EXPERIMENTAL STUDY

The effects of specific and non-specific phosphodiesterase inhibitors and N-acetylcysteine on oxidative stress and remote organ injury in two-hit trauma model

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ABSTRACT

BACKGROUND: Sepsis is a systemic inflammatory response to infection and is one of the leading causes of morbidity and mortality. The second hit after trauma causes increased inflammatory response and multiple organ failure (MOF). The infection which develops after burn injury is a suitable model for a two-hit trauma study. Sepsis causes the release of biochemical mediators, such as Free Oxygen Radicals (FORs), which may lead to lipid peroxidation, which may play a key role in multiple organ failure. In this study, we aimed to investigate the effects of phosphodiesterase (PDE) inhibitors (sildenafil, milrinone, pentoxifylline) and N-acetylcysteine (NAS) on oxidative stress and organ damage in two-hit models.

METHODS: In this experimental study, peritonitis was created by cecal ligation and puncture (CLP) method in 40 rats, 72 hours after creating a 30% scalding injury. Rats were divided into five groups of eight rats each as follows: Group I: No treatment; Group II: 10/mg/kg/day dosage of intraperitoneal (i.p) sildenafil treatment was applied for 72 hours after CLP; Group III: 1/mg/kg/day dosage of i.p milrinone treatment was applied for 72 hours after CLP; Group IV: 150/mg/kg/day dosage of i.p NAS treatment was applied for 72 hours after CLP; Group V: 50/mg/kg/day dosage of i.p pentoxifylline treatment was applied for 72 hours after CLP. All rats were sacrificed on the seventh day of this study. Malondialdehyde (MDA), Glutathione Peroxidase (GPx), Superoxide Dismutase (SOD), catalase, Tumor Necrotic Factor-alpha (TNF-α) levels, and tissue (lung, kidney) and serum samples were taken for histopathological study.

RESULTS: When compared to the control group, the tissue damage score was found to be lower in all treatment groups. Sildenafil, milrinone and NAS groups had higher kidney GPx levels compared to the control group. Milrinone and pentoxifylline were higher in the lung tissue compared to the SOD control group. TNFα levels were lower in pentoxifylline and milrinone groups compared to the control group.

CONCLUSION: This experimental study has shown that PDE inhibitors and NAS have a decreasing effect on oxidative stress and distant organ damage in the two-hit model. Further clinical and experimental studies are needed on this subject.

Keywords: Antioxidant; free oxygen radicals; tissue damage; phosphodiesterase inhibitor; sepsis.

INTRODUCTION

Sepsis is a systemic infectious disease that may lead to shock, organ failure, and death and should be treated urgently.[1] In the presence of endotoxin, oxygen decreases in hypoxic and acidic environments, and free oxygen radicals (FOR) are formed; activation of leukocytes may also lead to FOR production.[2]

The occurrence of the second hit in trauma patients causes the inflammatory response to exacerbate and leads to the development of multiple organ damage. Infection after burn injury is a suitable model for two-hit trauma studies.[3]

Systemic inflammatory response syndrome (SIRS), which develops in some patients with the burn or severe trauma, may directly lead to early multi-organ failure.[4]

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Initial trauma (e.g., hemorrhagic shock, ischemia-reperfusion injury, burn) leads to the emergence of an abnormal immune or inflammatory response which brings about a potential secondary trauma (e.g., infection), it is said that the occurrence of a second hit leads to an exaggerated systemic inflammatory response.[5] These inflammatory changes are called the "two-hit trauma" hypothesis. Post-traumatic opportunistic infections that would not normally be mortal may cause serious SIRS and late MOF development in critically ill patients and these findings support the two-hit hypothesis.[6]

Many different models with consecutive traumas were used to create two-hit trauma models. Infection following burn injury is one of the most common trauma models in the clinic. In the literature, two-hit trauma models created by the application of CLP and intraperitoneal endotoxin after burn trauma were studied.[5–7]

In this study, a CLP after-burn model was chosen to create a two-hit trauma model. In this study, the effects of NAS and PDE inhibitors (pentoxifylline, sildenafil, milrinone) on oxidative stress and distant organ damage are investigated in two-hit trauma models consisting of sequential burn and sepsis

MATERIALS AND METHODS

This study was carried out in Cumhuriyet University Experimental Research and Animal Laboratory. The permission was obtained from Cumhuriyet University Ethics Committee dated 23.07.2012-335. Forty Wistar Albino rats weighing 180–250 grams were used in this study. Rats were fed with standard rat feed. They were fasted for 12 hours before the operation but allowed to drink water.

