Evaluation of the Effects of Empagliflozin on Acute Lung Injury in Rat Intestinal Ischemia-Reperfusion Model

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Abstract

Background: Empagliflozin is a selective sodium-glucose co-transporter (SGLT2) inhibitor that is accepted for the treatment of type 2 diabetes. Positive effects of empagliflozin on systems other than diabetes have been detected. The aim of this study is to evaluate the role of empagliflozin on acute lung injury induced by intestinal ischemia/ reperfusion (I/R).

Materials and methods: A total of 27 male Wistar albino rats were divided into 3 groups: sham, I/R, and I/R+ empagliflozin; each group contains 9 animals. Sham group animals underwent laparotomy without I/R injury. After I/R groups animals underwent laparotomy, 1 h of superior mesenteric artery ligation was followed by 2 h of reperfusion. In the empagliflozin group, 7 days before I/R, empagliflozin (30 mg/kg) was administered by gastric gavage. All animals were killed at the end of reperfusion and lung tissue samples were obtained for immunohistochemical staining and histopathological investigation in all groups.

Results: Serum glucose, AST, ALT, creatinine, native thiol, total thiol, and disulfide levels and disulfide–native thiol, disulfide–total thiol, and native thiol–total thiol ratios as well as the IMA levels were analyzed and compared among the groups. While intestinal I/R significantly increases serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatinine levels; Thiol/Disulfide did not cause any change in homeostasis parameters and IMA level. Empagliflozin treatment had no significant effect on biochemical parameters. Empagliflozin treatment induced a significant decrease in positive immunostaining for IL-1, IL-6, TNF-alpha, caspase 3, caspase 8, and caspase 9 compared to the I/R group in lung tissue samples. Intestinal I/R caused severe histopathological injury including edema, hemorrhage, increased thickness of the alveolar wall, and infiltration of inflammatory cells into alveolar spaces. Empagliflozin treatment significantly attenuated the severity of intestinal I/R injury.

Conclusions: It was concluded that empagliflozin treatment may have beneficial effects in acute lung injury, and therefore has potential for clinical use Keywords: empagliflozin, intestinal ischemia/ reperfusion, acute lung injury, anti-inflammatory

Introduction

Intestinal ischemia-reperfusion may occur in conditions such as ischemic colitis, acute mesenteric ischemia, septic shock, hemorrhagic or traumatic shock, small intestine transplantation, severe burns; It is a condition in which systemic inflammation and multi-organ failure play an important role in its pathogenesis [1]. Acute respiratory failure is the most important component of multi-organ dysfunction after intestinal damage, and it is an important cause of morbidity and mortality in critically ill patients [2].

Pulmonary ischemia is a rapid complex sterile inflammatory condition with reperfusion injury, edema, and defective gas exchange resulting from damage to endothelial and epithelial barriers. In ischemia-reperfusion injury, there is a strong innate immune response involving alveolar macrophages, natural killer cells, and neutrophils, as well as the release of cytokines and injury-related molecules. The majority

of these responses are led by rapidly and strongly produced reactive oxygen products. Gielis and Chatterjee showed that acute oxidative stress products occur in peripheral blood in their lung ischemia-reperfusion models in rats [3].

Modified albumin is a sensitive marker for determining oxidative stress after ischemia. The oxidant-antioxidant balance plays a critical role in vascular homeostasis. Accordingly, antioxidant mechanisms that neutralize oxidative free radicals may be critical for cell survival. Thiol/disulfide homeostasis are important antioxidant in the defense mechanism against reactive oxygen radicals [4].

Programmed cell death, including apoptosis, also plays an important role in lung tissue damage after ischemia-reperfusion. Apoptosis is regulated by enzymes involving the caspase cascade. Caspase 8 and caspase 9 are the initiator of apoptosis; caspase 6 is from the effector caspase family [5, 6].

Empagliflozin is a selective sodium-glucose co-transporter (SGLT2) inhibitor that is accepted for the treatment of type 2 diabetes. In recent studies, positive effects of empagliflozin on systems other than diabetes have been detected. Empagliflozin reduced the risk of hospitalization for heart failure and cardiovascular deaths in patients with or without type 2 diabetes (T2DM) in large-scale clinical trials. It has been shown to have renoprotective effects in chronic kidney disease [7]. It has been shown that empagliflozin reduces the production of free oxygen radicals and upregulates antioxidant defense mechanisms. The antioxidant effects of empagliflozin depend on its effects on zinc transporters, matrix metalloproteinase, and oxidative stress. In addition, in animal studies, it has been observed that empagliflozin has an anti-apoptotic effect at the lung tissue level, as well as an anti-inflammatory effect by suppressing IL-6 and TNF-alpha expression [8–10]. Caspase inhibition may reduce ischemia-reperfusion injury and help improve lung function. Studies have shown that empagliflozin reduces hepatocyte apoptosis by inhibiting caspase 8 expression in the liver; It is not known whether it has a similar effect on lung tissue [5, 6, 11]. There is no study examining the effect of empagliflozin on apoptosis by affecting caspase 9 and 6 levels in lung tissue.

