various scavengers for reactions with OH radicals.

Statistical Evaluation

The micronucleus assay data were analyzed by the χ^2-test. The data obtained from the micronucleus assays, glutathione measurement and flow cytometry were analyzed using a *t*-test. Statistical significance was determined when *p* values were less than 0.05.

RESULTS

Radioprotective Effect of Cimetidine on Radiation-induced Micronucleus

The number of binucleated cells with micronuclei and the total number of micronuclei per 1000 binucleated cells for each group of pre-irradiation treatment of cimetidine are shown in Table I. The number of binucleate cells containing micronuclei and the total number of micronuclei per 1000 binucleated cells decreased with the concentration of cimetidine for each volunteer. The protective effects of pre-irradiation treatment of cimetidine on radiation-induced micronuclei are indicated in Fig. 1. "Protection rate" means a decreasing rate of binucleated cells with micronuclei following treatment with cimetidine compared to the control group

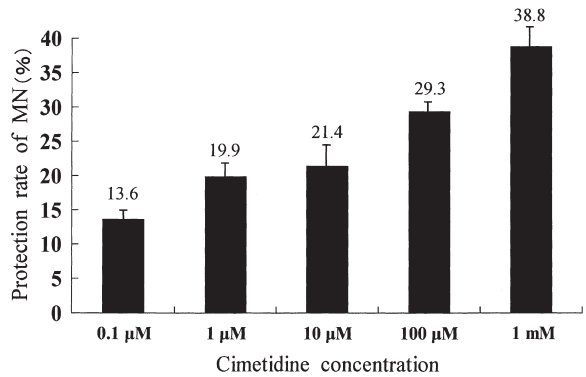

FIGURE 1 Protective effects of cimetidine on radiation-induced micronuclei. Protection rates indicate the percentage decrease of binucleated cells with micronuclei treated with cimetidine compared to controls. There is a significant dose-response relationship with $p < 0.05$. Bars in figure indicate the standard deviation of mean values ($n = 4$).

TABLE I Effects of pre-irradiation treatment of cimetidine on micronucleus frequencies in human peripheral blood lymphocytes

		The number of BN cells with MN/1000 BN cells				
		1MN	2MN	3MN	Total	Total MN*
Donor 1						
Control		9	1	0	10	11
Cimetidine	0.1 μM	9	0	0	9	9
	1 μM	6	2	0	8	10
	10 μM	9	0	0	9	9
	100 μM	8	0	0	8	8
	1 mM	9	0	0	9	9
+2.5 Gy		259	40	4	303	351
Cimetidine	0.1 μM	243	30	4	277	315
	1 μM	204	22	3	230	258
	10 μM	201	27	0	228	255
	100 μM	175	23	0	198	221
	1 mM	183	11	0	194	205
Donor 2						
Control		4	0	0	4	4
Cimetidine	0.1 μM	5	0	0	5	5
	1 μM	4	1	0	5	6
	10 μM	5	0	0	5	5
	100 μM	5	0	0	5	5
	1 mM	5	0	0	5	5
+2.5 Gy		228	35	3	266	307
Cimetidine	0.1 μM	204	18	4	226	252
	1 μM	203	28	0	231	259
	10 μM	197	22	2	221	247
	100 μM	169	23	2	194	221
	1 mM	180	10	0	190	200
Donor 3						
Control		7	1	0	8	9
Cimetidine	0.1 μM	7	0	0	7	7
	1 μM	6	1	0	7	8
	10 μM	7	0	0	7	7
	100 μM	7	1	0	8	9
	1 mM	7	1	0	8	9
+2.5 Gy		224	36	6	266	314
Cimetidine	0.1 μM	198	32	2	232	266
	1 μM	174	39	2	215	258
	10 μM	188	20	2	210	234
	100 μM	171	21	0	192	213
	1 mM	134	12	0	146	158
Donor 4						
Control		7	2	0	9	11
Cimetidine	0.1 μM	5	2	0	7	9
	1 μM	8	1	0	9	10
	10 μM	6	1	0	7	8
	100 μM	7	0	0	7	7
	1 mM	7	1	0	8	9
+2.5 Gy		208	37	2	247	288
Cimetidine	0.1 μM	183	18	0	201	219
	1 μM	165	21	2	188	211
	10 μM	173	14	2	189	207
	100 μM	165	12	0	177	189
	1 mM	132	2	0	134	136

* The total number of micronucrei per 1000 binucleated cells, MN: Micronucleus; BN: Binucleated cells.

