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Captopril protects mice bone marrow cells against genotoxicity induced by gamma irradiation

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The radioprotective effects of captopril were investigated by using the micronucleus test for anticlastogenic and cell proliferation activity. A single intraperitoneal administration of captopril at doses of 10, 25 and 50 mg/kg 1 h prior to gamma irradiation (2 Gy) reduced the frequencies of micronuleated polychromatic erythrocytes (MnPCEs). All three doses of captopril significantly reduced the frequencies of MnPCEs and increased polychromatic erythrocytes (PCE)/PCE + NCE (normochromatic erythrocyte) ratio in mice bone marrow compared to the non-drug-treated irradiated control ($p < 0.001$). The optimum dose for protection in mouse was 10 mg/kg to protect mice bone marrow 2.18-fold against the clastogenic effects of γ-irradiation with respect to the non-drug-treated irradiated control. There was a drug dose-response effect of captopril in increasing the PCE/PCE + NCE ratio in bone marrow cells. The maximum protective effect of captopril was at a dose of 25 mg/kg for increasing the PCE/PCE + NCE ratio. Captopril exhibited concentration-dependent antioxidant activity, scavenging > 96% of the 1,1-diphenyl-2-picryl hydrazyl free radicals when used at a concentration of 0.2 mM. In this study captopril reduced lipid peroxidation induced by hydrogen peroxide in mice liver. It appears that captopril, due to its free radical scavenging properties, protects mice bone marrow cells from radiation-induced DNA damage and genotoxicity.

KEY WORDS — captopril; radioprotective; micronuclei; radiation

ABBREVIATIONS — PCE (polychromatic erythrocyte); NCE (normochromatic erythrocyte); Mn (micronuclei); BHT (butylated hydroxy toluene); DPPH (1,1-diphenyl-2-picyl hydrazyl); MDA (malondialdehyde)

INTRODUCTION

Biological molecules are susceptible to damage whenever they are exposed to ionizing radiation. When ionizing radiation passes through a cell, it is capable of generating free radicals that can damage critical molecules such as DNA.[1,2] Cells have numerous protective mechanisms to reduce the incidence of severe oxidative damage. Enzymes, such as superoxide dismutase, catalase and glutathione peroxidase, scavenge a variety of free radicals.[1,3] If these mechanisms are not capable of scavenging free radicals in a cell, DNA becomes damaged. With respect to radiation damage to humans, it is important to protect biological systems against genotoxicity or lethality induced by irradiation. Although many radioprotective agents have been tested for efficacy, most of these compounds are of limited use in practice due to side effects and toxicity.[4,5] The search for less-toxic radiation protectors has spurred interest in the development of different compounds. Thiols, molecules containing a free or easily liberated sulfhydryl group (SH) in their structure have received a great deal of attention as radioprotective agents for animals.[5,6]

Captopril, an angiotensin-converting enzyme inhibitor is widely used in the treatment of hypertension. Experimental studies have indicated that some beneficial effects of captopril could also be due to antioxidant mechanisms, apparently related to the sulfhydryl group in this drug molecule.[7,8] Captopril may increase antioxidant enzymes and nonenzymic

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antioxidant defences in mouse tissues.[9] Captopril is known to have a radioprotective effect in intestines and heart, but its effect in mice bone marrow cells is unclear.[10,11] In this study we investigated the effects of captopril administration in modulating the genetic damage induced by gamma irradiation in the mouse erythropoietic system by determining the frequency of micronuclei in immature erythrocytes of bone marrow.

MATERIALS AND METHODS

Animal

Male NMRI mice (7–9 weeks old) weighing 25 ± 3 g were purchased from the Razi Institute (Tehran, Iran). Mice were housed in a good condition in the university animal house and given standard mouse pellets and water *ad libitum*. All animals were kept under controlled lighting conditions (light: dark, 12:12 h) and temperature ($22 \pm 1^{\circ}$C).

Chemical and treatment

Captopril was obtained from Darupakhsh Pharmaceutical Company (Tehran, Iran) and was dissolved in normal saline. Mice were injected intraperitoneally (i.p.) in all experiments. Three doses (10, 25, 50 mg/kg) of Captopril were administered to the animals 1 h before gamma irradiation. The control animals received the same volume of normal saline.

Irradiation

Whole-body irradiation was performed with a cobalt-60 γ-radiation source (Teratron 780, Canada). Mice were placed in ventilated Plexiglas cages and irradiated in groups of five mice simultaneously. The source-to-skin distance was 80 cm with a dose rate of 1.35 Gy/min at room temperature ($23 \pm 2^{\circ}$C). The mice were irradiated with a total dose of 2 Gy γ-rays.