Experiment Protocol

The animals were divided into five groups, each containing eight rats. A 3x4 cm area of the back of all rats was shaved. A third-degree 30% scald burn was performed, and 72 hours later, peritonitis was induced by the CLP method.

Anesthesia

General anesthesia was applied to all groups. For this purpose, a mixture of intramuscular 5 mg/kg xylazine (Rompun®) and 30 mg/kg ketamine hydrochloride (Ketalar®) was used. The burn model, CLP model and sacrifice were all made under general anesthesia.

Burn Model

Under general anesthesia, a 3x4 cm area representing 30% of the body surface was shaved in the dorsal region of the animals. The remaining body parts were placed in previously prepared molds, leaving the shaved area out. The shaved area was kept in 96C water for 12 seconds to obtain a third-degree standard burn. Water temperature was measured using a thermometer. The rats received no treatment for their burn injury during the experiment (Fig. 1).

CLP Model

A midline laparotomy was performed under general anesthesia. After the cecum was isolated, the ileocecal valve was ligated with 3/0 silk underneath. The cecum was perforated from two separate points by a 22 Gauge needle and was then slightly stroked, and intraperitoneal feces contamination was achieved. 4 ml of saline was given subcutaneously to the back area for resuscitation and the abdominal wall was closed in two layers (Figs. 2a-c).

Animals were divided into five groups, each containing eight rats:

Group I: No treatment;

Group II: 10/mg/kg/day dosage of intraperitoneal (i.p) sildenafil treatment was applied for 72 hours after CLP;

Group III: 1/mg/kg/day dosage of i.p milrinone treatment was applied for 72 hours after CLP;

Group IV: 150/mg/kg/day dosage of i.p NAS treatment was applied for 72 hours after CLP;

Group V: 50/mg/kg/day dosage of i.p pentoksifilin treatment was applied for 72 hours after CLP.

All rats were sacrificed on the seventh day of this study, under general anesthesia. Laparotomy was performed on the sacrificed rats, and lung and kidney tissue samples were obtained for the histopathological study and the determination of tissue MDA, GPX, SOD, catalase and TNF-α levels. The same biochemical parameters were also studied from serum samples taken from rats. 5 ml of blood was collected with intracardiac technic.

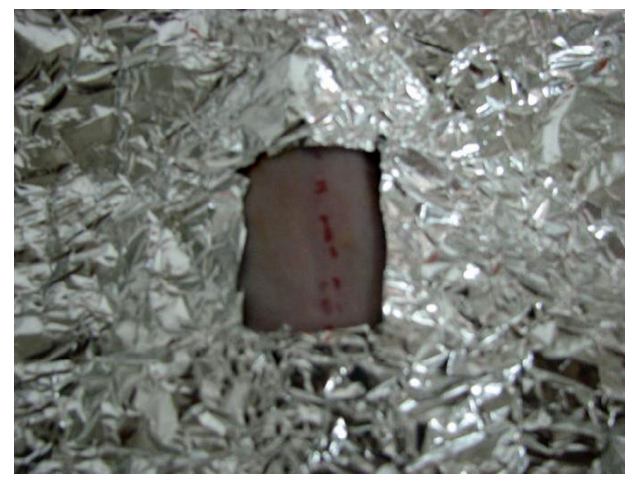

Figure 1. The shaved area was kept in 96°C water for 12 seconds and a third degree standard burn was obtained

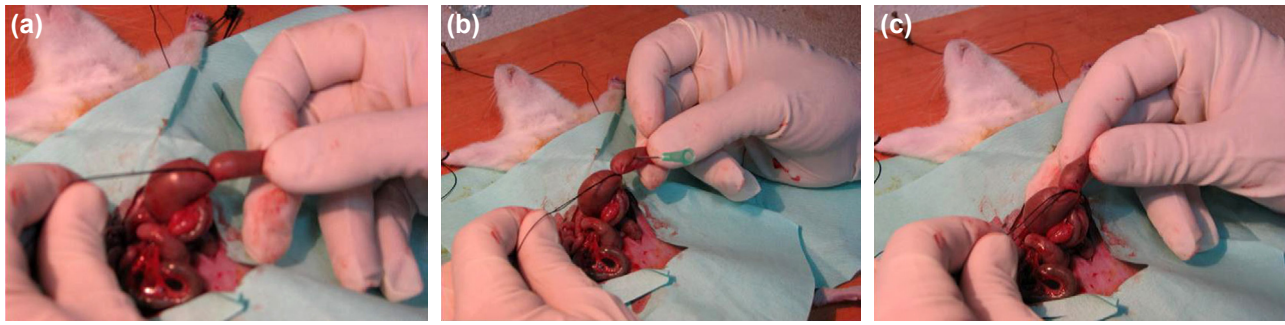

Figure 2. (a) The cecum was perforated from two separate points by a 22 Gauge needle, and was then slightly stroked and intraperitoneal feces contamination was achieved. 4 ml of saline was given subcutaneously to the back area. (b) The cecum was perforated from two separate points by a 22 Gauge needle, and was then slightly stroked and intraperitoneal feces contamination was achieved. 4 ml of saline was given subcutaneously to the back area. **(c)** The cecum was perforated from two separate points by a 22 Gauge needle, and was then slightly stroked and intraperitoneal feces contamination was achieved. 4 ml of saline was given subcutaneously to the back area.

