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RESEARCH ARTICLE

Metformin attenuates cisplatin-induced genotoxicity and apoptosis in rat bone marrow cells

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ABSTRACT

Metformin is widely used as an oral hypoglycemic drug in the management of type 2 diabetes mellitus. This study evaluated the possible protective effects of metformin against cisplatin-induced genotoxicity and apoptosis in rat bone marrow cells. Two different doses of metformin (50 and 100 mg/kg b.w.) were administered orally to experimental animals for seven consecutive days. On the seventh day, the rats were exposed to cisplatin (5 mg/kg, i.p.) 1 h after the last oral metformin administration. Rats in the control group were treated orally with 10 ml/kg PBS for 7 consecutive days and a single intraperitoneal injection of saline (0.9%) on the 7th day. The antagonistic effects of metformin against cisplatin were evaluated using micronucleus assay, reactive oxygen species (ROS) level analysis, hematological analysis, and flow cytometry. Treatment with 50 and 100 mg/kg metformin before cisplatin injection produced a significant reduction in the frequencies of micronucleated polychromatic erythrocytes (MnPCEs) and micronucleated normochromatic erythrocytes (MnNCEs) 24 h after cisplatin treatment with a corresponding increase in the PCE/(PCE + NCE) ratio. Moreover, metformin markedly elevated the levels of both red and white blood cells in peripheral blood and decreased the percentage of apoptotic cells and the ROS level in bone marrow cells of rats treated with cisplatin. The data suggest that metformin has potential chemoprotective properties in rat bone marrow after cisplatin treatment, which support its candidature as a potential chemoprotective agent for cancer patients undergoing chemotherapy.

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Introduction

Bone marrow is particularly sensitive to chemotherapeutic drugs due to the high numbers of proliferative cells undergoing DNA synthesis. Bone marrow toxicity and cumulative myelosuppression are the most common complications of cisplatin therapy (Das *et al*. 2008, Basu *et al*. 2017). Therefore, protecting the bone marrow may reduce the side effects of cisplatin-based chemotherapy.

DNA is one target of cisplatin, damage to which can lead to serious consequences. One of the most important effects of cisplatin in the organism is disorder in the synthesis of DNA, which affects mainly the blood, germ cells, and young cells. Furthermore, cisplatin produces reactive oxygen species (ROS) such as superoxide anion and hydroxyl radical by interacting with DNA (Masuda *et al*. 1994). The accumulation of these reactive species causes cellular oxidative stress which, if not repaired, can lead to the damage of important biomolecules (Dizdaroglu *et al*. 2002). Cisplatin also induces a decrease in plasma concentrations of antioxidants in cancer patients (Weijl *et al*. 1998, Srivastava *et al*. 2010). Therefore, agents with direct free radical scavenging properties may have the potential to reduce the side effects of chemotherapy.

Metformin is an oral hypoglycemic drug that is widely used in the treatment of type 2 diabetes mellitus (Viollet *et al*. 2012). It has been established that metformin has an excellent safety profile and no mutagenic or genotoxic effects in therapeutic doses (Aleisa *et al*. 2007, Attia *et al*. 2009, Sant'Anna *et al*. 2013). Metformin has many pharmacological effects, including immune stimulation, anti-inflammatory, anti-oxidant, free-radical scavenging, antimicrobial, and antiviral activities (Bonnefont-Rousselot *et al*. 2003, Hou *et al*. 2010, Hajihashemi *et al*. 2013, Wang *et al*. 2017, Pollak 2017, Najafi *et al*. 2018). Data obtained from both *in vitro* and in vivo studies have shown that metformin has preventive effects against toxicity induced by a variety of agents, including carbon tetrachloride (CCl4), doxorubicin, gentamicin, ethanol, adriamycin, and paraquat (Poon *et al*. 2003, Aleisa *et al*. 2007, Morales *et al*. 2010, Asensio-López *et al*. 2011, Chang *et al*. 2011, Ullah *et al*. 2012, Algire *et al*. 2012). The current authors have previously reported that metformin protects against radiation-induced genotoxicity and apoptosis in cultured human blood lymphocytes (Cheki *et al*. 2016). Several investigations have further shown that the administration of metformin can reduce cancer risk in diabetic patients. Metformin has also been shown to

