ORIGINAL ARTICLE

Recovery of Burn Stasis Zone Using Pentoxifylline and Milrinone in an Experimental Burn Model Established For Rats

Muratcan Mollaoğlu[1], Mustafa Atabey[1], Meriç Emre Bostanci[1], Mukadder Mollaoğlu[2], Kürşat Karadayi[1]

[1]Department of General Surgery, Cumhuriyet University, Sivas, Turkey, [2]Department of Health sciences , Health Sciences Faculty, Cumhuriyet University, Sivas, Turkey

ABSTRACT

Aim: The aim of this study is to investigate the effect of pentoxifylline (PTX) and milrinone on the stasis zone in rats for which an experimental burn model was established. **Method:** In this study with an experimental design, PTX and milrinone - 5-phosphodiesterase inhibitors - were administered orally and intraperitoneally to rats for which an experimental burn model was created. The effects of these pharmacological agents on the stasis zone and comparison of these effects with each other were investigated. In the study, 56 4-month-old female rats of Wistar albino type with a weight of 200 ± 10% g were used. The rats were divided into seven groups. The burn model defined by Regas and Erlich was applied to 48 rats under anesthesia. Groups 1, 2, and 3 were defined as the sham group, intraperitoneal control group, and oral control group, respectively. Drugs were administered to groups for 10 days as follows: PTX intraperitoneally daily at a dose of 50 mg/kg to Group 4, PTX orally daily at a dose of 50 mg/kg to Group 5, milrinone intraperitoneally daily at a dose of 1 mg/kg to Group 6, and milrinone orally daily at a dose of 1 mg/kg to Group 7. All rats were sacrificed on the 10th day of the experiment. Serum samples for the biochemical levels of malondialdehyde (MDA), glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase were taken as well as tissue samples for the histopathological study of these. **Results:** Pathologically, edema, hyperemia, epithelial degeneration, necrosis, inflammatory filtration, 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, while SOD, catalase, and GPx levels were higher. MDA levels were lower in the intraperitoneal and oral milrinone-administered group than in the control group, and SOD, catalase, and GPx levels were higher. In addition, it was found that MDA levels were lower and SOD, catalase, and GPx levels were higher in the intraperitoneal PTX-administered group than in the intraperitoneal milrinone-administered group. It was found that MDA levels were lower and SOD, catalase, and GPx levels were higher in the oral PTX-administered group than in the oral milrinone-administered group. **Conclusion:** As a result of our study, it was shown that PTX and milrinone reduce oxidative stress and have positive effects, improving wound healing in the burn model established for the rats.

Key words: 5-Phosphodiesterase inhibitor, burn, milrinone, pentoxifylline, stasis zone

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

Address for correspondence:
Muratcan Mollaoğlu, Department of General Surgery, Cumhuriyet University, Sivas, Turkey.
Fax: 0-346-219-10-10. E-mail: muratcanmollaoglu@gmail.com

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]

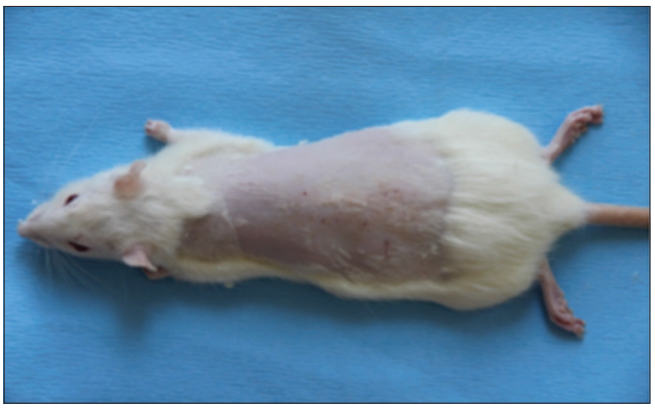

Figure 1: Image of shaved rat

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 nestled in 10% formaldehyde, 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.