Histopathological Study

Tissues harvested for histopathological examination were fixed in 10% buffered formalin solution. The fixated tissues were monitored according to known methods and blocked in paraffin. Serial sections of 5 µm thick paraffin blocks were stained with Hematoxylin Eosin and examined under a light microscope.

The evaluation of pathological lesions was done according to the following scores; edema in the lung tissue 1 point, hyperemia 1 point, thickening in intra-alveolar septum 2 points, mononuclear cell infiltration 2 points, shedding alveolar epithelium 3 points, hemorrhage 3 points; hyperemia in kidney tissue 1 point, mesangial cell hyperplasia in glomerulus 2 points, expansion in glomerular space 1 point, degeneration in tubular epithelium 2 points, necrosis in tubular epithelium 3 points, mononuclear cell infiltration 2 points, hemorrhage 3 points.

Study Methods of Biochemical Parameters

MDA (nm/mg) was measured spectrophotometrically by a method modified from Satoh and Yagi.[8]

Superoxide dismutase enzyme was determined by the method modified by Sun et al.[9]

Glutathione peroxidase activity was studied according to the method of Paglia et al.[10]

Catalase activity was measured by the Aebi method.[11]
TNF-α was determined by the single-step sandwich ELISA (TNF-α Trousse De Dosage Immunoennzymatique Immunoassay Kit, Immunoech, France) method.

Statistical Analysis

All data were given as mean ± standard error of the mean (SEM). Statistical analysis was performed using SPSS 11.5 program. Kruskal-Wallis test was used to evaluate statistical differences between independent groups, and Mann-Whitney U test was used for the comparison of groups. P<0.05 was considered statistically significant.

RESULTS

A total of five rats, one rat in the control group, two rats in the milrinone and two rats in the NAS groups, died during the experiment period. All deaths occurred within the first 72 hours after the burn. The dead rats were not replaced by new rats.

There was no statistically significant difference between the groups concerning mortality.

Tissue and serum malondialdehyde levels and the distribution of MDA values measured in lung, kidney tissues and serum are shown in Table 1.

When MDA levels were compared based on organs in all groups, the difference in lung and kidney tissues were found to be statistically significant (p=0.000 for lung; and 0.01 for kidney). MDA levels in the lung tissue was significantly higher in milrinone, NAS and pentoxifylline groups compared to the control group (p=0.007, p=0.015, p=0.001, respectively).

Table 1. MDA (nmol/mg) distribution of tissue and serum levels by groups

	Lung MDA value	Kidney MDA value	Serum MDA value
Control	0.060±0.018	0.223±0.077	0.6±0.2
Sildenafil	0.081±0.049	0.298±0.082	0.6±0.2
Milrinon	0.124±0.047	0.582±0.499	0.7±0.4
NAS	0.170±0.066	0.124±0.030	1.0±0.5
Pentoxyfilin	0.327±0.094	0.303±0.206	0.4±0.2
P value	0.000	0.010	0.093

* The values in the table are shown as mean±standard deviation.
MDA: Malondialdehyde; NAS: N-acetylcysteine.

Table 2. GPX (U/mg) distribution of tissue and serum levels by groups

	Lung GPx value	Kidney GPx value	Serum GPx value
Control	0.184±0.037	0.055±0.022	1.1±0.7
Sildenafil	0.206±0.080	0.267±0.058	1.0±0.4
Milrinon	0.279±0.091	0.148±0.055	0.9±0.5
NAS	0.311±0.062	0.166±0.014	1.3±0.3
Pentoxyfilin	0.157±0.121	0.127±0.077	1.4±0.4
P value	0.021	0.000	0.436

* The values in the table are shown as mean±standard deviation.
GPx: Glutathione Peroxidase; SOD: Superoxide Dismutase; NAS: N-acetylcysteine.

MDA decreased in the lung tissue in the sildenafil group more than all other groups and in the binary comparisons conducted; in the lung tissue, sildenafil group showed a significant decrease when compared to the NAS group (p=0.039) and pentoxyphyllin group (p=0.001). No significant difference was observed between the groups concerning MDA serum levels.

