Demonstration of ameliorating effect of papaverine in sepsis-induced acute lung injury on rat model through radiology and histology

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ABSTRACT

BACKGROUND: Our target was to show the role of high mobility group box-1/receptor for (HMGB1/RAGE) interaction in feces intraperitoneal injection procedure (FIP)-induced acute lung injury (ALI) pathophysiology, to investigate the effect of papaverine on RAGE associated NF-κB pathway by determining the level of soluble RAGE (sRAGE) and HMGB1, and to support this hypothesis by evaluating inflammatory biochemical, oxidative stress markers, Hounsfield unit (HU) value in computed tomography (CT), and histopathological results.

METHODS: FIP was performed on 37 Wistar rats for creating a sepsis-induced ALI model. The animals were assigned into four groups as follows: Normal control (no treatment), placebo (FIP and saline), and receiving 20 mg/kg and 40 mg/kg per day papaverine. Twenty h after FIP, CT examination was performed for all animals, and HU value of the lung parenchyma was measured. The plasma levels of tumor necrosis factor (TNF)-α, HMGB1, sRAGE, C-reactive protein (CRP) and malondialdehyde (MDA), and lactic acid (LA) were determined and PaO_2 and $PaCO_2$ were measured from arterial blood sample. Lung damage was assessed by histopathological.

RESULTS: TNF-, IL-6, CRP, HMGB1, MDA, LA levels, histopathologic scores, and HU values of CT were significantly increased and sRAGE levels were decreased in the saline-treated group against normal group (all $P<0.05$). Papaverine significantly reversed all results regardless of the dose (all $P<0.05$) and demonstrated inhibition of HMGB1/RAGE interaction through increasing sRAGE levels and suppresses the pro-inflammatory cytokines.

CONCLUSION: We concluded that papaverine has ameliorating effects in rat model of ALI.

Keywords: Acute lung injury; HMGB-1; inflammation; papaverine; RAGE; sepsis.

INTRODUCTION

Sepsis, which results from shock, trauma, major surgery, and systemic infection is a potentially fatal process, affecting millions of individuals worldwide, resulting multiple organ failure concurrent high morbidity and mortality.[1] The lung is a highly sensitive organ to sepsis and related complications, which are acute respiratory distress syndrome (ARDS) and acute lung injury (ALI). These complications are the most common causes of death in sepsis.[2] Sepsis-induced ALI/ARDS changes

the permeability of pulmonary capillaries causing perivascular edema, alveolar congestion (AC) and the formation of hyaline membrane, and progressing to the pulmonary interstitial fibrosis at the end stage.[3] Despite previously studies about animal model of sepsis-induced ALI/ARDS, the pathophysiological mechanism of it still has not been clarified literally.[4] Releasing a lot of number of inflammatory cytokines such as interleukins, tumor necrosis factor-α (TNF-α), and prostaglandins, which is triggered by the inflammatory response, is a main mechanism in the development of sepsis-induced ALI/ARDS.[5] Some studies have shown that decreasing the levels of pro-inflammatory cytokines in the plasma may ameliorate the damage of the lung. High mobility group box-1/receptor for advanced glycation end products (HMGB1/RAGE) pathway is other mechanism of sepsis-induced organ injury.[6]

The RAGE, which is classified in the immunoglobulin superfamily, is a glycoprotein located on the cell surface with the size of 35–kDa.[7] RAGE is associated with some intracellular processes, such as microtubule stabilization, cell proliferation and migration, apoptosis, and inflammation.[8] Soluble form of RAGE (sRAGE), which is production of membrane bound RAGE's cleavage, is released to extracellular compartment and can be detected in plasma.[9] In several animal models, such as model of colitis, streptozotocin-treated diabetic mice model, and thioglycollate-induced peritonitis model, modulation of RAGE expression has reduced neutrophil migration and inflammatory response.[10-12] The various RAGE ligand, such as the β-amyloid peptide, S100 family proteins, HMGB1, and advanced glycation end products (AGE), bind to the extracellular domains of membrane-bound RAGE and stimulate RAGE pathway.[13,14] Inflammatory cells, such as macrophages, release HMGB1, which are basically a nuclear protein and most important ligand of RAGE. The activation of nuclear factor-κB (NF-κB) is stimulated by the binding of extracellular HMGB1 to RAGE.[15] According to prior studies, NF-κB has an important function in expression of pro-inflammatory cytokines (TNF-α, interleukin-1ß [IL-1 ß], and interleukin 6 [IL-6], etc.) causing ALI/ARDS and other inflammatory diseases (diabetes, psoriasis, Alzheimer disease, etc.).[16,17] Thus, anti-RAGE antibody and sRAGE were examined for inhibiting the interaction between RAGE and its ligands and for using as therapeutic agents in ALI, by Lutterloh et al. and Yang et al. In these studies, increased survival rate was showed by treatment with anti-HMGB1 antibody or anti-RAGE antibody in the caecal ligation puncture sepsis model.[18,19] Consequently, variety of animal models about sepsis-induced lung injury focus on inhibiting RAGE/HMGB1 interaction.[20]

