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High dose zoledronic acid increases ischemia-reperfusion damage of the liver

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Abstract. – OBJECTIVE: Zoledronic acid (ZA), a nitrogen-containing bisphosphonate, has been reported to exhibit a protective effect against cancers and prevent bone fractures. It also induces apoptosis by increasing proinflammatory cytokines and oxidative stress. Oxidative stress increases significantly during ischemia-reperfusion (IR) injury. The liver is highly sensitive to IR injury. In this study, we aim to investigate whether high-dose ZA treatment affects the liver during IR.

MATERIALS AND METHODS: We used twenty-one Sprague-Dawley male rats in our study, and they were subdivided randomly into three groups, each containing seven rats. A single dose of 100 μg/kg ZA was administered via the intraperitoneal route in the ZA group. Forty-eight hours after the ZA administration, infrarenal abdominal aortic cross ligation was performed on the ZA and IR groups. After 2 hours of ischemia, 2 hours of reperfusion was applied.

RESULTS: The malondialdehyde (MDA) level of the control group was significantly lower than the IR (p = 0.006) and ZA (p<0.001) groups. However, the superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) values of the control group were significantly higher than the values of the IR group (p<0.05, p<0.001, and p<0.05) and ZA group (p = 0.002, p<0.001, and p<0.001). Caspase-3 activity was significantly higher in the IR group as compared to the control group (p<0.001). The caspase-3 activity in the ZA group, on the other hand, was higher than both the control (p<0.001) and IR groups (p<0.001).

CONCLUSIONS: High-dose ZA may exacerbate liver injury during IR by increasing reactive oxygen species production and apoptosis.

Key Words:
Zoledronic acid, Ischemia-reperfusion injury, Liver, Caspase.

Introduction

The liver plays a significant role in many important functions of the body, including metabolism, regulation of red blood cells, and glucose synthesis. However, it is extremely sensitive to ischemia in addition to other factors such as drugs, toxins, and viruses, and can be easily damaged. Ischemia associated with shock, transplantation, and liver surgery and followed by reperfusion causes severe liver injury which may lead to morbidity and mortality[1]. Ischemia occurs due to the absence of oxygen in the tissue, while in the reperfusion phase, the release of reactive oxygen species (ROS) and intense proinflammatory cytokine worsen the damage that occurred during ischemia[2].

Oxidative stress or increased ROS can play an important role in various biological reactions. It has also been reported to have an effect on the pathogenesis of many diseases. ROS causes tissue damage by affecting the cell membrane, genetic material, enzymatic pathways, and connective tissue structures[3]. The relationship between ROS and diseases depends on the balance between ROS and antioxidants[4]. Cells are protected against oxidative damage by various systems (enzymatic and non-enzymatic). Cleansing enzymes such as superoxide dismutase

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(SOD), catalase (CAT), and glutathione peroxidase (GPx) protect the cell from the harmful effects of oxidative stress[5]. Malondialdehyde (MDA) is also a good marker indicating increased oxidative stress and ROS[6]. Furthermore, MDA and antioxidant enzyme levels in blood or tissues along with histopathological markers are useful in detecting increased oxidative stress and damage in tissues and cells[7].

Zoledronic acid (ZA), a nitrogen-containing bisphosphonate, has been reported to exhibit a protective effect against breast cancer, prostate cancer, and multiple myeloma and prevent osteoporosis and bone fractures[8-11]. It is also known to increase the apoptosis of osteoclasts while decreasing bone resorption and preventing hypercalcemia[12-14]. ZA activates the caspase pathway by increasing oxidative stress and the release of cytokines[15,16], leading to apoptosis in osteoclasts and tumor tissue. Besides, it shows antitumor effects in different types of cancers through the release of growth factors, cell adhesion, and autophagy[16]. The half-life of ZA is 146 hours and it has a long duration of action[17]. ZA is administered once every 4 weeks in cancer patients and once a year in osteoporosis patients. Previous studies[18,19] have demonstrated that ZA increases the formation of proinflammatory cytokines and ROS[18]. Additionally, it causes osteonecrosis of the jaw by increasing the formation of peroxynitrite and other ROS[19]. Moreover, it is clinically important to know the side effects and toxic effects of this molecule which has a long duration of action.