In this study, we aimed to investigate the effects of empagliflozin on lung tissue and whether it reduces acute lung injury following ischemia-reperfusion (I/R) of the superior mesenteric artery in rats.

Materials And Methods

Animals Male albino Wistar albino rats (200–250 g) were used in the present study. All the animals were kept under optimum conditions (21 + 1C, 40–70% humidity, 12/12 dark–light cycle) and were fed ad libitum with a standard pellet diet and water. The experimental protocol was approved by the local ethic committee for Animal Research. The study was achieved in Ankara Training and Research Hospital animal experiment laboratory (Ankara, Turkey) by the National Laboratory Animal Use and Care Guidelines.

Experimental groups

A total of 27 male Wistar albino rats were divided into 3 groups: sham, I/R, and I/R + empagliflozin; each group contains 9 rats. Animals were pretreated with empagliflozin by gastric gavage (in a dose of 30 mg/kg body weight) for 7 days before intestinal I/R as described for I/R + empagliflozin group. In the I/R group, saline solution was given as a placebo by gastric lavage. Rats were weighed daily from the beginning of the experiment.

The Technique of intestinal I/R

Feeding of the animals was stopped 12 h before the start of the intestinal I/R procedure and they received only water. The rats were anesthetized with ketamine hydrochloride (40 mg/kg) and xylazine hydrochloride (10 mg/kg) intraperitoneally (i.p.) and their temperature was regulated using a lamp light bulb during the test. Intestinal I/R was induced as follows: the rats were placed in the supine position and secured in the dissection tray. The abdominal region was shaved and cleaned with antiseptic solutions. The intestinal region was reached using midline laparotomy. The superior mesenteric artery was subjected with care and occluded with an atraumatic microvascular clamp, thus intestinal ischemia was created in 1 h when the existence of pulseless or pale color of the intestine was recognized. The abdominal region was then closed. Following ischemia, the clamp was removed and 2 h reperfusion was induced. The return of the pulses and the reestablishment of the pink color were assumed to be due to the reperfusion of the intestine. At the end of reperfusion, blood will be drawn from the right carotid artery. The lungs of the euthanized rats were taken out and placed in formalin.

Histological analysis

To remove the fixed tissue samples from formalin, they were washed in running water overnight. Then, it was subjected to routine pathological tissue follow-up and passed through graded alcohol (50%, 75%, 96%, 100%) and xylol series and blocked in paraffin. 5 µ thick sections from the prepared blocks were taken on slides with Leica RM 2125 RT microtome (Leica, Biosystems, Nussloch, GmbH, Nussloch, Baden-Württemberg, Germany) the first three sections, and every tenth section. The prepared preparations were passed through alcohol and xylol series and stained with hematoxylin-eosin (HE). All samples were examined under a high-resolution light microscope (Olympus DP-73 camera, Olympus BX53-DIC microscope; Tokyo, Japan). The slides were examined for the presence of peribronchial inflammatory cell infiltration (PICI), alveolar septal infiltration (ASI), alveolar edema (AED), alveolar exudate (AEX), and interstitial fibrosis (IF). These changes were scored according to the 4-point scale used by Takil et al. (2003; Table 1). All the changes detected in tissues structures were noted and graded according to the presence and severity of any particular finding as in some pre- studies: 0: none, 1: mild, 2: moderate, and 3: severe. These changes were scored according to the 4-point scale used by Takil et al. 2003 (12); Table 1

Table 1
The 4-point scale was used for histopathologic assessment

	0	1	2	3
Peribronchial inflammatory cell infiltration	No	Prominent germinal centres of lymphoid follicules	Infiltration between lymphoid follicles	Confluent band-like form
Alveolar septal infiltration	No	Minimal	Moderate	Severe, impending of lumen
Alveolar edema	No	Focal	In multiple alveoli	Widespread, involving lobules
Alveolar exudate	No	Focal	In multiple alveoli	Prominent, widespread
Interstitial fibrosis	No	Focal, minimal	Focal, prominent fibrous thickening	Widespread, prominent fibrous thickening

Immunohistochemical stainings

For the immunohistochemical studies, 4-μm thick sections were obtained from the paraffin-embedded tissue blocks and placed on poly-L-lysine-coated glass slides. They were stained with the streptavidin–biotin–peroxidase complex (ABC) technique after routine deparaffinization and rehydration procedures. Antigen retrieval was performed in a microwave oven with citrate buffer (pH 6.0) (700 W, 20 min). Endogenous peroxidase activation in the tissues was blocked for 15min with 0.3% hydrogen peroxide in 0.01mol/l PBS in methanol at room temperature. Before applying the primary antibody, the tissues were incubated for 20 min with 5% normal goat serum for protein blocking. Then, the sections were incubated with caspase-3 (5 μg/mL, PA5-16335; Invitrogen, California, USA), Caspase-8 (ab4052; Abcam, USA), Caspase-9 (ab52298; Abcam, USA), TNF alpha (1:50, ab6671; Abcam, Cambridge, USA), IL-1β (1:200, sc-52012, Santa Cruz Bıotechnology, Inc. Texas, U.S.A.) and IL-6 (10 μg/mL, RPA079Ga01-Recombinant Interleukin 6; Cloud-Clone Corp. (CCC, USA)) primer antibodies for 1 h at room temperature. Sections were then reacted with a biotinylated secondary antibody for 30 min after removing the unbound primary antibody. Then, the sections were reacted with horseradish peroxidase streptavidin for 30 min. After washing with PBS, the sections were treated and incubated with DAB (3,3'-Diaminobenzidine, Dako, Glostrup, Denmark) for 5 min. Finally, the background of the tissue sections was stained with hematoxylin. For negative controls, PBS was used instead of the primary antibody. All staining steps were carried out at 37°C and in humidity cabinets. PBS solution was used as a wash-away solution during all the staining steps. Staining indexes were calculated as follows on the basis of the percentages of the