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suppress proliferation of a wide variety of cancer cell lines, including prostate, lung, breast, glioma, and ovarian cancer cells (Evans *et al*. 2005, Rizos and Elisaf 2013, Kasznicki *et al*. 2014). Furthermore, synergistic antitumor effects were found when metformin was administered in combination with cisplatin (Lin *et al*. 2013, Teixeira *et al*. 2013, Wang and Wu 2015, Zhu *et al*. 2016, Qi *et al*. 2016). Metformin has also been shown to attenuate hepatotoxicity, nephrotoxicity, ototoxicity, peripheral neuropathy, cognitive impairment, and brain damage induced by cisplatin (Chang *et al*. 2014; Mao-Ying *et al*. 2014; Li *et al*. 2016; Zhou *et al*. 2016; Mansour *et al*. 2017). Thus, the combination of cisplatin and metformin may be of therapeutic benefit and may have an impact on the treatment of cancer patients. However, the influence of metformin on cisplatin-induced genotoxicity and apoptosis in bone marrow has not yet been reported. Therefore, the purpose of this work was to study the effects of metformin on cisplatin-induced genotoxicity and apoptosis in rat bone marrow cells.

Materials and methods

Chemicals

Cisplatin (Cis-dichlorodiammineplatinum(II); CAS number: 15663–27-1), metformin (1,1-dimethylbiguanide hydrochloride; CAS number: 1115–70-4), 2′,7′-dichlorofluorescin diacetate (DCFH-DA; CAS number: 4091–99-0), May-Grünwald stain (CAS number: 68988–92-1), Giemsa stain (CAS number: 51811–82-6), fetal bovine serum (FBS; CAS number: 9014–81-7), phosphate-buffered saline (PBS; CAS number: 1314–87-0), Annexin-V-FLUOS Staining Kit (CAS number: 11828681001) were purchased from Sigma chemicals Co. (St. Louis, CA, USA).

Animals

Adult male Wistar rats weighing 120–180 g were used throughout the study. All of them were kept in the same room under a constant temperature (22 ± 2 °C) and humidity (55%–60%), illuminated 7:00 a.m. to 7:00 p.m., and given free access to food pellets and water. The rats were acclimatized to the laboratory conditions one day before the experimental session. All animal experiments were carried out in accordance with the NIH Guide for Care and Use of Laboratory Animals. All study protocols were approved by the Institutional Animal Ethical Committee of Ahvaz Jundishapur University of Medical Sciences (IR.AJUMS.REC.1396.269).

Drug and experimental protocol

Metformin was administered in doses of 50 and 100 mg/kg daily by gavage. According to the Reagan-Shaw method for dose conversion from animal to human studies (Reagan-Shaw *et al*. 2008), the human equivalents of rat doses of 50 and 100 mg/kg are 486 and 972 mg, respectively, for an average sized 60 kg adult human. Therefore, the selected dose in this study is within the safe therapeutic range reported in humans (1000–2500 mg daily; Wang *et al*. 2017). Also, according to previous reports, these doses of metformin have no detectable toxicity in experimental animals (Aleisa *et al*. 2007). Furthermore, the dose of cisplatin (5 mg/kg b.w., i.p.) was selected on the basis of its effectiveness in inducing genotoxicity in rodent bone marrow (Mora Lde *et al*. 2002, Yilmaz *et al*. 2010, Khandelwal and Abraham 2014).

The animals were divided into five groups, each containing five rats, as follows:

- Control group: oral treatment with 10 ml/kg PBS (metformin solvent) for 7 consecutive days and a single intraperitoneal (i.p.) injection of normal saline (cisplatin solvent; 0.9%) on the 7th day.
- 100 mg/kg metformin group: oral treatment with 100 mg/kg metformin for 7 consecutive days and a single i.p. injection of normal saline (0.9%) on the 7th day.
- Cisplatin-alone group: oral treatment with PBS for 7 consecutive days and a single i.p. injection of cisplatin (5 mg/kg) on the 7th day.
- 50 mg/kg metformin + cisplatin group: oral treatment with 50 mg/kg metformin for 7 consecutive days and a single i.p. injection of cisplatin (5 mg/kg) on the 7th day, 1 h after the last metformin administration.
- 100 mg/kg metformin + cisplatin group: oral treatment with 100 mg/kg metformin for 7 consecutive days and a single i.p. injection of cisplatin (5 mg/kg) on the 7th day, 1 h after the last metformin administration.

All animals were deeply anesthetized and sacrificed 24 h post-treatment with cisplatin.