There is no study in the literature investigating the effects of high-dose ZA on liver damage during ischemia-reperfusion (IR). However, a previous study[20] has shown that ZA can have a toxic effect on the liver. In this study, intense histopathological damage, an increase in ROS, and a decrease in antioxidant enzymes were detected in the liver of rats that are given 100 μg/kg ZA every day for 28 days[20]. In a study[21] where the rats were given 100 μg/kg ZA before IR, an increase in histopathological damage, cytokines such as tumor necrosis factor-alpha and interleukin-6, and ROS such as peroxynitrite radicals was observed in the kidney tissue.

In the current study, we aim to investigate whether ZA causes damage to the liver tissue during abdominal IR through histopathological and biochemical examination. Our secondary objective is to investigate whether ZA causes liver damage by increasing ROS formation and triggering apoptosis during IR.

Materials and Methods

Animals

Twenty-one male albino Sprague Dawley rats of 14-16 weeks of age weighing 250-300 mg were divided into 3 groups of 7 rats. These rats were kept and fed in a sterile experimental animal unit set to 12-hour light and 12-hour dark cycle, at a humidity of 55-60%, and a room temperature of 22± 3°C. They were allowed to consume ad libitum, unlimited feed, and tap water. All materials and methods in this study were designed according to the protocols of the National Institute of Health Guide for the Care and Use of Laboratory Animals. Necessary approval was also obtained from the Local Ethics Committee for Animal Experiments (RTEU, Approval no: 2014/48).

Group 1. The untreated animals (n = 7) were named as the Control group.

Group 2. The animals that underwent 120 minutes of reperfusion (n = 7) after 120 minutes of abdominal ischemia were named as the IR group.

Group 3. The animals that were given 100 μg/kg ZA intraperitoneally and underwent IR were named as the ZA group.

ZA Dosage

ZA reaches peak concentration in plasma after infusion. The plasma concentration of ZA decreases by 10% within the first 4 hours after infusion and 1% in each subsequent day[17]. Since it is in stable concentration in plasma, no difference is expected in the effect of ZA in the first 6 days after infusion[22]. We could have conducted our experiment on one of these days. In a previous IR model, ZA was used 48 hours before the IR[21]. Additionally, a previous study considered 100 μg/kg a high dose for ZA, and this dose was chosen for our study to see its toxic effects[15].

Application of the Experiment

Firstly, 4 mg of ZA (Zometa®, Novartis, Istanbul, Turkey) was diluted with isotonic sodium chloride solution. The ZA group was then administered with a single dose of 100 μg/kg of ZA through the intraperitoneal route. The application phase of the experiment started 48 hours after the administration of ZA to the ZA group[21]. All animals were subjected to the experiment under anesthesia by administering intraperitoneal Ketamine (50 mg/kg Ketalar®; Parke-Davis, Istanbul, Turkey) + Xylazine (5 mg/kg Rompun®; Bayer, Istanbul, Turkey). A

midline laparotomy was performed on the rats in the control group to dissect their infrarenal aorta (IRA). Subsequently, saline solution was poured into the peritoneal cavity and the laparotomy was closed without performing any obstruction procedure. On the other hand, in the IR group, the IRA was dissected and clamped after laparotomy. After 120 minutes, the clamp was released, and reperfusion was achieved for 120 minutes. It is important to note that IR was induced in the ZA group in the same way as the IR group. All rats were sacrificed at the end of the experiment. The liver tissues were taken after decapitation and evaluated immunohistochemically and histopathologically using conventional light microscopic methods. Furthermore, MDA and antioxidant enzymes in the liver tissues were analyzed in the biochemistry laboratory using an appropriate method.

Clamping of the Infrarenal Aorta

To perform midline laparotomy, the ambient temperature was provided with a heating lamp. The rats were placed in a supine position and their skin was washed with an aseptic solution with the surgical procedure. The fluid balance was maintained by pouring 10 mL saline solution into the peritoneal cavity. The bowel loops were then gently rotated to the left to expose the abdominal aorta and the interrupted aortic arch became visible. Subsequently, the IRA was clamped with an atraumatic microvascular clamp to achieve complete obstruction. Heat and fluid loss were prevented by covering the abdomen with a plastic cover. Reperfusion was achieved by releasing the clamp after 120 minutes.