Figure 2: Image of plate made of brass

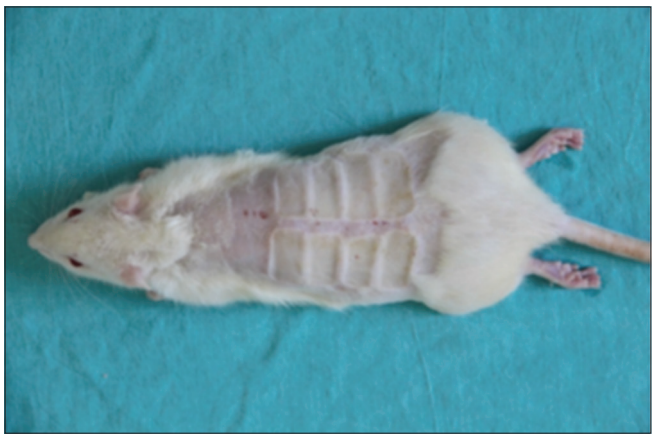

Figure 3: Image of the rat after being burned

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,

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

Groups	MDA	SOD	CAT	GPX
	X±S	X±S	X±S	X±S
Sham	2.64±1.67	12.58±7.57	14.15±10.09	78.04±55.03
Minimum–Maximum	1.22–6.54	6.43–30.12	3.23–30.32	30.31–200.21
Oral-control	14.01±7.45	16.02±5.77	12.75±5.54	82.24±26.51
Minimum–maximum	5.10–24.63	8.86–26.58	4.10–20.23	36.71–107.21
intraperitoneal control	16.75±2.40	8.45±3.23	11.80±7.82	77.73±25.92
Minimum–maximum	14.23–22.24	5.20–14.21	3.21–25.40	40.44–120.32
Statistical tests	KW=15.540	KW=7. 077	KW=0. 375	KW=1.205
	P=0.000	*P*=0. 029	*P*=0. 829	*P*=0.547

MDA: Malondialdehyde, SOD: Superoxide dismutase, CAT: Catalase, GPX: Glutathione peroxidase

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 ($\mu = 77.73$). The mean scores of the sham group and the oral control group were calculated as $\mu = 78.04$ 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 $\mu = 8.73$ and $\mu = 16.75$, 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 ($\mu = 8.45$). The mean scores of the milrinone intraperitoneal and the PTX intraperitoneal groups were calculated as $\mu = 35.41$ and $\mu = 75.03$, respectively. On the other hand, 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 intraperitoneal control group ($\mu = 11.80$). The mean scores of the milrinone intraperitoneal and the PTX intraperitoneal groups were calculated as $\mu = 32.59$ and $\mu = 56.80$, respectively. According to the GPx values, the difference between the groups was statistically significant ($P < 0.05$), and the group with the lowest mean score was the intraperitoneal control group ($\mu = 77.73$). The mean scores of the milrinone intraperitoneal and the PTX intraperitoneal groups were calculated as $\mu = 121.93$ and $\mu = 223.03$, 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 ($\mu = 12.75$). 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 ($\mu = 82.24$). The mean scores of the milrinone oral and the PTX oral groups were calculated as $\mu = 114.85$ and $\mu = 157.19$, respectively [Table 3].

DISCUSSION

A good experimental burn model should be simple, repeatable, reliable, easy to implement and, if possible, cost-effective.[10] There is no consensus standardization in burn modeling. In

Table 2: Distribution of serum levels of MDA (U/mg), SOD (U/mg), CAT (U/mg), and GPx (U/mg) by intraperitoneal groups

Groups	MDA	SOD	CAT	GPx
	X±S	X±S	X±S	X±S
Intraperitoneal	16.75±2.40	8.45±3.23	11.80±7.82	77.73±25.92
Control (minimum–maximum)	14.23–22.24	5.20–14.21	3.21–25.40	40.44–120.32
Milrinone	8.73±3.82	35.41±5.49	32.59±8.90	121.93±21.81
Intraperitoneal (minimum–maximum)	5.21–14.69	28.86–45.20	22.07–45.32	101.75–152.44
Pentoxifylline	4.48±2.35	75.03±12.72	56.80±15.98	223.03±278.32
Intraperitoneal (minimum–maximum)	2.18–8.13	56.58–87.59	33.70–85.55	85.24–908.96
Statistical tests	KW=17.645	KW=20.507	KW=18.060	KW=11.065
	P=0.000	*P*=0.000	*P*=0.000	*P*=0.004

MDA: Malondialdehyde, SOD: Superoxide dismutase, CAT: Catalase, GPX: Glutathione peroxidase