Micronucleus assay

The mouse bone marrow micronucleus test was carried out according to Schmid for evaluation of the chromosomal damage in experimental animals.[12,13] The animals were sacrificed by cervical dislocation 24 h after irradiation. The bone marrow from both femurs was flushed out with two ml foetal calf serum, in the form of a fine suspension into a centrifuge tube containing foetal calf serum (FCS). FCS was used without dilution. The cells were dispersed by gentle pipetting and collected by centrifugation at 2000 rpm for 5 min at 4°C. The cell pellet was resuspended in a drop of FCS and bone marrow smears were prepared. The slides were coded to avoid observer bias. After 24 h air-drying, the smears were stained with May-Grunwald/Giemsa as described by Schmid.[12] With this method polychromatic erythrocytes (PCEs) stain reddish-blue and normochromatic erythrocytes (NCEs) stain orange, while nuclear material is dark purple. For each experimental point, five mice were used to determine the percentage of micronucleated polychromatic erythrocytes (MnPCEs), and the ratio of PCE to (PCE + NCE). The ratio of PCE to (PCE + NCE) was determined for each experimental group to assess radiation effects with or without captopril on bone marrow proliferation.

Measurement of free radical scavenging activity

The free radical-scavenging capacity of captopril was determined as bleaching of the stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH).[14] Different concentrations of captopril (0.02–0.2 mM) were added, at an equal volume, to a methanolic solution of DPPH (100 mM). After 15 min at room temperature, the absorbance was recorded at 517 nm. The experiment was performed in triplicate. BHT (butylated hydroxy toluene) was used as an antioxidant standard. The percentage of scavenging was calculated using the formula [(Control-Test)/Control] $\times$ 100.

Lipid peroxidation

Thiobarbituric acid-reactive substance (TBARS) was determined in liver homogenates by the method of Ohkawa *et al.*,[15] in which malondialdehyde (MDA), an end-product of fatty acid peroxidation, reacts with thiobarbituric acid (TBA) to form a coloured complex. Briefly, animals were sacrificed and their livers were rapidly removed and chilled in ice cold 0.9% NaCl. The tissue was homogenized in phosphate buffer (0.05 M, pH 7.4) at 10% w/v. The tissue was oxidized with H_2O_2 as reported earlier.[16] One ml homogenate was incubated for 60 min at 37°C with 100 µl hydrogen peroxide (50 mM) in the presence or absence of 100 µl of captopril (1 mM). The reaction was stopped by the addition of 2 ml of stopper solution (15% trichloroacetic acid (w/v), 0.375% TBA (w/v), 2.08 ml concentrated HCl, 0.05% butylated hydroxy toluene (BHT) (w/v)) and 100 µl sodium dodecyl sulphate (8.1%). The mixture was heated in a boiling water bath for 30 min, cooled, centrifuged and the supernatant was used to measure the MDA level

spectrophotometrically at 532 nm with reference to a standard curve prepared with 1,1,3,3-tetraethoxypropane. The results were expressed as nmol MDA formed/g of liver.

Statistical analysis

The data are presented as means ± SD. One-way ANOVA analysis and Tukey's HSD test were used for multiple comparisons of data.

RESULTS

Effects of captopril on radiation-induced micronuclei

The effect of gamma irradiation with or without captopril on the induction of MnPCEs and the PCE/PCE + NCE ratio in bone marrow cells, 24 h after γ-irradiation, is shown in Table 1. The frequency of micronuclei was increased in all groups of mice irradiated with γ-irradiation at a dose 2 Gy compared with the control treated with normal saline ($p < 0.001$). The frequency of MnPCE found in the captopril treated groups was significantly much lower than the treated group with radiation alone. The total MnPCE values were 2.18-, 2.35-, and 1.55- fold less in the 10, 25 and 50 mg/kg captopril groups respectively after exposure to 2 Gy of γ-rays than in the respective irradiated control. All three doses were effective in significantly reducing ($p < 0.001$) the frequency of MnPCE induced by 2 Gy irradiation. Although with a further increase in the captopril dose from 10 to 25 mg/kg, a decreased MnPCE was observed, it was not statistically significant. The maximum decrease in MnPCE was observed in mice treated with captopril at a dose of 25 mg/kg (Table 1). These data showed a suppressive effect of captopril on radiation induced-clastogenic effects. The ratio of PCE/PCE + NCE decreased significantly after exposure to 2 Gy of γ-irradiation ($p < 0.0001$). Treatment of mice with captopril arrested the radiation-induced decline in the PCE/PCE + NCE ratio (Table 1), and the increased PCE/PCE + NCE ratio in the captopril + irradiated group (at doses of 10, 25, 50 mg/kg) was higher than that of the irradiated-alone group ($p < 0.001$). There was a dose-dependent effect of captopril at doses of 10 and 25 mg/kg in increasing the PCE/PCE + NCE ratio in bone marrow cells. In this study, captopril did not show any genotoxicity and toxicity effects at a dose of 50 mg/kg.