For evaluating ALI/ARDS, best imaging modality is the computed tomography (CT). Symmetric or diffuse consolidation and/or ground glass opacities, caused by AC, interstitial edema and alveolar wall thickening, are the typical CT findings of early exudative phase of ALI/ARDS and this causes increasing lung density, which is measured in the Hounsfield unit (HU) scale.[21] Despite different values were defined in the literature, normal, early ARDS, and late ARDS lung HU scale was accepted commonly between −950 HU and −700 HU, between −550 HU and −450 HU, and between −420 HU and −300 HU, respectively, for human, but there was no threshold value of HU for rats.[22] Consolidation or ground glass opacities increase the density of the lung parenchyma, and there are several studies showed that we may use HU value of lung parenchyma in chest CT as a predictive indicator of ALI/ARDS.[23]

Papaverine is a non-narcotic opium substance, derived from the plant of Papaver somniferum, and relaxes directly smooth muscle and provides vasodilatation. Its mechanism of action is explained with inhibiting phosphodiesterases non-selectively and direct blockage of calcium channels.[24,25] Some studies showed that papaverine directly inhibits the binding HMGB1 to RAGE and suppresses HMGB1-mediated inflammatory response by inhibiting RAGE associated NF-κB pathway, causing decreased plasma levels of inflammatory cytokines. Furthermore, decreased levels of LA by treatment of papaverine were found and this effect was associated with ameliorating impaired microcirculation.[26-28]

The primary target of our study was to assess the role of HMGB1/RAGE molecular interaction in mechanism of ALI and to investigate the effect of papaverine on RAGE-associated NF-κB pathway by determining the level of sRAGE and HMGB1. The other aim of this study was to analyze ameliorating effect of papaverine in ALI by the evaluation of inflammatory and oxidative stress biomarkers (C-reactive protein [CRP], TNF-α, malondialdehyde [MDA], and lactic acid [LA]) and to support this effect with HU values in CT and histopathological results. In our knowledge, this is the first study show that papaverine has an ameliorating effect in ALI rat model by evaluating CT changes, histopathological results, and inflammatory biomarkers.

MATERIALS AND METHODS

Animals

This study used 37 Wistar adult male rats with an average weight of 300–350 g. Animals were placed in steel cages (41×28.2×15.3 cm) and they remained on 12 h light/dark cycle. The mean temperature of the room was 22±2 °C and the humidity was 40–50%. Animals were fed with standard chow and given water ad libitum during the study. All experiments were performed in line with the Guide for the Care and Use of Laboratory Animals rules adopted by the National Institutes of Health (U.S.A).[29] The Animal Research Ethics Committee confirmed all procedures (Demiroğlu Science University, number: 20210635).