Tissue Homogenates

The liver tissues were weighed, homogenized in 10 X ice-cold pH 7.4 phosphate-buffered saline (PBS) (50 mM), and centrifuged at 10,000 g for 20 minutes. The supernatant was taken and aliquoted into tubes. They were frozen at -80 degrees. The parameters were then analyzed within one month.

Protein Measurement

The Lowry protocol was used to measure protein levels in tissue homogenate. In this method, the total protein concentration was observed through a change in color in the sample solution. Biuret reaction and Folin-Ciocalteu reaction form the basis of this protocol. The reaction of copper ions with peptide bonds in an alkaline environment (Biuret test) is combined with the oxidation of aromatic protein units in this method. It is also worth noting that the Lowry method is based on the reaction of Cu^{+} ions reduced by peptide bonds with Folin-Ciocalteu reagent[23].

Malondialdehyde (MDA)

MDA levels were measured using the double heating method of Draper and Hadley. In this method, the color formed as a result of the reaction of thiobarbituric acid (TBA) with MDA is measured spectrophotometrically. The MDA levels are given in micromoles/L. This value was then divided into protein values to obtain the MDA level in nmol/mg protein[24].

Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) Activity

Cayman colorimetric assay kit (Cayman Chemical Company, MI, USA) was used for measuring the activities of SOD and GPx which are presented in U/ml. The results for both enzyme activities were obtained using an appropriate method, strictly following the manufacturer's instructions. Absorbance was measured at a wavelength of 450 ηm using a plate reader.

Catalase (CAT) Activity

The spectrophotometric method was used to determine the catalase activity. It is based on the monitoring of the resulting absorbance at 240 nm while catalase in the activity measurement medium is converts H_2O_2 into H_2O and O_2[25].

Histopathological Examination

The liver tissues taken from rats in all groups were divided into four groups, given label code numbers, and placed into a 10% neutral formaldehyde solution. After placing them in a fixative for 24 hours and passing through ethanol (50-100%) and xylene series in automatic tissue monitoring (Citadel 2000, Thermo Fisher Scientific, Waltham, MA, USA), the tissues were washed under running water for about 6 hours and embedded in paraffin. For hematoxylin-eosin staining, 4-6 μm thick sections were taken from the tissues and kept in an incubator before staining. After staining, they were examined under a light microscope and the photos of the sites that were deemed appropriate were taken at different magnifications.

We chose the sections cut at a thickness of 3-4 μm for immunohistochemical staining. They were kept in xylene twice for 10 minutes

at a time and passed through alcohol series. Afterward, they were kept in 3% hydrogen peroxide (H_2O_2) solution for 30 minutes (50-100%). After washing with PBS twice, they were heated in a buffer solution of Antigen Retrieval-Citrate 4 times for 5 minutes at a time at 700-800 Watt and kept in the secondary blocking agent for 30 minutes. Each preparation was held in different dilutions of the primary antibody [in Anti-Caspase-3; 1/200] for 75 minutes for staining with Anti-Caspase-3 (code: ab4051, Abcam plc, Cambridge CB4 0FL UK). Diaminobenzidine (DAB) solution was used as a chromogen and the preparations were stained with Mayers' hematoxylin for 3-5 minutes for counterstaining. Here, PBS was used as negative controls. The preparations were covered with suitable covering materials and their photographs were taken. Immunopositive reactions in the tissues were divided into 4 categories as mild (1, +), moderate (2, ++), severe (3, +++), and very severe (4, ++++) according to the % values of the area resulting from immunohistochemical staining[26]. Two of the sections were chosen randomly in each preparation and fifteen areas were determined in each of the selected sections. A histologist and pathologist interpreted histopathological and immunohistochemical scores using the blind grading method, and their percentage rates were determined based on the above-mentioned categories. The results were evaluated through statistical comparisons of all data within and between the groups.

Statistical Analysis

All statistical tests were conducted using SPSS 20 (SPSS Inc., Chicago, IL, USA). Data were given as mean ± SD. ANOVA test and Bonferroni post-hoc test were performed in sequence to compare the data. A *p*-value less than 0.05 was accepted as significant.