Bone marrow micronucleus assay

The micronucleus assay in bone marrow was carried out according to the method described by Schmid (1975). The bone marrow from both femurs was flushed in the form of a fine suspension into a centrifuge tube containing FBS. The cells were collected by centrifuge at 1000 rpm for 10 min. Bone marrow smears were prepared and the slides were placed in room temperature. After 24 h air-drying, the smears were fixed with methanol and stained with May-Grunwald/Giemsa. With this method, polychromatic erythrocytes (PCEs) stain reddish-blue and normochromatic erythrocytes (NCEs) stain orange, while nuclear material is dark purple. Cells were counted by light microscopy with 1000× magnification under oil immersion. For each experimental group, five rats were used, and a total of 5000 PCEs and corresponding NCEs (1000 PCEs and 1000 NCEs per animal) were scored to determine the number of micronucleated polychromatic erythrocytes (MnPCEs), micronucleated normochromatic erythrocytes (MnNCEs), and the ratio of PCE to (PCE + NCE). The ratio of PCE to (PCE + NCE) was determined for each experimental group to assess cisplatin effects with and without metformin on bone marrow proliferation.

ROS determination

The generation of intracellular ROS was quantified using the oxidation-sensitive fluorescent probe DCFH-DA.

DCF was used as a general probe for ROS. DCFH-DA enters the cell and is easily hydrolyzed by intracellular esterases to the nonfluorescent form DCFH, which is rapidly converted to fluorescent DCF in the presence of a variety of ROS (Halliwell and Whiteman 2004). Briefly, the bone-marrow cells were collected in tubes containing 1.5 ml FBS, centrifuged, and washed with ice-cold PBS. The bone-marrow cells were harvested by centrifugation, washed twice with cold PBS, and finally resuspended in PBS. Bone marrow cells (1×10^6) were loaded with 10 μM DCFH-DA and incubated in darkness for 30 min to allow the formation of DCF. Then, fluorescence intensities were measured by using a Perkin-Elmer LS50B Fluorescence Spectrometer (Beaconsfield, UK) at an excitation wavelength of 485 nm and an emission wavelength of 529 nm. Results were expressed as fold-difference with the control.

Hematological study

Blood samples from all groups were gathered in K_2EDTA-coated microvette tubes through cardiac puncture. Complete blood cell counts were measured using a Sysmex KX-21N Automated Hematology Analyzer and included white blood cells (WBCs), red blood cells (RBCs), and platelets as well as hemoglobin concentration.

Quantification of apoptosis by flow cytometry

Apoptosis and necrosis were measured using an Annexin-V-FLUOS Staining Kit according to the manufacturer's instruction. Briefly, the bone marrow cells were washed with PBS and incubated with Annexin-V FLUOS labeling solution (containing 2 μl Annexin-V-FLUOS labeling reagent and 2 μl propidium iodide solution in 100 μl incubation buffer for each sample) at room temperature and in darkness for 15 min. A negative control bone marrow sample was obtained without the staining procedure for use in identifying the quadrant. Typically, each bone marrow sample consisted of an initial density of 1×10^6 bone marrow cells/ml. The bone marrow samples were analyzed for the presence of apoptotic and necrotic cells by flow cytometry on an FACS Calibur flow cytometer (Becton-Dickinson, San Jose, CA, USA). Data were analyzed using the FlowJo software (FlowJo LLC, Ashland, OR, USA). For each group, five independent bone marrow samples were analyzed. In each sample, a minimum of 10 000 events were counted and analyzed.

Statistical analysis

The data values are presented as means ± standard error of mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) and *post hoc* Tukey tests. A p value <0.05 was considered to be significant.

Results

Micronucleus assay

The MnPCE/1000PCE and MnNCE/1000NCE induced by metformin alone or in combination with cisplatin are shown in Table 1. Treatment with 100 mg/kg metformin alone did not lead to a significant increase in the number of MnPCE/1000PCE and MnNCE/1000NCE when compared to the control group (p values $=0.990$ and 0.950, respectively), indicating the nongenotoxic nature of metformin. A significant increase in the number of MnPCE/1000PCE and MnNCE/1000NCE was observed in the group treated with cisplatin alone compared to the control group ($p < 0.001$). The data demonstrate that pretreatment with 50 and 100 mg/kg metformin caused a decrease in the number of MnPCE and MnNCE compared with the cisplatin-alone group ($p < 0.001$). The protective effect of metformin on the cisplatin-induced micronuclei formation in PCEs and NCEs was significantly increased when treatment dose was increased from 50 to 100 mg/kg ($p < 0.05$).

The PCE/(PCE + NCE) ratio was used as a measure of cell proliferation (Table 1). The PCE/(PCE + NCE) ratio in the group treated with 100 mg/kg metformin alone was within range of the control group (p value $=0.991$). On the other hand, a significant reduction in the PCE/(PCE + NCE) ratio was found in the cisplatin-treated group when compared to the groups not treated with cisplatin ($p < 0.001$). The PCE/(PCE + NCE) ratio was significantly increased by about 58% and 79% in groups treated with metformin doses of 50 and 100 mg/kg, respectively, compared with the cisplatin-alone group.

