Effect of Opium Dependency on Burn Healing in a Rat Model: An Experimental Study

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Key Words

Burn • Opium • Rat model • Thermal injury • Wound healing

Abstract

Objective: This study aimed to investigate the effect of opium dependency on the healing of third-degree burns in rats.

Material and Methods: Twenty-four rats were randomly divided to experimental and control groups. In the experimental group, opium was added to the drinking water for 21 days at increasing concentrations. The control group did not receive opium. To prove dependency on opium in the rats, naloxone was injected intraperitoneally. Full-thickness burn wounds were inflicted by applying an iron cuboid preheated to 94°C to the flank of all rats for 20 s. On day 14 after burn injury, full-thickness biopsies were taken. Blind histopathologic evaluation was performed to assess length and thickness of the re-epithelialization area, number of neutrophils, fibroblasts, mononuclear cells and new vessels, and percentage of tissue in repair (neutrophilic exudate, and granulation and fibrous tissue). Findings were analyzed using SPSS software. **Results:** The wound surface area was $95 \pm 43.35 \text{ mm}^2$ in the control group and $120.4 \pm 50.12 \text{ mm}^2$ in the experimental group ($p = 0.224$). The findings show that opium de-

pendency has no significant effect on the healing of burn wounds in rats except for the number of monocytes on day 14 ($p < 0.05$). **Conclusion:** Morphine dependency does not seem to be as effective on third-degree burn healing.

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Introduction

The prevalence of thermal injury is high in Iran, insofar as different studies have indicated incidence rates of burn hospitalization ranging from 13.5 to 21.6 per 100,000 person-years [1, 2]. Many intrinsic and extrinsic factors affect healing of burn wounds. Physiologic or biochemical defects can delay or impair the healing process and cause later complications [3].

Opioids are the preferred treatment for controlling pain in burn patients both for the initial injury and for procedures such as wound debridement and changing dressings [4, 5]. On the other hand, opium is a common form of substance abuse in Iran. In 2004, a study using unlinked anonymous urine drug testing was performed in a medical center in Kerman, the biggest province in Iran. In this study 14.4% of urine samples were positive

for opioid metabolites [6]. A study by Santos et al. [7] showed that burn patients were more likely to have drug abuse.

Previous studies have investigated the effect of opioids on the healing process of wounds, with both positive and negative effects. Opioids mediate wound healing probably by altering the inflammatory milieu of wounds [8–10]. Morphine suppresses monocyte chemotaxis and inhibits cytolytic activity of natural killer cells and proliferative responses of lymphocytes to mitogens, which can potentially impair wound healing [11–13]. However, there is little research on the effect of opioids on the healing of burn wounds. As a better understanding of the relationship between opioid dependency and burn healing will likely contribute towards the understanding of the role of opioids, we decided to study the effect of opium dependency on the healing of burns in an animal model.

Materials and Methods

Experimental Animals

Twenty-four female non-pregnant Sprague-Dawley rats with an average weight of 210 ± 20 g were randomly divided into two groups, experimental and control groups, with 12 rats in each group. The temperature, food and rearing conditions of both groups were similar. Experimental procedures on animals, which were approved by the Ethics Committee, Kerman University of Medical Sciences, were in accordance with the guidelines for the care and use of laboratory animals [14].

Opium Dependency

The experimental group of rats received opium tablets dissolved in drinking water, while rats in the control group received water without any additive. Opium tablets were produced by Daroupakhsh, Tehran, Iran (containing 100 mg opium, equivalent to 10% morphine). In the first 48 h, opium tablets (1 mg/ml) were added to the rats' drinking water. The concentration of opium in water was increased every 48 h to 2, 3 and 4 mg/ml and continued at 4 mg/ml to the end of the study. The rats drank opium-containing water without adding sucrose or sodium chloride [15].

Opium Withdrawal Syndrome

To confirm opium dependency in our rat samples, we used naloxone – a morphine antagonist – and detected the withdrawal signs in all rats sampled. The withdrawal symptoms consisted of: writhing, ptosis, diarrhea, jumping, teeth chattering, paw tremor, chewing, and head and wet-dog shaking [16]. To achieve this, on day 21, 1 rat was selected randomly from each group and naloxone (2 mg/kg; Daroupakhsh) was injected intraperitoneally. The rats were observed for 20 min for the naloxone-precipitated withdrawal syndrome. The aforementioned symptoms were assessed in experimental and control samples. Opium dependency of rats in experimental and control groups was verified.