Results

Biochemical Parameters

The MDA level of the control group was significantly lower than the IR ($p = 0.006$) and ZA ($p<0.001$) groups. The MDA level of the ZA group was higher than the IR group, but it was not significant. The SOD, CAT and GPx values of the control group were significantly higher than that of the IR group ($p<0.05$, $p<0.001$, and $p<0.05$, respectively) and the ZA group ($p = 0.002$, $p<0.001$, and $p<0.001$, respectively). On the other hand, the SOD, CAT, and GPx values of the ZA group were lower than the values of the IR group, but they were not significant. The results of the biochemical values are presented in Table I.

Histopathological Parameters

No degenerative changes in the tissue structure were found in the livers of the control group. It was observed that the area surrounding the vena centralis was regular, and the cords formed by hepatocytes and the sinusoidal spaces between them were small (Figure 1A).

In the IR group, both intense necrosis and degeneration of hepatocytes ($p<0.001$ *vs.* control) and degenerative disorders such as cell swelling and cell shedding of endothelial cells ($p = 0.002$ *vs.* control) were observed (Figure 1B). Vasoconstriction in the portal area vessels and vacuolization and degeneration in the muscular structure of the vessels were observed in the IR group. Intensive dilatations ($p<0.001$ *vs.* control), eosinophils, and neutrophils ($p<0.001$ *vs.* control) were found to be increased in the portal area and the sinusoids near the portal area as compared to the control group. A small amount of edema fluid in the dilated sinusoids, endothelial cell swelling and shedding in their walls due to intracellular edema, and sporadic platelet aggregation were detected. In addition, deformations with indentations and protrusions and degenerated cells with

Table I. MDA and antioxidant enzyme levels of the groups.

	Control (n = 7)	IR (n = 7)	Za (n = 7)	¥*p*-value
MDA	5.9 ± 0.8	9.9 ± 2.0††	11.2 ± 3.6**	0.002
SOD	70.2 ± 23.1	60.9 ± 22.4*	34.6± 3.3†	0.006
CAT	72.6 ± 21.9	70.7 ± 29.4**	17.9 ± 6.7**	0.001
GPx	226.6 ± 48.1	177.1 ± 42.4*	119.2 ± 48.8**	0.002

Abbreviations: MDA: malondialdehyde: IR, ischemia-reperfusion injury: ZA, zoledronic acid: SOD, superoxide dismutase: CAT, catalase: GPx, glutathione peroxidase. *p <0.05, †p =0.002, ††p =0.006, **p <0.001 *vs*. control group with Bonferroni post-hoc test. ¥Anova test.

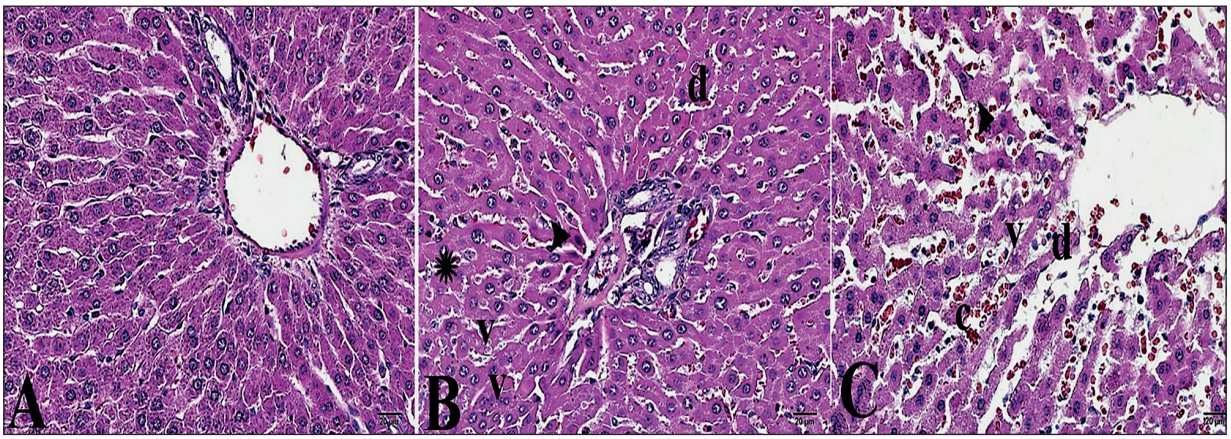

Figure 1. A, control **B,** IR group, **C,** IR + ZA group. c: congestion, v: vacuolization, d: dilatation, asterisk: degenerative cells, arrowhead: pyknotic and apoptotic cells, Hematoxylin-eosin staining, ×400.