Experimental Procedures

The feces intraperitoneal-injection procedure (FIP) was performed according to the model previously described by Shrum et al. and Tyml et al. to induce sepsis.[30,31] For preparing saline-feces mixture, the fresh feces were collected from rat, which is not included in this study, suspended in 0.9% NaCl (saline) at the concentration of 75 mg/mL. Then, the
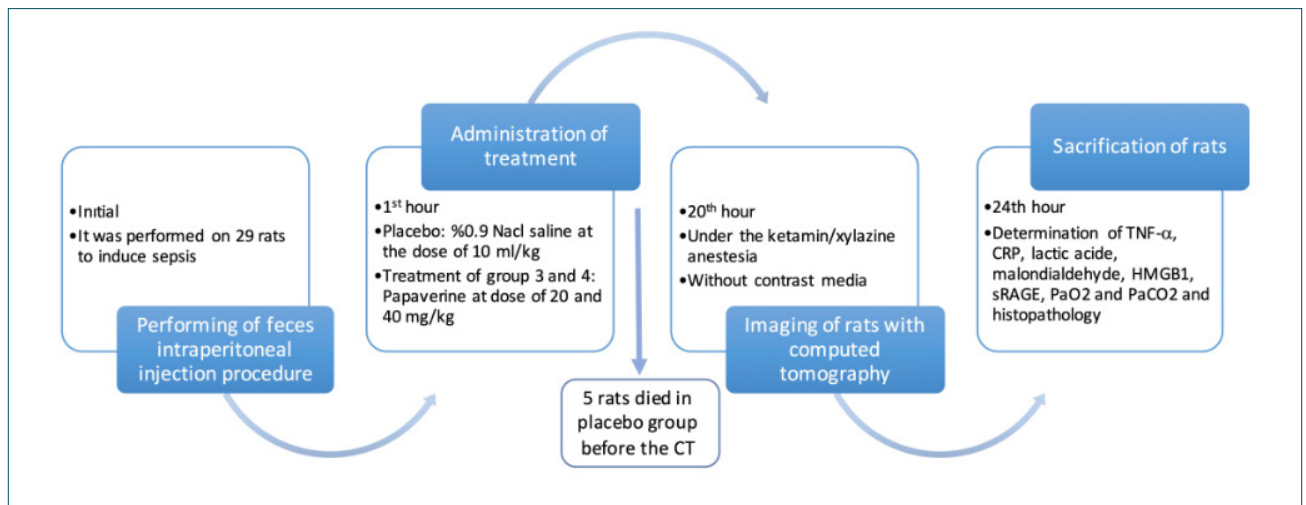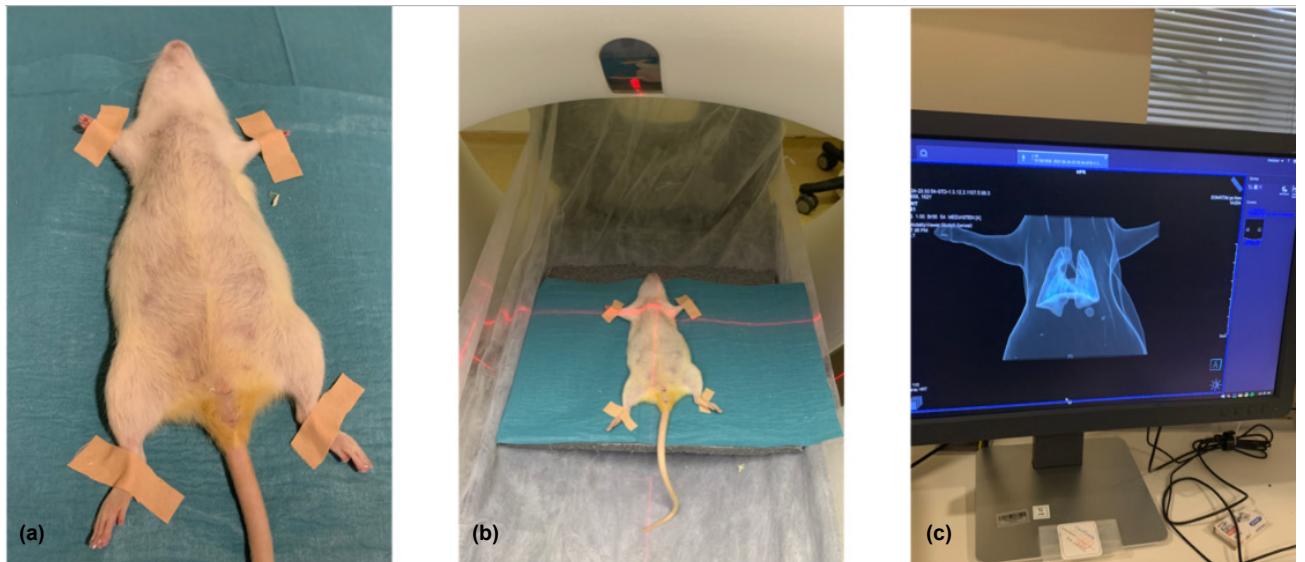
Figure 1. Design and timeline of the study.

Figure 2. CT procedure. **(a)** All rats were bound on scanning table with suitable materials for preventing motion artifacts. **(b)** Examinations were performed in the supine position without contrast media. **(c)** Reformat images were created by software.

mixture was injected with 21 Gauge needle intraperitoneally (i.p.) at 0.1 mg/kg body weight once. Animals were assigned into four equal groups randomly. Group 1 (n=8) served as the control group (CG), and no treatment was administered. FIP was performed to Group 2 (n=8), Group 3 (n=8), and Group 4 (n=8), and 1 h after FIP, rats were treated with saline at a dose of 10 ml/kg/day i.p. (FIP+Saline, FIPS), papaverine (Papaverine 50 mg/2 mL, Galen) at a dose of 20 mg/kg/day i.p. (FIP+Papaverin 20, FIPP20), and at a dose of 40 mg/kg per day i.p. (FIPP40), respectively. Five rats of Group 2 died before the CT examination (within the first 20 h after FIP) and were excluded from the study. Twenty h after FIP, all animals were sedated with ketamine, and CT examination was performed. The study was ended at the 24th h. At the end of the study, high-dose anesthesia was administered to all animals for euthanasia (xylazine [50 mg/kg, Rompun, Bayer]/ketamine [100

mg/kg, Ketasol, Richter Pharma]). The animals were then sacrificed by cervical dislocation. The cardiac puncture was used for obtaining blood of animals and these blood samples were analyzed to evaluate the level of serum biomarkers (Fig. 1).