(not treated). The protection rates of micronucleus cells increased with the concentration of cimetidine.

Table II shows the frequencies of micronuclei after post-irradiation treatment of 1 mM cimetidine for three volunteers' PBL. The frequencies of binucleated cells with micronuclei for the groups with post-irradiation treatment of cimetidine were significantly lower than in the groups without treatment ($p < 0.05$). The average decrease of binucleated cells with micronuclei was 30.2% for the groups treated with cimetidine. These results indicate that cimetidine protects PBL from chromosomal damage induced by irradiation.

Radioprotective Effect of Cimetidine on Radiation-induced Apoptosis

To investigate the effect of cimetidine on radiation-induced apoptosis, isolated PBL were irradiated, harvested after 24 h and subsequently flow cytometrically analyzed with annexin-V/FITC and PI. Representative flow cytometric data are shown in

TABLE II Effects of post-irradiation treatment of cimetidine on micronucleus frequencies in human peripheral blood lymphocytes

	BN cells with MN/1000 BN cells				
	1MN	2MN	3MN	Total	Protection rate* (%)
Donor 1					
Control	9	0	0	9	
+Cimetidine 1 mM	8	0	0	8	
2.5 Gy	249	41	5	295	
+Cimetidine 1 mM	200	16	3	219	25.5‡
Donor 2					
Control	5	0	0	5	
+Cimetidine 1 mM	5	0	0	5	
2.5 Gy	228	37	3	268	
+Cimetidine 1 mM	176	20	2	198	26.1‡
Donor 3					
Control	7	0	0	7	
+Cimetidine 1 mM	6	0	0	6	
2.5 Gy	216	35	8	259	
+Cimetidine 1 mM	133	19	3	155	39.9‡
Total 3 donors†					
Control	21	0	0	21	
+Cimetidine 1 mM	19	0	0	19	
2.5 Gy	693	113	16	822	
+Cimetidine 1 mM	509	55	8	572	30.2‡

*Protection rate indicates the percentage decrease of binucleated cells with micronucleus treated with cimetidine compared to control. †A total of 3000 binucleated cells were examined in each culture. ‡$p < 0.05$, with respect to control values, MN: Micronucleus; BN: Binucleated cells.

Fig. 2. The proportions of cells spontaneously undergoing early apoptosis and secondary necrosis were 4.8 and 1.6%, and no effect of cimetidine was observed. In the samples of irradiated PBL without cimetidine treatment, the percentages of the early apoptotic cells (annexin-V/FITC +, PI−) were 34.9 and 40.1%, and those of the secondary necrosis (annexin-V/FITC +, PI+) were 7.3 and 9.1% with 2.5 and 5.0 Gy exposures, respectively. When the cells were treated with cimetidine prior to or after irradiation, the percentages of the early apoptotic cells were significantly decreased. The percentages of the early apoptosis for the pre-irradiation treatment groups were 26.4 ± 2.8% and 32.1 ± 3.7%, and those for the post-irradiation treatment groups were 23.0 ± 1.3% and 26.8 ± 0.8% (mean ± SD, $n = 3$) with 2.5 and 5.0 Gy exposures, respectively. These results show that pre- and post-irradiation treatment of cimetidine inhibited the radiation-induced apoptosis of PBL.

Caspase-3 Activity of PBL

To investigate the caspase-3 activity of PBL, flow cytometric analysis was performed using PhiPhiLux-G1D2. Figure 3 indicates the fraction of cells showing