ROS generation

ROS generation in bone marrow was investigated by measuring the fluorescence intensity of DCF. As shown in Figure 1, DCF fluorescence was not significantly different after treatment with 100 mg/kg metformin compared to the control group (p value $=0.999$). DCF fluorescence in the group treated with cisplatin alone was significantly increased by about 2.1-fold

Table 1. Effects of metformin on the formation of cisplatin-induced micronulei in PCEs and NCEs and the ratio of PCE/PCE + NCE in rat bone marrow.

Treatment groups	MnPCE/1000PCE	MnNCE/1000NCE	PCE/PCE + NCE
Control	5.20 ± 0.86	2.60 ± 0.67	0.45 ± 0.01
100 mg/kg metformin	6.60 ± 0.67	3.60 ± 0.67	0.44 ± 0.02
Cisplatin-alone	73.40 ± 3.23*	26.20 ± 1.71*	0.24 ± 0.02*
50 mg/kg metformin + cisplatin	44.20 ± 2.63#	14.60 ± 0.81#	0.38 ± 0.02+
100 mg/kg metformin + cisplatin	26.80 ± 2.00#	10.20 ± 0.58#	0.43 ± 0.02#

MnPCE: micronucleated polychromatic erythrocyte; MnNCE: micronucleated normochromatic erythrocyte; PCEs: polychromatic erythrocytes; NCEs: normochromatic erythrocytes.
*$p < 0.001$ compared to control.
#$p < 0.001$ compared to cisplatin.
+$p < 0.05$ compared to cisplatin.

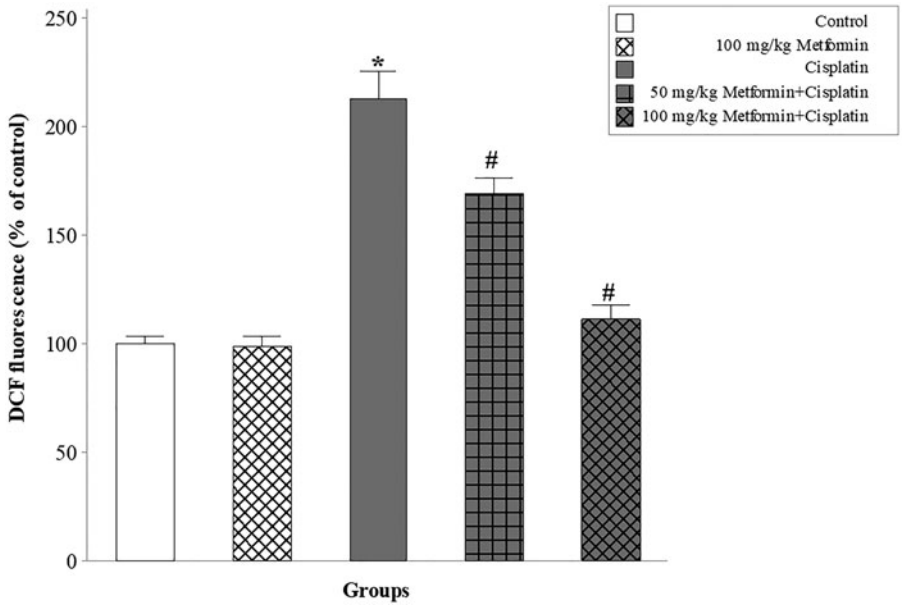

Figure 1. Effect of metformin on cisplatin-induced reactive oxygen species (ROS) level in rat bone marrow cells. Values are expressed as mean ± SEM of five experiments in each group. $^{*}p < 0.001$: Cisplatin-alone group compared to control, $^{\#}p < 0.001$: 50 and 100 mg/kg metformin + cisplatin groups compared to cisplatin-alone.

