INTRODUCTION

Changes in the stasis zone are reversible in the early phase of the burn. The patient’s supportive treatment should not be delayed so that the cells in the stasis zone - characterized by inflammation and edema around the burned coagulation zone and with less damage and a potential for recovery - do not lose their viability. If appropriate conditions are provided, the cells in the stasis zone regenerate within a week.[1] Dehydration, pressure, hypovolemia, excessive fluid intake, and infection may cause necrosis in the stasis zone, which may, therefore, cause the burn to...
deepen. Especially, in the 1st week, avoiding dryness in the burn wound, the use of topical antimicrobial agents, and proper resuscitation are the necessary treatment approaches for stopping the progress of necrosis. Various vasoactive inflammatory mediators, especially thromboxane A2 and prostaglandin F2, are released in burned tissues. Some studies have shown that the use of specific thromboxane inhibitors significantly improves dermal perfusion by inhibiting platelet adhesion and vasoconstriction, thereby reducing necrosis development in burn injuries.[2,3]

The peripheral part of the stasis zone is called the hyperemia zone. A significant amount of vasodilatation is observed in this zone, accompanying an increase in blood flow with the effect of vasoactive mediators that are revealed as a result of the inflammatory response. The hyperemia zone is characterized by minimal cell damages. If complications such as trauma and infection do not develop, this zone shows a complete cellular improvement. Phosphodiesterase (PDE) inhibitors have critical control in the intracellular signaling system because they hydrolyze cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). They are involved in many pathological events such as inflammation, cancer, neurodegeneration, and oxidative stress.[4]

Agents for inhibiting the synthesis and release of cytokines also include PDE inhibitors. A methylxanthine derivative, pentoxifylline (PTX), inhibits tumor necrosis factor (TNF-α) gene transcription by increasing intracellular cAMP levels.[5]

Milrinone is a PDE 3 inhibitor with inotrope and vasodilator effects. It acts by increasing intracellular cAMP.[6] It was emphasized in studies that milrinone has an anti-inflammatory effect, independent of its vasodilator effect.[7] The establishment of experimental burns in animal models is believed to be useful for understanding the pathogenesis of burn injury to human skin and for designing and developing new treatment methods. Several animal models have been proposed to investigate the pathology of burn wounds, their local and systemic treatments, and the effect of burn trauma on the whole organism.[8] Based on the above information, in this study, a research was carried out on the effects of PDE inhibitors, PTX and milrinone, on the burn stasis zone in rats and its rescue.

**METHOD**

A total of 56 4-month-old female rats of Wistar albino type with a weight of 200 ± 10% g and with similar characteristics in size and age were included in the study. The animals were maintained and fed in metal cages fitted over with mats, at 22–24°C room temperature, 55% humidity, 12 h light/12 h dark, with water and feeds given ad libitum.

**Experimental protocol: Anesthesia**

At the end of the 10th days, the rats were sacrificed with 200 mg/kg pentothal sodium (thiopental sodium) (IV), and tissue samples were taken.

**Groups**

The experimental animals were divided into seven groups.

Group 1: Sham group - the group without any action.

Group 2: This group was selected as an intraperitoneal control group, and no treatment was applied by creating a comb model.

Group 3: This group was selected as an oral control group; a comb model was created, and no treatment was applied.

Group 4: This is the group to which 5-PDE inhibitor (PTX) was administered intraperitoneally; PTX was administered intraperitoneally at a dose of 50 mg/kg daily for 10 days.

Group 5: This is the group to which 5-PDE inhibitor (PTX) was administered orally; PTX was administered orally at a dose of 50 mg/kg daily for 10 days.

Group 6: This is the group to which 5-PDE inhibitor (milrinone) was administered intraperitoneally; milrinone was administered intraperitoneally at a dose of 1mg/kg daily for 10 days.

Group 7: This is the group to which 5-PDE inhibitor (milrinone) was administered orally; milrinone was administered orally at a dose of 1mg/kg daily for 10 days.