stained nuclei for these three markers; negative: 0 (< 1% positive); weak: 1 (1–25% positive); moderate: 2 (> 25–75% positive); and strong: 3 (> 75% positive).

Laboratory analysis

The samples were centrifuged at 3000 rpm for 10 minutes to separate the plasma and serum. Serum was immediately frozen and stored at 80 C until the analysis was performed for the Thiol/Disulfide homeostasis and IMA. Serum levels of native and total thiol and the ratio of disulfide to native and total thiol were measured by using a simple novel fully automated colorimetric method in the same manner as the method developed by Erel and Neselioglu (12). In this method, the reducible dynamic disulfide bonds (–S–S–) were reduced to functional thiol groups (–SH) using NaBH4. Following this procedure, the residual NaBH4 materials were entirely removed from the biochemical environment with formaldehyde. Ellman's reagent was used to measure the amount of total thiol in the sample. The dynamic disulfide content was calculated by taking half of the difference between the amount of total thiol and native thiol. In this way, the native thiol, total thiol, and disulfide levels were measured, and the percentage of disulfide–native thiol, disulfide–total thiol, and native thiol–total thiol were calculated in all subjects. Serum IMA levels were measured by using the colorimetric assay method previously described by Bar-Or et al. (14). This colorimetric method is based on the biochemical properties of albumin to bind exogenous cobalt. In brief, 200 lL of a subject serum was added to 50 lL of 0.1% cobalt II chloride (CoCl2, 6H2O) (Sigma-Aldrich Chemie GmbH Riedstrasse 2, Steinheim, Germany) followed by mixing and 10 minutes of incubation in the dark at 37 C to allow for cobalt albumin cobalt binding. Then, a total of 50 lL dithiothreitol (DTT) were added as a coloring agent. After 2 minutes of incubation, 1 mL of 0.9 sodium chloride was added to reduce the binding capacity. The blank was prepared similarly with distilled water instead of DTT. The absorbance of samples was measured at 470 nm using a spectrophotometer (Jenway 6315 UV/visible Scanning Spectrophotometers, United Kingdom). IMA results were expressed in absorbance units (ABSUs). Each sample was measured in duplicate and the mean value was reported.

Statistical analysis

Shapiro-Wilk test was used to understand that the distribution was normal. Normally distributed data were reported using mean ± standard deviation (SD) and non-normally distributed data using descriptive statistical methods such as median (min-max). $\chi 2$ or Fisher's exact test was used to compare categorical variables. A comparison of continuous variables was performed using One Way Anova for normally distributed data and Kruskal Wallis test for non-normally distributed data. The differences between the continuous variables were evaluated by a two-tailed t-test. All statistical analyzes were done using SPSS, v.26 program. $p < 0.05$ value was considered statistically significant.

Results

Biochemical findings

All rats survived until the end of the study period. Demographics and laboratory characteristics of participants and statistical differences of these variables are shown in Table 2. There was no significant difference between the groups in terms of first and final weights and serum glucose levels of the rats ($p = 0.84$, $p = 0.23$, $p = 0.11$, respectively). Intestinal I/R significantly increased the serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatinine levels ($p < 0.001$, $p < 0.001$, $p < 0.009$, respectively) when compared to the sham group. There was no statistically significant difference between the I/R + empagliflozin group and the I/R group in terms of these laboratory variables ($p = 0.89$, $p = 0.54$, $p = 0.06$). Comparison of Thiol/Disulfide homeostasis parameters and IMA levels among the groups were similar in all groups ($p > 0.05$). Data is shown in Table 3.

Table 2
Demographics and laboratory characteristics of participants

	Sham (n = 9)	I/R group (n = 9)	I/R + Empa group (n = 9)	p
First weight (gram)	229.11 ± 6.86	230.88 ± 6.25	230.44 ± 7.17	0.84
Final weight (gram)	269.88 ± 24.20	279.11 ± 28.33	297.33 ± 44.86	0.23
Serum Glucose (mg/dl)	309.11 ± 120.44	469.44 ± 226.62	371.66 ± 89.71	0.11
AST (U/L)	95.80 (73.70-214.80)	1000.00 (460.60–1000.00)	1000.00 (134.30–1000.00)	**< 0.001** **< 0.001[a]**, **<0.001[b]**,0.89[c]
ALT (U/L)	58.00 (50.00-145.00)	1000.00 (391.00-1000.00)	1000.00 (1000.00-1000.00)	**< 0.001** **< 0.001[a]**, **< 0.001[b]**,0.54[c]
Creatinine (mg/dl)	0.39 (0.32–0.78)	0.91 (0.46–1.18)	1.23 (1.04–1.34)	**< 0.001** **0.009[a]**,**<0.001[b]**,0.06[c]

AST: Aspartate transaminase, ALT: Alanine transaminase. I/R: Ischemia-reperfusion, I/R + Empa: Ischemia-reperfusion + Empagliflozin.

