EXPERIMENTAL STUDY

Effects of amifostine against blunt chest trauma-induced cardiac injury in rats

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ABSTRACT

BACKGROUND: This study aimed to examine whether two different doses of dexamethasone (DXM), which is a corticosteroid, and amifostine (AMI), which reduces cumulative tissue toxicity induced by cisplatin in advanced-stage cancer patients, have ameliorative effects on pathologic changes associated with cardiac contusion (CC) induced in rats.

METHODS: Forty-two Wistar albino rats were equally divided into six groups (n=7): C, CC, CC+AMI 400, CC+AMI 200, CC+AMI+DXM, and CC+DXM. Tomography images and electrocardiographic analyzes were performed, mean arterial pressure was measured from the carotid artery, and blood and tissue samples were obtained for histopathological and biochemical analyses after trauma-induced CC.

RESULTS: While the total oxidant status and disulfide parameters in the cardiac tissue and serum were significantly higher ($p<0.05$), the total antioxidant status, total thiol, and native thiol parameters were significantly lower ($p<0.01$) in rats with trauma-induced CC. The most frequently observed finding in the electrocardiography analyze was ST elevation.

CONCLUSION: According to evaluation based on histological, biochemical, and electrocardiographic examinations, we believe that only 400 mg/kg dose of AMI or DXM can be effective in the treatment of myocardial contusion in rats. Evaluation based on histological findings.

Keywords: Amifostine; cardiac contusion; trauma.

INTRODUCTION

Cardiac traumas developing with thoracic traumas are important because they can cause life-threatening complications. Cardiac contusion (CC) is a potentially deadly complication of blunt thoracic trauma.[1] The rate of blunt cardiac injuries after thoracic traumas has been reported in the range of 5–50%.[2,3] Cardiac and major vascular injuries occurring in both blunt and penetrating trauma cases result in death mostly before patients reach the hospital and within minutes or hours among patients who can reach the hospital.

CC, which causes cardiac injury following blunt thoracic trauma, can lead to the disruption of hemodynamic flow and arrhythmias by causing injuries such as hematomas, ventricular ruptures, ventricular septal defects, vascular lesions, thrombo-

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sis, vasoconstriction, and valve defects.[4] On the other hand, although several methods are used in the accurate diagnosis of CC s forming as a consequence of blunt thoracic traumas today, definitive diagnostic methods have not been determined yet.[5] The most frequently used diagnostic methods may be listed as physical examination, electrocardiography (ECG), examining cardiac enzyme levels, echocardiography (ECHO), scintigraphy, and radionuclide angiography. ECG, which is vitally important for diagnosis, is an imaging method that is also used at the follow-up stage of the disease. The follow-ups of the disease are carried out by ECG and ECHO tests. ST elevation and inverted T waves are among the frequently encountered ECG findings. The identification of cardiac injury and fast intervention is crucial. Mainly supplementary treatments, fluid replacement, and inotropic and antiarrhythmic drugs are used at the clinic in the treatment of CC.

Although there are protocols applied in such emergency cardiac trauma cases, a specific agent against CC has not been developed yet.[6] Therefore, studies designed with histopathological and molecular methods may contribute to the development of effective drugs in the treatment of CC. It is known that amifostine (AMI), which is an organic thiophosphate, shows a protective effect by repairing the DNA damage caused by free oxygen radicals and scavenging free radicals.[7] Corticosteroids are also known to have beneficial effects such as reducing tissue edema and inflammatory response.[8]

Therefore, it was aimed to investigate whether two different doses of AMI, which reduces the cumulative tissue toxicity caused by cisplatin in patients with advanced cancer, and dexamethasone (DXM), a frequently used corticosteroid in traumas, have a mitigating effect on the pathologic changes associated with CC.

MATERIALS AND METHODS

Animals

Forty-two male Sprague-Dawley rats weighing 370–400 g each were obtained from the Experimental Research Centre of the Faculty of Medicine at Kahramanmaras Sutcu Imam University (Kahramanmaras, Turkey). To facilitate the habituation of the animals to their environment, they were kept in a room at a constant temperature of 21–22°C with a light/dark cycle of 12–12 h for 7 days, and they had ad libitum access to food. The design and experimental procedures of the study were approved by the Faculty of Medicine Ethics Committee of Kahramanmaras Sutcu Imam University (Approved no: 2021/04). All experimental procedures carried out with the animals were in strict compliance with the principles of the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes and the National Institutes of Health Policy on the Humane Care and Use of Laboratory Animals.

Allocation of the Groups in the Trial

The 42 rats were randomly divided into six groups that included seven rats each. The groups were designed as explained below.

- Control (C) Group: Normal saline was intraperitoneally (i.p.) injected at a single dose of 1 mL
- CC Group: CC was induced
- CC+AMI 400 Group: AMI was administered intraperitoneally at a dose of 400 mg/kg 45 min after inducing CC[9,10]
- CC+AMI 200 Group: AMI was administered intraperitoneally at a dose of 200 mg/kg 45 min after inducing CC[11]
- CC+AMI+DXM Group: 10 mg/kg DXM was administered intraperitoneally in addition to 400 mg/kg AMI 45 min after inducing CC
- CC+DXM Group: DXM was administered intraperitoneally at a dose of 10 mg/kg 45 min after inducing CC.

Blunt Cardiac Trauma

CC was induced based on the model used in the study conducted by Raghavendran et al.[12,13] This model involves dropping a cylindrical mass at a certain weight (400 g) vertically from a certain height (50 cm) in a hollow cylindrical pipe directly onto the chest. The impact energy created with this procedure is calculated with the formula E=mgh (E: energy; g: Gravity (9.8 m/s2); h: Height from the platform (50 cm); m: Mass of the cylindrical object (0.40 kg)). The total energy transferred to the thoracic wall of a rat was calculated as 1.96 J.

