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Febuxostat Mitigates Genotoxicity Induced by Ionizing Radiation in Human Normal Lymphocytes

BRIEF REPORT

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ABSTRACT

Febuxostat (BF) is a xanthine oxidase inhibitor that has been used to treat chronic gout. FB exhibits an anti-inflammatory effect. In the present study, the radioprotective effect of BF was investigated against genotoxicity induced by ionizing radiation (IR) in healthy human lymphocytes. Peripheral blood samples were treated with BF at various concentrations for 3 h. Then, the whole blood samples were exposed to IR at a dose of 1.5 Gy. Lymphocytes were cultured to determine the frequency of micronuclei in binucleated lymphocytes. The frequency of micronuclei was significantly lower in human lymphocytes that were exposed to IR and treated with BF than in irradiated lymphocytes without BF treatment. The maximum radioprotection as decreasing in the frequency of micronuclei was observed at 100 μM of BF treatment (43% decrease). The anti-inflammatory property is probably involved in the mechanism of FB exerting radioprotective effect in human lymphocytes.

Keywords: Febuxostat, DNA damage, radioprotective, micronucleus test, radiation biology

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INTRODUCTION

Ionizing radiation (IR) produces free radicals and reactive oxygen species (ROS) when passing into cells. These reactive substances destroy cellular organelles mostly DNA. Extensive DNA damages lead to cell death. However, the killing effect of IR has benefit for cancer treatment, and it limits normal organs that are surrounded by tumor tissue. IR causes side effects in patients during radiotherapy for their cancer treatment (1). It is important to find a radioprotective agent that is able to protect patients or people against side effects induced by IR. DNA is a crucial and sensitive macromolecule to IR, and its protection is important. Antioxidant and anti-inflammatory properties are mechanisms involved in radioprotective agents (1, 2).

Febuxostat (FB) is a xanthine oxidase inhibitor that has been used to treat chronic gout. It is an orally active and selective xanthine oxidase inhibitor that is used for treatment of hyperuricemia or gout. Gout is the most common cause of inflammatory arthritis (3). Anti-inflammatory effect is one of the mechanisms of this drug. FB markedly protects doxorubicin-induced cardiotoxicity (4), lipopolysaccharide (LPS)-induced lung inflammation (5), and myocardial ischemia/reperfusion injury (6) in animals. Since IR causes oxidative stress and inflammation in cells, we hypothesized that FB could also have beneficial effects against genotoxicity induced by IR in normal lymphocytes in addition to its anti-gout indication.

MATERIAL AND METHODS

Materials

FB was obtained from Chimidaru, Iran. Cytochalasin B was purchased from Sigma Chemicals Co., USA. Phytohemagglutinin (PHA), Roswell Park Memorial Institute (RPMI) 1640 medium, fetal bovine serum (FBS), penicillin, and streptomycin were purchased from Gibco Co., USA. Giemsa stain, methanol, and acetic acid were obtained from Merck, Germany.

Blood Treatment

The study was approved by the research and ethical committees of Mazandaran University of Medical Sciences (ID no. 3178, 2017-7-30). Three healthy, non-smoking male volunteers aged 20–24 years old participated in the present study. Ten milliliter of whole blood was collected from participants using a heparinized syringe, transferred into tubes, and then divided into microtubes at 0.9 mL each. One hundred microliter solutions of FB at final concentrations of 1, 10, 50, or 100 μM (6, 7) were added to blood samples. The plasma concentration of FB was 10–12 μM after oral ingestion of FB at a dose of 80 mg in healthy humans (8), whereas it was 15–25 μM at an oral dose of 120 mg (9, 10). FB as powder was dissolved in dimethyl sulfoxide (DMSO) as solvent and diluted in RPMI culture medium. Control samples received DMSO (0.1%) the same as other treated groups.

Irradiation Protocol and Micronucleus Assay

Microtubes containing blood samples were kept on a plastic box containing water as phantom, and then samples were irradiated with X-ray (6 MV) beam produced by a linear accelerator (Siemens, Primus, Germany) at a dose of 1.5 Gy. After irradiation, half milliliter of each sample was then added to 4.4 mL of RPMI 1640 culture medium, which was completed with 10% FBS, and then PHA (100 µL) was added to the mixture. All samples were in duplicate. All culture samples were incubated at 37°C in a cell culture incubator. After culturing for 44 h, cytochalasin B was added in the cell culture medium. After culturing for 72 h, blood samples were centrifuged and removed from the supernatant medium. The cellular sediment was re-suspended in cold potassium chloride, and then lymphocytes were collected from blood samples. Lymphocytes were immediately fixed in methanol/acetic acid (5/1 v/v) as a fixative solution. The fixed lymphocytes were smeared onto clean microscopic slides. The cells were stained with Giemsa solution (10%). All slides were observed under a microscope with 1000x magnification to determine the frequency of micronuclei in the cytokinesis-blocked binucleated lymphocytes. Overall, 10 control and treated groups were evaluated in this experiment.