Antioxidant activity

The method of scavenging DPPH free radical can be used to evaluate the antioxidant activity of a specific compound. As shown in Figure 1, captopril exhibited a concentration-dependent antioxidant activity. There was a greater than 96% DPPH scavenging activity at a concentration of 0.2 mM. Captopril exhibited an antioxidant activity that was greater than that obtained by the same concentrations of a synthetic antioxidant (BHT).

Effect of captopril on lipid peroxidation

MDA level is a common parameter of oxidative status of tissue. The effect of captopril on hydrogen peroxide-induced MDA formation was investigated using captopril and/or hydrogen peroxide treated mice liver (Figure 2). The MDA level was significantly increased by hydrogen peroxide in tissue compared to the control, whereas captopril treatment significantly decreased the MDA level compared to hydrogen peroxide alone ($p < 0.05$). These results indicated that

Table 1. Effects of captopril on the formation of radiation-induced micronuclei PCE and the ratio of PCE/PCE + NCE in mice bone marrow exposed to 2 Gy γ-radiation

Group	Treatment	MnPCE/PCE (%)*	PCE/PCE + NCE (%)*
1	Control	1.06 ± 0.13	61.63 ± 1.49
2	Irradiation	9.65 ± 0.98[a]	38.28 ± 1.09[a]
3	10 mg/kg Captopril + Irradiation	4.43 ± 0.26[b]	44.18 ± 1.9[c]
4	25 mg/kg Captopril + Irradiation	4.10 ± 0.30[b]	53.33 ± 2.08[c,d]
5	50 mg/kg Captopril + Irradiation	6.20 ± 0.46[b]	53.63 ± 2.76[c]
6	50 mg/kg Captopril	0.94 ± 0.07	62.00 ± 2.65

*Values are means ± SD for each group of five mice.
[a] $p < 0.0001$ compared to control.
[b] $p < 0.0001$ compared to irradiation alone.
[c] $p < 0.001$ compared to irradiation alone.
[d] $p < 0.001$ compared to group 3.

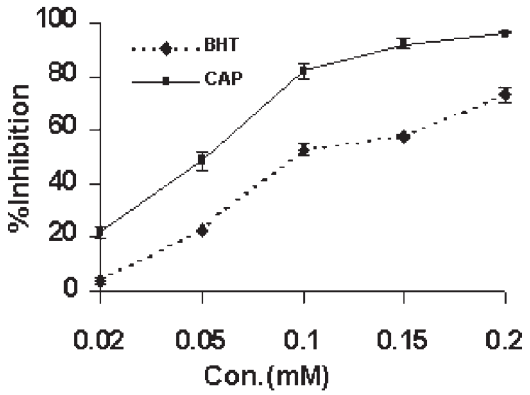

Figure 1. Scavenging effect of different concentration of Captopril (CAP) (■) and BHT (▲) on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical

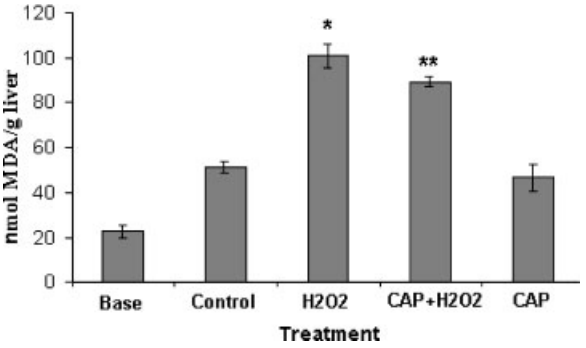

Figure 2. The effect of captopril (CAP) on the malondialdehyde (MDA) concentration induced by hydrogen peroxide (H_2O_2) in mice liver. MDA was assayed according to the method described in Materials and Methods. Values are means ± SD ($n = 5$). *$p < 0.001$ compared with control; **$p < 0.05$ compared with H_2O_2 group

captopril attenuated the lipid peroxidation induced by hydrogen peroxide in mice liver.