After inducing contusion, all groups were observed in their cages for 6 h. At the end of this observation period, the rats were given O2 to breathe for 5 min. Then, midsternotomy was performed on all animals in all groups, and blood and cardiac tissue samples were collected.

CT Imaging and Electrocardiographic Analysis

Images were obtained with the Somatom Definition Flash dual-source spiral computed tomography (CT) device (Siemens Healthcare, Forchheim, Germany). The scanning parameters were as follows: 120 kV; 250 mAs: Rotation time, 0.35 s; step 1.5. Images were reconstructed at a slice thickness of 1 mm using a high-frequency reconstruction algorithm.

Electrocardiogram alterations were made using a data acquisition system (BIOPAC MP36 Systems, Santa Barbara, CA, USA [United States of America]).

Blood Pressure Measurement

The arterial blood pressure values of the rats were measured as previously described.[14] In this process, a cannula was inserted into the carotid artery of the rats under anesthesia. The mean carotid artery pressure was then measured with this arterial cannula connected to a pressure transducer (May.

com; Commat Ltd., Ankara, Turkey) coupled with a hemodynamic recorder (BIOPAC MP35 System Inc., Santa Barbara, CA, USA).

Arterial Blood Gas Measurements

Blood samples were collected from the descending aorta in a heparinized syringe during midsternotomy performed 6 h after blunt trauma. Analysis was performed using a blood gas analyser (Medica Easystat/USA) to assess and compare the effects of the drugs administered in the early period of blunt injury on pulmonary shunting.

Histopathological Examination and Scoring

Heart tissues were fixed with 10% buffered formalin and then embedded in paraffin, and the hematoxylin and eosin staining procedure was applied. All serial tissues were examined under a microscope (Olympus, BX51, Japan), and histopathological changes were scored as follows: normal epithelium without degeneration, edema, or signs of inflammation, necrosis, or bleeding (0); mild degeneration, edema, and inflammation, no bleeding or necrosis (1); moderate edema, necrosis, and inflammation, flattening of epithelium and regeneration, degeneration, and mild bleeding (2); and severe degeneration, severe edema, and inflammation, necrosis, extensive ulceration, and bleeding (3).

Biochemical Assay

All tissue samples were washed with 0.9% NaCl to remove the haematoma and then dried. The samples were stored in plastic bottles at −20°C until the biochemical analyses. The TNF-α levels of the rats were determined using the ELISA (enzyme-linked immunosorbent assay) method with a commercial kit (Shanghai Coon Koon Biotech), an automated ELISA microplate reader (Thermo Scientific, Finland), and a computer program (Skanlt for Multiscan FC 2.5). The sensitivity of the test was 1.0 pg/mL, and its detection range was 10–320 pg/mL. The intratest CV value was <7%, and the intertest CV value was <10%. The results were recorded in units of pg/mL. The serum thiol disulfide measurements were made according to the method developed by Erel and Neşelioğlu using an autoanalyser (Roche, cobas 501, Mannheim, Germany). In this method, the disulfide bonds in the sample were transformed into functional thiol bonds by NaBH4. The total thiol (TT) content of the sample was calculated using the Ellman method. Serum disulphide concentrations were determined using the formula: (serum TT-serum native thiol [NT])/2.[15]

The total antioxidant status (TAS) levels of the cardiac tissue homogenates and serum samples were determined using a Rel Assay brand commercial kit (Rel Assay Kit Diagnostics, Turkey, REF. No: RL0017, LOT No: JE 14042 A). The results are presented as mmol Trolox equiv./L. The total oxidant status (TOS) levels of the samples were determined using a Rel Assay brand commercial kit (Rel Assay Kit Diagnostics, Turkey, REF. No: RL0024, LOT No: JE 14048Og). The results are presented as μmol H2O2 equiv./L.[14] The OSI value, which is expressed as the ratio of TOS levels to TAS levels, was calculated. The results are presented as "arbitrary units" (AU).

Statistical Analysis

To calculate the sample size of animals required for the study, the G*Power 3.1 program (Germany) was used. The sample size suitable for the study was calculated by taking the significance level of 0.5, the power of 0.80, and the effect size of 0.80.[16] In the G*Power 3.1 analysis, based on the mean values obtained from the previous studies,[6] a power estimation of 86.18% was found. Thus, it was sufficient to select a total of 42 rats, including seven rats for each group. The data that were obtained were analyzed using GraphPad Prism version 5 (GraphPad Software Inc., San Diego, CA, USA). A one-way analysis of variance (ANOVA) test was performed, followed by Bonferroni and Tukey's multiple comparison tests to determine the differences between the six groups. The data are presented as mean±standard error. Values are given as mean and 95% confidence interval. P<0.05 were considered statistically significant.

RESULTS

Macroscopic and Microscopic Histopathological Findings

In the macroscopic examinations of the heart tissues in all groups in which trauma was induced, superficial hemorrhages

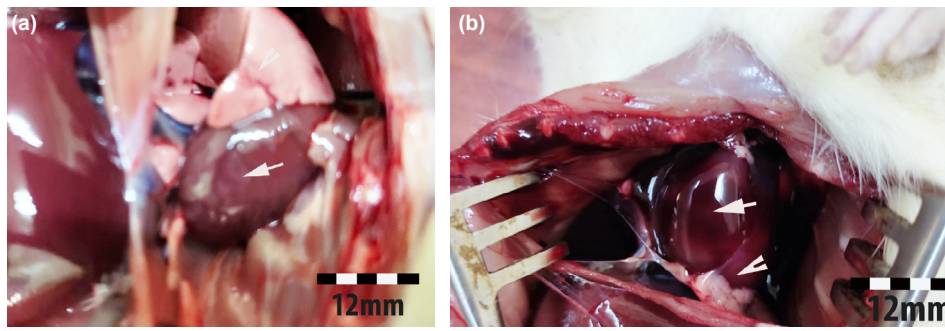

Figure 1. Macroscopic images of control **(a)** and cardiac contusion rat **(b)** groups 6 h after trauma (Arrow: Heart, arrowhead: Lung).