Data with a normal distribution (glucose, first weight, final weight) were presented as mean ± SD, and data without normal distribution (AST, ALT, creatinine) were presented as median (min-max). A comparison of continuous variables was performed using One Way Anova for normally distributed data and Kruskal Wallis test for non-normally distributed data.

[a]Significance comparison between sham and I/R group.

[b]Significance comparison between sham and I/R + Empa group.

[c]Significance comparison between I/R group and I/R + Empa group.

Table 3
Comparison of Thiol/Disulfide homeostasis parameters and IMA levels among the Groups

	Sham (n = 9)	I/R group (n = 9)	I/R + Empa group (n = 9)	p
Native thiol (µmol/L)	330.12 ± 58.82	298.70 ± 99.17	240.40 ± 81.93	0.08
Total thiol (µmol/L)	358.62 ± 63.45	322.41 ± 111.44	275.60 ± 84.25	0.15
Disulfide (µmol/L)	14.00 (7.95–20.60)	11.15 (0.80–32.40)	18.20 (2.50–41.00)	0.31
Disulfide/Native thiol (%)	4.40 (2.80–5.80)	3.20 (0.40–7.50)	6.60 (2.30–24.30)	0.13
Disulfide/Total thiol (%)	4.00 (2.70–5.20)	3.00 (0.40–6.50)	5.80 (2.20–16.40)	0.13
Native thiol/Total thiol (%)	91.90 (89.60–94.60)	94.00 (86.90–99.20)	88.40 (67.30–95.60)	0.13
IMA (ABSU)	1.09 (1.04–1.16)	1.12 (1.04–1.17)	1.14 (0.69–1.20)	0.30

IMA: Ischemia modified albumin. I/R: Ischemia-reperfusion ve I/R + Empa: Ischemia-reperfusion + Empagliflozin

Data with a normal distribution (native thiol, total thiol) were presented as mean ± SD, and data without normal distribution (disulfide, disulfide/native thiol, disulfide/total thiol, native thiol/total thiol, IMA) were presented as median (min-max). A comparison of continuous variables was performed using One Way Anova for normally distributed data and Kruskal Wallis test for non-normally distributed data.

Histopathologic findings

The histopathological examinations of the study groups stained with hematoxylin and eosin are shown in Fig. 1. Intestinal I/R resulted in the characteristic features of pulmonary injury, whereas rats in the sham group showed normal lung architecture (Fig. 1a). In the intestinal ischemia-reperfusion group, severe edema in the lung tissue, alveolar hemorrhage, an increase in alveolar wall thickness, many inflammatory cells infiltrating the interstitium and alveoli, and fibrosis were observed (Fig. 1b). The pathological damage was apparently less in the empagliflozin pretreatment group in comparison to the I/R group. The interstitium of the lungs appeared thinner and the number of inflammatory cells and fibrosis is apparently reduced (Fig. 1c). Histopathological results of study groups are presented in Table 4. Histopathological parameters including PICI, ASI, AED, AEX and IF were decreased significantly in all treated intestinal I/R with empagliflozin groups compared to untreated groups ($p < 0.001$).

Table 4
Histopathological evaluation of pulmonary tissue between groups

	Sham (n = 9)	I/R group (n = 9)	I/R + Empa group (n = 9)	p
PICI	0.00 (0.00–0.00)	3.00 (3.00–3.00)	0.00 (0.00–1.00)	**< 0.001** **< 0.001**[a],0.53[b],**<0.001**[c]
ASI	0.00 (0.00–0.00)	3.00 (2.00–3.00)	0.00 (0.00–1.00)	**< 0.001** **< 0.001**[a],0.54[b],**<0.001**[c]
AED	0.00 (0.00–0.00)	2.00 (2.00–3.00)	0.00 (0.00–1.00)	**< 0.001** **< 0.001**[a],0.75[b],**<0.001**[c]
AEX	0.00 (0.00–1.00)	3.00 (2.00–3.00)	0.00 (0.00–1.00)	**< 0.001** **< 0.001**[a],0.77[b],**<0.001**[c]
IF	0.00 (0.00–0.00)	3.00 (2.00–3.00)	0.00 (0.00–1.00)	**< 0.001** **< 0.001**[a],0.75[b],**<0.001**[c]

PICI: Peribronchial inflammatory cell infiltration, ASI: Alveolar septal infiltration, AED: Alveolar edema, AEX: Alveolar exudate, IF: Interstitial fibrosis. I/R: Ischemia-reperfusion, I/R + Empa: Ischemia-reperfusion + Empagliflozin.