**Experimental burn model**

The skin on the back of the rats was shaved and made hairless [Figure 1].

The experimental burn model defined by Regas and Erlich was applied to these 56 rats under anesthesia.[9] A coagulation zone forms on the area exposed to direct heat according to the model. In the vicinity of the coagulation zone, a transition zone (stasis zone) is formed in which progressive ischemia and necrosis develop over time due to reduced blood flow, where dynamic changes in blood flow can be examined. The model was designed in accordance with the original model so that if the stasis zone is treated within the first 24 h, the blood flow and tissue viability can be sustained, and if it is not treated, it will turn into a coagulation zone.[9]

![Figure 1: Image of shaved rat](image-url)
To create this burn model, a comb-shaped brass tool with four rectangular contact areas of 1 cm × 2 cm which have three intermediate zones of 0.5 cm × 2 cm was used [Figure 2]. The described brass comb was soaked in boiling water at 100°C for 3 min and then contacted with its own weight for 30 s without applying pressure to the left and right side of the shaved dorsum of the anesthetized rat. Thus, the burns were formed in such a way that four full-thickness burned areas of rectangular shape each 1 cm × 2 cm in size were produced on both sides 0.5 cm lateral to the middle line of the hairless dorsum area of the rats, and there would be three intact zones of 0.5 cm × 2 cm in size in between these burned areas [Figure 3]. The 1 cm × 2 cm burned areas that were created were described as the coagulation zone, and the healthy tissue areas of 0.5 cm × 2 cm between these were described as the ischemic (stasis) zone.

**Histopathological study**

Tissue samples in the form of a ribbon were taken from the burn line of the rats. The samples were nested in 10% formaldehdy, and paraffin blocks were prepared by passing the coagulation zone and ischemia (stasis) zone through routine tissue monitoring procedures. Sections with a thickness of 6 μ taken from the prepared paraffin blocks were stained with hematoxylin and eosin for histopathological examination. Based on the intensity of histopathological lesions, healing of the burn wound was scored as 0 = none, 1 = mild, 2 = moderate, and 3 = dense by checking the amount of edema, hyperemia, epithelial degeneration necrosis, MNH infiltration, polymorphonuclear neutrophil leukocytes (PMNL) infiltration, and fibroblast-fibrocyte on microscopic examinations.

**Study methods of biochemical parameters:**

**Catalase nm/mg assay**

Catalase activity was calculated using Aebi’s method. A 50 mM of pH 7.0 phosphate buffer was prepared. A 30 mM hydrogen peroxide solution was also prepared, and the solutions were prepared at the ratios given in table. The sample was diluted 50-fold. As soon as hydrogen peroxide was added, the absorbance values were read kinetically at 240 nm.

**Malondialdehyde (MDA) nm/mg assay**

This was studied according to Yagi K. method. 0.084 M sulfuric acid, 10% phosphotungstic acid, and thiobarbituric acid solutions were prepared.

**Superoxide dismutase (SOD) nm/mg assay**

The serum samples that were taken were prepared according to the method of McCords and Fridowich. Pipetting was performed according to the following table. The cuvettes were again compared, reading the initial absorbance (A1) at 505 nm and 37°C after a 30 s of delay phase, and reading the final absorbance after 3 min (A2).

**Glutathione peroxidase (GPx) nm/mg assay**

This was prepared according to the method of Goldberg and Spooner. The catalytic activity was determined by following the reduction kinetically at 340 nm due to the oxidation of the absorbance of NADPH.

**Ethical considerations**

This study was conducted with the approval of the Ethics Committee of Cumhuriyet University Hospital.

**Statistical analyses**

All data were expressed as mean ± standard error of mean. Statistical evaluation was performed using the SPSS 22.0 program. The Kruskal–Wallis test was used to assess the statistical differences between the independent groups, and the Mann–Whitney U-test was used to compare the groups. Values of $P < 0.05$ were considered statistically significant.