Statistical Analysis

The micronuclei frequency is presented as mean±standard deviation. One-way analysis of variance (ANOVA), as well as post hoc Tukey's multiple comparison tests, was used for statistical analysis using Prism 7 software (2016; GraphPad Software Inc., La Jolla, CA, USA).

RESULTS

Binucleated lymphocyte with the presence of the micronucleus is shown in Figure 1. In the present study, the mean percentage of micronuclei in irradiated blood samples without any drug treatment was 7.63±0.08, whereas it was 0.41±0.15 in non-irradiated control samples. Exposure of blood samples to IR significantly increased the percentage of the micronucleus (18-fold increase) in irradiated lymphocytes ($p<0.001$) (Table 1). In FB pretreatment and irradiated samples, the percentages of micronuclei at various concentrations of FB as 1, 10, 50, or 100 µM were 6.7±0.1%, 6.4±0.3%, 5.4±0.1%, and 4.4±0.2%, respectively (Table 1). The findings demonstrated that pre-treated blood with FB at all concentrations had significantly lower percentages of micronuclei than irradiated samples without FB treatment ($p<0.01$). Total micronuclei frequencies in irradiated samples pre-treated with FB were lower at 11%, 16%, 30%, and 43% at concentrations of 1, 10, 50, or 100 µM, respectively, than those in irradiated samples alone. The maximum protection of lymphocytes was observed at a concentration of 100 µM with FB treatment. The percentages of micronuclei were significantly lower in irradiated samples treated with FB at concentrations of 50 and 100 µM than in irradiated samples treated with FB at concentrations of 1 and 10 µM ($p<0.01$). The frequency of micronuclei was insignificant between FB 1+IR and FB 10+IR groups. Non-irradiated samples with FB treatments at 1 µM and 10 µM have not shown any increased genotoxicity as compared with non-irradiated control group, whereas it was significant at 50 µM and 100 µM as compared with the control group ($p<0.05$)

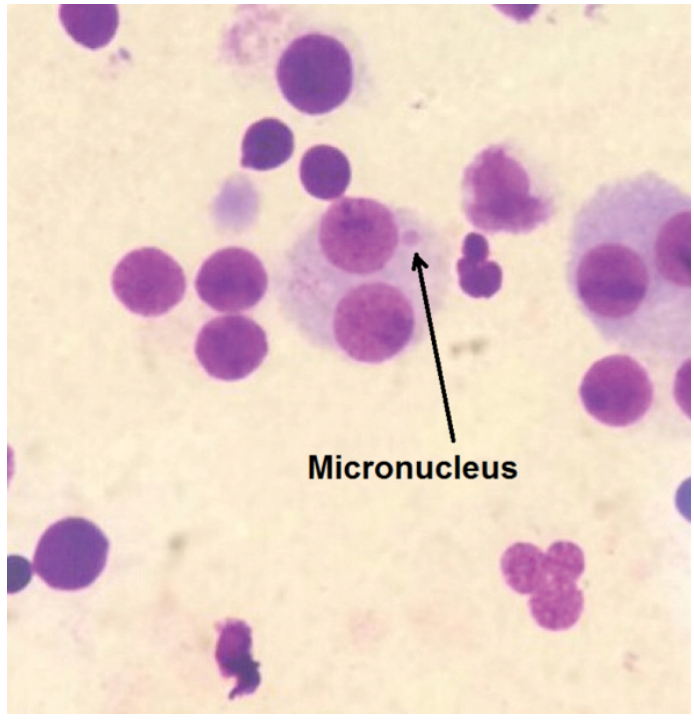

Figure 1. A typical binucleated lymphocyte with the micronucleus

Table 1. The frequency of micronuclei induced in vitro by 1.50 Gy X-ray radiation in cultured blood lymphocytes from human volunteers examined treated at different doses of febuxostat