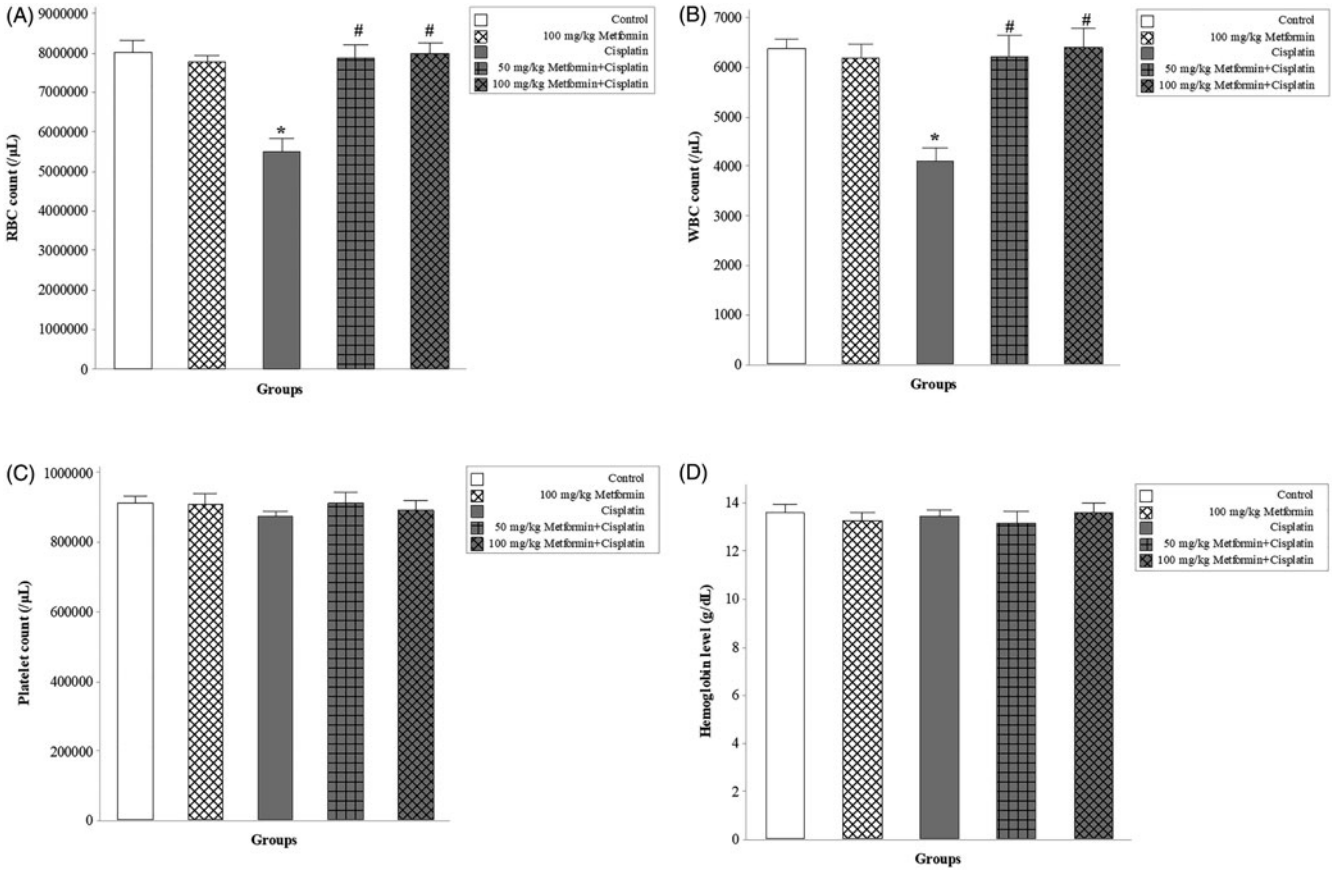

Figure 2. Effect of metformin on blood cell counts and hemoglobin concentration in the normal and cisplatin-treated rats. Red blood cells (A), white blood cells (B), platelets (C), and hemoglobin (D). Values are expressed as mean ± SEM of five experiments in each group. $^{*}p < 0.001$: Cisplatin-alone group compared to control, $^{\#}p < 0.001$: 50 and 100 mg/kg metformin + cisplatin groups compared to cisplatin-alone.

as compared with the control group ($p < 0.001$). Pretreatment with 50 and 100 mg/kg metformin prior to cisplatin injection significantly reduced DCF fluorescence compared with the cisplatin-alone group ($p < 0.001$).

Hematological analysis

As shown in Figure 2, a significant reduction in WBC and RBC counts was found in the cisplatin-alone group when compared

with the control group ($p < 0.001$). In contrast, pretreatment with 50 and 100 mg/kg metformin before the cisplatin injection showed a significant increase in WBC and RBC counts when compared to the cisplatin-alone group ($p < 0.001$). No difference was observed in platelets count or hemoglobin level after the cisplatin injection at 24 h (Figure 2).