Table 2 shows the distribution of tissue and serum glutathione peroxidase levels between the groups and GPx values measured in the lung, kidney tissues and serum. The difference in the increase in GPX levels was found to be significant in the lung and kidney tissues compared to all groups (p=0.021 for lung; p=0.000 for kidney). Bilateral comparisons showed significantly higher levels of milrinone and NAS groups in lung tissue than the control group (p=0.046, p=0.004, respectively). Sildenafil, milrinone and NAS groups were significantly increased in kidney tissue compared to the control group (p=0.001, p=0.022, p=0.003, respectively). In addition, the increase in the sildenafil group in kidney tissue was significantly higher than milrinone, NAS and pentoxifylline groups (p=0,005, p=0,002, p=0,003, respectively). No significant difference was observed between the groups concerning GPX serum levels.

Table 3 shows the distribution of SOD values between the groups, and SOD levels in lung, kidney tissue and serum. The difference in the change of SOD levels was found to be significant in the lung tissue among all groups (p=0.03). In the comparisons with the control group, the SOD value of the milrinone and pentoxifylline groups were significantly higher in the lung tissue (p=0.032, p=0.049, respectively). It was significantly higher in the pentoxifylline group than the control group in the kidney tissue (p=0.025). SOD serum levels were not significantly different between groups.

The distribution of tissue and serum catalase levels, catalase values in the lungs, kidney tissues and serum are given in Table 4. Kidney tissue levels were found to be significantly lower in the pentoxyfylline group compared to the control group (p=0.018). Catalase serum levels were not significantly different between groups.

Table 3. Distribution of SOD (U/mg protein) tissue and serum levels by groups

	Lung SOD value	Kidney SOD value	Serum SOD value
Control	1.567±0.604	1.126±0.102	11.5±2.1
Sildenafil	1.530±0.673	1.446±0.647	10.6±3.5
Milrinon	2.353±0.407	1.293±0.380	7.9±3.4
NAS	2.146±0.521	1.184±0.133	7.5±4.2
Pentoxyfilin	2.259±0.471	1.343±0.184	11.4±1.8
P value	0.034	0.243	0.307

* The values in the table are shown as mean±standard deviation.
SOD: Superoxide Dismutase; NAS: N-acetylcysteine.

Table 4. Catalase (k/g) distribution of tissue and serum levels by groups

	Lung Catalase value	Kidney Catalase value	Serum Catalase value
Control	41.557±70.382	14.214±4.984	0.0±0.0
Sildenafil	2.938±2.190	15.800±4.646	0.0±0.0
Milrinon	40.283±83.395	12.450±8.225	0.0±0.0
NAS	5.767±2.915	16.117±12.028	0.0±0.0
Pentoxyfilin	9.100±11.999	6.800±3.894	0.0±0.0
P value	0.319	0.082	0.562

* The values in the table are shown as mean±standard deviation.
NAS: N-acetylcysteine.

Table 5. Distribution of TNF-a tissue and serum levels by groups

	Lung TNF-a value	Kidney TNF-a value	Serum TNF-a value
Control	104.100±41.417	755.114±57.571	0.0±0.0
Sildenafil	91.275±20.448	544.250±249.469	0.0±0.0
Mİlrinon	194.367±278.229	445.883±263.447	0.0±0.0
NAS	77.267±12.232	736.650±112.953	0.0±0.0±
Pentoxyfilin	69.350±31.202	457.029±150.208	0.0
P value	0.228	0.016	1.00

* The values in the table are shown as mean±standard deviation.
NAS: N-acetylcysteine. TNF-α: Tumor Necrotic Factor alpha.

The distribution of tissue and serum TNF-a levels among groups and TNF-α values measured in lung, kidney and serum are shown in Table 5. In the kidney tissue, milrinone and penthoxyfylline groups were significantly lower than the control group (p=0.046, p=0.002, respectively). No significant difference was observed in TNF-a serum levels between the groups.

Table 6. Distribution of histopathological damage score by groups

	Lung Pathologic score	Kidney Pathologic score
Control	9.4±1.0	10.0±1.2
Sildenafil	4.6±1.4	4.8±1.3
Milrinon	5.1±2.0	5.1±1.5
NAS	5.6±2.1	5.1±1.5
Pentoxyfilin	7.3±1.3	8.4±2.1
P value	0.000	0.000

*The values in the table are shown as mean±standard deviation.
NAS: N-acetylcysteine.