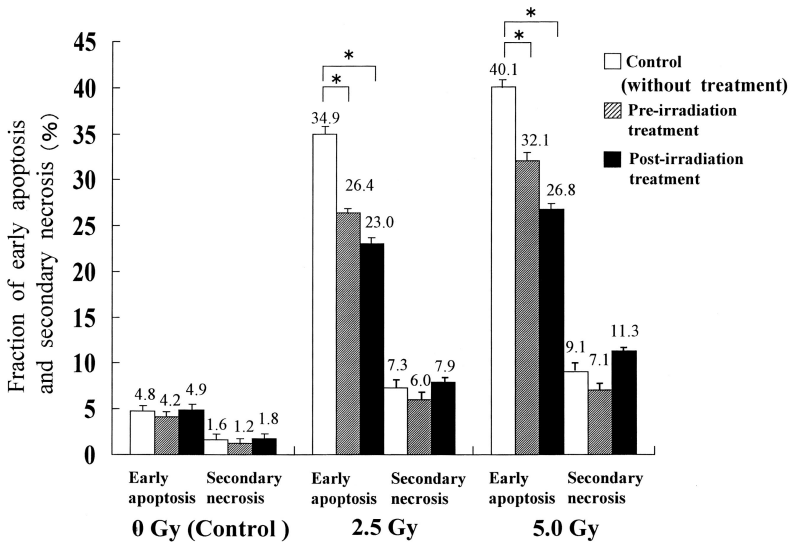

FIGURE 2 Protective effects of cimetidine on radiation-induced early apoptosis and secondary necrosis. Cimetidine conc., 1 mM. *$p < 0.05$, with respect to control values. Bars in figure indicate the standard deviation of mean values ($n = 3$).

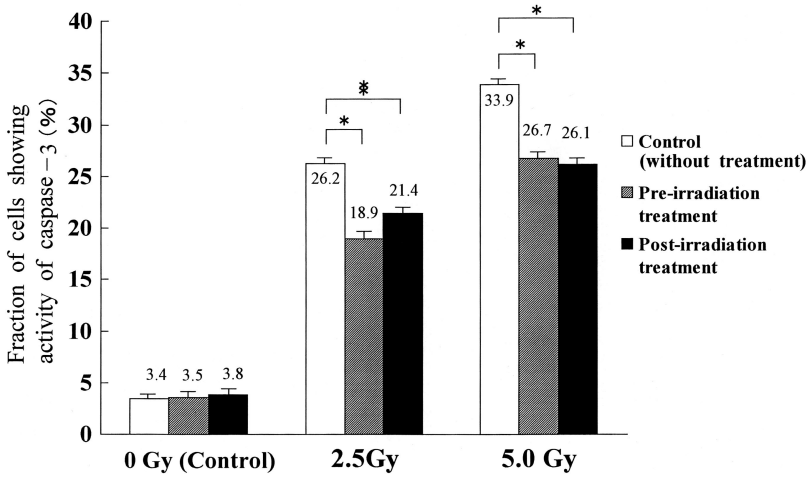

FIGURE 3 Protective effects of cimetidine on radiation-induced activity of caspase-3. Cimetidine conc., 1 mM. $*p < 0.05$, with respect to control values. Bars in figure indicate the standard deviation of mean values ($n = 3$).

the caspase-3 activity by pre- and post-irradiation treatment of cimetidine. The fractions of cells with high activation of caspase-3 were 26.2 and 33.9% after 2.5 and 5.0 Gy without treatment of cimetidine, respectively. When the cells were treated with cimetidine prior to or after irradiation, caspase-3 activity was significantly decreased compared with the cells without cimetidine treatment.

Measurement of Intracellular Glutathione

Intracellular glutathione levels were measured by the glutathione assay kit as described in the previous section. PBL were treated with 1 mM cimetidine for 2 h, irradiated (2.5 Gy), and the intracellular GSH levels were measured. The quantities of glutathione per 1×10^6 cells were 6.1 ± 0.1, 5.7 ± 0.7 and 5.0 ± 0.1 nmol with pre-irradiation treatment of cimetidine, and 6.2 ± 1.0, 6.6 ± 0.1 and 4.9 ± 0.2 nmol (mean $\pm$ SD, $n = 3$) without cimetidine treatment at 0, 6 and 24 h after irradiation, respectively. No significant difference in the intracellular glutathione levels was observed as a result of the pre-irradiation treatment of cimetidine.

ESR Measurement for Rate Constant With OH Radicals

The $C\frac{1}{2}$ value is a scavenger concentration at which the DMPO–OH adducts yield is decreased by 50%. The $C\frac{1}{2}$ value of cimetidine was compared with those of various OH radical scavengers, and the rate constant of cimetidine for reactions of OH radicals was obtained (Fig. 4). The estimated rate constant is about 4.5×10^9 l/mol/s, which is similar to that of sodium formate. The results indicate that cimetidine has a potent activity to scavenge radiation-induced OH radicals.