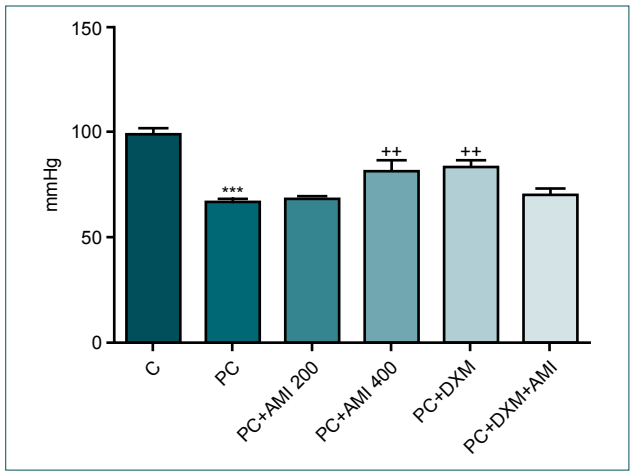

Figure 2. Effects of amifostine on the blood pressure. The statistical comparison among groups was conducted using one-way analysis of variance (ANOVA) (F=24.37, p<0001 for Blood pressure. Blood pressure were compared in all groups; **p<0.001 Significant compared Control (C) to Cardiac Contusion rat (CC), ++p<0.01 significant compared CC to treatment groups rat (one-way ANOVA and Bonferroni post hoc contrast, p<0.05).

on the heart, increased pericardial fluid levels, and hemorrhage-related color changes in the pericardial fluid were observed. It was seen that the severity of the bleeding sites in the treatment groups decreased, and there was a lightening of color in the pericardial fluid (Fig. 1).

In the examination performed using CT, typical trauma signs were found in the post-traumatic CC group. However, in the CT imaging examination performed after the treatment, findings similar to the CC group were observed in all treatment groups (Fig. 2).

In the macroscopic examinations, it was determined that the pericardial fluid was darker and had higher volumes in all trauma and treatment groups in comparison to the control. In the observation made 6 h later, occasional decreases in the amount of the pericardial fluid and lightening of its color were determined. Nevertheless, pericardial rupture was not encountered in any of the groups. In the microscopic examination, there was no anomaly in the rats in the control group. There were no degenerative or necrotic cells among their

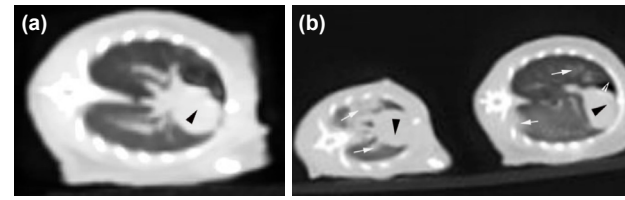

Figure 3. Macroscopic computed tomography images of control **(a)** and cardiac contusion rat **(b)** groups 6 h after trauma (black arrow head; heart, white arrow head; Pneumothorax, white arrow head.

cardiac tissue cells. Degeneration, necrotic sites, and diffuse hemorrhages were encountered in the CC group (Fig. 3a).

Myofibrillar degeneration, necrosis, and hemorrhage were present in all treatment groups. Although histopathological scoring improved in the DXM and AMI+400 groups, there was no remarkable or significant difference between the CC and treatment groups (Table 1 and Fig. 3b).

As shown in Figure 4, there was a significant difference between the C and CC groups in the blood pressure analysis. There was also a significant difference between CC and all treatment groups, except for the CC+DXM+AMI and AMI+200 groups.

ECG Findings

Non-selective and widespread ST elevation was observed in the rats in the CC group after trauma due to CC with a damaged pericardium and this increase was statistically significant compared to the rats in the C group. In addition, ST elevation, depression, Q wave development, and ECG changes due to ischemia were observed (Table 2 and Fig. 5).

Biochemical Findings

After troponin, NT, TT, and disulfide levels were determined, the values were divided by protein levels, and the resulting values are shown in Figure 6. The TNF-α, IL-6, TAS, and TOS results are illustrated in Figure 7. In comparison to the C group, CC had significantly higher TOS and disulphide levels (p<0.05), as well as significantly lower TAS, TT, and NT levels (p<0.01). There was no significant difference between the CC+AMI 200 and CC+AMI+DXM groups and the CC

Table 1. Histopathological evaluation in heart tissue

	C	CC	CC+DXM	CC+AMI 400	CC+DXM+AMI 400	CC+AMI 200	P (ANOVA)	F
Degeneration	0.2857±0.4880	2.714***±0.4880	2.429+±0.5345	2.001±0.570	2.000±0.4880	2.571±0.5345	<0.0001	19.40
Necrosis	0.1429±0.3780	2.857***±0.3780	2.429++±0.5345	2.278+±0.5345	2.143±0.69	2.571±0.5345	<0.0001	25.47
Hemorrhage	0.1429±0.3780	3.00±0.4880	2.714±0.4880	2.143±0.3780	2.490±0.8165	2.429±0.7868	<0.0001	24.91

C: Control; CC: Cardiac contusion; DXM: Dexamethasone; AMI400: Amifostine 400 mg/kg; AMI200: Amifostine 200 mg/kg. Data are expressed as mean±SD. One-way ANOVA was used at 0.05 level of significance. ***P<0.001 compared to the control group, +p<0.01, ++p<0.001 for AMI- or DXM-treated groups (CC+AMI400, CC+DXM+AMI400, CC+AMI200) compared to the CC groups (one-way ANOVA and Tukey post hoc contrast, p<0.05).