The histopathological findings and the distribution of histopathological damage score values between the groups in the lung and kidney tissues are shown in Table 6. When the comparison of renal tissue damage scores was made, pathological damage was significantly lower in sildenafil, milrinone and NAS groups compared to the control group (p=0.001, p=0.001, p=0.001, respectively).

The pathological damage score of all groups was significantly lower than the control group for the lung tissue (sildenafil, milrinone, NAS and pentoxyfylline, p=0.001, p=0.001, p=0.002, p=005, respectively). Histopathological damage developed in kidney tissue (A) Control group: Severe hyperemia (star), necrosis in tubule epithelia (red arrows) and expansion in the glomerular space (white arrow). (B) Sildenafil group: Mild expansion of glomerular spaces (white arrow) (Fig. 3).

Histopathological damage developed in lung tissue (A) Control group: Thickening of interalveolar septum and severe hyperemia (arrrows). (B) NAS group: Thickening of interalveolar septum and severe hyperemia (arrows). (C) Milrinone group: edema fluid in alveol lumens (white arrows), mild thickening of interalveolar septum (black arrows) (Fig. 4a-c).

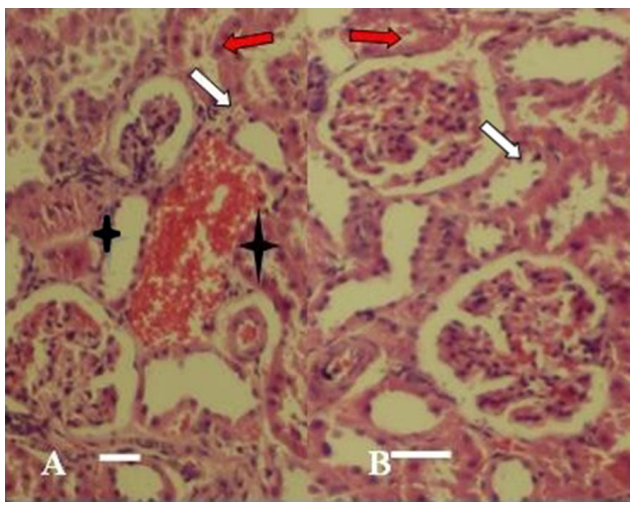

Figure 3. Histopathological damage developed in kidney tissue (A) Control group: Severe hyperemia (star), necrosis in tubule epithelia (red arrows) and expansion in the glomerular space (white arrow). (B) Sildenafil group: Mild expansion of glomerular spaces (white).

DISCUSSION

Two-hit trauma models are known to be effective in the development of systemic complications, such as post-traumatic respiratory failure syndrome.[12,13]

Common components of sepsis, such as increased lactate level, thrombocytopenia and hyperdynamic-hypodynamic shock, are observed more densely in the experimental two-hit trauma model.[14] In the two-hit models with CLP after a burn injury, it was shown that the first trauma (burn) caused a decrease in resistance to peritoneal sepsis in experimental animals and the mortality increased.[7] In this model, the second trauma causes maximum mortality after the seventh day of burn injury alone. This finding is consistent with clinical information indicating that infection resistance in burn patients is very low after one week.[7,15,16] In other studies in the literature, it was shown that the second hit performed by giving intraperitoneal endotoxin in experimental animals with burn trauma, increased Toll-like receptor-4, IL-1β, TNFα and IL-6 levels.[5,6] Similarly, neutrophil infiltration increases in the lung and liver.[5] CLP-induced polymicrobial sepsis model is the most similar model to the progression and characteristic

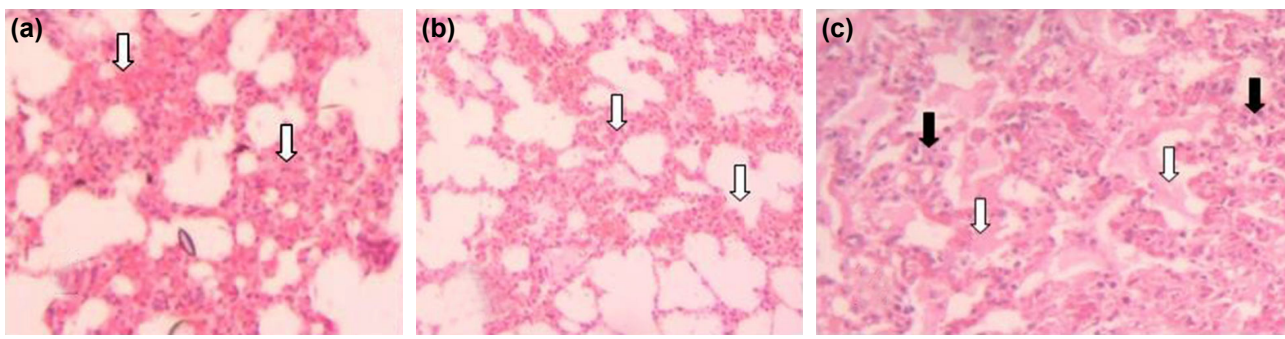

Figure 4. Histopathological damage developed in lung tissue **(a)** Control group: Thickening of interalveolar septum and severe hyperemia (arrrows). **(b)** NAS group: Thickening of interalveolar septum and severe hyperemia (arrows). **(c)** Histopathological damage developed in lung tissue Milrinon group: edema fluid in alveol lumens (white arrows), mild thickening of interalveolar septum (black arrows).