surrounding vacuolar areas were observed on the surfaces of the endothelial and Kupffer cells. It was determined that the muscle cells of the great vessels in the portal area were basophilic and the surrounding eosinophil and neutrophil infiltrations were greater than the area surrounding the small vessel. It was observed that the dilatations in the sinusoids ($p<0.001$ *vs.* control) increased towards the portal area and the Kupffer cells present on their walls sometimes formed flat, round, or shuttle-like protrusions bulging in the middle towards the lumen. Further, more apoptotic (arrowhead) and vacuolized cells were found in hepatocytes around the central vein. Additionally, cytoplasmic as well as nuclear vacuole formations were detected in hepatocytes near the portal area. Cellular degenerations resulting from the vacuolization, concentrated around the portal area, were also detected in the form of necrotic cell losses (Figure 1B, Table II).

Tissue and cellular disorders in the liver were found to be higher in the ZA group than in the IR group (Table II, Figure 1C). In the IR group, sinusoidal dilatations ($p<0.001$ *vs.* IR group) were observed to be intense at all locations, especially around the vena centralis. In the ZA group, the occurrence of more intense sinusoidal area enlargements was observed as compared to the IR group. Additionally, it was determined that the Kupffer cells in the sinusoidal walls were more flat-shaped and densely stained basophilic as compared to that of the IR group. It was also observed that the cells around the central vein were more dispersed and the number of apoptotic-shaped and pyknotic cells increased heavily. In the control group, it was found that edema decreased in the portal area, and the morphological appearance of the ducts and vessels were similar to the nearly normal cells. In this group, vacuolization ($p<0.001$ *vs.* IR group) occurring in hepatocytes was more intense in the areas close to the portal area during the examination of the cells. However, the vacuolization type was found to be smaller in size, intensely granular, and homogeneously distributed in the cytoplasm. It was also determined that eosinophilic staining decre-

Table II. Histopathological results of liver tissue.

	Control (n = 7)	IR (n = 7)	Za (n = 7)	¥*p*-value
Sinusoid dilation	0.7 ± 0.5	2.1 ± 0.7*	3.7 ± 0.5*†	0.001
Hepatocyte degeneration	1.0 ± 0.5	2.2 ± 0.5*	3.8 ± 0.3*†	0.001
Neutrophil infiltration	0 ± 0	1.4 ± 0.8*	2.0 ± 0.6*	0.001
Vacuolization	1.1 ± 0.4	1.6 ± 0.9	3.7 ± 0.5*†	0.001
Epithelial cell shedding	1.0 ± 0.6	2.1 ± 0.7˟	3.7 ± 0.5*†	0.001
Anti-caspase-3	0.8 ± 0.3	2.1 ± 0.7*	3.8 ± 0.3*†	0.001

Abbreviations: IR: ischemia-reperfusion injury; ZA: zoledronic acid. ˟$p = 0.002$, *$p < 0.001$ *vs.* control group with Bonferroni post-hoc test. †$p < 0.001$ *vs*. IR group with Bonferroni post-hoc test. ¥ANOVA test.

ased in areas where vacuolization increased, and their nuclei were in the form of a heterochromatic ring (Figure 1C).

Immunohistochemical Staining

Anti-caspase-3 was immunopositive in the liver tissue at a rate of 57% (+), 29% (++), and 14% (+++) in the Control group; 57% (++), 29% (+++), and 14% (++++) in the IR group; and 29% (+++) and 71% (++++) in the ZA group. Caspase-3 activity was significantly higher in the IR group than in the control group ($p<0.001$). On the other hand, the caspase-3 activity in the ZA group was higher than both the control group ($p<0.001$) and the IR group ($p<0.001$). All histochemical results are shown in Table II and Figure 2.

Discussion

In this study, we observed a significant increase in oxidative stress and histopathological damage in the liver tissue in the ZA and IR groups compared to the control group. It is imperative to note that histopathological damage and apoptosis were more prominent in the ZA group compared to the IR group. Oxidative stress markers were also found to be higher in the ZA group than in the IR group, but there was no statistical significance. Consequently, our study results showed that ZA increases IR damage in the liver.