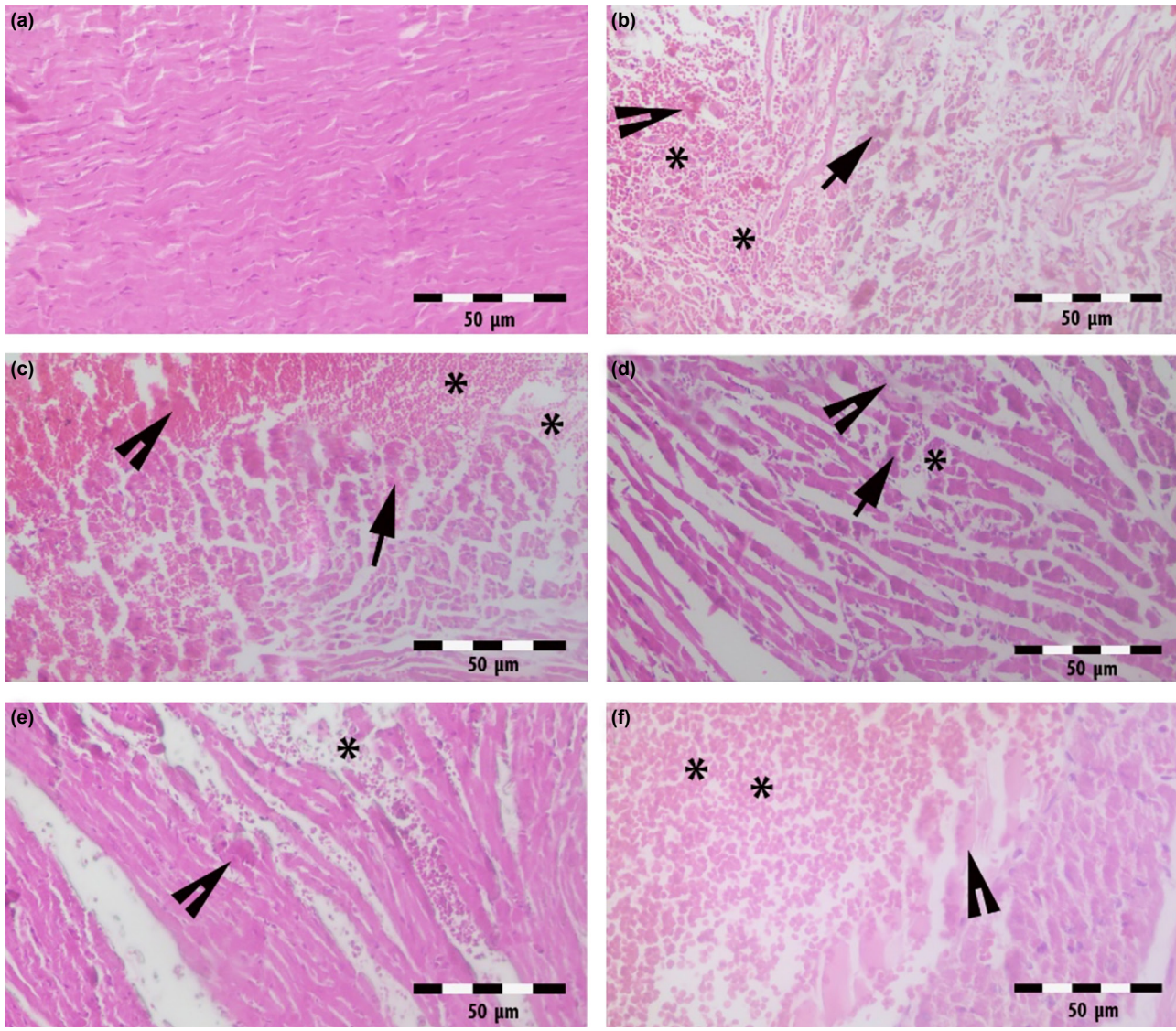

Figure 4. Microscopic images of heart rat control **(a)** and cardiac contusion rat (CC); **(b)**, CC+AMI200 **(c)**, CC+AMI400 **(d)**, CC+DXM **(e)** and CC+AMI400+DXM **(f)** 6 h after trauma (H-E x20) hemorrhage; *, degeneration; arrow, necrosis; arrow had.

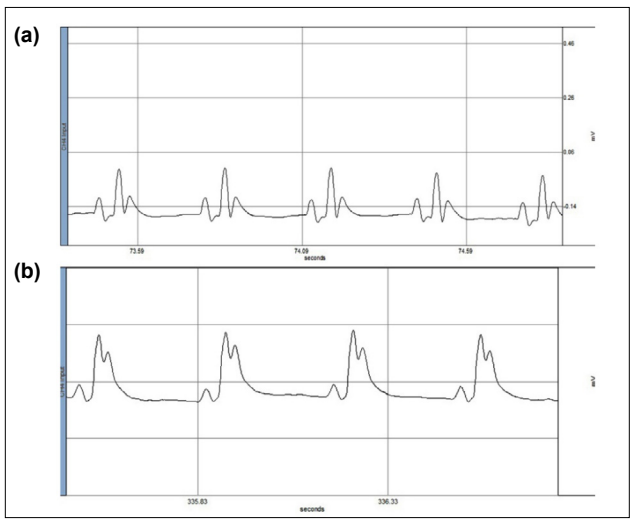

Figure 5. Electrocardiograms of the rats in control **(a)** and computed tomography **(b)** 6 h after trauma.

group (p>0.05). However, in the comparison of the CC group and the treatment groups, which were the CC+AMI 400 and CC+DXM groups, TOS and disulfide levels in the tissue samples were found to be significantly lower (p<0.01), while the TT and NT levels were significantly higher. The serum TNF-α, IL-6, and troponin levels in the CC group were significantly higher than those in the C group. Moreover, there were statistically significant differences between the CC group and all treatment groups after the treatment.