of sepsis in humans. The CLP model is now considered the gold standard for the experimental sepsis model.[17]

In clinical and experimental studies, burn injury has been shown to lead to an increase in SOR and thus induce lipid peroxidation, leading to local tissue damage, systemic complement activation and inflammation in distant organs.[18,19] Hypovolemia that develops in burn injury leads to splanchnic vasoconstriction and causes mucosal ischemia. Fluid resuscitation causes mucosal ischemia/reperfusion injury and thus excessive SOR production, leading to systemic tissue damage progression.[20,21] Increased levels of oxidative stress in lung and liver tissues as a result of burn and ischemia/reperfusion injury are thought to cause GSH to decrease due to excessive consumption.[22]

In experimental studies, it has been found that allopurinol, superoxide dismutase, deferoxamine, GSH, NAS, sildenafil and other PDE inhibitors and antioxidants, such as Vit-C reduce oxidative stress and tissue damage.[23–25] In clinical studies performed in patients with septic shock, antioxidant and PDE inhibitor therapy has been shown to reduce lipid peroxidation, maintain cardiac hemodynamic stability, and reduce the number of days spent with a ventilator and in the intensive care unit.[26,27] However, antioxidant and PDE inhibitor therapy did not have any effects on mortality in these studies.

This study aimed to investigate the efficacy of antioxidant and PDE inhibitor therapy in two-hit trauma models that would better mimic clinically evolving sepsis. In the literature, to our knowledge, there is no study investigating the efficacy of antioxidant and phosphodiesterase inhibitor therapy in two-hit models.

PDE inhibitors are one of the agents for inhibiting the synthesis and release of cytokines.[28] Pentoxifylline, a methylxanthine derivative, inhibits TNF-α gene transcription by increasing intracellular cAMP levels.[28] Sildenafil is an increasingly common PDE-5 enzyme inhibitor used for the treatment of erectile dysfunction and pulmonary hypertension because of its vascular dilator effect.[29,30]

In addition to its vasodilation effect, sildenafil inhibits platelet aggregation and has anti-inflammatory and antioxidative properties.[31,32] Milrinone is a PDE 3 inhibitor with inotropic and vasodilatory action.[33] Milrinone shows its effect by increasing intracellular cAMP.[33] It has been emphasized in the studies that milrinone has an anti-inflammatory effect independent of its vasodilator effect.[34]

Sildenafil is a specific PDE5 inhibitor.[35] It inhibits cGMP specifically and potently.[35] Sildenafil has been reported to have healing effects on inflammation and oxidative stress in the lungs and other organs. It has been proven in studies that it suppresses inflammatory events by reducing oxidative stress.[36] In their study, Yildirim et al.[37] showed a significant reduction in tissue MDA level and a maintained GSH level in the group of lung fibrosis patients treated with 10 mg/kg sildenafil. In another study, sildenafil has been shown to have a renoprotective effect against oxidation and inflammation in diabetic rats.[38] In our study, the GPx level in the kidney tissue in the sildenafil group was not only higher than the control group but was significantly higher than all other groups. No significant effect of sildenafil on other parameters was observed. The MDA value was lower in the sildenafil group than in the other groups. However, this difference was not significant when compared with the control group but was significantly lower than NAS and pentoxifylline.

The damage score of all tissues was significantly lower in the sildenafil group compared to the control groups. This situation suggests that sildenafil shows its protective effect on tissue damage by increasing GPx activity and decreasing lipid peroxidation. The results of our study support other studies in the literature. In the literature, different treatment doses were used in sildenafil studies. Pentoxifylline, a methylxanthine derivative, has been used for many years for its circulatory regulating effect, it has been shown to have a strong inhibitory effect on neutrophils in recent years, especially by inhibiting the release of free oxygen radicals, primarily superoxide, and lysosomal enzymes[39] and clearing hydroxyl radicals from damaged tissues.[40]

PTX is also well known to reduce the TNF-α release from inflammatory cells.[41] Different results have been obtained in antioxidant studies. Sulkowska et al.[42] reported that it prevents lung injury caused by free oxygen radicals due to cyclophosphamide. PTX has been shown to have beneficial effects on sepsis in human and animal experiments.[43] PTX has been shown to improve the hemodynamic state in sepsis.[44] It prevents the passage from hyperdynamic response to hypodynamic response, improving renal blood flow.[44] Inflammatory lung injury after endotoxemia has also been shown to be improved by PTX.[45] In the study conducted by Zeni et al.,[44] it was observed that PTX decreased TNF-a and IL-1 levels in adults and newborns.