**Findings**

Table 1 shows serum MDA, SOD, CAT, and GPx levels of sham and control groups. Based on this, when the groups were compared,
the difference between the groups according to MDA levels was statistically significant ($P < 0.05$). The group with the lowest mean score was the sham group ($\mu = 2.64$). The mean scores of the oral control and the intraperitoneal control groups were calculated as $\mu = 14.01$ and $\mu = 16.75$, respectively. The mean scores of the sham group and the oral control group were calculated as $\mu = 12.58$ and $\mu = 16.02$, respectively, according to SOD values. When the groups were compared, there was no statistically significant difference between the groups according to catalase values ($P > 0.05$). The group with the lowest mean score was the intraperitoneal control group ($\mu = 11.80$). The mean scores of the oral control and the sham groups were calculated as $\mu = 12.75$ and $\mu = 14.15$, respectively. There was no statistically significant difference between the groups according to the GPx values ($P > 0.05$). The group with the lowest mean score was the intraperitoneal control group ($\mu = 78.04$). The mean scores of the sham group and the oral control group were calculated as $\mu = 78.45$ and $\mu = 82.24$, respectively. The difference between the groups was statistically significant ($P < 0.05$). The group with the lowest mean score was the intraperitoneal control group ($\mu = 8.45$) [Table 1].

Serum MDA, SOD, CAT, and GPx levels of the intraperitoneal groups are listed in Table 2. When the groups were compared, there was a statistically significant difference between groups according to MDA values ($P < 0.05$). The group with the lowest mean score was the PTX intraperitoneal group ($\mu = 4.48$). The mean scores of the milrinone intraperitoneal and the intraperitoneal control groups were calculated as $\mu = 7.98$ and $\mu = 16.02$, respectively. There was a statistically significant difference between the groups according to SOD values ($P < 0.05$). The group with the lowest mean score was the intraperitoneal control group ($\mu = 14.01$). The mean scores of the milrinone intraperitoneal and the PTX intraperitoneal groups were calculated as $\mu = 114.85$ and $\mu = 157.19$, respectively. There was a statistically significant difference between the groups according to catalase values ($P < 0.05$). The group with the lowest mean score was the oral control group ($\mu = 12.75$). The mean scores of the milrinone oral and the PTX oral groups were calculated as $\mu = 24.49$ and $\mu = 43.38$, respectively. There was a statistically significant difference between the groups according to GPx values ($P < 0.05$). The group with the lowest mean score was the oral control group ($\mu = 14.01$). The mean scores of the milrinone oral and the PTX oral groups were calculated as $\mu = 25.14$ and $\mu = 36.21$, respectively. There was a statistically significant difference between the groups according to catalase values ($P < 0.05$). The group with the lowest mean score was the oral control group ($\mu = 16.02$). The mean scores of the milrinone oral and the PTX oral groups were calculated as $\mu = 223.03$, respectively [Table 2].

The serum MDA, SOD, and GPx values of the oral groups are shown in Table 3. When the groups were compared, there was a statistically significant difference between the groups according to MDA values ($P < 0.05$). The group with the lowest mean score was the PTX oral group ($\mu = 6.00$). The mean scores of the milrinone oral and the oral control groups were calculated as $\mu = 7.98$ and $\mu = 14.01$, respectively. There was a statistically significant difference between the groups according to SOD values ($P < 0.05$). The group with the lowest mean score was the oral control group ($\mu = 16.02$). The mean scores of the milrinone oral and the PTX oral groups were calculated as $\mu = 25.14$ and $\mu = 36.21$, respectively. There was a statistically significant difference between the groups according to catalase values ($P < 0.05$). The group with the lowest mean score was the oral control group ($\mu = 14.01$). The mean scores of the milrinone oral and the PTX oral groups were calculated as $\mu = 8.45$ and $\mu = 157.19$, respectively. There is no consensus standardization in burn modeling. In