DISCUSSION

Although the heart is well-protected inside the thorax, blunt cardiac trauma is encountered in 15% of transportation vehicle accident deaths.[17] Blunt cardiac trauma can result from motor vehicle collisions, explosions, falls, crush injuries, or cardiovascular resuscitation.[18] Especially, the presence of a rib fracture or sternal fracture at the anterior of the thoracic

Table 2. Effects of Dexamethasone, Amifostine, and their combination on rats with pulmonary contusion ECG alterations

	C	CC	CC+DXM	CC+AMI 400	CC+DXM+AMI 400	CC+AMI 200	P (ANOVA)	F
P wave duration (ms)	0.039±0.003	0.038±0.003	0.036±0.003	0.038±0.003	0.040±0.004	0.040±0.007	0.5165	0.864
P-R interval duration (ms)	0.047±0.004	0.043±0.003	0.048±0.004	0.050±0.004	0.053±0.003	0.046±0.003	<0.0001	14.26
QRS complex duration (ms)	0.035±0.004	0.036±0.005	0.033±0.004	0.033±0.007	0.033±0.005	0.035±0.003	0.0625	2.377
QT interval duration (ms)	0.121±0.022	0.141±0.019	0.120±0.013	0.138±0.025	0.145±0.013	0.135±0.023	0.0057	4.121
QRS amplitude (mV)	0.220±0.041	0.210±0.050	0.278±0.062	0.360±0.055^{++}	0.345±0.045^{+}	0.331±0.051^{+}	0.0080	3.864
Heart rate (per min.)	180.0±8.295	200.0±18.63	193.2±14.78	136.7±4.502^{++}	134.3±22.03^{++}	159.0±17.13^{+}	<0.0001	10.64
ST-segment elevation (mV)	0.011±0.003	0.084±0.009*	0.040±0.006^{++}	0.053±0.016^{+}	0.051±0.020^{+}	0.060±0.020	0.0326	3.123

C: Control; CC: Cardiac contusion; DXM: Dexamethasone; AMI400: Amifostine 400 mg/kg; AMI200: Amifostine 200 mg/kg. Values are expressed as mean±S.D. One-way ANOVA was used at 0.05 level of significance. *P<0.05 compared to the control group, $^{+}$p<0.01; $^{++}$p<0.001 for AMI- or DXM-treated groups (CC+AMI400, CC+DXM+AMI400, CC+AMI200) compared to the CC group (one-way ANOVA and Tukey post hoc contrast, p<0.05).

wall of the patient, the form of injury, ecchymosis in the thoracic wall, seatbelt and steering wheel marks, and the severity of injury may indicate accompanying cardiac injury. The diagnosis of cardiac damage and prompt intervention is crucial. In the treatment of CC at the clinic, mainly supportive treatments, fluid replacement, and inotropic and antiarrhythmic drugs are used. A specific agent against CC has not been developed yet.[19] As in this study, histopathological and molecular studies may contribute to the development of effective drugs in the treatment of CC cases. In this study, which was planned with AMI and DXM, the drug doses were adjusted based on the previous animal experiments.[20] The possibility of accompanying cardiac injury must be kept in mind in high-impact injuries. In these cases, injuries can be seen in the myocardium, coronary arteries, cardiac valves, and pericardium. As a consequence of this trauma, CC, laceration, rupture, interventricular septal defects (traumatic VSD), and traumatic aneurysm in the ventricles can occur, while lacerations and/or thrombosis can be seen in the coronary arteries.[21–25] The trauma model in this study was created by considering a previous study as a basis, and no contusion-related interventricular septal defect, traumatic aneurysm in the ventricles, atrial rupture, or ventricular rupture was identified.[26] On the other hand, structural dysfunction associated with the magnitude of the trauma was observed in the myocardium. In blunt traumas, especially crushing types, left atrial rupture occurs, or contusion may develop in the ventricular myocardium. Depending on its severity, this contusion can affect all layers of the heart or only a part of the myocardium.[27] Injury covering a broad area from subepicardial petechia to the full-thickness injury of the ventricular wall may be encountered. In this study, no mortality was seen in the rats in which trauma was inflicted, whereas there were superficial bleeding sites on the myocardium in their hearts.

A contused myocardium has different electrical characteristics compared to a normal myocardium.[28] Acid-base imbalance and changing serum potassium and calcium levels that can be encountered with contusion also affect the electrical activity of the heart negatively. Thus, tachyarrhythmias, ventricular ectopic beats, bradyarrhythmia, atrial fibrillation, atrial flutter, blocks, or all types of rhythm disorders can be seen in a shorter time in patients with CC, within 24–48 h after trauma.[29] In this study, it was observed that the heart rate was higher in the CC group than in the C group, in the measurements performed 6 h after the trauma. In addition to this, in the groups treated with the 400 and 200 mg/kg doses of AMI, the heart rates were lower than those in the CC group.

To exclude underlying cardiac injuries or injuries of the major blood vessels in the thorax in haemodynamically stable trauma patients, diagnostic tools such as ECHO, CT, and magnetic resonance imaging (MRI) can be utilized.[30] After the vital functions of polytrauma patients are stabilized, the most reliable diagnostic method is CT. With CT, the pleural fluid, alveolar bleeding, rib fractures, pulmonary contusion and laceration, mediastinal pathologies, pneumothorax, cardiac tamponade, and major vascular injuries can be examined.[31] To understand the thorax damage of the trauma model applied on rats in this study, all groups were scanned with