In our study, the levels of SOD in the kidney tissue were significantly higher and TNF-α and catalase values were significantly lower in the PTX group. SOD levels were significantly higher in the lung tissue. The pathological damage score was found to be low only in the lung tissue. The results support previous studies in the literature. In addition, the effect of PTX on TNF-α was observed more clearly than other groups. Although the significant increase in SOD is thought to be compensatory, SOD activity may be increased by PTX. There are a limited number of studies investigating the use of milrinone in SIRS and sepsis in the literature.[46,47] Although it improves cardiac performance in these patients, it is not recommended for the treatment of sepsis because of its vasodilator effect.[48] However, the anti-inflammatory effects of milrinone, as well as cardiovascular effects, have been shown.[49,50]

Ming Gong et al.[51] to investigate the effect of milrinone on cardiopulmonary bypass associated inflammation, randomized 30 patients before cardiopulmonary bypass by inhalation of milrinone and saline. TNF-α, IL-6 and matrix metalloproteinase levels were significantly lower in the milrinone group after the operation. In our study, a significant increase in lung SOD and GPx levels, a significant decrease in TNF-α levels in kidney tissue and significant increase in GPx value were observed in the milrinone group. In addition, the damage score of all organs in the milrinone group was significantly lower than the control group.

N-Acetylcysteine (NAS) is a thiol compound with potent antioxidant and anti-inflammatory properties. NAS is also a well-known glutathione (GSH) precursor.[52] NAS shows its effects by transforming into an endogenous FOR retainer, glutathione.[53,54] Phosphodiesterase (PDE) inhibitors have critical control of the intracellular signal transduction system because they hydrolyze cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP).[31] They play a role in many pathological events, including inflammation, cancer, neurodegeneration and oxidative stress.[31]

NAS is a mucolytic commonly used in the clinic and its effects on burn injury as a precursor of GSH have been studied in the literature. For example, the use of NAS in animals with burn injury has been shown to improve cellular immunity.[55,56] Similarly, short-term 24-hour post-burn NAS treatment decreases MDA levels in lung tissue and increases GSH levels and thus decreases oxidative stress.[54] These effects of NAS are attributed to correcting cellular immunity in thermal damage.[56] NAS treatment in animals with experimental peritonitis, strengthens peritone defense mechanisms by correcting suppressed neutrophil activation.[57] On the other hand, the level of GSH increases with NAS therapy in the lung tissue, thereby reducing neutrophil infiltration.[57] It has been shown that NAS treatment acts both as a FOR retainer and also increases the level of GSH, thereby suppressing production and activity of proinflammatory cytokines and chemokines, while increasing cytotoxic T cell activity and IL-2 production.[58]

These anti-inflammatory and immunostimulatory effects may explain the effects of NAS on both peritoneal defense mechanisms and the reduction of distant organ damage. In our study, a significant increase was observed in the level of GPx in the lung tissue in the group given NAS; and again in the group treated with NAS, there was a significant decrease in MDA value in the kidney tissue and a significant increase in the GPx level. In addition, the histopathological damage score of all tissues was significantly lower than the control group. Our results were similar to other studies in the literature.

The effectiveness of NAS in preventing tissue damage was observed. Our results suggest that the antioxidant effect of NAS is produced by increasing the activity of GPx and decreasing lipid peroxidation, similar to sildenafil. This can be explained by saying that the diagnostic value of the tissue levels of antioxidant enzymes and free radicals, which have a shorter life and are effective in the tissue in which they are formed, are more valuable than their serum levels. In this study, it was found that antioxidant and phosphodiesterase inhibitor treatment decreased oxidative stress level in lung and kidney tissues in a consecutive two-hit trauma model performed with CLP peritonitis after burn. Generally, the results of our study reflect the strong anti-inflammatory and antioxidant properties of PDE inhibitors.

The SOD and GPx values were mostly influenced by the treatments we provided. Our results suggest that PDE inhibitors and NAS can exhibit antioxidant properties by increasing SOD and GPx enzyme activities. The role of PDE inhibitors and NAS in reducing tissue damage was evident. The antioxidant and anti-inflammatory effects of PDE inhibitors and NAS on lung tissue were found to be stronger than other organs. Milrinone was the most potent in the groups. Again, PTX and milrinone were found to be most effective on TNF-a, which played an important role in the course of sepsis.