Data without normal distribution (PICI, ASI, AED, AEX, IF) were presented as median (min-max). A comparison of continuous variables was performed using the Kruskal Wallis test for non-normally distributed data.

[a]Significance comparison between sham and I/R group.

[b]Significance comparison between sham and I/R + Empagliflozin group.

[c]Significance comparison between I/R group and I/R + Empagliflozin group.

Immunohistochemical findings

Intestinal ischemia-reperfusion induced a significant increase in positive immunostaining for IL-1, IL-6, TNF-alpha, caspase-3, caspase-8, caspase-9 compared to the control group (Fig. 2a, 2b), Immunohistochemical findings were significantly improved in the empagliflozin I/R group when compared to the I/R group (Fig. 2c).

A comparison of immunohistochemical staining of lung tissue between groups is given in Table 5. There was a statistically significant difference between the groups in terms of immunohistochemical staining of IL-1, IL-6, TNF-alpha, caspase 3, caspase 8, and caspase 9 in the lung tissue ($p < 0.001$). The number of cells stained immunohistochemically showed a statistically significant increase in the I/R group

compared to the sham group ($p < 0.001$). There was a statistically significant decrease in the number of cells stained for IL-1, IL-6, TNF-alpha, caspase 3, caspase 8, and caspase 9 in I/R empagliflozin compared to the I/R group ($p < 0.001$). Immunohistochemical findings were similar between the sham group and the I/R empagliflozin group.

Table 5
Comparison of immunohistochemical staining of lung tissue between groups

	Sham (n = 9)	I/R group (n = 9)	I/R + Empagliflozin group (n = 9)	p
IL-1 Negative(n)	6	0	5	**< 0.001**
Weak (n)	3	0	4	**< 0.001**[a],1.00[b],**<0.001**[c]
Moderate(n)	0	0	0	
Strong(n)	0	9	0	
IL-6	6	0	5	**< 0.001**
Absent (n)	3	0	4	**< 0.001**[a],1.00[b],**<0.001**[c]
Weak(n)	0	0	0	
Moderate(n)	0	9	0	
Strong (n)				
TNF-α	9	0	5	**< 0.001**
Absent (n)	0	0	4	**< 0.001**[a],0.08[b],**<0.001**[c]
Weak(n)	0	3	0	
Moderate(n)	0	6	0	
Strong(n)				
Caspase 3	9	0	5	**< 0.001**
Absent (n)	0	0	4	**< 0.001**[a],0.08[b],**<0.001**[c]
Weak(n)	0	3	0	
Moderate(n)	0	6	0	
Strong(n)				

IL-1: Interleukin-1, IL-6: Interleukin-6, TNF-α: Tumor necrosis factor alpha. I/R: Ischemia-reperfusion, I/R + Empa: Ischemia-reperfusion + Empagliflozin.

Categorical variables were given as n. $\chi 2$ or Fisher's exact test was used to compare categorical variables.

[a]Significance comparison between sham and I/R group.

[b]Significance comparison between sham and I/R + Empa group.

[c]Significance comparison between I/R group and I/R + Empa group.

Discussion

Acute respiratory failure is the most important component of multi-organ dysfunction (MODS) after intestinal damage and is an important cause of morbidity and mortality in critically ill patients. Intestinal ischemia-reperfusion causes a widespread systemic inflammatory response, resulting in MODS with subsequent acute lung injury. Disturbance in the intestinal epithelial barrier following intestinal ischemia causes activation of proinflammatory cytokines and circulating leukocytes [2]. TNF alpha, reactive oxygen substrate(ROS), and IL-6 are present in tissue damage that occurs during ischemia-reperfusion, these toxic molecules cause changes in the structure of cellular proteins, lipids, and ribonucleic acids that cause cell dysfunction or death [5]. Tissue hypoperfusion secondary to ischemia results in an increase in adhesion molecules on the endothelial cell surface in addition to these changes. The interaction between activated leukocytes and endothelial cells causes the migration of leukocytes and the production of proteases and ROS. Failure to control this inflammatory response causes tissue damage [2]. In our study, there were significant elevations of AST, ALT, and creatinine due to damage to the liver and kidney tissue in rats treated with I/R. Reperfusion following intestinal ischemia is associated with acute lung injury characterized by increased microvascular permeability, histological evidence of alveolar-capillary endothelial cell damage, and lung deposition of neutrophils. Typical histological features in the I/R group were edema, hemorrhage, increased alveolar wall thickness, and inflammatory cell infiltration in the alveolar spaces of the lung tissue.

In clinical and experimental studies, it has been shown that there is a rapid release of proinflammatory cytokines in ischemia-reperfusion of solid organs such as the lung. TNF alpha, ROS, and IL-6 are present in tissue damage that occurs during ischemia-reperfusion, these toxic molecules cause changes in the structure of cellular proteins, lipids, and ribonucleic acids that cause cell dysfunction or death [15].

