

## Celecoxib mitigates genotoxicity induced by ionizing radiation in human blood lymphocytes

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### Abstract

Ionizing radiation causes DNA damage and chromosome abbreviations on normal cells. The radioprotective effect of celecoxib (CLX) was investigated against genotoxicity induced by ionizing radiation in cultured human blood lymphocytes. Peripheral blood samples were collected from human volunteers and were incubated at different concentrations at 1, 5, 10 and 50  $\mu\text{M}$  of CLX for two hours. At each dose point, the whole blood was exposed *in vitro* to 150 cGy of X-ray, and then the lymphocytes were cultured with mitogenic stimulation to determine the micronucleus frequency in cytokinesis blocked binucleated lymphocytes. Incubation of the whole blood with CLX exhibited a significant decrease in the incidence of micronuclei in lymphocytes induced by ionizing radiation, as compared with similarly irradiated lymphocytes without CLX treatment. The maximum reduction on the frequency of micronuclei was observed at 50  $\mu\text{M}$  of CLX (65% decrease). This data may have an important possible application for the protection of human lymphocytes from the genetic damage induced by ionizing irradiation in human exposed to radiation.

**Keywords:** Celecoxib; Genotoxicity; Ionizing radiation; Lymphocyte

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### INTRODUCTION

Ionizing radiation (IR) produces reactive oxygen species (ROS) and free radicals when is passing through cells. DNA is the most susceptible macromolecule to damage induced by IR. Cell death, apoptosis and autophagy are induced in the cells in response to radiation in cancer cells and in normal cells (1,2). Cyclooxygenase-2 (COX II) is an enzyme induced by a variety of factors such as tumor promoters. It causes radiation-induced G2/M arrest and leads to resistance to IR. COX II is overexpressed by IR in the cells, and it is associated to carcinogenesis and maintenance of progressive tumor growth. COX II and its products may act as radioprotector against cell damage and lead to tumor resistance to IR (3,4). COX II selective inhibitors can radiosensitize cancer cells in a COX II

expression-dependent manner. Several studies showed that celecoxib (CLX), as a COX II selective inhibitor, sensitized cancer cells to IR (5-7). CLX, as a non-steroid anti-inflammatory drug, is widely used in inflammation-related diseases. It improved tumor response to radiotherapy in some clinical studies (8,9). While the potential of radiosensitizing effect of CLX on tumor cells is established, its effect on normal cells exposed to IR is unclear. DNA double strand breaks (DSBs) occur when the two strands of the DNA are closely broken. DSBs can be induced by genotoxic agents such as IR, chemical and anti-cancer agents. These agents mainly produce ROS on cells result in chromosome breaks and genotoxicity.

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High frequency of chromosome damage and genotoxicity has been observed in patients and personnel after exposure to IR (10-12). Lymphocytes are sensitive cells to IR. IR exposure to lymphocyte can lead to chromosome abbreviations and DNA breaks. Micronuclei in nucleus arise during cell division and produce from chromosome fragments.

The detection of micronucleus in binucleated lymphocyte cell has been performed as reliable biomarker of *in vitro/in vivo* exposure to ionizing radiation and other genotoxic agents (13). The aim of this study was to evaluate the effect of CLX against genotoxicity induced by IR in normal human peripheral blood lymphocytes *in vitro*.

## MATERIALS AND METHODS

### *Human blood treatment*

Three healthy nonsmoking human male volunteers, aging 25-30 years, were enrolled in this study. The study protocol was approved by the Research and Ethical Committee of the Mazandaran University of Medical Sciences NO. 91-77. CLX was obtained from Daroupakhsh Pharmaceutical Company (Tehran, Iran). A specified quantity of CLX was dissolved in 800  $\mu$ L DMSO and made up to 10 mL volume with culture medium to produce a stock solution of CLX at concentration of 1 mM. Working standard concentrations of CLX were prepared by dilution of CLX stock solution with culture medium. The final concentration of DMSO was maintained at 0.4% in all cell culture experiments. Control and irradiated samples were treated with culture medium containing 0.4% DMSO. After overnight fasting, 10 mL of the whole blood were collected from the volunteers in heparinized tubes and divided in tubes each containing 0.9 mL of blood. Blood samples were treated with 100  $\mu$ L solution of CLX to yield final concentrations of 1, 5, 10 or 50  $\mu$ M. These samples were incubated for two h at 37 °C in a humidified atmosphere of 5% CO<sub>2</sub> and 95% air.