### Table 1: Distribution of serum levels of MDA (U/mg), SOD (U/mg), CAT (U/mg), and GPx (U/mg) by control and sham groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA</th>
<th>SOD</th>
<th>CAT</th>
<th>GPx</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X±S</td>
<td>X±S</td>
<td>X±S</td>
<td>X±S</td>
</tr>
<tr>
<td>Sham</td>
<td>2.64±1.67</td>
<td>12.58±7.57</td>
<td>14.15±10.09</td>
<td>78.04±55.03</td>
</tr>
<tr>
<td>Oral-control</td>
<td>14.01±7.45</td>
<td>16.02±5.77</td>
<td>12.75±5.54</td>
<td>82.24–26.51</td>
</tr>
<tr>
<td>intraperitoneal control</td>
<td>16.75±2.40</td>
<td>8.45±3.23</td>
<td>11.80±7.82</td>
<td>77.73±25.92</td>
</tr>
</tbody>
</table>

Table 1: Distribution of serum levels of MDA (U/mg), SOD (U/mg), CAT (U/mg), and GPx (U/mg) by control and sham groups

**Table 2**: The serum MDA, SOD, and GPx values of the oral groups are shown in Table 3. When the groups were compared, there was a statistically significant difference between the groups according to MDA values ($P < 0.05$). The group with the lowest mean score was the PTX oral group ($\mu = 6.00$). The mean scores of the milrinone oral and the oral control groups were calculated as $\mu = 7.98$ and $\mu = 14.01$, respectively. There was a statistically significant difference between the groups according to SOD values ($P < 0.05$). The group with the lowest mean score was the oral control group ($\mu = 16.02$). The mean scores of the milrinone oral and the PTX oral groups were calculated as $\mu = 25.14$ and $\mu = 36.21$, respectively. There was a statistically significant difference between the groups according to catalase values ($P < 0.05$). The group with the lowest mean score was the oral control group ($\mu = 14.01$). The mean scores of the milrinone oral and the PTX oral groups were calculated as $\mu = 8.45$ and $\mu = 157.19$, respectively. There is no consensus standardization in burn modeling. In

### DISCUSSION

A good experimental burn model should be simple, repeatable, reliable, easy to implement and, if possible, cost-effective.[10] The serum MDA, SOD, and GPx values of the oral groups are shown in Table 3. When the groups were compared, there was a statistically significant difference between the groups according to MDA values ($P < 0.05$). The group with the lowest mean score was the PTX oral group ($\mu = 6.00$). The mean scores of the milrinone oral and the oral control groups were calculated as $\mu = 7.98$ and $\mu = 14.01$, respectively. There was a statistically significant difference between the groups according to SOD values ($P < 0.05$). The group with the lowest mean score was the oral control group ($\mu = 16.02$). The mean scores of the milrinone oral and the PTX oral groups were calculated as $\mu = 25.14$ and $\mu = 36.21$, respectively. There was a statistically significant difference between the groups according to catalase values ($P < 0.05$). The group with the lowest mean score was the oral control group ($\mu = 14.01$). The mean scores of the milrinone oral and the PTX oral groups were calculated as $\mu = 8.45$ and $\mu = 157.19$, respectively. There is no consensus standardization in burn modeling. In
As our study, we tried to create a cost-effective work, which was easy to understand, and easy to analyze.

Pigs, rabbits, mice, dogs, guinea pigs, rats, and sheep were used as model animals in different studies. The most preferred animal was rat because of its advantages such as being small, easy to obtain, cheap, and having a high reproductive rate. It should be emphasized that metabolic, physiological characteristics, and anatomy of rats are different from those of the human body. For example, rats have thin epidermis and dermis, they have a denser hair layer, and their primary way of healing is through contraction, contrary to reepithelization. We also preferred to use rats in our study, considering the advantages that were mentioned.