Quantification of apoptosis by flow cytometry

Representative data of the Annexin V/PI flow cytometry analysis is shown in Figures 3 and 4. The average percentage of spontaneous apoptosis in bone marrow showed no significant variation in animals treated with 100 mg/kg metformin alone compared with the control group (p value = 0.917). The percentage of apoptotic cells (Annexin V^+ and PI^-) observed in animals treated with cisplatin-alone were significantly increased compared with the control group ($6.30 \pm 0.15\%$ vs. $1.26 \pm 0.08\%$; $p < 0.001$). In animals treated with 50 and 100 mg/kg metformin before cisplatin injection, the percentage of apoptotic cells was significantly decreased in comparison with the cisplatin-alone group ($3.42 \pm 0.21\%$ and $2.10 \pm 0.16\%$ vs. $6.30 \pm 0.15\%$, respectively; $p < 0.001$). The protective effect of metformin on the percentage of apoptotic cells induced by cisplatin was significantly increased when treatment dose was increased from 50 to 100 mg/kg ($p < 0.001$). Furthermore, in all groups, the percentage of necrotic cells (Annexin V^+ and PI^+) and necrotic cell debris or apoptotic bodies (Annexin V^- and PI^+) was too low and negligible (<1%, Figure 3).

Discussion

It has been reported that the direct interaction of cisplatin with DNA generates superoxide anion and hydroxyl radical (Masuda *et al*. 1994). The hydroxyl radical is one of the most reactive and aggressive chemical species; it severely damages the bases and sugars of DNA and induces strand breakage (Dizdaroglu *et al*. 2002). The cytotoxic effects of cisplatin may be associated, at least in part, with free radical-induced DNA damage. Rios *et al*. (2009) showed that the ROS generated by DNA–cisplatin interaction is inhibited by both lycopene and bixin in a concentration-dependent manner. Silva *et al*. (2001) reported that pretreatment with bixin reduced the number of chromosomal aberrations and inhibited lipid peroxidation in rats treated with cisplatin. Several reports have shown that the use of antioxidants attenuated genome instability in rodent bone marrow after cisplatin injection (Mora Lde *et al*. 2002, Serpeloni *et al*.