Table 3: Serum MDA, SOD, CAT, and GPx levels of oral groups

Groups	MDA	SOD	CAT	GPX
	X±S	X±S	X±S	X±S
Oral control	14.01±7.45	16.02±5.77	12.75±5.54	82.24±26.51
Minimum–maximum	5.10±24.63	8.86±26.58	4.10±20.23	36.71±107.21
Milrinone oral	7.98±3.69	25.14±9.92	24.49±12.44	114.85±52.20
Minimum–maximum	2.21±15.20	13.20–38.86	10.20±41.30	26.99–165.24
Pentoxifylline oral	6.00±0.62	36.21±18.20	43.38±37.22	157.19±55.87
Minimum–maximum	4.73±6.67	13.29±68.86	10.09±128.49	44.43–232.48
	KW=6.140	KW=7.888	KW=8.705	KW=7.950
Statistical tests	*P*=0.046	*P*=0. 019	*P*=0.013	*P*=0.019

MDA: Malondialdehyde, SOD: Superoxide dismutase, CAT: Catalase, GPX: Glutathione peroxidase

our study, we tried to create a cost-effective work, which was easy to understand, and easy to analyze.

Pigs,[11] rabbits,[12] mice,[10,13] dogs,[14] guinea pigs,[15] rats,[16] and sheep[17] 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.[18] 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.[10] 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.[19] 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.[20] 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.[21] In addition, IL-1 and, especially, IL-6 and IL-8 levels increase significantly in the first 5 days following the burn.[22] Serum

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.[1] 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.[4] 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.[32] 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.[36] In another experimental burn study conducted on rats by Özer *et al*.,[38] 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 TOS level 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*.[44] 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*.,[38] 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.

MDA levels were lower in the oral PTX-administered group than in the oral milrinone-administered group. SOD, CAT, and GPx levels were higher in the oral PTX-administered group than in the oral milrinone-administered group. When the literature was reviewed, no study was found investigating the effects of PTX as a wound care product with milrinone. As the PTX and milrinone groups were compared at the end of the study, it was observed that the recovery findings were histopathologically better in the PTX group. Again, when the PTX and milrinone groups were compared, PTX was shown to have better effects that reduced oxidative stress levels and healed wounds than milrinone. A limitation of this study was the lack of a number of animals used and its being an experimental study. We believe that prospective randomized, large-scale clinical trials are necessary to recommend the use of PTX as a standard wound care product.

CONCLUSION

It was determined in our study that oral and intraperitoneal PTX administration for healing wounds was superior to oral and intraperitoneal administration of milrinone in the experimental burn model developed for rats. To use oral and intraperitoneal PTX as a wound care product, we consider that its effects on the stasis zone, its most effective treatment dose, its side effects, and its duration of treatment should be investigated in new studies and considered as a molecule that can go into the active clinical use; and active prospective randomized clinical trials should be performed for this purpose.

ACKNOWLEDGMENT

We thank Dr. Ziynet Çınar, Department of Biostatistics, Cumhuriyet University for her help in analyzing the data.