The liver is extremely sensitive to IR damage. The hepatic and abdominal aorta IR models revealed intensive histopathological and biochemical damage to the liver tissue. The damage occurred due to anoxia during ischemic damage and intense migration of neutrophils and macrophages and reoxygenation in the early stages of the reperfusion phase[27]. Neutrophils and macrophages particularly lead to an increase in superoxide and hydroxyl radicals and the formation of ROS at the site of damage[28]. The increased levels of these radicals along with hydrogen peroxide are extremely toxic to the cells. Exposure to ROS can cause oxidative damage to mitochondrial and cellular proteins, lipids, and nucleic acids, while free radicals directly attack the complexes in the mitochondrial respiratory chain. On the other hand, superoxide radicals, formed by electron reduction from O_2[29], cause disruption to DNA, cellular structure, and lipid preoksidasyon[29]. Likewise, the highly reactive hydroxyl radical damages structures within mitochondria such as proteins, lipids, and DNA[30]. As a result, permanent cell damage and apoptosis may occur. Measurement of the end products of lipid peroxidation is a widely accepted method to determine free radical production. And the degree of lipid peroxidation is commonly determined using MDA measurement[31]. Lipid peroxidation of cell membranes where free radicals react with lipids containing polyunsaturated fatty acids results in the formation of MDA as the final products[32]. Increased MDA level in the liver tissue reflects the severity of IR damage.

The SOD activity is measured by detecting superoxide radicals produced by the xanthine-xanthine oxidase system, and it indirectly reflects the level of superoxide radicals[32-34]. CAT enzyme, on the other hand, converts hydrogen peroxide into water and O_2. It prevents the formation of hydroxyl radicals by removing the hydrogen peroxi-

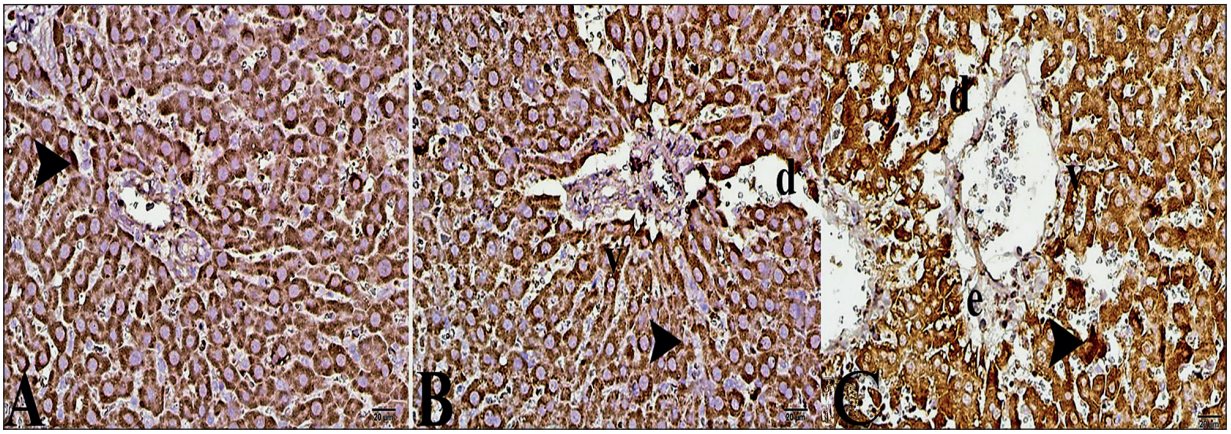

Figure 2. A, control **B,** IR group, **C,** IR + ZA group. Arrowhead: dense positive cells, v: vacuolization, d: dilation, arrow: swollen endothelial cell, e: edema, Anti-Caspase-3 immunoperoxidase staining, ×400.

de formed in the cell[32-34]. GPx converts reduced glutathione to oxidized glutathione in the presence of hydrogen peroxide. It reduces membrane phospholipid hydroperoxides to alcohols[32-34]. Free radicals can be detected directly measure, or indirectly by measuring antioxidant enzyme activities. In the current study, we determined the increase in ROS indirectly by measuring antioxidant enzyme activities[32-34]. MDA reflects excessive ROS in cells and tissues in IR models. Consequently, there is an occurrence of gene downregulation of antioxidant enzymes such as SOD, CAT, and GPx that removes the excess ROS and lowers the levels of these enzymes[35,36]. Decreased antioxidant level protects the organism from severe oxidative stress. In our study, increased MDA and decreased SOD, CAT, and GPx levels indicate that the liver tissue is exposed to excessive oxidative stress during IR. The increased antioxidant system is aimed at reducing liver damage; however, ZA further increases IR damage in the liver.