DISCUSSION

Radiation-induced micronuclei formed in interphase cells are the consequence of DNA damage and repair, and arise mainly from acentric chromosomal fragments that cannot be attached to the spindle apparatus during mitosis.[17,32] Since the micronucleated cells lose their clonogenecity, the frequency of cells with micronuclei reflects the cytogenetic damage fraction induced by ionizing radiation. Fenech and Morley[15] developed a cytokinesis-block micronucleus assay based on identification of binucleated cells by blocking cytokinesis with cytochalasin-B, an inhibitor of actin polymerization required for the formation of the microfilament ring, and scoring micronuclei in binucleated cells that have undergone only one division. The method is widely used when testing for genotoxicity and cytogenetic damage in mitogen-stimulated PBL. In this study, we measured the radiation-induced micronuclei in PBL from four healthy volunteers to assess the cytogenetic effects of X-irradiation and the radioprotective effects of cimetidine.

The results presented here demonstrated the protective effects of cimetidine against radiation-induced micronucleus formation. The protective effect was dose dependent, since increasing the concentration of cimetidine resulted in significant reduction of the number of binucleated cells with micronuclei and the total number of micronuclei per 1000 binucleated cells.

Although amplification of the glutathione system has been reported as the mechanism of radioprotective effects of cimetidine,[8,9] our results indicated that intracellular glutathione levels did not significantly increase as a result of the treatment of cimetidine. It has been shown that Cu(II)/cimetidine complexes and Fe(III)/cimetidine complexes have higher superoxide dismutase-like activities

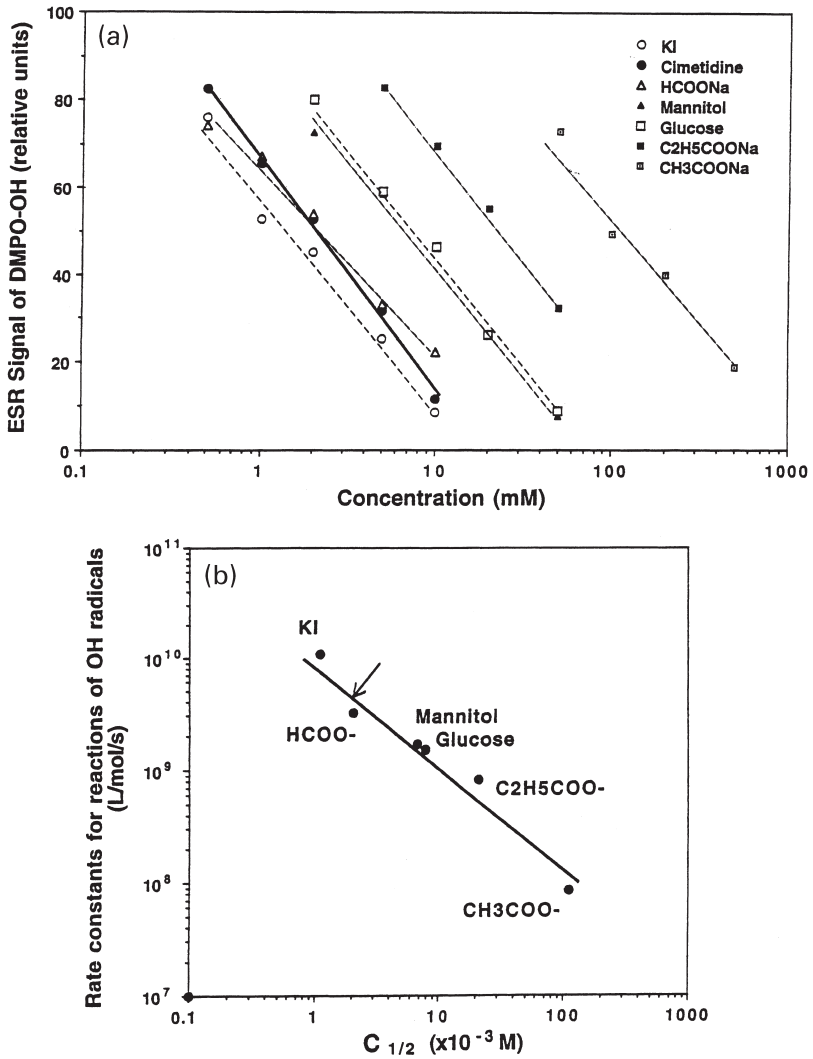

FIGURE 4 Relationships between ESR signal intensity of DMPO–OH adducts induced by X-irradiation and concentration of OH radical scavengers. (a) Relationships between rate constants for reactions of OH radicals and $C\frac{1}{2}$, (b) Aqueous 20 mM DMPO solutions containing various OH radical scavengers were X-irradiated at a dose of 150 Gy and the ESR signal intensity of DMPO–OH adducts was measured with an ESR spectrometer. A comparison of $C\frac{1}{2}$ values, the scavenger concentration at which the DMPO–OH adducts yield is decreased by 50% and rate constants for reactions of OH radicals from J. Phys. Chem. Ref. Data[31] was performed. Arrow in figure shows C1/2 value of cimetidine.