REFERENCES

1. Zawacki BE. Reversal of capillary stasis and prevention of necrosis in burns. Ann Surg 1974;180:98-102.
2. DelBeccaro EJ, Robson MC, Heggers JP, Swaminathan R. The use of specific thromboxane inhibitors to preserve the dermal microcirculation after burning. Surgery 1980;87:137-41.
3. Robson MC, Del Beccaro EJ, Heggers JP. The effect of prostaglandins on the dermal microcirculation after burning, and the inhibition of the effect by specific pharmacological agents. Plast Reconstr Surg 1979;63:781-7.
4. Iseri SO, Ersoy Y, Ercan F, Yuksel M, Atukeren P, Gumustas K, *et al.* The effect of sildenafil, a phosphodiesterase-5 inhibitor, on acetic acid-induced colonic inflammation in the rat. J Gastroenterol Hepatol 2009;24:1142-8.
5. Doherty GM, Jensen JC, Alexander HR, Buresh CM, Norton JA. Pentoxifylline suppression of tumor necrosis factor gene transcription. Surgery 1991;110:192-8.
6. Santhosh KT, Elkhateeb O, Nolette N, Outbih O, Halayko AJ, Dakshinamurti S, *et al.* Milrinone attenuates thromboxane receptor-mediated hyperresponsiveness in hypoxic pulmonary arterial myocytes. Br J Pharmacol 2011;163:1223-36.
7. Baumann A, Derelle AL, Mertes PM, Audibert G. Seeking new approaches: Milrinone in the treatment of cerebral vasospasm. Neurocrit Care 2012;16:351-3.
8. Campelo AP, Campelo MW, Britto GA, Ayala AP, Guimarães SB, Vasconcelos PR, *et al.* An optimized animal model for partial and total skin thickness burns studies. Acta Cir Bras 2011;26 Suppl 1:38-42.
9. Regas FC, Ehrlich HP. Elucidating the vascular response to burns with a new rat model. J Trauma 1992;32:557-63.
10. Dahiya P. Burns as a model of SIRS. Front Biosci Landmark Ed 2009;14:4962-7.
11. Singer AJ, Berruti L, Thode HC Jr. McClain SA. Standardized burn model using a multiparametric histologic analysis of burn depth. Acad Emerg Med 2000;7:1-6.
12. Knabl JS, Bayer GS, Bauer WA, Schwendenwein I, Dado PF, Kucher C, *et al.* Controlled partial skin thickness burns: An animal model for studies of burnwound progression. Burns 1999;25:229-35.
13. Liu Y, Zhou Q, Wang Y, Liu Z, Dong M, Wang Y, *et al.* Negative pressure wound therapy decreases mortality in a murine model of burn-wound sepsis involving *Pseudomonas aeruginosa* infection. PLoS One 2014;9:e90494.
14. Matsumura H, Yoshizawa N, Kimura T, Watanabe K, Gibran NS, Engrav LH, *et al.* A burn wound healing model in the hairless descendant of the Mexican hairless dog. J Burn Care Rehabil 1997;18:306-12.
15. Chu CS, McManus AT, Mason AD Jr., Okerberg CV, Pruitt BA Jr. Multple graft harvestings from deep partial-thickness scald wounds healed under the influence of weak direct current. J Trauma 1990;30:1044-049.
16. Kim DE, Phillips TM, Jeng JC, Rizzo AG, Roth RT, Stanford JL, *et al.* Microvascular assessment of burn depth conversion