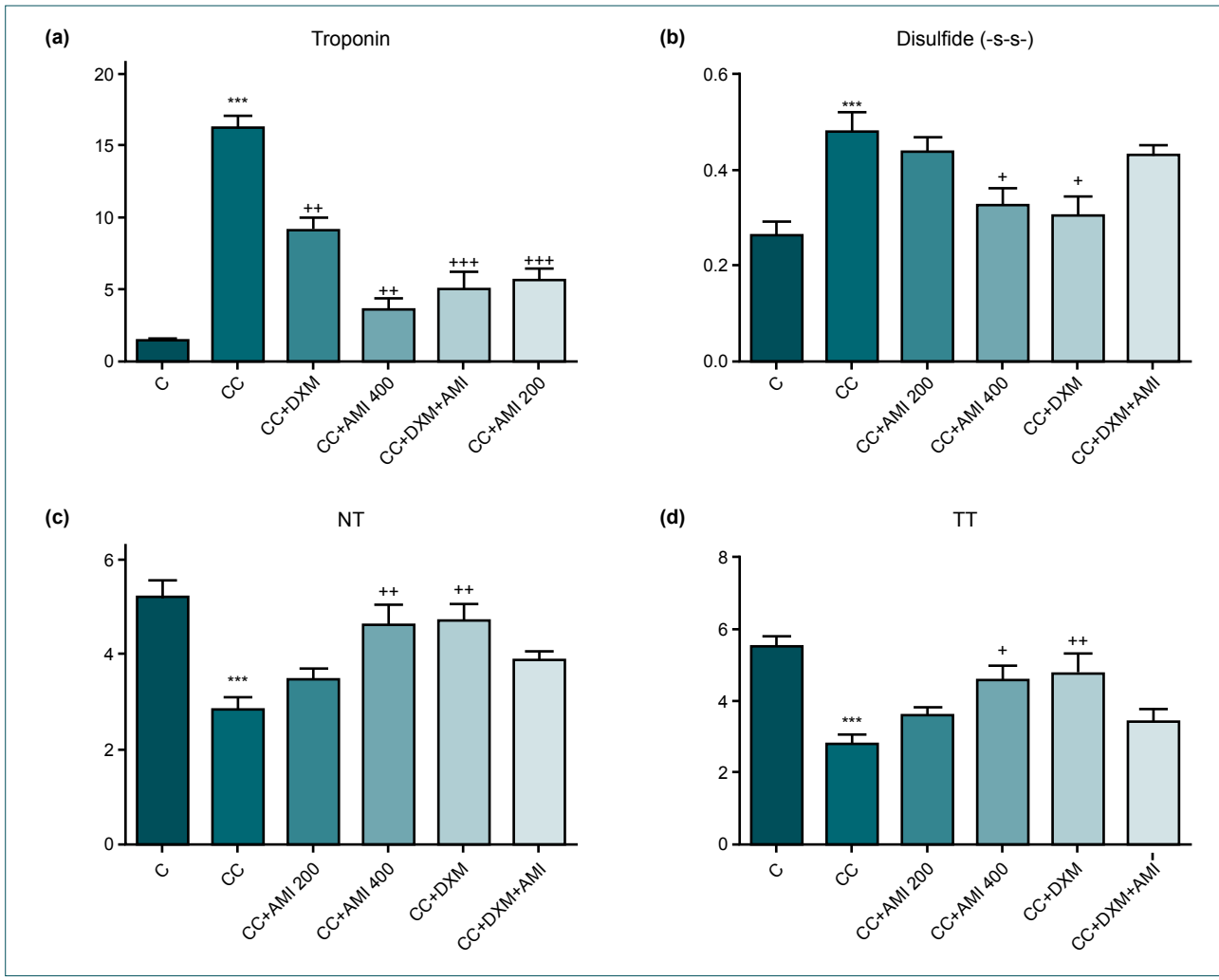

Figure 6. Effects of amifostine on serum Troponin **(a)** Disulphide **(b)** natural thiol **(c)**, and total thiol **(d)** 6 h after cardiac trauma The statistical comparison among groups was conducted using one-way analysis of variance (ANOVA) (F=40.81, p<0001 for Troponin; F=40.81; p<0001 for disulfide; F=8.368, p<0001 for NT and F=7.634, p<0001 for TT). ***P<0.001 significant compared to the control group and $^{+}$p<0.05, $^{++}$p<0.01, p<0.001 (one-way ANOVA and Bonferroni post hoc contrast, p<0.05).

CT. Although pathological changes related to trauma were observed in the thorax cavity after trauma in the evaluations, it was determined that these changes did not change after the treatment. This may be caused by the insufficient time after treatment to be seen in the CT scan. Therefore, methods that can detect damage at the cellular level were used.

The presence of contusion can be detected most accurately based on cardiac troponin levels.[32–35] Troponins that are not secreted by the skeletal muscles have high specificity in the diagnosis of cardiac injury. Troponin-I is found at high concentrations in patients with cardiac injuries.[36] It has been reported that in patients with high troponin levels among those who have contusion developing as a result of blunt cardiac trauma, the risk of adverse event development in the hospital and after discharge may increase.[37,38] This is why troponins are highly valuable parameters in the assessment of CC.[39,40] In support of this important pathognomonic marker, in this study, one of our important findings was the observation of lower troponin levels in the CC+DXM, CC+AMI400, and CC+AMI+DXM400 treatment groups in comparison to the CC group. Moreover, in the identification of heart-related complications developing as a result of blunt cardiac trauma, in addition to troponins, ECG changes are also highly important.[41] Following CC, non-selective and diffuse ST elevation can be observed due to the injury of the pericardium. In addition, as a result of injury in the myocardium, it is possible to observe ECG changes such as ischemic ST elevation or depression, as well as abnormal Q waves. Other potential ECG changes depending on the severity of contusion are bundle branch blocks, bradycardic events such as atrioventricular blocks at varying degrees, and tachycardic events ranging from sinus tachycardia to ventricular fibrillation.[42]

It was reported that even if ECG changes do not occur in the early period, CC can cause fatal cases of arrhythmia.[43] Therefore, the early detection of patients who have developed cardiac injuries in this patient group, their close obser-