Thorax CT Examination

CT scanner with a 128-slice multi-detector row (GE Healthcare, Optima, USA) was used for acquiring images. The combination of xylazine (10 mg/kg, Rompun, Bayer)/ketamine (50 mg/kg, Ketasol, Richterpharma) was used intraperitoneally for the anesthesia. No contrast media was used and all animals were fixed in the supine position on the scanning table with proper materials to prevent motion artifacts during examination (Fig. 2). Scanning parameters as follows: kV: 120, mAs: variable, slice thickness: 1 mm. The evaluating area was from the basis of the skull to the upper abdomen, including the lower and upper segments of the lung. After image
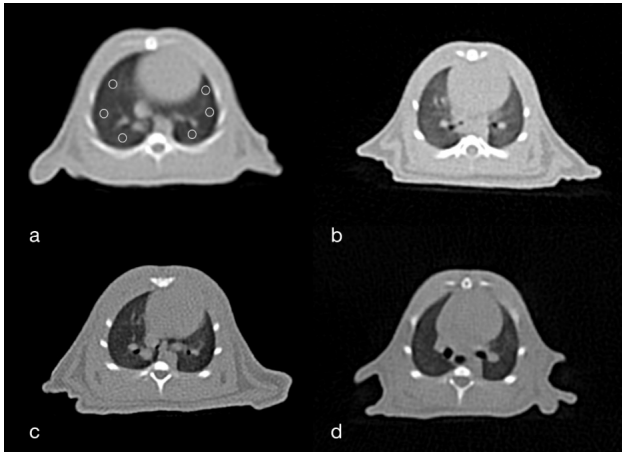
Figure 3. Axial CT images of lung at the level of the heart, six ROI placed with the same size at the same location. **(a)** Normal control group lung, **(b)** FIPS group showed increased density of lung, **(c)** FIP performed and 20 mg/kg papaverine-treated group showed decreased density of lung against FIP performed and saline-treated group, **(d)** FIP performed and 40 mg/kg papaverine-treated group showed density of lung very close to normal group.

acquisition, a sharp reconstruction kernel (KernelBr64) and 512×512 matrix size were used for reconstruction using raw data. Three radiologists, who were blinded to animals' groups, evaluated all images. For all animals, the same size (2.347 mm2) of regions of interests (ROI) was used and placed at the level of near the heart apex on axial images. The parenchymal window was used for placing ROI on the right and left lung (upper, middle, and lower zone). ROI was not placed on big vessels, bones, and airways to prevent bias (Fig. 3).

Biochemical Analysis

Plasma levels of TNF-α and CRP were determined with using of commercially available enzyme-linked immunosorbent assay (ELISA) kits (Biosciences, Abcam). ELISA kits were used to determine sRAGE plasma level with sensitivity of 16.14 pg/mL (R&D Systems, Cat #DRG00), and HMGB1 plasma level with sensitivity of 0.2 ng/mL (IBL International Cat#ST51011). All measurements were accomplished according to the guidelines provided by the manufacturer. Pursuant to the manufacturer instructions, the serum samples of blood were diluted 1–2; and CRP and TNF-α were determined in duplicate. The blood gas analyzer was used for determining LA levels.

The thiobarbituric acid reactive substances (TBARS) were used for detecting lipid oxidation by measuring MDA plasma levels as previously described by Wichterman et al.[32] The plasma sample was mixed with TBARS reagent and trichloroacetic acid, and the mixture was incubated at 100°C for 1 h. For 20 min, the mixture was centrifuged at 3000 rpm with the samples on ice. A reading of the absorbance at 535 nm was performed after centrifugation to determine the supernatant's absorbance. Tetra ethoxy propane was used to calibrate, and MDA concentrations were measured in nanometers (nM).

Histopathology of the Lung

All histological sections of the lung (5 μm) were fixed with formalin and evaluated after Hematoxylin and Eosin (H&E) staining. Digital camera (Olympus C-5050) mounted on the microscope (Olympus BX51) was used for photographing sections. The histopathological lung damage score, which was previously described by Kwon et al., was calculated.[33] Histopathological sections were evaluated by the histologist who was blinded to animals' groups. AC, aggregation or infiltration of leukocytes in vessel walls and/or air spaces (AL), hemorrhage (H), edema of perivascular and/or interstitial space (PE), and hyaline membrane formation and/or thickness of the alveolar wall (TA) were evaluated to score histopathological lung damage in all sections. Every item was graded from mild to severe as follows: 1 (0–25%), 2 (25–50%), 3 (50–75%), and 4 (75–100%).

PaO_2 and $PaCO_2$ in Arterial Blood Gas

At the end of all procedures, artery blood was sampled by cardiac puncture for all animals, and these samples were used for assaying PaO_2 and $PaCO_2$ with a blood gas analyzer.
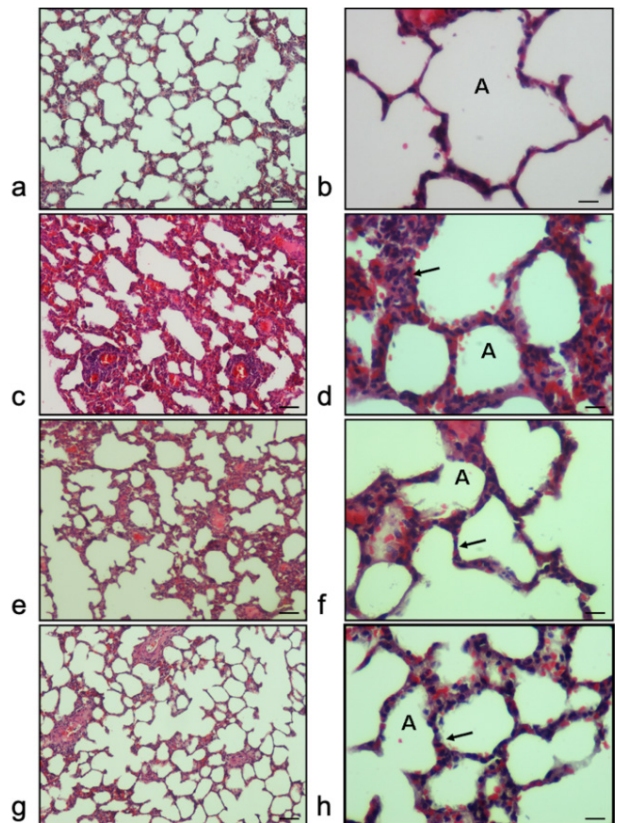