during varying resuscitation conditions. J Burn Care Rehabil 2001;22:406-16.
17. Stair JM, Bowser BH, Marvin TH, Stewart CL, Caldwell FT Jr. The effects of sodium nitroprusside on the hemodynamics of burn shock: Results of an experimental sheep model. J Trauma 1983;23:939-44.
18. Gould LJ, Leong M, Sonstein J, Wilson S. Optimization and validation of an ischemic wound model. Wound Repair Regen 2005;13:576-82.
19. Nisanci M, Ozturk S. Burn pathophysiology. J Plast Surg 2010;2:8-14.
20. Mozingo DW, Cioffi WG, Pruitt BA. Burns. In: Bongard FS, Sue DY, Vintch JR editors. Current Diagnosis and Treatment Critical Care. NewYork: The McGraw-Hill Companies; 2008. p. 723-51.
21. Gueugniaud PY, Carsin H, Bertin-Maghit M, Petit P. Current advances in the initial management of major thermal burns. Intensive Care Med 2000;26:848-56.
22. Hettiaratchy S, Dziewulski P. ABC of burns: Pathophysiology and types of burns. BMJ 2004;328:1427-9.
23. Dziewulski P, Juan P. Barret assessment, operative planning and surgery for burn wound closure. In: Wolf SE, Herndon DN, editors. Burn Care. Austin, TX: Landes Bioscience; 1999. p. 19-52.
24. Boykin JV, Eriksson E, Pittman RN. Microcirculation of scald burn: An *in vivo* experimental study of the hairless mouse ear. Burns 1980;7:335-8.
25. Johnson GS, Lineberger T, Hothem AL, Adams K, Hinson T, Winkenwerder W. Erythrocyte flexibility in the burned patient. Burns 1979;6:91-5.
26. Zawacki BE. The local effects of burn injury. In: Boswick JA, editor. The Art and Science of Burn Care. Rockwille: Aspen; 1987. p. 29.
27. Sener G, Akgün U, Satiroğlu H, Topaloğlu U, Keyer-Uysal M. The effect of pentoxifylline on intestinal ischemia/reperfusion injury. Fundam Clin Pharmacol 2001;15:19-22.
28. Pasquier C, Franzini E, Abedinzaldelr Z, Hakim J. Protective effect of pentoxyfilline against hydroxyl radical-induced damage to proteins in pentoxyfilline and analogues. In: Hakim J, Mandel GL editors. Effects On Leukocyte Function. Basel: Karger; 1988. p. 91-6.
29. Toda K, Kumagai N, Kaneko F, Tsunematsu S, Tsuchimoto K, Saito H, *et al.* Pentoxifylline prevents pig serum-induced rat liver fibrosis by inhibiting interleukin-6 production. J Gastroenterol Hepatol 2009;24:860-5.
30. Seifi B, Kadkhodaee M, Delavari F, Mikaeili S, Shams S, Ostad SN, *et al.* Pretreatment with pentoxifylline and N-acetylcysteine in liver ischemia reperfusion-induced renal injury. Ren Fail 2012;34:610-5.
31. Tireli GA, Salman T, Ozbey H, Abbasoglu L, Toker G, Celik A, *et al.* The effect of pentoxifylline on intestinal anastomotic healing after ischemia. Pediatr Surg Int 2003;19:88-90.
32. Oksuz H, Bulbuloglu E, Senoglu N, Ciralik H, Yuzbasioglu MF, Kilinc M, *et al.* Re-protective effects of pre- and post-laparoscopy conditioning, zinc, pentoxifylline, and N-acetylcysteine in an animal model of laparoscopy-induced ischemia/reperfusion injury of the kidney. Ren Fail 2009;31:297-302.
33. Herndon DN, editor. Total Burn Care. Edinburg: Elsevier Saunders; 2007. p. 829-43.
34. Pammi M, Haque KN. Pentoxifylline for treatment of sepsis and necrotizing enterocolitis in neonates. Cochrane Database Syst Rev 2015;3:CD004205.
35. Sulkowska M, Sulkowski S, Skrzydlewska E. The effect of pentoxifylline on ultrastructure andantioxidant potential during cyclophosphamide-induced liver injury. J Submicrosc Cytol Pathol 1999;31:413-22.
36. Zeni F, Pain P, Vindimian M, Gay JP, Gery P, Bertrand M, *et al.* Effects of pentoxifylline on circulating cytokine concentrations and hemodynamics in patients with septic shock: Results from a double-blind, randomized, placebo-controlled study. Crit Care Med 1996;24:207-14.
37. Kayaalp O. Tıbbi farmakoloji. Cilt 2, 3. Baski Haccettepe Yayinlari 1985;2:1079-2, 1382-3.
38. Özer Ö. (Specialized Thesis) Specific and Non-specific Phosphodiesterase Inhibitors and N-acetylcysteine Effects on Oxidative Stress and Distant Organ Damage. Cumhuriyet University School; 2014.
39. Vlahos NF, Gregoriou O, Deliveliotou A, Perrea D, Vlachos A, Zhao Y, *et al*. Effect of pentoxifylline on vascular endothelial growth factor cand flk-1 expression on endometrial implants in the rat endometriosis model. Fertil Steril 2010;93:1316-23.
40. Heinz G, Geppert A, Delle Karth G, Reinelt P, Gschwandtner ME, Neunteufl T, *et al.* IV milrinone for cardiac output increase and maintenance: Comparison in nonhyperdynamic SIRS/sepsis and congestive heart failure. Intensive Care Med 1999;25:620-4.
41. Barton P, Garcia J, Kouatli A, Kitchen L, Zorka A, Lindsay C, *et al.* Hemodynamic effects of i.v. milrinone lactate in pediatric patients with septic shock. A prospective, double-blinded, randomized, placebo-controlled, interventional study. Chest 1996;109:1302-12.
42. Yoshimura T, Kurita C, Nagao T. Effects of cAMP-phosphodiesterase isozyme inhibitor oncytokine production by lipopolysaccharide-stimulated human peripheral blood mononuclear cells. Gen Pharmacol 1997; 29: 633–8.
43. Nielson CP, Vestal RE, Sturm RJ, Heaslip R. Effects of selective phosphodiesterase inhibitors on the polymorphonuclear leukocyte respiratory burst. J Allergy Clin Immunol 1990;86:801-8.
44. Gong M, Lin XZ, Lu GT, Zheng LJ. Preoperative inhalation of milrinone attenuates inflammation in patients undergoing cardiac surgery with cardiopulmonary bypass. Med Princ Pract 2012;21:30-5.

How to cite this article: Mollaoğlu MC, Atabey M, Bostanci ME, Karadayi K, Mollaoğlu M. Recovery of Burn Stasis Zone Using Pentoxifylline and Milrinone in an Experimental Burn Model Established For Rats. Asclepius Med Res Rev 2019;2(1):1-8.