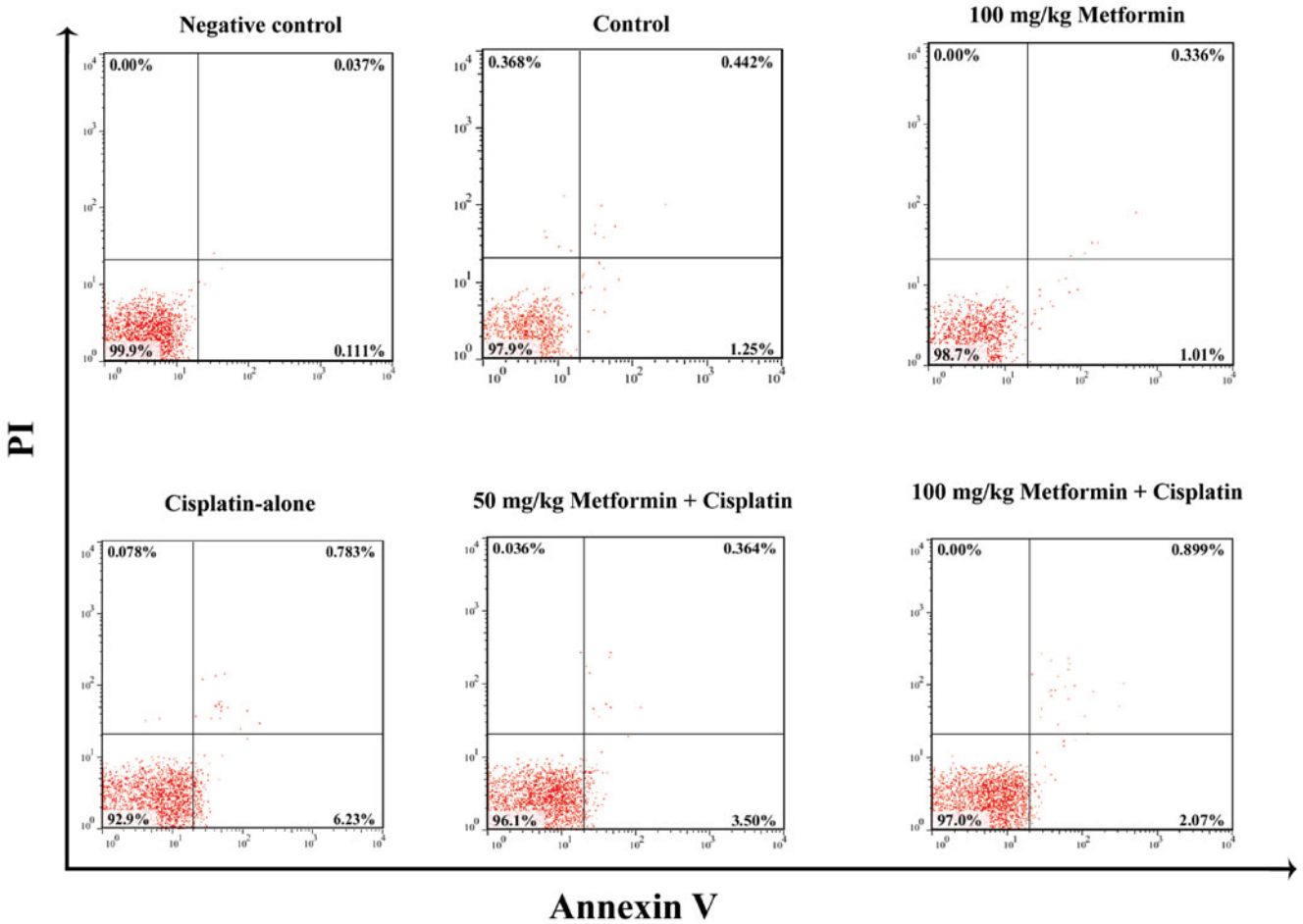

Figure 3. Flow cytometric analysis of Annexin V and propidium iodide-stained bone-marrow cells of rats treated with cisplatin and/or metformin. Representative dot plots of one set of five independent experiments of Annexin V and PI staining. The lower left quadrant (Annexin V^- and PI^-) was considered as live cells, the lower right quadrant (Annexin V^+ and PI^-) was considered as apoptotic cells, the upper right quadrant (Annexin V^+ and PI^+) was considered necrotic cells, and the upper left quadrant (Annexin V^- and PI^+) was considered as necrotic cells debris or apoptotic bodies.

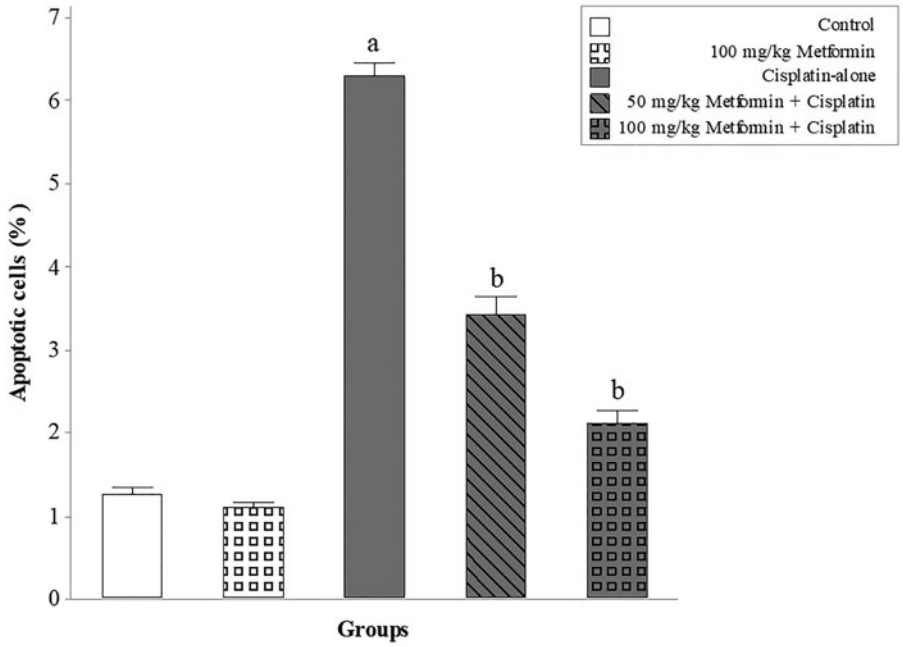

Figure 4. The percentage of apoptotic cells (Annexin V^+ and PI^-) in bone marrow of rats treated with cisplatin and/or metformin. Values are expressed as mean ± SEM of five experiments in each group. [a]$p < 0.001$: compared to control; [b]$p < 0.001$: compared to cisplatin-alone.