compared with other copper complexes such as Cu(II)/(*o*-phenanthroline)$_2$, Cu(II)/glycylglycine and Cu(II)/salicilate.[3,34] Therefore, the superoxide dismutase like activities of metal/cimetidine complex appear to contribute to the radioprotective effects of cimetidine in both pre- and post-irradiation treatment.

We demonstrated the hydroxyl radical scavenging activity of cimetidine with a higher rate constant than that of mannitol, and this evidence is consistent with the previous reports that cimetidine is a powerful hydroxyl radical scavenger.[4–7] These results indicate that the radioprotective effects of cimetidine administered prior to radiation are due to the OH radical scavenging activity.

The detailed molecular mechanism and signaling pathways for radiation-induced apoptosis remain unknown. Recent data suggest that plasma membrane processes are important in radiation-induced apoptosis, in contrast to the widely acknowledged role of DNA damage in inducing classical reproductive cell death.[26] For the quantitation of apoptosis, the phosphatidylserine externalization of apoptosis was determined by flow cytometry with annexin-V/FITC and PI staining, which can separately measure early apoptosis and secondary necrosis.[24] Our results showed that the fraction of radiation-induced early apoptosis but not that of secondary necrosis in PBL was reduced by the treatment with cimetidine, and cimetidine also inhibited the radiation-induced caspase-3 activity of PBL when administered both before and after irradiation. Activation of caspase-3 plays an important role not only in radiation but also in other forms of stress-induced apoptosis.[33,35–38] Therefore, cimetidine inhibited the upstream caspase-3 dependent pathways of radiation-induced apoptosis,

most likely due to scavenging of hydroxyl radicals and/or intracellularly-produced superoxide.

The detailed mechanism of the radioprotective effects of cimetidine on micronucleus formation when after irradiation is still unknown, but the intracellular antioxidation mechanism also may play a role in radiation-induced micronucleus formation.

Cimetidine is a potent histamine H_2 receptor antagonist and is currently in worldwide use for the clinical treatment of peptic ulcers. The potent radioprotective effects of cimetidine against X-irradiation when administered not only prior to irradiation but also after exposure suggest that cimetidine is a useful candidate for the future development of post-irradiation radioprotectors.

Acknowledgements

This study was supported in part by a Grant in Aid for Scientific Research on Priority Areas (C) (12217049) from the Ministry of Education, Science, Sports and Culture, Japan.

References

[1] Foye, W.O. (1998) "History of radioprotector development", In: Bump, E.A. and Malaker, K., eds, Radioprotectors: Chemical, Biological, and Clinical Perspectives (CRC Press, Boca Raton, Boston), pp 15–24.

[2] Brizel, D.M., Wasserman, T.H., Henke, M., Strnad, V., Rudat, V., Monnier, A., Eschwege, F., Zhang, J., Russell, L., Oster, W. and Sauer, R. (2000) "Phase III randomized trial of amifostine as a radioprotector in head and neck cancer", *Journal of Clinical Oncology* **18**, 3339–3345.

[3] Kimura, E., Koike, T., Shimizu, Y. and Kodama, M. (1986) "Complexes of the histamine H_2-antagonist cimetidine with divalent and monovalent copper ions", *Inorganic Chemistry* **25**, 2242–2246.

[4] Lapenna, D., De Gioia, S., Mezzetti, A., Grossi, L., Festi, D., Marzio, L. and Cuccurullo, F. (1994) "H_2-receptor antagonists are scavengers of oxygen radicals", *European Journal of Clinical Investigation* **24**, 476–481.

[5] Ching, T.L., Haenen, G.R. and Bast, A. (1993) "Cimetidine and other H_2 receptor antagonists as powerful hydroxyl radical scavengers", *Chemico-biological Interactions* **86**, 119–127.