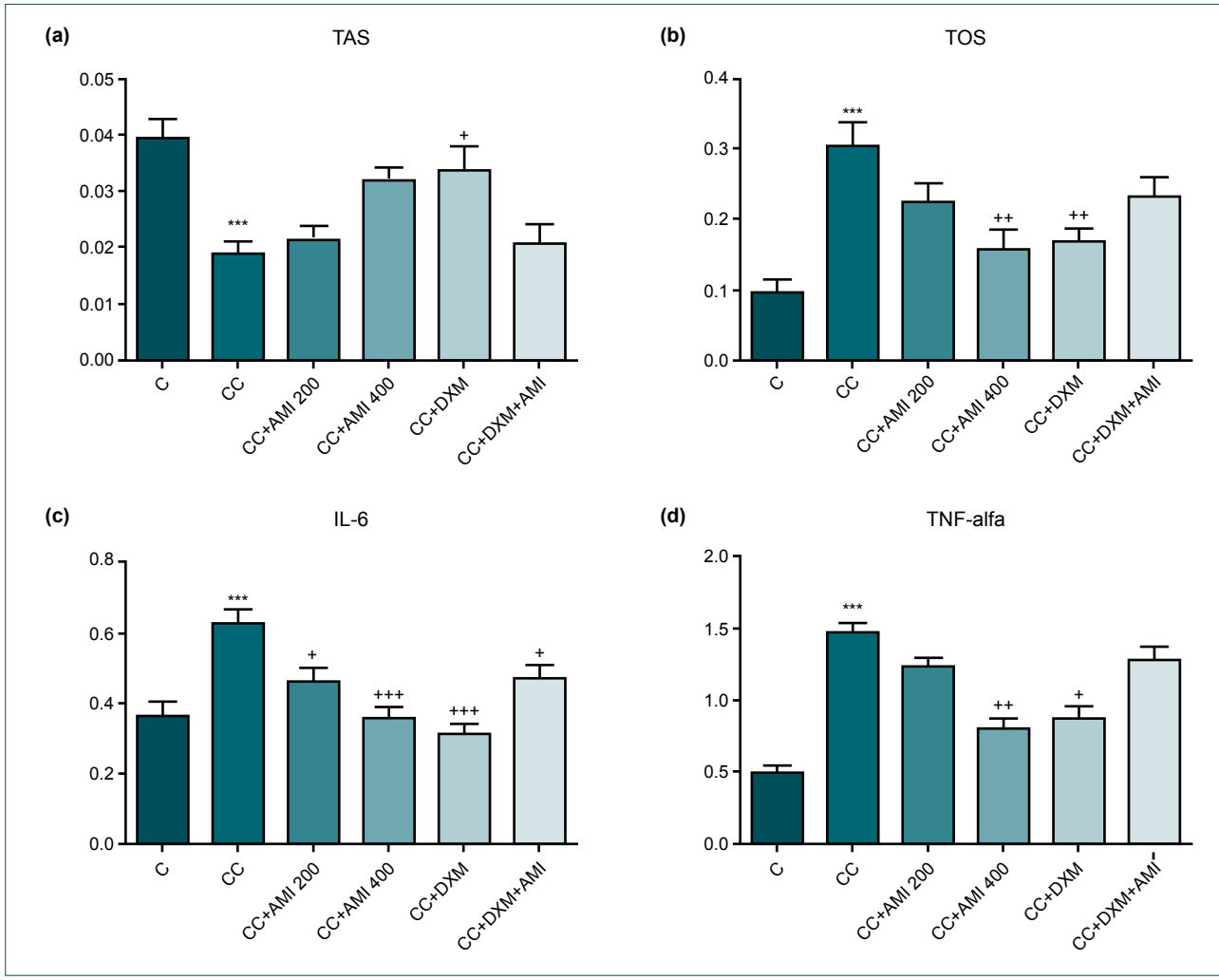

Figure 7. Effects of AMI on heart tissue TAS **(a)**, TOS **(b)**, IL-6 **(c)**, and TNF-alfa **(d)** 6 h after cardiac trauma. The statistical comparison among groups was conducted using one-way ANOVA (F=7.380, p<0001 for TAS; F=8.290, p<0001 for TOS; F=10.59, p<0001 for IL-6 and F=24.71, p<0001 for TNF-α). ***P<0.001 significant compared to the control group and $^{+}$p<0.05, $^{++}$p<0.01, $^{+++}$p<0.001 significant compared to the cardiac contusion group (one-way ANOVA and Bonferroni or Tukey post hoc contrast, p<0.05). AMI: Amifostine, TAS: Total antioxidant status, ANOVA: Analysis of variance.

vation, and the administration of treatments that can reduce the severity of the cardiac injury may have beneficial effects against mortality and morbidity. In our study, post-traumatic tachycardic response and ST elevation were the main ECG changes that we observed. The CC+DXM, CC+AMI400, and CC+AMI+DXM400 treatment groups showed reduced heart rates and ST segment resolution. A low QRS amplitude is one of the main ECG findings that can be observed in cardiac infarction patients.[44] In this study, an increase was seen in the QRS amplitudes of the CC+DXM, CC+AMI400, and CC+AMI+DXM400 treatment groups which may be an effect of the treatment. These results in our study showed that combining troponin testing with ECG in patients with suspected contusion may provide clinical benefits in the identification of contusion and for distinguishing patients with a high risk of cardiac complications.

Several different mediators are released in the organism as a result of cardiac injury. Recent studies in humans[45] and experimental animals[46] have shown that cytokines and chemokines such as TNF-α and IL-6 increase after trauma-induced injury. It is an important and reliable indicator that IL-6 and TNF-α measurement in the Ist h of the trauma helps in determining the severity of the trauma. However, measurements after 6 h were found to be clinically insignificant.[47,48]

After the trauma that was induced in the subjects in this study, increases were observed in their cardiac tissue and serum TNF-α and IL-6 values. In parallel with the rise in troponin values, the increases in pro-inflammatory markers (TNF-α and IL-6) also proved that the trauma model in this study was implemented correctly. Following the treatment, a significant drop was observed in TNF-α and IL-6 values, especially in the CC+AMI400 group.