Free radicals formed during ischemia-reperfusion are very important. Apoptosis occurs through two pathways, the mitochondrial-dependent intrinsic pathway activated by ROS, and the extrinsic pathway linked to inflammatory molecules such as TNF alpha. The intrinsic pathway is activated in the early phase of reperfusion, the extrinsic pathway is activated a few hours after reperfusion. Both pathways accelerate the activation of caspases and proteases responsible for the clearance of specific cellular substrates that cause cell death [5]. Using an experimental lung ischemia-reperfusion model, Forgiarini et al. showed that there is a large number of apoptotic cells with increased caspase 3 activity after ischemia [16]. It has been shown that caspase 3, 8, and 9 activities are increased in lung tissue samples in programmed cell damage after ischemia-reperfusion injury in lung transplantation [17]. In our study, significantly increased IL-1, IL-6, TNF-alpha staining, and diffuse caspase 3,8 and 9 activity were observed in the lung tissue after I/R.

Many treatment options have been tried to prevent or minimize cell death that occurs during ischemia-reperfusionfusion. Sodium-glucose cotransporter 2 (SGLT2) inhibitors-, have been shown to have anti-inflammatory, antioxidant, and antifibrotic properties in cardiovascular diseases and renal damage. In lung infections, it has been shown to effectively reduce Pseudomonas infection and increase antibiotic

effectiveness in diabetic rats. Therefore, the idea that SGLT2 inhibitors may show potential benefit in lung diseases has arisen. In the study of Lina et al. canagliflozin successfully reduced inflammatory cell infiltration, congestion, and edema in the lung [18]. Kıngır et al. reported that dapagliflozin provided mild histological improvement by reducing oxidative stress and inflammation (TNF-α) in lung tissue [19].

There are few studies in the literature on the pulmonary protective effect of empagliflozin. Ojima et al. (2015) also reported that empagliflozin exerts its anti-inflammatory and antifibrotic effects by inhibiting pro-inflammatory cytokine expression and by suppressing advanced glycosylation products and receptor axis [8]. In the study of Hess et al. it was shown that the number of pro-angiogenic CD133 + progenitor cells in the circulation increased, pro-inflammatory granulocyte precursors decreased, and the anti-inflammatory M2 polarization of monocytes increased with 6-month empagliflozin administration (20). Chowdhury et al. stated that empagliflozin treatment can reduce pulmonary artery remodeling by increasing apoptosis and decreasing the proliferation rate in the pulmonary artery vascular Wall. The effect of empagliflozin on pulmonary artery remodeling in Covid infection has been explained by a similar protective effect [21]. Histopathological and immunohistochemical improvements were observed in the lung tissue with empagliflozin treatment. Kabel et al. showed that empagliflozin can suppress the expression of TGF-β1, TNF-α, and IL-6 and caspase 3 expression in lung tissue in bleomycin-mediated lung injury. They observed histopathological and immunohistochemical improvements in the lung tissue with empagliflozin treatment. Elmaaboud et al. (2019) et al. also supported the view that empagliflozin inhibits apoptosis by suppressing caspase 3 expression [8, 22]. In our study, we observed that the lung damage developed after I/R in rats pre-treated with empagliflozin was significantly milder compared to the placebo group that was not given empagliflozin. In the immunohistochemical staining of the lungs of rats given empagliflozin, it was shown that the number of cells showing inflammatory cytokines such as IL-1, IL-6, and TNF-alpha and caspase 3,8 and 9 activity was lower. The relationship between lung injury due to ischemia-reperfusion injury and oxidative stress was investigated in our study. No significant decrease in thiol-related antioxidant capacity and an increase in the level of ischemia-modified albumin (IMA), the oxidant marker, were observed. The absence of the expected change led to the assumption that the main change after ischemia-reperfusion occurred in the precursor molecules that we could not measure, or that a different oxidant-antioxidant system was effective. Empagliflozin may have exerted its suppressive effect on inflammation and apoptosis in acute lung injury through inhibition of sodium-hydrogen exchange at the cellular level, ketone oxidation, and SGLT receptor inhibition, which have been proven as cardiorenal protective mechanisms in previous studies. It may have exerted its antioxidant effect at the tissue level that we could not measure, or through another system.

We conclude that empagliflozin therapy causes morphologic improvement in acute lung injury in rats after intestinal I/R injury. We believe that further preclinical research into the utility of empagliflozin may indicate its usefulness as a potential treatment for pulmonary damage after intestinal I/R injury in rats

Declarations

Acknowledgments: None

Conflict of Interest: The authors declare there are no conflicts of interest.

Funding: None

The study was conducted according to the ethical standards specified in the 1964 Declaration of Helsinki. Research and publication ethical rules were followed in our study. Our study was approved by the ethics committee of Ankara Training and Research Hospital Animal Experiments Local Ethics Committee, dated 26.07.2021, with a 0066 meeting and 668 decision number.