ZA is a third-generation bisphosphonate, which is used in the treatment of various conditions such as osteoporosis, high blood calcium levels due to cancer, cancer-related bone damage, and Paget's bone disease[37]. It inhibits the differentiation of osteoclasts while inducing their apoptosis. Additionally, it prevents the metastasis and angiogenesis of cancer cells[37]. Jiang et al[38] have reported that ZA may cause hepatotoxicity in humans, albeit rarely. Unlike these researchers[38], Mohamed et al[39] reported that the administration of 100 μg/kg ZA once a week for 12 weeks reduced non-alcoholic steatosis in rats. Zhao et al[40], in their study, reported that ZA liposome prevented the apoptosis of hepatocytes by inhibiting tumor necrosis factor-alpha and interleukin-1 in the hepatic IR model. It reduced vacuolar degeneration, congestion, and necrosis, thus protecting the liver from IR damage. In our study, the IR damage was aggravated in the ZA group; there was extensive damage to the liver, and apoptosis was significantly increased in the histopathological and biochemical examination. The most important difference between Zhao et al[40] study and our study was the doses applied and the pharmacological form of the drugs. They administered 1 μg ZA for 3 days i.e., 3 μg ZA in total. However, in our study, a single dose of 100 μg/kg ZA was administered intraperitoneally.

Liposomal technology, in general, reduces the toxic effect of the drug; it has been reported that liposomal ZA is not toxic to the liver at a dose of 30 μg/kg[41]. It has also been reported that a dose of 30 μg/kg ZA is a low dose[42]. In our study, we administered a high dose of ZA in its classical form[42], whereas Zhao et al[40] used liposomal ZA. ZA may protect the liver from IR damage at low doses and in liposomal form. However, a high dose of ZA can cause liver damage, according to our study. On the other hand, Mohamed et al[39] study was a steatosis model, not an IR model, in rats, and we may have found different results as the mechanisms of these two models are different. Similar to our results, Karabulut et al[20] demonstrated that 100 μg/kg ZA increased oxidative stress and MDA level in the liver of rabbits. Sehitoglu et al[21] also reported that 100 μg/kg ZA increased renal damage with an increase in cytokine and inflammation in the renal IR model. Furthermore, Tsai et al[42] reported that the administration of a high dose of ZA at 100 μg/kg caused damage to the bone tissue in the IR model. Contrary to the studies by Tsai et al[42] and Sehitoglu et al[21], Pocs et al[43] reported that ZA did not cause any significant damage to the periosteal microcirculation in the IR model. In this model, they administered ZA at 80 μg/kg weekly for 1 to 4 weeks to ovariectomized rats. In our study, we examined the hepatic structure, but not the periosteal structure. We may have also detected extensive hepatic damage, as the liver tissue is extremely sensitive to ischemia. Since ZA increases ROS formation and cytokine release, it is also expected to increase in IR damage, as shown in our study.

Activation of the Caspase pathway in the early reperfusion phase of IR causes apoptosis. Caspase-3 is an inactive zymogen found in many tissues[44]. Increased ROS and oxidative stress activate Caspase-3[15] that initiates apoptosis[44]. ZA, in particular, has been reported to activate Caspase 3, 5, and 7[45,46]. In our study, a greater increase in Caspase-3 activity in the ZA group than in the control and IR groups indicates that ZA increases IR damage. More intense histopathological damage was also observed in the ZA group than in the IR group. These findings may suggest that ZA increases IR damage. Our research is the first to show that ZA aggravates liver damage during IR injury. It also showed that the use of ZA before vascular trauma in the clinic may cause liver damage and suggested the liver operation be performed carefully after ZA administration. The current investigation expects to shed light on new studies on this subject.

Conclusions

The administration of ZA at a high dose of 100 μg/kg may increase oxidative stress in the liver during IR injury. During the injury, the antioxidant system is activated to prevent hepatic damage due to increased oxidative stress. It is important to note that increased oxidative stress can further aggravate damage to hepatocytes by inducing the caspase pathway.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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