Figure 4. Lung histopathology ×10 and ×40 magnification H&E staining. **(a, b)** Normal control group lung, (A) Alveol, **(c, d)** FIP performed and saline-treated group showed severe histopathologic alteration related to increased alveolar inflammation and septal thickness (arrow), **(e, f)** FIP performed and 20 mg/kg papaverine-treated group showed decreased inflammation and septal thickening (arrow), **(g, h)** FIP performed and 40 mg.

Table 1. Levels of inflammation and oxidative stress markers in the groups

	CG	FIPS	FIPP20	FIPP40
MDA (nM)	68.6±9.4	184.1±14.5[**]	105.8±9.04[#]	87.6±11.5[##]
TNF alfa (pg/ml)	24.5±3.2	217.8±21.7[**]	154.2.1±6.6[#]	133.9±7.7[##]
CRP (mg/dl)	0.31±0.42	1.65±0.3[*]	0.8±0.4[#]	0.74±0.2[#]
Lactic acid (mmol/L)	1.3±0.5	7.2±1.8[**]	6.3±1.8[#]	4.05±1.1[#]

Results were presented as mean ± SEM. one-way ANOVA was used for statistical analysis and post hoc Bonferroni correction was done. [*]P<0.05, [**]P<0.001 different from CG; [#] P<0.05, [##] P<0.0001 different from FIPS. CG: Control group, FIPS: saline-treated feces intraperitoneal-injection procedure performed group; FIPP20: 20 mg/kg papaverine treated FIP performed group; FIPP40: 40 mg/kg papaverine treated and FIP performed group; MDA: Malondialdehyde; TNF: Tumor necrosis factor; CRP: C-reactive protein; HMGB1: High mobility group box 1; sRAGE: soluble Receptor for advanced glycation end products.

Statistical Analysis

The statistical evaluation was performed using Statistical Package for the Social Sciences (SPSS, version 15.0). Variables were presented as mean ± standard error of the mean. Whether the Shapiro–Wilk test determined the normal or non-normal distribution of variables. Student's t-test and one-way analysis of variance were used for evaluating parametric variables, and post hoc Bonferroni correction was done for subgroup evaluation. P≤0.05 was considered as statistically significant.

RESULTS

Biochemical Findings

Plasma levels of sRAGE were lower in the FIPS (1425.2±65.7 pg/mL) against the CG (2110.6±81.9 pg/mL) and statistically significance was found (P<0.05); moreover, sRAGE plasma levels of both groups treated with papaverine (FIPP20; 2463.1±103.7 pg/mL and FIPP40; 2517.8±110.4 pg/mL) were higher than FIPS and there were statistically significant differences (both group P<0.0001).

Plasma levels of HMGB1 were (P<0.05) found higher in the FIPS (2.55±0.82 pg/mL) against the CG (1.3±0.3 pg/mL); besides, HMGB1 plasma levels in both groups that treated with papaverine (FIPP20; 1.57±0.4 pg/mL and FIPP40; 1.16±0.5 pg/mL) were found lower than FIPS and these results were statistical significant (P<0.05 for both group) (Figure 4).

While TNF-α and CRP levels were significantly increased in the FIPS group than the CG (P<0.0001, P<0.05, respectively), these inflammatory markers in both groups that administered papaverine (for FIPP20, both P<0.05, and for FIPP40, P<0.05, P<0.0001, respectively) were found significantly decreased against to the FIPS group.