[6] Miura, Y., Anzai, K., Ueda, J. and Ozawa, T. (2000) "Novel approach to *in vivo* screening for radioprotective activity in whole mice: *in vivo* electron spin resonance study probing the redox reaction of nitroxyl", *Journal of Radiation Research (Tokyo)* **41**, 103–111.

[7] Uchida, K. and Kawakishi, S. (1990) "Cimetidine anti-ulcer drug as a powerful hydroxyl radical scavenger", *Agricultural and Biological Chemistry* **54**, 2485–2487.

[8] Mozdarani, H. and Gharbali, A. (1993) "Radioprotective effects of cimetidine in mouse bone marrow cells exposed to γ-rays as assayed by the micronucleus test", *International Journal of Radiation Biology* **64**, 189–194.

[9] Mozdarani, H. and Khoshbin-Khoshnazar, A.R. (1998) "*In vivo* protection by cimetidine against fast neutron-induced micronuclei in mouse bone marrow cells", *Cancer Letters* **124**, 65–71.

[10] Al-Mustafa, Z.H., Al-Ali, A.K., Qaw, F.S. and Abdul-Cader, Z. (1997) "Cimetidine enhances the hepatoprotective action of *N*-acetylcystein in mice treated with toxic doses of paracetamol", *Toxicology* **121**, 223–228.

[11] Mozdarani, H. and Kamali, S. (1998) "Antigenotoxic effects of cimetidine against benzene induced micronuclei in mouse bone marrow erythrocytes", *Toxicology Letters* **99**, 53–61.

[12] Shaw, S. and Jayatilleke, E. (1992) "Cimetidine as a scavenger of ethanol-induced free radicals", *Alcohol* **9**, 363–367.

[13] Mitchell, M.C., Schenker, S., Avant, G.R. and Speeg, Jr., K.V. (1981) "Cimetidine protects against acetaminophen hepatotoxicity in rats", *Gastroenterology* **81**, 1052–1060.

[14] Mera, E., Muriel, P., Castillo, C. and Mourelle, M. (1994) "Cimetidine prevents and partially reverses CCl_4-induced liver cirrhosis", *Journal of Applied Toxicology* **14**, 87–90.

[15] Fenech, M. and Morley, A.A. (1985) "Measurement of micronuclei in lymphocytes", *Mutation Research* **147**, 29–36.

[16] Fenech, M. (1993) "The cytokinesis-block micronucleus technique: a detailed description of the method and its application to genotoxicity studies in human populations", *Mutation Research* **285**, 35–44.

[17] Fenech, M. (2000) "The *in vitro* micronucleus technique", *Mutation Research* **455**, 81–95.

[18] Thierens, H., Vral, A. and De Ridder, L. (1991) "Biological dosimetry using the micronucleus assay for lymphocytes: interindividual differences in dose response", *Health Physics* **61**, 623–630.

[19] Vral, A., Thierens, H. and De Ridder, L. (1992) "Study of dose-rate and split-dose effects on the *in vitro* micronucleus yield in human lymphocytes exposed to X-rays", *International Journal of Radiation Biology* **61**, 777–784.

[20] Silva, M.J., Carothers, A., Dias, A., Luis, J.H., Piper, J. and Boavida, M.G. (1994) "Dose dependence of radiation-induced micronuclei in cytokinesis-blocked human lymphocytes", *Mutation Research* **322**, 117–128.

[21] Verhaegen, F. and Vral, A. (1994) "Sensitivity of micronucleus induction in human lymphocytes to low-LET radiation qualities: RBE and correlation of RBE and LET", *Radiation Research* **139**, 208–213.

[22] Littlefield, L.G., Sayer, A.M. and Frome, E.L. (1989) "Comparisons of dose-response parameters for radiation-induced acentric fragments and micronuclei observed in cytokinesis-arrested lymphocytes", *Mutagenesis* **4**, 265–270.

[23] Louagie, H., Philippe, J., Vral, A., Cornelissen, M., Thierens, H. and De Ridder, L. (1998) "Induction of micronuclei and apoptosis in natural killer cells compared to T lymphocytes after γ-irradiation", *International Journal of Radiation Biology* **73**, 179–185.

[24] Hertveldt, K., Philippe, J., Thierens, H., Cornelissen, M., Vral, A. and De Ridder, L. (1997) "Flow cytometry as a quantitative and sensitive method to evaluate low dose radiation induced apoptosis *in vitro* in human peripheral blood lymphocytes", *International Journal of Radiation Biology* **71**, 429–433.