It is well-known that superoxide radicals (SORs) cause oxi-

dative stress in trauma patients. These free radicals lead to reversible or irreversible damage to biomolecules. There is strong evidence that SORs play a key role in the injury occurring in acute traumas, and they initiate tissue injury by causing lipid peroxidation.[49] The causes of this imbalance include not only the excess production of SORs but also their insufficient elimination by antioxidant mechanisms. In this case, various cell and tissue structures are damaged. The antioxidant system has a protective effect against oxidative stress. TAS shows the total effects of all antioxidants in the plasma, while TOS shows the total effects of all oxidants.[50–52]

The balance between the oxidant capacity and the antioxidant capacity on the cellular level is crucial for cellular functions. To investigate the severity of injuries or therapeutic efficacy, many researchers have utilized the TOS and TAS parameters.[53–56] The degree of oxidative damage can be lowered with the help of free radical scavenging systems. Thiol molecules constitute an important part of this antioxidant system in the body. They have a critical role in preventing oxidative stress in cells. Thiols are organic compounds that contain sulfhydryl groups (-SH) and can react with free radicals against the harmful effects of reactive oxygen species.[57] Based on the trauma that was induced in this study, in comparison to the control group, the CC group had higher TOS and disulfide levels, as well as lower TAS, TT, and NT levels. In the CC+AMI 200 and CC+AMI+DXM groups, there was no significant difference in these parameters. We think that the treatment effect of the dose in the group that was only given 200 mg/kg AMI was insufficient. Furthermore, in our opinion, in the group where AMI at 400 mg/kg and DXM were used together, the drugs suppressed each other's efficacy in the treatment of CC. In the CC+AMI400 and CC+DXM groups, there were significant decreases in the TOS and disulfide parameters ($p<0.01$), while their TT and NT parameters were significantly increased ($p<0.01$).

The lowered TOS and disulfide values and increased TT and NT values in the group that was given only 400 mg/kg AMI and the group that was given only DXM demonstrated the effectiveness of these two drugs in the treatment of CC in rats. The reduced heart rates, ST segment resolution, and increased QRS amplitudes in these groups in the ECG findings also supported the finding on the contribution of these drugs to the treatment.

Conclusion

We believe that AMI is an option that may be tested in the treatment of CC based on the biochemical and electrocardiographic evidence we present in this study. Initiating preventive treatments before secondary injury starts following trauma is highly important in terms of reducing mortality and morbidity rates.

This study is valuable as it is one of the rare studies about CC in the literature, and AMI, which was used in this study as a treatment agent, had not been tested in the treatment of CC before.

To the best of our knowledge, this study is the first study based on the biochemical and electrocardiographic evidence that reflects the protective effect of AMI against CC. AMI, also known to have a protective effect against toxicities of radiotherapy, chemotherapy, or other drugs, needs to be further studied to shed light on its potential clinical use in acute CC.

We believe that the positive contributions of AMI in the treatment of contusion in rats will guide future studies with larger series, and it will be more accurate to decide after more detailed studies for the dose adjustment of its effect on CC in humans. In light of this information, our findings also support the efficacy of AMI on the treatment of CC, but more comprehensive studies are needed on this subject.

Ethics Committee Approval: This study was approved by the Kahramanmaraş Sütçü İmam University Faculty of Medicine Animal Experiment Ethics Committee (Date: 15.09.2021, Decision No: 04).

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Authorship Contributions: Concept: A.A., F.İ.T., N.E.; Design: N.E., A.Y., A.A.; Supervision: İ.C.K., E.A., A.A.; Resource: A.E.Y., F.İ.T., A.Y.; Materials: A.E.Y., A.A.; Data: N.E., E.A., A.A.; Analysis: İ.C.K., F.İ.T., A.A.; Literature search: N.E., A.E.Y., E.A.; Writing: A.Y., E.A., A.A.; Critical revision: İ.C.K., A.Y., E.A., A.A.

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DENEYSEL ÇALIŞMA - *ÖZ*

Amifostinin sıçanlarda künt göğüs travmasına bağlı kardiyak yaralanmaya karşı etkileri

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AMAÇ: Bu çalışmada, ileri evre kanser hastalarında kullanılan bir kortikosteroid olan deksametazon (DXM) ve sisplatinin kümülatif doku toksisitesini azaltan amifostin'in (AMI), sıçanlarda oluşturulan kardiyak kontüzyon (KK) modelinde kalp ile ilişkili patolojik değişiklikler üzerinde iyileştirici etkilerinin olup olmadığının incelenmesi amaçlandı.
GEREÇ VE YÖNTEM: Toplamda kırk iki Wistar albino sıçan, eşit olarak altı gruba ayrıldı: K (Kontrol), KK, KK+AMI 400, KK+AMI 200, KK+AMI+DXM and KK+DXM. Travma sonucu oluşan kontüzyon sonrası tomografi görüntüleri ile elektrokardiyografik analizleri yapılarak karotid arterden ortalama arteryel basıncı (OAB) ölçülüp, histopatolojik ve biyokimyasal analizler için kan ve doku örnekleri alındı.
BULGULAR: Künt travma ile kalp kontüzyonunun oluşturulduğu sıçanlarda, kalp dokusu ve serumdaki toplam oksidant status (TOS) ve disülfid parametreleri anlamlı olarak yüksek ($p<0.05$), toplam antioksidant status (TAS), toplam tiyol (TT) ve native thiol (NT) parametreleri anlamlı olarak daha düşük bulundu ($p<0.01$). Elektrokardiyografi testlerinde en sık gözlenen bulgu ST yükselmesi idi.
TARTIŞMA: Histolojik, biyokimyasal ve elektrokardiyografik incelemeler sonucunda sıçanlarda miyokard kontüzyonunun tedavisinde sadece 400 mg/kg dozda amifostin veya DXM'nin etkili olabileceği kanaatindeyiz.
Anahtar sözcükler: Amifostine; kardiyak kontüzyon; travma.

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