Similar results were found in oxidative stress biomarkers. Plasma levels of MDA and LA were found increased in FIPS group compared to the CG (both P<0.0001). These biomarkers in both groups that received papaverine (for FIPP20, both P<0.05, and for FIPP40, P<0.05, P<0.0001, respectively) were significantly decreased compared to the FIPS group. Plasma levels of all biochemical markers and P values of the comparisons are shown in Table 1.
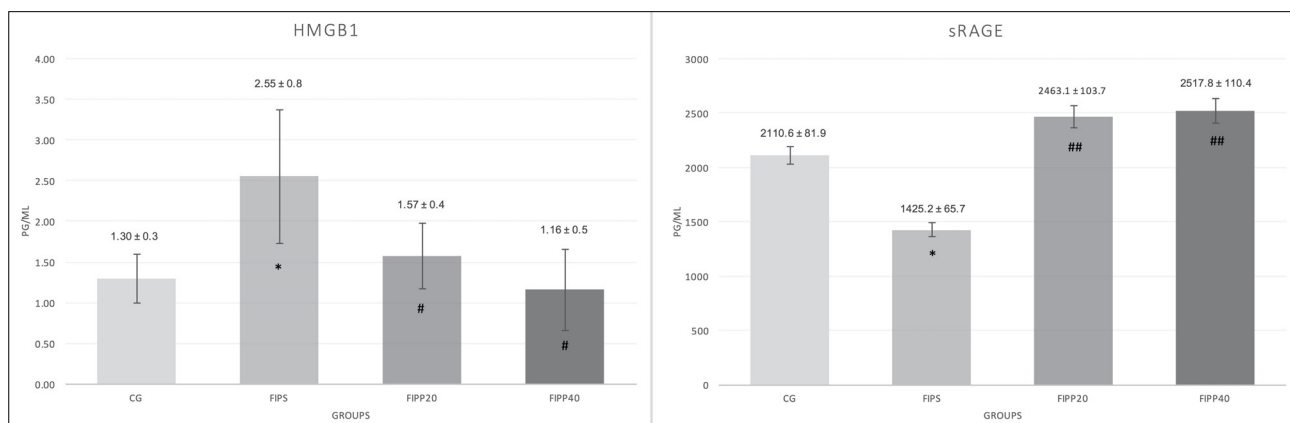

Figure 5. Comparison of HMGB1 and sRAGE levels between groups; HMGB, High mobility group box; sRAGE, soluble receptor for advanced glycation end products; CG, Control group; FIPS, Feces intraperitoneal injection procedure performed and saline-treated group; FIPP20, FIP performed and 20 mg/kg papaverine-treated group; FIPP40, FIP performed and 40 mg/kg papaverine-treated group; Results were presented as mean ± SEM. one-way ANOVA was used for statistical analysis and post hoc Bonferroni correction was done. * P<0.01, different from CG; #P<0.05, ##P<0.001 different from FIPS.

Table 2. Histopathological scores according to the groups

	CG	FIPS	FIPP20	FIPP40
AC	0.3±0.1	3.1±0.2**	2.2±0.3#	1.0±0.2##
AL	0.4±0.2	3.8±0.2*	1.8±0.3#	1.2±0.2#
H	0.5±0.2	3.5±0.2**	1.8±0.3#	1.3±0.2##
PE	0.5±0.2	3.3±0.3**	1.9±1.3#	1.3±1.3#
TA	1.4±0.2	3.1±0.2*	1.7±0.4#	1.2±0.3#

Results were presented as mean ± SEM. one-way ANOVA was used for statistical analysis and post hoc Bonferroni correction was done. *P<0.01; **P<0.001 different from CG; #P<0.05; ##P<0.001 different from FIPS; CG: Control group; FIPS: Saline-treated feces intraperitoneal-injection procedure performed group; FIPP20: 20 mg/kg papaverine-treated FIP performed group; FIPP40: 40 mg/kg papaverine-treated and FIP performed group; AC: Alveolar congestion, AL: aggregation or infiltration of leukocytes in vessel walls/air spaces; H: Hemorrhage; PE: perivascular/interstitial edema; TA: Thickness of the alveolar wall/ hyaline membrane formation; HU: Hounsfield unit.

Histopathological Lung Damage Score and CT Findings

FIP model was successful in our study and septal thickening and inflammation are clearly seen on histopathology sections (Figures 4a-d). Papaverine altered the inflammation results on the FIP model (Figures 4e-h). AC (P<0.0001), H (P<0.0001), AL (P<0.05), PE (P<0.0001), and TA (P<0.05) scores, which represents lung damage, were found significantly higher in
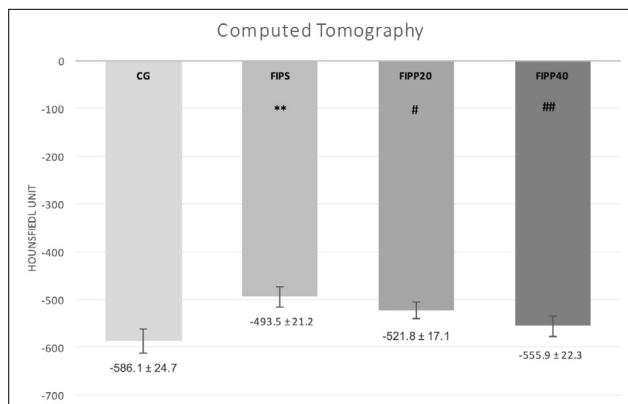

Figure 6. Comparison of CT density results of lung between groups; CG, Control group; FIPS, Feces intraperitoneal injection procedure performed and saline-treated group; FIPP20, FIP performed and 20 mg/kg papaverine-treated group; FIPP40, FIP performed and 40 mg/kg papaverine treated group; Results were presented as mean ± SEM. one-way ANOVA was used for statistical analysis and post-hoc Bonferroni correction was done. *P<0.05, **P<0.001 different from CG; #P<0.05, ##P<0.0001 different from FIPS.