Table 1. Results of the withdrawal test with naloxone in experimental and control rats

Withdrawal signs	Opium-dependent group	Control group
Writhing	0/10	0/10
Ptosis	10/10	0/10
Diarrhea	8/10	0/10
Jumping	0/10	0/10
Teeth chattering	9/10	0/10
Paw tremor	0/10	0/10
Chewing	10/10	0/10
Head shaking	10/10	0/10
Wet-dog shaking	8/10	0/10

Thermal Injury Model

On day 21, the rats were anesthetized using 25 mg of intraperitoneal ketamine (Daroupakhsh). The rats were completely anesthetized for 10 min and got full consciousness after 30–45 min. This time was enough for shaving hair and inducing burn. The hair in the flank area was cut using an electric hair clipper (Moser, model 1400) to fully remove hair. A preliminary pilot study was performed on 3 rats to define the anesthetic drug dosage and the temperature needed to induce a third-degree burn.

For burn induction, four iron cuboids measuring $2 \times 2 \times 3$ cm with a 7-cm handle weighing 100 g were used. The cuboids were heated for 2 h in a water bath containing boiling water at 94°C (the temperature of boiling water in Kerman). Cuboids were heated for 30 min after burn induction and were used again. The anesthetized rat was kept on one side on a board. The heated and wet cuboid was put on the shaved flank area vertically. It was kept for 20 s with a minimum pressure in place without any extra pressure. This led to a third-degree burn measuring 2×2 cm (400 mm^2). The temperature and duration of the contact of the cube with the skin for inducing a third-degree burn were defined in the pilot study and verified histologically.

Histological Examination

After burn induction, the rats were kept for 14 days. During this time, 2 rats from each experimental group died. On day 14, the remaining rats were anesthetized using ketamine again. Then the surface area of the wound was measured using transparent checkerboard paper with a precision of 1 mm^2 in a double-blind manner. The burn area was excised in full thickness with a 1-cm margin and sent for histological study.

The samples were fixed in 10% formalin solution for 24 h. Paraffin blocks were made and a $5\text{-}\mu\text{m}$ longitudinal slice of the wound was provided by the tissue processor and stained with hematoxylin-eosin. Polymorphonuclear leukocyte (PMN), fibroblast, new vessel and monocyte counts, and percent of tissue in repair were recorded.

Statistical Analysis

The analysis was done by SPSS (version 15; SPSS, Chicago, Ill., USA). Values were expressed as means $\pm$ SD; the sample code

Table 2. Characteristics of burn wounds 14 days after burn injury in control and experimental rats (1 unit = 24 μm)

Characteristics	Sample code										mean $\pm$ SD
	1	2	3	4	5	6	7	8	9	10	
Wound surface area ^a , mm ²											
Control group	50	90	78	57	80	181	48	95	139	132	95 $\pm$ 43.35
Opium-dependent group	144	45	138	88	123	66	95	124	203	188	120.4 $\pm$ 50.12
Length of re-epithelialized area ^b , units											
Control group	13	22	60	43	40	44	35	33	38	48	37.6 $\pm$ 13.17
Opium-dependent group	90	40	48	30	65	41	50	12	80	88	54.4 $\pm$ 25.82
Thickness of re-epithelialized area ^c , units											
Control group	13	22	60	43	40	44	35	33	38	48	7.3 $\pm$ 2.4
Opium-dependent group	90	40	48	30	65	41	50	12	80	88	6.1 $\pm$ 2.18

^a t = 1.26, d.f. = 18, p = 0.224. ^b t = 1.83, d.f. = 18, p = 0.08. ^c t = 1.16, d.f. = 18, p = 0.25.

Table 3. Percentage of tissue repair in control and experimental rats

Tissue repair	Sample code										mean $\pm$ SD
	1	2	3	4	5	6	7	8	9	10	
<i>Control group</i>											
Neutrophilic exudate	10	10	10	5	20	25	20	20	20	20	16 $\pm$ 6.58
Granulation tissue	90	90	90	95	80	75	80	80	80	80	84 $\pm$ 6.58
Fibrous tissue	0	0	0	0	0	0	0	0	0	0	0
<i>Opium-dependent group</i>											
Neutrophilic exudate	10	20	10	10	10	10	20	30	20	20	16 $\pm$ 6.9
Granulation tissue	90	80	90	90	90	90	80	70	80	80	84 $\pm$ 6.9
Fibrous tissue	0	0	0	0	0	0	0	0	0	0	0

No significant differences were found between both groups.

represented the number of animals in experimental and control groups. A two-sample t test was applied to compare means, and Fisher's exact probability test for nominal variables was used to compare two groups. Significance was accepted at $p < 0.05$. To compare naloxone-precipitated withdrawal symptoms, the χ^2 test was used.