Antioxidant and phosphodiesterase inhibitor treatment is a promising treatment option for the prevention of late-stage organ damage and multiple organ failure caused by the second hit, in cases where the two-hit models in MOF development, such as burn, hemorrhagic shock, and sepsis is thought to be functional. In patients who have experienced major trauma, antioxidant and phosphodiesterase inhibitor therapy may have a potential prophylactic effect in the treatment of immune deficiency, especially in the first week, before the development of the second trauma, such as an infection. Further experimental and clinical studies are needed.

In the experimental sequential two-hit trauma model, antioxidant and phosphodiesterase inhibitor treatment suppress the level of tissue oxidative stress, and the treatment of PDE inhibitors and NAS initiated after the formation of the second trauma reduces distant organ tissue damage.

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DENEYSEL ÇALIŞMA - *ÖZET*

İki darbe modelinde spesifik ve non-spesifik fosfodiesteraz inhibitörleri ve N-asetilsisteinin oksidatif stres ve uzak organ hasarına etkisi

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AMAÇ: Sepsis enfeksiyona karşı oluşan sistemik bir enflamatuvar yanıttır ve morbidite ve mortalitenin ana nedenlerinden biridir. Travma sonrası ikinci darbe artmış enflamatuvar yanıta ve çoklu organ yetersizliğine (ÇOY) neden olur. Yanık hasarı sonrası gelişen enfeksiyon iki darbe travma çalışması için uygun bir modeldir. Sepsis çoklu organ yetersizliğinde anahtar rol oynayabilecek lipid peroksidasyonuna neden olan serbest oksijen radikalleri (SOR) gibi biyokimyasal mediyatörlerin salınımına neden olur. Biz bu çalışmada iki darbe modelinde fosfodiesteraz (PDE) inhibitörleri (sildenafil, milrinon, pentoksifilin) ve N-asetilsisteinin (NAS) oksidatif stres ve organ hasarı üzerindeki etkilerini araştırmayı amaçladık.

GEREÇ VE YÖNTEM: Bu deneysel çalışmada 40 sıçanda %30'luk haşlama yanığı oluşturulduktan 72 saat sonra çekal ligasyon ve ponksiyon (CLP) yöntemi ile peritonit oluşturuldu. Sıçanlar her biri sekiz sıçandan oluşan beş gruba ayrıldı. Grup I: Tedavi uyulanmadı; Grup II: CLP sonrasında 72 saat boyunca 10/mg/kg gün dozunda intraperitoneal (i.p) sildenafil tedavisi uygulandı; Grup III: CLP sonrasında 72 saat boyunca 1/mg/kg gün dozunda i.p milrinon tedavisi uygulandı; Grup IV: CLP sonrasında 72 saat boyunca 150/mg/kg gün dozunda i.p NAS tedavisi uygulandı; Grup V: CLP sonrasında 72 saat boyunca 50/mg/kg gün dozunda i.p pentoksifilin tedavisi uygulandı. Tüm sıçanlar deneyin yedinci gününde sakrifiye edildi. Malondialdehit (MDA), glutatyon peroksidaz (GPx), süperoksit dismutaz (SOD), katalaz, tümör nekröz faktör alfa (TNF-α), düzeyleri ve histopatolojik çalışma için doku (akciğer, böbrek) ve serum örnekleri alındı.

BULGULAR: Kontrol grubu ile karşılaştırıldığında tedavi edilen tüm gruplarda doku hasar skoru düşük bulundu. Sildenafil, milrinon ve NAS gruplarında böbrek GPx düzeyi kontrol grubuna göre yüksek bulundu. Akciğer dokusunda milrinon ve pentoksifilin gruplarında SOD kontrol grubuna göre yüksek bulunurken milrinon ve NAS ile GPx düzeyi yüksek bulundu. Böbrekte pentoksifilin ve milrinon gruplarında TNF-α düzeyi kontrol grubuna göre düşük bulundu.

TARTIŞMA: Bu deneysel çalışma iki darbe modelinde PDE inhibitörleri ve NAS'nin oksidatif stres düzeyini ve uzak organ hasarını azaltıcı etkileri olduğunu göstermiştir. Bu konu üzerinde ileri klinik ve deneysel çalışmalara ihtiyaç vardır.

Anahtar sözcükler: Antioksidan; doku hasarı; fosfodiesteraz inhibitörü; sepsis; serbest oksijen radikalleri.

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