FIPS than the CG. All scores in both groups that administered papaverine (for FIPP20, all scores P<0.05 and for FIPP40, P<0.001, P<0.001, P<0.05, P<0.05, and P<0.005, respectively) were found significantly lower against to FIPS (Fig. 5). Histopathological scores were provided in Table 2.

HU value of lung in FIPS (−493.5±11.2) was higher than the CG (−586.1±14.7), and there was a statistically significant (P<0.001). HU value of lung in both groups that received papaverine (−521.8±7.1 and −555.9±12.3) was lower than FIPS, and there was a statistically significant (P<0.001, Fig. 6).

Arterial PaO$_2$ and PaCO$_2$

When arterial blood gas was investigated, although PaO$_2$ in FIPP20 (P<0.05) and FIPP40 (P<0.05) was higher against FIPS and statistically significant, there was no difference in PaCO$_2$. Mean and P values of PaO$_2$ and PaCO$_2$ were provided in Table 3.

DISCUSSION

In this study, we used the FIP-induced sepsis model to investigate ameliorating effect of papaverine in sepsis-induced ALI. The principal finding of our study was that papaverine increases sRAGE plasma levels in FIP-induced sepsis rat model, regardless of the dose. Moreover, we found that papaverine decreases HMGB1 levels and pro-inflammatory cytokines including CRP and TNF-α, oxidative stress biomarkers, such as MDA and LA, and HMGB1 levels, while increasing PaO$_2$. Final,

Table 3. Levels of arterial blood gas according to the groups

	CG	FIPS	FIPP20	FIPP40
PaO$_2$ (mmHg)	118.3±4.9	77.5±6.1*	89.6±10.4#	98.3±5.6#
PaCO$_2$ (mmHg)	41.3±6.7	35.3±2.6*	33.7±1.8	37.2±5.1

Results were presented as mean ± SEM. one-way ANOVA was used for statistical analysis. *P<0.05, different from CG; #P<0.05 different from FIPS. CG: Control group; FIPS: Saline-treated feces intraperitoneal-injection procedure performed group; FIPP20: 20 mg/kg papaverine treated FIP performed group; FIPP40: 40 mg/kg papaverine treated and FIP performed group.

our study was showed that papaverine decreases histopathological lung damage score according to scoring system previously described and HU value of the lung parenchyma derived from CT images, in the sepsis-induced ALI. In our knowledge, this is the first study showing that papaverine reduced the severity of ALI by inhibiting HMGB1 translocation and decreasing binding to RAGE through increased sRAGE level and evaluating HU values of CT and histopathological findings.

Despite the lack of exactly knowing of RAGE's physiologic role on inflammatory response, studies continue to investigate this mechanism. An interesting finding was showed that, although many systems designed to maintain homeostasis, RAGE expression is increased in inflammatory process when RAGE/HMGB1 interaction is complete. This positive feedback mechanism may play a role to function against to homeostatic mechanism in severe inflammatory process.[18,34,35] The previous studies showed that molecules which targeted HMGB1/RAGE pathway, such as ulinastatin, lidocaine, salidroside, and RAGE antibody, inhibiting HMGB1/RAGE interaction, increasing survival rate in animal models and they have a very strong potential to suppressing inflammatory response by inhibiting RAGE associated NF-κB pathway in ALI. This hypothesis was supported by showing increased plasma levels of sRAGE and sRAGE bind RAGE ligands, resulting decreased HMGB1 plasma levels. These levels have been used in some studies as reliable marker of RAGE/HMGB1 interaction in pathophysiology of sepsis.[20,36-38] Consistently with the literature, we found that sRAGE plasma levels were decreased and HMGB1 plasma levels were increased in FIPS group (placebo) in our study. Increased HMGB1 level induced sepsis-dependent inflammatory and organ injury in placebo group.[39]

The anti-inflammatory effects of papaverine, suppressing the binding of HMGB1 to RAGE, have been evaluated by the limited studies, which were investigated glioblastoma.[40,41] Only two study in the literature, designed by Tamada et al. and Solmaz et al., focuses on anti-inflammatory effect in sepsis model.[28,42] Similar to our study, they reported that papaverine was found inhibit the binding between RAGE and HMGB1, hence decreasing the release of IL-6 and TNF-α, which were pro-inflammatory cytokines, in the cecal ligation puncture and FIP sepsis model. Despite, 1st time investigation of protective effect of papaverine in ALI/ARDS, according to decreased pro-inflammatory cytokines, CT findings, and histopathological results, our study showed that papaverine, similarly like investigated molecules, blocks the HMGB1/RAGE pathway efficiently and decreases severity of ALI/ARDS. In the ALI/ARDS, fluid replaces instead of air, which represents AC, interstitial edema and consolidation, and decreases the well-aerated lung parenchyma, thereby these changes may quantitatively measure according to HU scale.[43] We think that papaverine inhibits HMGB1/RAGE interaction and increased plasma sRAGE level resulting decreased HMGB1 plasma level. Effect of papaverine is decreased plasma level of inflammatory cytokines and this prevents change of vascular permeability and fluid leakage to alveoli, hereby CT HU value decreases.