### *Irradiation, lymphocyte culture and micronuclei analysis*

At each concentration and for each volunteer, tubes containing blood plus CLX

were irradiated at 37 °C with 6 MV X-ray beam produced by a radiotherapy machine (Linear accelerator, Siemens, Primus, Germany) at a dose of 150 cGy with a dose rate of 200 cGy/min (14-18). In irradiation protocol, the blood samples in microtubes were placed in a box containing water that acted as a phantom for uniformity of radiation exposure to blood samples. Three series of three blood samples, one from each volunteer, were used as the non-irradiated control, irradiated samples with no CLX, and non-irradiated samples containing 50  $\mu$ M CLX also were included in the experiment. Subsequently, cell culture were performed in duplicate and 0.5 mL of each sample (control and irradiated samples) was added to 4.4 mL of RPMI 1640 culture medium (Gibco, USA), which contained a mixture of 10% fetal calf serum, 100  $\mu$ L phytohaemagglutinin M (Gibco, USA) and antibiotics (50 U/mL penicillin, 50  $\mu$ g/mL streptomycin). All cultures were incubated at 37 °C in a humidified atmosphere of 5% CO<sub>2</sub> and 95% air. Cytochalasin B (Sigma, USA) (final concentration 6  $\mu$ g/mL) was added 44 h from cell culture establishment. Following 72 h from incubation, the cells were collected by centrifugation, re-suspended in cold potassium chloride. Cells were immediately fixed in a fixative solution of methanol: acetic acid (6:1). The fixed cells were dropped onto clean microscopic slides, air-dried and stained with Giemsa solution.

All slides were evaluated at  $\times$  100 magnification in order to determine the frequency of micronuclei in the cytokinesis-blocked binucleated cells with a well-preserved cytoplasm.

The criteria for scoring micronuclei were a diameter between 1/16<sup>th</sup> and 1/3<sup>rd</sup> of the main nuclei, non-refractile, not linked to the main nuclei and not overlapping the main nuclei (19). At each concentration and for each volunteer, 1000 binucleated lymphocyte cells were examined from the irradiated and control cultures in duplicate to record the frequency of micronuclei. Twenty one thousands binucleated lymphocytes were examined. In our study, a typical micronucleus in binucleated lymphocyte is shown in Fig. 1.

**Statistical analysis**

For each volunteer, at each concentration, the incidence of radiation-induced micronuclei was recorded. The data were analyzed with student *t*-test. A probability value of 0.05 was accepted to denote significance.

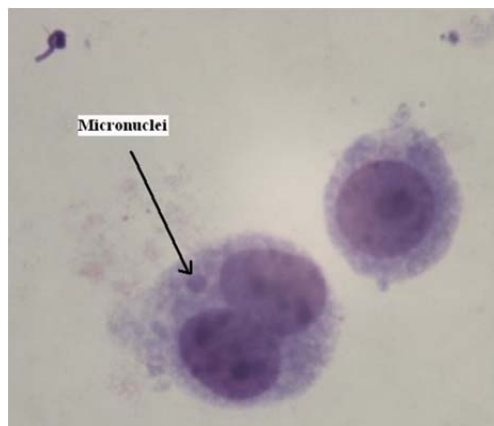
**RESULTS**

The percentage of micronucleus in binucleated lymphocytes exposed to 1.5 Gy of X-ray was  $3.57 \pm 0.4$ , while the percentage in non-treated control lymphocytes was  $0.33 \pm 0.07$ . There was a significant increase of 10-fold in frequency of micronuclei in samples treated with X-ray ( $P < 0.001$ ) in comparison with the control non-irradiated samples (Fig. 2).

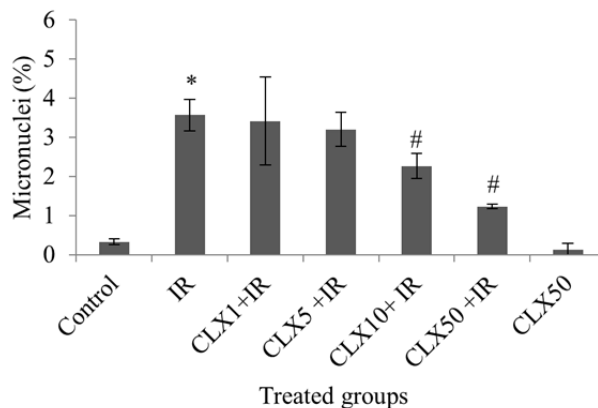
The frequency of micronuclei after pre-treatment with CLX at doses of 1, 5, 10 and

50  $\mu\text{M}$  was  $3.42 \pm 1.13$ ,  $3.20 \pm 0.44$ ,  $2.27 \pm 0.32$  and  $1.23 \pm 0.06$ , respectively (Fig. 2).