Group / Sample	% micronuclei in binucleated human lymphocyte			
	I#	II	III	Mean±SD
Control	0.45	0.25	0.54	0.41±0.15
IR	7.67	7.54	7.68	7.63±0.08[a]
FB 1+IR	6.73	6.86	6.69	6.76±0.09[b]
FB 10+IR	6.50	6.68	5.99	6.39±0.36[b]
FB 50+IR	5.38	5.47	5.27	5.37±0.09[b,c]
FB 100+IR	4.57	4.26	4.35	4.39±0.16[b,c]
FB 1	0.49	0.59	0.58	0.55±0.05
FB 10	0.74	0.87	0.76	0.79±0.06
FB 50	0.89	0.85	0.93	0.89±0.04[d]
FB 100	0.94	1.03	1.02	0.99±0.05[d]

#I, II, and III are the number of volunteers who participated in the study, and whole blood was obtained here. 1000 BN cells were examined in each sample. [a]$p<0.001$ compared with control; [b]$p<0.01$ compared with IR; [c]$p<0.01$ FB 1+IR with FB 50+IR and FB 100+IR; [d]$p<0.05$ compared with control (ANOVA analysis with Tukey's multiple comparison test was applied for comparison of data). SD: Standard deviation; IR: Ionizing radiation; FB: Febuxostat

DISCUSSION

In the present study, the radioprotective effect of FB was demonstrated against DNA damage induced by IR in normal human lymphocytes. A dose relevance between FB concentrations and

its radioprotective effect was observed. The maximum radioprotection was observed at a concentration of 100 µM of FB with 43% efficacy. However, FB showed a significant radioprotective effect at a concentration of 100 µM, and this dose of FB exhibited higher micronucleus than control. Hence, the percentage of the micronucleus in FB treatment samples (100 µM) was <1%. The micronucleus is a gene toxicity biomarker that is increased in oxidative stress condition in cells. However, IR-produced free radicals and ROS are the most toxic substances involved in cellular damage, and it is a crosstalk between oxidative stress and inflammation process in the cellular environment. IR-produced inflammation activates signaling pathways into the cell that induces DNA damage (2). Mefenamic acid exhibited a radioprotective effect through anti-inflammatory property (11). In the present study, FB exhibited radioprotective effect, but its mechanism is unclear. Several studies have been reported that FB had protective effects on normal tissues against high dose of drugs or toxic agents. FB markedly ameliorated the doxorubicin-induced cardiac toxicity in rats. It prevented the reduction of non-protein thiol levels through increased manganese superoxide dismutase (SOD) level and reduced cardiac damage markers and malondialdehyde (MDA) level as a lipid peroxidation marker. It also exhibited an anti-inflammatory effect through decreasing the expression of nuclear factor kappa and tumor necrosis factor (TNF)-α. It exerted an anti-apoptotic effect through increased Bcl-2 (a cell survival protein) expression and decreased caspase-3 and Bax expressions (4). It was able to decrease LPS-induced lung inflammation and edema. It attenuated the elevated levels of TNF-α in the lung tissue of LPS-treated rats. In addition, it exhibited an antioxidant activity through decreasing MDA levels in the lung tissue (3.4-fold) as well as enhancing SOD activity (by 34%) (5). It inhibited acute pulmonary inflammation triggered by lung injury induced by acid injection. Moreover, it inhibited inflammation in the liver in response to acetaminophen-induced hepatic injury (12). The free radical scavenging and reducing power activities of FB were evaluated by diphenyl-picrylhydrazyl radical and ferric reducing antioxidant power methods, respectively. FB did not exhibit any antioxidant activity in vitro up to 200 µM, whereas a percentage of >95% antioxidant activity with ascorbic acid were observed (data not shown). Then, free radical scavenging and reducing power activities are not possibly contributed in the radioprotection of FB. Since the anti-inflammatory effects of FB have been approved in the above stated studies, then this mechanism is mostly involved in the radioprotection of FB. The maximum radiation protection of FB (100 µM) was 43% efficacy in our study. In a previous study, mefenamic acid (100 µM) with mainly anti-inflammatory effect was 38% (11).

CONCLUSION

In the present study, FB reduced genotoxicity induced by IR in human normal lymphocytes, with regard to drug approval; it will help defense normal tissue against toxicity induced by IR in humans.

Ethics Committee Approval: This study was approved by the ethics committee of Mazandaran University of Medical Sciences, Sari, Iran (ID no. 3178, 2017-7-30).

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

Author Contributions: Conceived and designed the experiments or case: SJH. Performed the experiments or case: SA, AG. Analyzed the data: SA, SJH. Wrote the paper: SJH. All authors have read and approved the final manuscript.

Conflict of Interest: The authors declare that they have no conflict of interest.

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