Moreover, we supported this hypothesis with histopathological results and plasma levels of sRAGE and HMGB1.

Microcirculation supplies nutrients and oxygen for aerobic metabolism and removes cellular waste products. There is an inconsistency between macrocirculatroy and microcirculatory hemodynamics in sepsis.[44] Impaired microcirculatory perfusion in septic shock with contribution of vasoconstriction increases anaerobic glycolysis in tissue, causing increased plasma LA levels which are indirect method of microcirculatory assessment. Plasma LA levels and their changes in time are reliable markers of sepsis severity and mortality.[45] Li et al. reported that papaverine improves the microcirculation after fluid resuscitation combined with vasopressor drug treatment in septic shock.[46] In our study, LA levels were decreased after papaverine treatment and we think that papaverine ameliorates microcirculatory perfusion in tissue.

MDA is frequently used for evaluating oxidative stress and it is sensitive for indicating oxidative stress but not specific. Few experimental studies, such as sepsis-induced neuropathy and ethanol-induced hepatotoxicity, have been reported antioxidant effects of papaverine.[47,42] Our study showed that MDA and LA levels are significantly decreased in both groups treated with papaverine similar with the literature, and consequently, anti-oxidant and anti-inflammatory and micro-circulator regulator effect of papaverine may more efficient in suppressing the development of ALI.

Limitation of our study is that papaverine has vasodilator effect and arterial tension of rats is not monitored during the study.

CONCLUSION

Our study is the first study showing that papaverine has antioxidant and anti-inflammatory effect by inhibiting HMGB1/RAGE interaction in rat model and supporting this effect by CT findings and histopathological results. In addition, results of our study showed that this molecule may be use as an efficient therapeutic agent, as it is capable of ameliorating severe inflammatory injury in the lungs of sepsis animal model. Therefore, we think that the benefit of papaverine will be clarified with long-term prospective studies in humans.

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Papaverinin sepsis kaynaklı akut akciğer hasarında iyileştirici etkisinin sıçan modelinde radyoloji ve histoloji yoluyla gösterilmesi

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AMAÇ: High mobility group box-1/receptor for advanced glycation end products (HMGB-1/RAGE) etkileşiminin, periton içine feces enjekte edilerek (FIP) indüklenen akut akciğer hasarındaki (AAH) rolünün gösterilmesi, çözülebilir RAGE (sRAGE) ve HMGB-1 seviyelerinin tespit edilerek papaverinin RAGE ilişkili NF-κB yolağı üzerindeki etkisinin araştırılması ve bu etkilerin enflamatuvar ve oksidatif stres biyobelirteçlerin, toraks BT'de Hounsfield Unit (HU) değerlerinin ve histopatolojik sonuçların incelenerek desteklenmesi çalışmamızın hedefleridir.

GEREÇ VE YÖNTEM: Sepsisle indüklenen AAH modeli oluşturmak üzere 37 Wistar sıçana FIP uygulandı. Hayvanlar aşağıdaki gibi dört gruba ayrıldı: Kontrol grubu (tedavi almayan), plasebo grubu (FIP ve 1 saat sonra salin uygulanan), FIP ve 1 saat sonra 20 mg/kg/gün ve 40 mg/kg/gün papaverin uygulanan gruplar. FIP uygulanmasından 20 saat sonra tüm hayvanlara BT çekildi ve akciğer parankiminde HU değerleri ölçüldü. Tümör nekrosis faktör (TNF)-, HMGB1, sRAGE, C- reaktif protein (CRP), malondialdehit (MDA) ve laktik asit (LA) plazma değerleri belirlendi ve arteryel kan gazından PaO$_2$ and PaCO$_2$ ölçüldü. Akciğer hasarı histopatolojik olarak değerlendirildi.

BULGULAR: TNF-, IL-6, CRP, HMGB1, MDA, LA seviyeleri, histopatoloji skorları ve BT'den elde edilen HU değerleri normal gruba göre plasebo grubunda anlamlı derecede artmış ve sRAGE seviyeleri düşmüştü (Tüm bulgularda p<0.05). Papaverine uygulanan dozdan bağımsız olarak tüm bulguları anlamlı olarak tersine çevirmiş ve sRAGE seviyelerini artırarak ve proinflamatuvar sitokinleri baskılayarak HMGB1/RAGE etkileşiminin inhibisyonunu göstermiştir.

SONUÇ: AAH sıçan modelinde papaverinin iyileştirici etkileri olduğu sonucuna vardık.

Anahtar sözcükler: Akut akciğer hasarı; enflamasyon; HMGB-1; papaverin; RAGE; sepsis.