The data demonstrated that human blood incubated with CLX for two h, and then exposed *in vitro* to 1.5 Gy of X-ray radiation, exhibited a significant reduction in micronuclei frequency compared to blood samples incubated with X-ray alone. CLX significantly mitigated the frequency of micronuclei at doses 10 and 50  $\mu\text{M}$  in lymphocytes exposed to IR ( $P < 0.05$ ). Total micronuclei values were reduced by 4%, 10%, 36% and 65% in samples treated with CLX and irradiation at concentrations of 1, 5, 10 and 50  $\mu\text{M}$ , respectively, compared to irradiated sample control (Fig. 2). CLX by itself without radiation exposure did not induce any genotoxicity effect in cultured lymphocytes treated with 50  $\mu\text{M}$  concentration.



**Fig. 1.** A typical binucleated lymphocyte with micronuclei in our experiments.



**Fig. 2.** In vitro protection by celecoxib (CLX) at different concentrations (1, 5, 10 and 50  $\mu\text{M}$ ) against radiation-induced genetic damage induced by X-ray (IR; 1.5 Gy) in cultured blood lymphocyte. The data represent average  $\pm$  standard deviation of three human volunteers. \* $P < 0.001$ ; Irradiated lymphocytes from the blood sample treated with IR compared to non-irradiated control samples. # $P < 0.05$ ; CLX10 + IR and CLX50 + IR samples compared to IR sample.

## DISCUSSION

In this study we showed that CLX has a radioprotective effect against genotoxicity induced by IR in human lymphocytes. CLX reduced the frequency of micronuclei in binucleated lymphocytes which was induced by IR. CLX exhibited a protective effect at concentrations of 10 and 50  $\mu\text{M}$  by factors 1.6 and 2.9, respectively. It was not observed any protective effect of CLX at low concentrations (1 and 5  $\mu\text{M}$ ). In this study, CLX concentrations were selected based on plasma concentration of CLX after oral administration of this drug. CLX showed a plasma concentration of 1-8.5  $\mu\text{M}$  at a single dosage administration of 200 mg or 400 mg (20-22), then we selected the concentrations of 1, 10  $\mu\text{M}$  and also two different concentrations as 5 and 50  $\mu\text{M}$  for comparing with CLX plasma concentrations. In this study, CLX showed radioprotective effect at high concentrations then it is recommended that patients receive at least 400 mg of CLX for radioprotection. COX II expression levels are increased in several cancer cells such as breast, colorectal and prostate cancers. Previous studies exhibited that CLX had beneficial effects through killing of cancer cells. CLX effectively inhibited the cell survival and colony formation and increased IR-induced apoptosis in cancer cells. CLX significantly enhanced tumor radiosensitivity which is related to COX II inhibition by CLX (23,24). Cancer cells increase expression of COX II in response to IR, and it contributes in pro-inflammatory process and resistance to killing effect of IR by tumor cells. CLX has a synergistic effect with IR on killing of cancer cells (3, 25). IR can cause side effects on normal tissues. IR can activate inflammatory process that causes dysfunction in normal cells (26). The cytokinesis-block micronucleus assay in peripheral blood lymphocytes is one of the best standard and validated techniques for assessment of the absorbed radiation doses in human (27,28). Human T lymphocytes express the COX II enzyme and involves in the inflammatory responses. Pro-inflammatory processes have cross-talk with COX II. Inflammatory processes induced by IR have different effects in cancer cells and normal

cells. In cancer cells, enhanced inflammatory process activates some cellular signaling pathways that lead to tumor growth. Then CLX as a COX II inhibitor can reduce tumor proliferation or increase cell death. While in normal cells, inflammatory processes activate cellular signaling pathways involved in cell death, so CLX as COX II inhibitor can lead to protect normal cells against toxicity induced by IR. Recently it was reported that mefenamic acid as an anti-inflammatory drug exhibited radioprotective effects on genotoxicity induced by IR (14). CLX probably attenuates DNA damage induced by IR through suppression of pro-inflammatory process in lymphocytes. CLX was reported to reduce oxidative stress (29) and also enhanced the activities of antioxidant enzymes, superoxide dismutase, glutathione peroxidase and the total antioxidant status in hypercholesterolemic rabbits (30). CLX exhibited a protective effect against DNA fragmentation induced by hydrogen peroxide as an oxidative stress agent on normal mucosa cell (31). These results show CLX probably has anti-oxidative stress properties on reduction of DNA damage.

## CONCLUSION

In this study, we showed that CLX significantly reduced DNA damage induced by IR on human normal lymphocytes. Although CLX can sensitize cancer cells to IR, it likely protects normal cell against genotoxicity induced by IR. This result provides a new indication of CLX on normal cells exposed to IR and is promising in treatment of cancer patients during radiation therapy.

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