Results

Opium Dependency

Rats in the experimental group showed symptoms of naloxone withdrawal. Only 3 of 9 behavioral parameters were absent: writhing, jumping and paw tremor (table 1). The control group did not exhibit any of these symptoms.

Wound Characteristics and Microscopic Evaluation

The surface area of wounds was $95 \pm 43.35 \text{ mm}^2$ in the control group and $120.4 \pm 50.12 \text{ mm}^2$ in the experimental group ($t = 1.26$, d.f. = 18, $p = 0.224$). The length of the re-epithelialization area was 37.6 ± 13.17 units in the controls and 54.4 ± 25.82 units in the experimental rats. One unit is equal to $24 \mu\text{m}$ ($t = 1.83$, d.f. = 18, $p = 0.08$). The thickness of the re-epithelialized area was $7.3 \pm 2.4 \mu\text{m}$ in the controls and $6.1 \pm 2.18 \mu\text{m}$ in the experimental group ($t = 1.16$, d.f. = 18, $p = 0.25$) (table 2).

PMN count was 8.54 ± 1.1 in the controls and 5.41 ± 3.9 in the experimental group ($t = 1.38$, d.f. = 18, $p = 0.18$). Fibroblast count was 71.44 ± 16.47 in the control group and 72.59 ± 13.09 in the experimental group ($t = 0.86$, d.f. = 18, $p = 0.17$). Mononuclear cell count was $9.02 \pm$

2.71 in the control and 6.69 ± 1.94 in the experimental group ($t = 2.2$, d.f. = 18, $p = 0.041$). The number of blood vessels was 4.55 ± 1.76 in the control group and 3.23 ± 1.84 in the experimental group ($t = 1.63$, d.f. = 18, $p = 0.11$).

Neutrophilic exudates, granulation tissue and fibrotic tissue were measured as indicators of healing. In the control group, $16 \pm 6.58\%$ of the healing wounds were formed by neutrophilic exudate and $84 \pm 6.58\%$ with granulation tissue. In the experimental group, $16 \pm 6.9\%$ of the healing wound was formed by neutrophilic exudate and $84 \pm 6.9\%$ with granulation tissue ($t = 0$, d.f. = 18, $p = 1$). The wounds did not contain fibrotic tissue. Mononuclear cell count was significantly higher in the control group ($p < 0.05$) (table 3).

Discussion

This study investigated the effect of opium dependency on the healing process of third-degree burns in rats. A model for inducing a third-degree burn in rats was also offered. The model was verified histopathologically. Our findings indicate that opium dependency did not have a significant effect on the healing of burn wounds in rats. However, the number of monocytes was significantly different between both groups.

In a study by Lam et al. [8] in a mouse model of excisional wound injury, high-dose morphine impaired angiogenesis, increased systemic oxidative stress and impaired mobilization of endothelial progenitor cells. These findings reveal the potentially detrimental effect of morphine on wound healing. In another study in rats, morphine delayed wound closure when applied during the first 3 days, while no significant delay was observed when morphine was started after 3 days. Treatment of wounds with morphine reduced the number of myofibroblasts and macrophages in the closing wound significantly [9], being similar to our study in which the number of monocytes was lower in the opium-dependent group. However, in another study, topical opioids exerted positive effects on the healing of ischemic wounds in rats, especially during the first 4 days. Opioids also induced a 45–87% increase in angiogenesis [10]. Our findings did not support increased angiogenesis in opium-dependent rats.

However, an important difference between our and the above studies should be borne in mind when comparing results. The experimental rats in our study were opium dependent, thus differing from animals receiv-

ing opium for a short term or at low doses. So any comparison of our findings with other studies must be made cautiously. Also, some other factors might have affected the results of our study. Whether degree and extent of burn, or dosage and type of opioids have influenced the results is to be clarified. Evidence indicates the impact of such parameters on the healing of wounds. For example, morphine is the main component of opium and morphine has been found to have the least healing property relative to fentanyl and hydromorphone [10]. Schwacha et al. [17] proposed that at a special size, a maximal level of burn injury may be reached so that opiates have no further detrimental effect. In this regard, studies comparing the effect of different sizes and grades of burn on wound healing can clarify the role of burn size and grade on the healing of burn wounds in opium-dependent rats.

Conclusion

Our findings did not reveal any association between opium dependency and third-degree burn healing in the rat, and morphine dependency does not seem to be as effective on third-degree burn healing.

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