[25] Philippe, J., Louagie, H., Thierens, H., Vral, A., Cornelissen, M. and De Ridder, L. (1997) "Quantification of apoptosis in lymphocytes subsets and effect of apoptosis on apparent expression of membrane antigens", *Cytometry* **29**, 242–249.

[26] Ramakrishnan, N., Kalinich, J.F. and McClain, D.E. (1998) "Radiation-induced apoptosis in lymphoid cells: induction, prevention, and molecular mechanisms", In: Bump, E.A. and Malapert, K., eds, Radioprotectors: Chemical, Biological, and Clinical Perspectives (CRC Press, Boca Raton, Boston), pp 253–273.

[27] Seki, H., Kanegane, H., Iwai, K., Konno, A., Ohta, K., Yachie, A., Taniguchi, N. and Miyawaki, T. (1994) "Ionizing radiation induces apoptotic cell death in human TcR-γ/δ + T and natural killer cells without detectable p53 protein", *European Journal of Immunology* **24**, 2914–2917.

[28] Vandeputte, C., Guizon, I., Genestie-Denis, I., Vannier, B. and Lorenzon, G. (1994) "A microtiter plate assay for total glutathione and glutathione disulfide contents in cultured/isolated cells: performance study of a new miniaturized protocol", *Cell Biology and Toxicology* **10**, 415–421.

[29] Griffith, O.W. (1980) "Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine", *Analytical Biochemistry* **106**, 207–212.

[30] Baker, M.A., Cerniglia, G.J. and Zaman, A. (1990) "Microtiter plate assay for the measurement of glutathione and glutathione disulfide in large numbers of biological samples", *Analytical Biochemistry* **190**, 360–365.

[31] Buxton, G.V., Greenstock, C.L., Phillip Helman, W. and Ross, A.B. (1988) "Critical review of rate constants for reactions of hydrated electrons, hydrogen atoms and hydroxyl radicals

(·OH/·O$^-$) in aqueous solution", *Journal of Physical and Chemical Reference Data* **17**, 513–886.
[32] Miller, B.M., Werner, T., Weier, H.-U. and Nusse, M. (1992) "Analysis of radiation-induced micronuclei by fluorescence *in situ* hybridization (FISH) simultaneously using telomeric and centromeric DNA probes", *Radiation Research* **131**, 177–185.
[33] Louagie, H., Schotte, P., Vral, A., Cornelissen, M., Thierens, H., Beyaert, R., De Ridder, L. and Philippe, J. (1999) "Apoptosis induced by γ irradiation in peripheral blood mononuclear cells is not mediated by cytochrome-c release and only partially involves caspase-3-like proteases", *Cell Biology International* **23**, 611–617.
[34] Kirkova, M., Atanassova, M. and Russanov, E. (1999) "Effects of cimetidine and its metal complexes on nitroblue tetrazolium and ferricytochrome c reduction by superoxide radicals", *General Pharmacology* **33**, 271–276.
[35] Zhao, Q.-L., Kondo, T., Noda, A. and Fujiwara, Y. (1999) "Mitochondrial and intracellular free-calcium regulation of radiation-induced apoptosis in human leukemic cells", *International Journal of Radiation Biology* **75**, 493–504.
[36] Kimura, C., Zhao, Q.-L., Kondo, T., Amatsu, M. and Fujiwara, Y. (1998) "Mechanism of UV-induced apoptosis in human leukemia cells: roles of Ca^{2+}-Mg^{2+}-dependent endonuclease, caspase-3, and stress-activated protein kinase", *Experimental Cell Research* **239**, 411–422.
[37] Li, F.-J., Kondo, T., Zhao, Q.-L., Tanabe, K., Ogawa, R., Li, M. and Arai, Y. (2001) "Enhancement of hyperthermia-induced apoptosis by a free radical initiator, 2,2′-azobis(2-amidino-propane)dihydrochloride, in human histiocytic lymphoma U937 cells", *Free Radical Research* **35**, 281–299.
[38] Li, M., Kondo, T., Zhao, Q.-L., Li, F.-J., Tanabe, K., Arai, Y., Zhou, Z.-C. and Kasuya, M. (2000) "Apoptosis induced by cadmium in human lymphoma U937 cells through Ca^{2+} calpain and caspase-mitochondria-dependent pathways", *Journal of Biological Chemistry* **275**, 39702–39709.