The sudden increase in body surface temperature causes simultaneous local responses aimed at fending off heat by vasodilatation in vascular structures in this area. A further increase in tissue temperature initiates the inflammatory response, leading to local release of inflammatory mediators, followed by a reaction chain. Injury initiates a response involving cytokines and all immune system cells originating from active leukocytes.

Inflammation is a reaction in which the immune system members are directed to the injury site against tissue damage, infection, or antigenic stimulation. Of the active complement products, C3a and C5a, platelet factor 4 and various bacterial products are chemotactic factors that attract phagocytic leukocytes to the injury site. The leukocytes that first reach the wound and rapidly phagocyte and kill bacteria are the PMNL. PMNLs are slowly and gradually replaced by macrophages that respond to the same chemotactic factors as themselves. Macrophages, on the other hand, produce many cytokines for multiple wound healings when stimulated. Infection in burn patients continues to be a major cause of death.

The cellular and humoral immune response is suppressed in direct proportion to the size of the burn. The number of leukocytes is high in the 1st week after the burn event, while the number of lymphocytes is low. Lymphocytes in solid organs are lost by apoptosis. Interleukin (IL)-2 levels decrease in proportion to the size of the burn. IL-2 production is further reduced if septic complications develop. In addition, IL-1 and, especially, IL-6 and IL-8 levels increase significantly in the first 5 days following the burn.
IgG levels decrease in the period after the burn. Cytosolic oxidase activities and normal oxidase activities of the granulocytes increase, and consequently, their oxidative potential increases; if neutrophils are activated, their capacity to cause tissue and organ damage also increases.[21]

Thermal damage causes complex local and systemic responses. Local inflammatory response results in an increase in vasodilatation and vascular permeability.[22] A burn is basically a three-dimensional ischemic wound, with a necrosis (coagulation) zone, an ischemic (stasis) zone, and an inflammation (hyperemia) zone. The necrosis (coagulation) zone: It is the area with the most damage. There is irreversible tissue loss caused by the coagulation of structural proteins.[23,24]

There is a stasis zone around the coagulation zone where there is less damage with the potential for rescue, characterized by inflammation and edema. The circulation in this region progressively deteriorates, causing ischemia and death of cells. Disruption of the blood flow in this zone occurs as a result of pathologic changes in the microvascular level.[25] These changes include the formation of microthrombi, adhesion of the neutrophils to the vessel wall, fibrin deposition, edema in endothelium, and vasoconstriction.[26] Disruption of blood flow occurs within 2–3 h of severe burns. In less severe burn areas, this time span may be delayed up to 16–24 h.[11] This process can take up to 48 h. The patient’s supportive treatment should not be delayed so that the cells of the stasis zone do not lose their vitality.

If appropriate conditions are provided, the cells in the stasis zone regenerate within a week.[26] However, even if healing occurs, the epithelial cell loss is high in this zone. During recovery, cells are hypersensitive to injury. Dehydration, pressure, hypovolemia, excessive fluid intake, and infection may cause necrosis in the stasis zone, which may therefore cause the burn to deepen. Especially, in the 1st week, avoiding dryness in the burn wound, the use of topical antimicrobial agents, and proper resuscitation are the necessary treatment approaches for stopping the progress of necrosis. Right at this point, we tried to figure out how much the agents we used influenced various mediators in the stasis zone.

The peripheral part of the stasis zone is called the hyperemia zone. A significant amount of vasodilatation is observed in this zone, accompanying an increase in blood flow with the effect of vasoactive mediators that are revealed as a result of the inflammatory response. The hyperemia zone is characterized by minimal cell damages. If complications such as trauma and infection do not develop, this zone shows a complete cellular improvement. PDE inhibitors have critical control in the intracellular signaling system because they hydrolyze cAMP and cGMP. They are involved in many pathological events such as inflammation, cancer, neurodegeneration, and oxidative stress.[27]

The circulation regulatory effect of PTX, a methylxanthine derivative, has been benefited for many years. In recent years, it has been determined to have a strong inhibitory effect on neutrophils, to inhibit the release of free oxygen radicals and lysosomal enzymes, especially superoxide radicals, from neutrophils in damaged tissues,[28] and to clear the hydroxyl radicals in damaged tissues.[29] PTX is also well known to reduce TNF-α release from inflammatory cells.[30] Different results were obtained in studies where it was used as an antioxidant.