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Figures

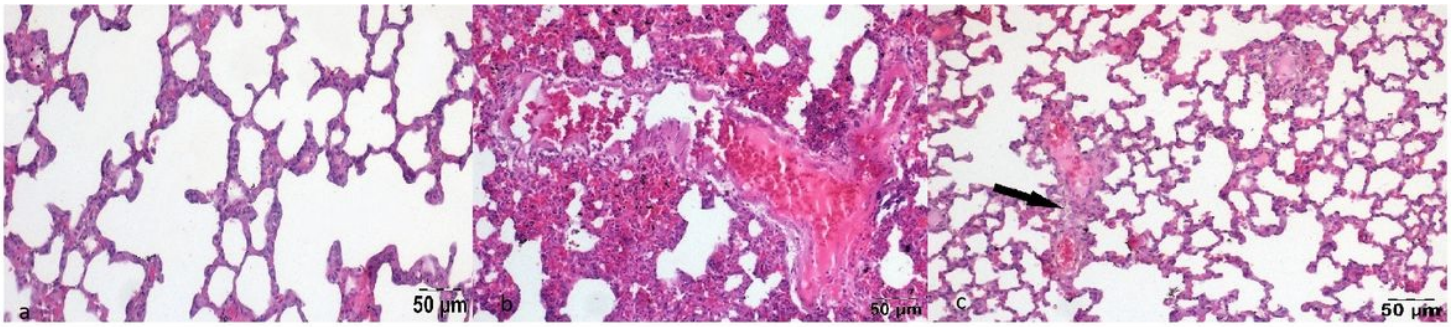

Figure 1

Histopathological examination of the lung tissues of the groups

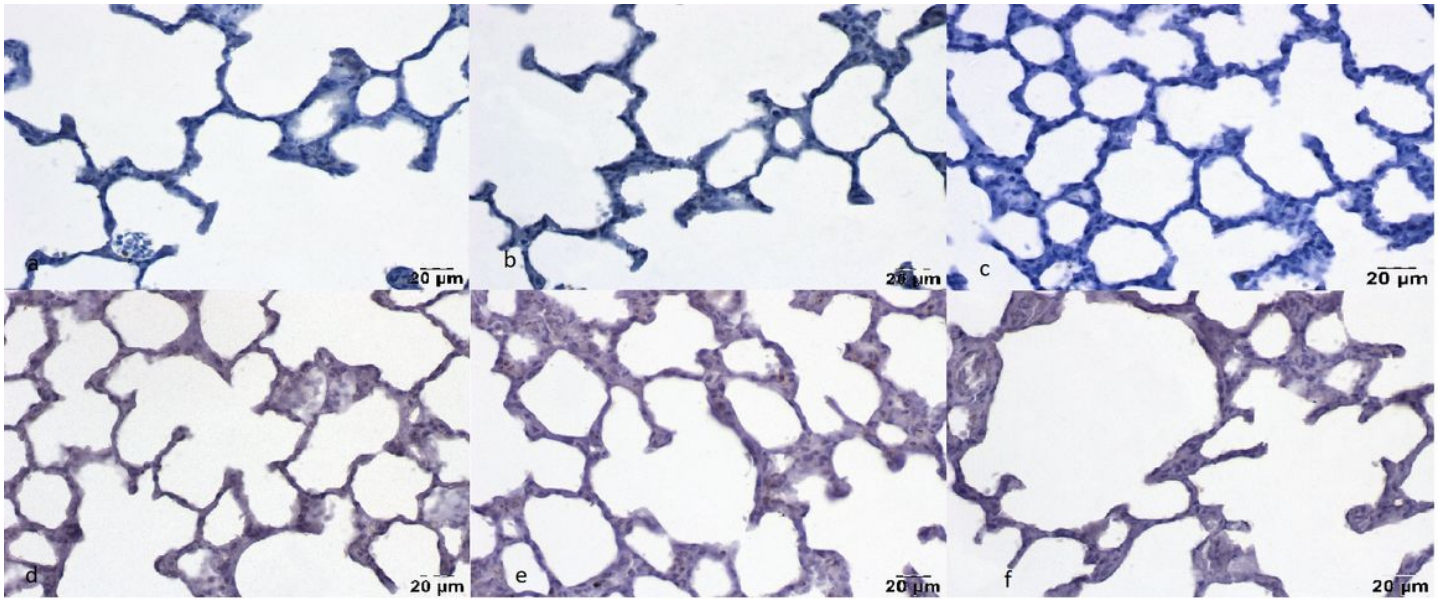

Figure 2

Immunohistochemical staining of lung tissue of the sham group