DISCUSSION

The results of our present study show the protective effect of captopril on genotoxicity induced by γ-irradiation analysed by determination of the frequency of MnPCEs in the bone marrow cells, 24 h after gamma irradiation. In our study, a single i.p. administration of captopril resulted in inhibition of micronuclei formation induced by gamma rays in mouse bone marrow cells. Captopril reduced the frequency of MnPCEs significantly and showed protective and anticlastogenic action against the effects of irradiation. The ratio of PCE to PCE + NCE was decreased in irradiated mice, and this ratio gives a direct index of cell division. Captopril protected mice against the radiation-induced decline in cell proliferation as evidenced by the increased PCE/PCE + NCE ratio. Ionizing radiation generates free radicals that can damage DNA and induce genotoxic effects and death in the cells.[17] Scavenging of reactive molecules represents one of the most important approaches in antimutagenesis and anticarcinogenesis.[3] We previously reported that citrus extract, with antioxidant activity, protected mice bone marrow cells against gamma irradiation when injected prior to exposure.[13]

In the present study, captopril by scavenging properties has excellent antioxidant activity. Therefore, protective effects of captopril against clastogenic induced by irradiation could arise from the scavenging ability of captopril possessing an SH group, to trap hydroxyl radicals. Lipid peroxidation, mediated by oxygen free radicals, is believed to be an important cause of destruction and damage to cells. MDA has been extensively studied in biological and medical sciences due to its reactivity with biological macromolecules and possible connection to cancer and other diseases.[18] Our results showed that the antioxidant captopril significantly reduced MDA elevations induced by hydrogen peroxide. Thus captopril could protect against organ damage in oxidative stress. A similar decrease in MnPCE induced by γ-irradiation has been described by other antioxidants such as amifostine, glutathione and cimetidine.[19,20] Treatment of mice with captopril at dose of 10 mg/kg before exposure to 2 Gy radiation reduced the frequency of MnPCE almost 2.18 fold. Among radioprotective agents, amifostine has been evaluated as a powerful radioprotector, but this drug is effective only at high doses which are close to the toxic level (i.e. two-thirds of the LD_{50} value) and induce side effects.[19] In this study we showed captopril protected mice bone marrow at the relatively low dose 10 mg/kg. This dose is very much lower than the LD_{50} value (iv, $LD_{50} > 1600$ mg/kg) of captopril.[21]

Captopril provides protection from acute radiation damage to the jejunal mucosa and heart in animals. Cytokines probably play roles in the radioprotective mechanism of captopril from the radiation-induced heart toxicity.[10,11] Captopril and Enalapril, two angiotensin-converting enzymes (ACE) inhibitors protected the renal parenchyma from development of radiation-induced fibrosis in rats. Angiotensin II played an important role in this

protection.[22] Sulfhydryl compounds as a class have antioxidant properties by an ability to neutralize oxygen radicals by either a hydrogen donating or electron transferring mechanism The mechanism of oxygen radical scavenging mediated by sulfhydryl compounds may also involve carbon-centred radical production. It also appears that the protective effects of sulfhydryl agents correlate better with their direct hydroxyl radical scavenging abilities than with their antiperoxidative potency.[23] Captopril possesses antioxidant activity and this effect is due to the SH-group in the chemical structure. It has previously been reported that captopril is very effective in scavenging free radicals, in a manner similar to glutathione, N-2-mercaptoproppionylglycine, and N-acetylcysteine, but this effect was not mimicked by enalapril.[24]

Potentially, captopril has also been demonstrated to chelate metals and increase CuZn-superoxide dismutase activity and nonenzymic antioxidant activity *in vivo*.[7–9] Administration of captopril increased superoxide dismutase activity, an antioxidant enzyme, in mice. The increase in the activity of this enzyme may be one the mechanism responsible for the protective effects shown by captopril *in vivo* in different pathologies.[25] Captopril increases antioxidant defences in mouse tissue and enhances protection against oxidative stress. Captopril was able to enhance total glutathione content in various tissues; it can induce substantial increases in erythrocyte total glutathione content. The concurrent captopril induced enhancement of glutathione and superoxide dismutase in tissues explains the higher protection against oxidant damage in mice.[26] We recently showed administration of captopril prior to cyclophosphamide significantly reduced the frequency of MnPCEs. Captopril, due to its antioxidant activity and by increasing GSH level, modulated the decreased cellular thiol content induced by cyclophosphamide and reduces the genotoxicity of cyclophosphamide in bone marrow cells.[27]

It appears that sulfhydryl containing antioxidants such as captopril scavenge the free radicals generated by γ-rays and decrease damage to DNA and thus reduce genotoxicity induced by γ-irradiation in bone marrow cells.

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