PTX has been shown to improve endothelial function and tissue oxygenation, to decrease pro-inflammatory cytokine release, and to be beneficial in ischemic reperfusion injury.[31,32] These effects include increasing prostaglandin I2 synthesis, increasing SOD activity, inhibiting PMNL migration and myeloperoxidase release to the ischemic area, inhibiting xanthine oxidase activity, and reducing factor production that activates platelets.[32,33] PTX has been shown to have beneficial effects on sepsis and NEC in human and animal experiments.[34] Sulkowska et al.[35] reported to have found that it inhibited lung damage caused by cyclophosphamide-induced free oxygen radicals, but it was inadequate to prevent oxidative damage of the liver. PTX has been shown to improve hemodynamics in sepsis.[35] It inhibits transition from the hyperdynamic response to hypodynamic response and improves renal blood flow.[36,37] In a study by Zeni et al., PTX has been shown to reduce TNF-α and IL-1 levels in adults and newborns.[38] In another experimental burn study conducted on rats by Özer et al.,[39] SOD levels in kidney tissue were found to be significantly higher, and TNF-α and catalase values were significantly lower in the group that received PTX. SOD and TAS levels in lung tissue were significantly higher and the LOS value was lower. It was observed that it significantly reduced catalase values in the ileum. The pathological damage score was low only in lung tissue. Moreover, the effect of PTX on TNF-α was more pronounced than in the other groups. In a study conducted by Vlahos et al.[39] PTX has also been shown to suppress angiogenesis in rats by reducing VEGF-C in the endometriosis model. All-cause mortality is reported to be reduced in PTX-treated groups in addition to antibiotic therapy in newborns with sepsis.[34]

There are a limited number of studies in the literature investigating the use of milrinone.[40,41] In addition to the cardiovascular effect of milrinone, its anti-inflammatory effects have also been demonstrated.[42,43] Ming Gong et al.[42] randomized 30 patients before they underwent a cardiopulmonary bypass to investigate the effect of milrinone on cardiopulmonary bypass-related inflammation, giving them milrinone inhalation and saline. TNF-α, IL-6, and matrix metalloproteinase levels were found to be significantly lower in the group administered milrinone after the operation than in the other group. In a burn study performed by Özer et al.,[39] there were a significant decrease in the ileum MDA level, a significant increase in lung SOD, GPx, and TAS levels, and a decrease in the TOS value in the milrinone-administered group.
There were a significant decrease in the TNF-α level and a significant increase in the GPx value in kidney tissue, as well. Moreover, the damage score of all organs was also found to be significantly lower in the milrinone-administered group than in the control group. There were a decrease in the MDA level, an increase in SOD and GPx levels, and a decrease in the tissue damage score, all being statistically significant in our study, as well. This supports the study that was done. In our study, pathologically, edema, hyperemia, epithelial degeneration, necrosis, inflammatory infiltration, and fibrosis measurements were taken. Compared to the control group, the tissue damage score was lower in all treated groups. MDA levels were lower in the intraperitoneal and oral PTX-administered group than in the control group. SOD, CAT, and GPx levels were higher in the intraperitoneal and oral PTX-administered group than in the control group. The MDA level was lower in the intraperitoneal and oral milrinone administered group than in the control group. SOD, CAT, and GPx levels were higher in the intraperitoneal and oral milrinone-administered group than in the control group. The results support previous studies in the literature. MDA levels were lower in the intraperitoneal PTX-administered group than in the intraperitoneal milrinone-administered group. SOD, CAT, and GPx levels were higher in the intraperitoneal PTX-administered group than in the intraperitoneal milrinone-administered group.

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