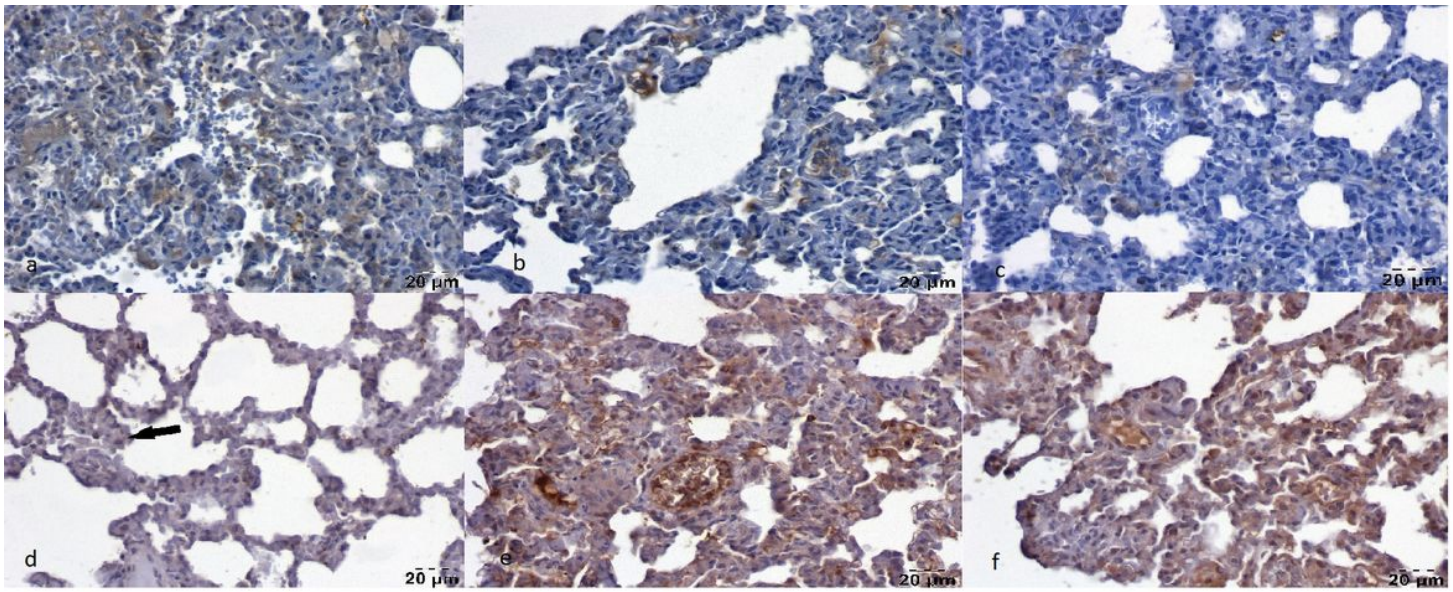

Figure 3

Immunohistochemical staining of lung tissue of intestinal ischemia-reperfusion group

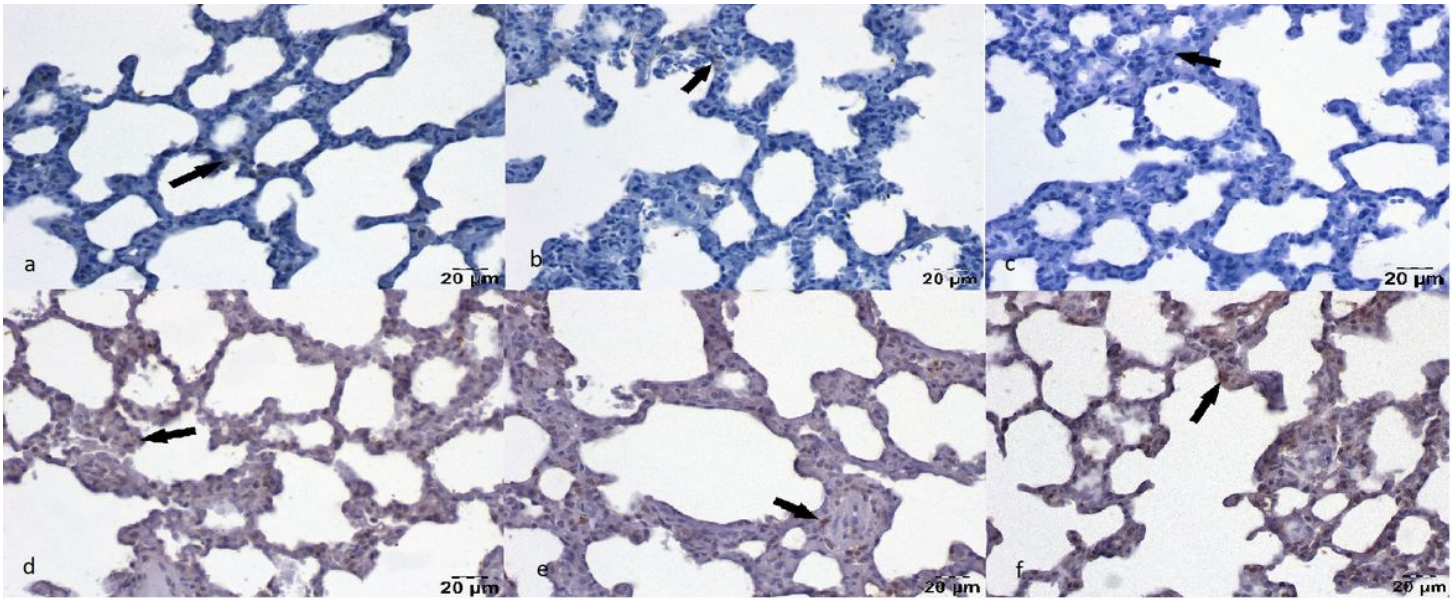

Figure 4

Immunohistochemical staining of lung tissue of intestinal ischemia-reperfusion+empagliflozin grou