2010, Yilmaz *et al*. 2010, Rjiba-Touati *et al*. 2012, Khandelwal and Abraham 2014). Hence, the use of any agent with free-radical scavenging and antioxidant activity may be useful in modulating the genotoxicity of cisplatin. Several studies have reported that metformin exerts strong antioxidant activity (Bonnefont-Rousselot *et al*. 2003, Hou *et al*. 2010, Hajihashemi *et al*. 2013). Metformin protects mouse bone marrow against MnPCEs induced by adriamycin by increasing glutathione (GSH) and decreasing malondialdehyde (MDA) (Aleisa *et al*. 2007). It has been reported that metformin decreases the frequency of micronuclei in PCEs and NCEs and improves the changes of catalase (CAT) and superoxide dismutase (SOD) in rats damaged by nicotinamide-streptozotocin (Rabbani *et al*. 2010). Xu *et al*. (2015) showed that the administration of metformin to mice significantly mitigated irradiation-induced increases in ROS production and DNA damage and upregulated NADPH oxidase 4 expression in bone-marrow hematopoietic stem cells. They also observed a significant increase in enzymatic activities of SOD, CAT, and glutathione peroxidase-1 (GPX1) in irradiated rats in the presence of metformin. Algire *et al*. (2012) in an *in vitro* study showed that metformin attenuates paraquat-induced elevations in ROS and DNA damage in mouse embryonic fibroblasts. Sahu *et al*. (2013) reported that metformin elevated GSH levels, CAT, SOD, and glutathione-s transferase (GST) and decreased MDA and ROS levels in rat kidneys damaged by cisplatin. In addition, the administration to rats of metformin before cisplatin reduced MDA and total nitrate/nitrite (NOx) levels and restored changes in the activities of GSH and SOD in liver tissue (2017). GSH has been implicated in the metabolism of cisplatin (Suzuki *et al*. 1990, Cavaletti *et al*. 1994, Brozovic *et al*. 2010, Zhao *et al*. 2015). Experimental studies have also shown that increased glutathione levels reduce the genotoxicity of cisplatin in rodent bone marrow cells (Attia 2010, 2012, Basu *et al*. 2017). Hence, one possible protective mechanism of metformin against cisplatin can be its ability to increase glutathione levels. The results of this study showed that metformin has potent chemoprotective effects against genotoxicity induced by cisplatin in rat bone marrow. Metformin reduced the frequency of cisplatin-induced MnPCEs and MnNCEs. Also, the administration of metformin significantly reduced ROS levels in bone marrow cells after cisplatin injection, which could be attributed to its free radical scavenging activity. On the other hand, the data of the present study demonstrated that metformin at doses of 50 and 100 mg/kg is not a genotoxic drug. The current results are in agreement with those of Aleisa *et al*. (2007, 2009).

The PCE/(PCE + NCE) ratio is an index of the rate of proliferation, and a decrease in the ratio of PCE/(PCE + NCE) at 24 h after cisplatin treatment is a sign that erythropoiesis has been suppressed. The significant increment of this ratio after treatment with metformin showed its protective effect against cisplatin. These findings are consistent with those of previous studies which have shown that metformin enhanced the ratio of PCE/(PCE + NCE) which had been reduced by adriamycin, nicotinamide-streptozotocin, and hyperglycemia in rodent bone marrow cells (Aleisa *et al*. 2007, Attia *et al*. 2009, Rabbani *et al*. 2010).

Protection of the hematopoietic system against cisplatin is the key strategy for the development of chemoprotective agents. It has been reported that metformin plays an important role in improving hematopoiesis (Zhang *et al*. 2016). Bikas *et al*. (2016) reported that metformin attenuated the radioactive iodine-induced decrease in complete blood count parameters, and its radioprotective properties are more prominent in WBCs. Recently, a preclinical study showed that metformin improves peripheral blood counts and hematopoiesis in a murine model of Fanconi anemia (Zhang *et al*. 2016). It has also been reported that metformin

administration improves leukocyte counts in women with polycystic ovary syndrome (Orio *et al*. 2007). Moreover, metformin ameliorates ionizing irradiation-induced long-term hematopoietic stem cell injury in mice (Xu *et al*. 2015). The analysis of hematological parameters in rats treated with cisplatin has shown a significant decrease in WBC and RBC counts, whereas preadministration of metformin tends to mitigate this cisplatin-induced reduction.

Cisplatin damage to DNA, if not repaired, can lead to cell death through apoptosis and other modes. So far, several scavengers of free radicals and antioxidant agents have been found to mitigate cisplatin-induced apoptosis in rodent bone marrow (Attia 2010, 2012). In this study, it was observed that the administration of metformin significantly reduced apoptosis in bone marrow cells after cisplatin injection. Chang *et al*. (2014) showed that metformin protects against cisplatin-induced ototoxicity by inhibiting the increase in intracellular calcium levels, preventing apoptosis, and limiting ROS production. Furthermore, metformin attenuates cisplatin-induced tubular cell apoptosis and acute kidney injury (Li *et al*. 2016). Regarding the close relationship between free radicals, particularly ROS, and apoptosis, this anti-apoptotic effect in this study is supposed to have resulted from the action of metformin as a direct free-radical scavenger of ROS generated by cisplatin.

Conclusions

This study has shown that the administration of metformin prior to cisplatin decreases the harmful effects of cisplatin on bone marrow cells. Hence, metformin could be of excellent benefit in mitigating cisplatin toxicity to bone marrow in cancer patients undergoing chemotherapy.

Disclosure statement

The authors report no conflict of interest.

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