Original Article

The Effects of Pentoxifylline on the Wound Healing Process in a Rat Experimental Pressure Sore Model

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Abstract

Introduction: The present study used a histological evaluation method to examine the effects of pentoxifylline (PTX) on healing an experimentally-induced pressure sore in a rat model.
Materials and Methods: There were 36 adult male rats used in this study. Under general anesthesia and sterile conditions, we used forceps to create one pressure sore on each rat. A double layer of folded skin from the dorsal region was held with the highest forceps pressure grade for two hours, followed by 30 minutes of relaxation. This was repeated 12 times over three consecutive working days, and created a pressure sore after seven days. Next, rats were randomly divided into three control and three experimental groups. The experimental groups received intraperitoneal injections of PTX (50 mg/kg) for 14, 21, and 28 days after the pressure sore was created. Control groups received a similar volume of saline solution. Rats were euthanized, after which samples were extracted from the wound area and prepared for light microscopy examination. We calculated the number of neutrophils, macrophages, fibroblasts, blood vessel sections, and thicknesses of the newly formed epidermis and dermis.
Results: Although the values of some studied parameters were higher in the experimental group, there were no significant differences noted between the experimental and control groups.
Conclusion: In this study PTX did not increase any histological parameters. Thus, the effects of PTX on the pressure sore model seem to result from different mechanisms.
Keywords: Pentoxifylline, Histology, Rats

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Introduction

The ability of a wound to heal is a topic of concern in medicine, such that the tissue healing process and medications used are important areas for medical research. Materials and medications that can lead to healing or at least accelerate wound healing have been reviewed by various studies [1-3]. One medication that has been used experimentally to accelerate the wound healing process is pentoxifylline (PTX).

PTX is a xanthine derivative. As with other methylated xanthine derivatives, PTX is a competitive nonselective phosphodiesterase inhibitor [4] that raises intracellular cyclic AMP (c AMP), activates protein kinase A (PKA), inhibits tumor necrosis factor-alpha [5,6] and leukotriene synthesis [7], and reduces inflammation and innates immunity [7]. In addition, PTX improves red blood cell deformability, reduces blood viscosity and decreases the potential for platelet aggregation and thrombus formation [8]. PTX increases blood flow through peripheral blood vessels and therefore improves blood circulation in the arms and legs (intermittent claudication), and in the brain (vascular dementia) [3]. A large volume of studies have identified the positive effects of PTX administration on healing skin flaps [9-11], venous ulcers [12-15], cutneous wounds in healthy and diabetic mice [16], colitis, stomach ulcers, and small and large bowel anastomoses under experimentally created ischemic conditions [17-20].

According to the literature, administration of PTX has been shown to positively impact the wound healing process, including those that arise from pressure sores. The pressure sore is a major health problem that currently affects approximately 3 million adults [21].

Research on pressure sores in humans is difficult due to variations in both internal (fever, anemia, infection, ischemia, hypoxia, malnutrition, low body mass, neurologic disease) and external factors (shear forces, friction and immobility). Due to a lack of knowledge and wide variety of influencing factors, thus the use of several animal models is necessary to increase our knowledge of the role of PTX in wound healing. Any method generated to study the pressure ulcer, by itself, cannot show all the features of the pathology of chronic wounds. Only certain types of pressure sore models can mimic certain aspects of wound evolution [22-25].

Pressure sores mostly occur in immobile patients or those who are unable to change their body position. Under these circumstances the patient's dermal tissues are at increased risk for necrosis of the skin, subcutaneous tissue, and muscles. Pressure sores are defined as areas of skin discoloration or damage that persist after the removal of pressure and which are likely to be due to the effects of pressure on the tissues [3,26].

Pressure sores have also been described as decubitus ulcers and bed sores [26]. Scientists have attempted to use these terms to identify and describe the pathophysiology of wounds caused by physical stress. However, this term could not justify the existence of pressure sores that result from other reasons, such as the sores that occur in wheelchair patients. Currently, the term pressure sore is the best description for these lesions, since they are multifactorial and may occur anywhere on the body [3].

Stress, time, spasticity, infection, edema, nerve transaction, and poor nutrition are considered main factors that can lead to the formation of pressure sores or play a role in their development. Over 60% of pressure sores occur in hospitalized patients [21,27-29]. A possible explanation for this increase in pressure

sores prevalence is due to the increasing older population who need hospital services. Approximately one third to one half of the elderly population have limited mobility. Health care providers and hospital administrators are legally obligated to prevent patients from acquiring bed sores and physical weakness [3]. In order to prevent pressure sores, intensive preventative measures should be undertaken [30,31].

As mentioned earlier, the primary cause of pressure sores is based on the exerted pressure and permanent forces on the patient's dermal tissues, whereby the supply of oxygen is reduced or cut-off, thereby causing tissue necrosis [32,33]. Pressure sores remain a major challenge in the medical world [3,33].

Pressure sores result from the ischemia-reperfusion cycle. An important issue in ischemia-reperfusion cycles is cellular impairment. Reperfusion controversially accelerates cell damage in the tissue, which is followed by more cell damage and tissue destruction. Ischemia-reperfusion ulcers are such as the pressure sores that caused by the same pathomechanical and pathophysiological conditions [23,25,34].

Pressure sores are clinically divided as follows: i) grade I (similar to intact skin with a pale pink or red appearance); ii) grade II (presence of an injured epidermis); iii) grade III (damage develops in the dermis layer of the skin); and grade IV (the whole skin and underlying muscle to the bone's surface are damaged) [23,29].

Due to an increase in the elderly population and prevalence of pressure sores, it seems necessary to research the pathophysiological, prevention and treatment of pressure sores in both basic science and the clinic setting [3,9].

The prevalence rate of pressure sores among adult patients in hospitals in the UK ranged from 6.9% to 9.11%. The incidence rate among those who underwent surgery was 12%, whereas for the elderly it was 22%. Statistics taken from the entire British population indicated a prevalence rate of 4.4% for adults and 6.8% in children.

According to research, worldwide, the prevalence and incidence of pressure sores varies according to environment conditions. For example, in the acute care setting the prevalence ranges from 4.7% to 29.7% [1, 3]. A similar study in European hospitals has shown a prevalence of 18.1% [2-4]. According to reports by the Consultation Secretariat of the National Association of Pressure Ulcers, the range of prevalence in the US is 10% to 18% in general acute care units [34,35].

It is known that ischemia and reperfusion are the most important factors in the pathogenesis of pressure sore development[36,37]. Numerous recent studies have shown the positive effects of PTX in cases of ischemic conditions and wound healing [3,10,], however studies pertaining to the influence of PTX on pressure sores are lacking. The present study aims to use a histomorphometrical evaluating method to investigate the effects of PTX administration on the wound healing process in a grade III experimental pressure sore in rats.

Materials and Methods

We obtained 36 adult male Wistar rats (mean ages: twelve weeks) from Pasteur Institute of Iran. Rats were individually housed in clean cages in an animal house on a light-dark cycle (12 hours light: 12 hours dark) with access to water and food ad libitum. All study procedures were approved by the Medical Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Generation of pressure sores

To create a grade III pressure sore [38] we anesthetized the rats by administering intra-

muscular injections of ketamine (50 mg/kg) and diazepam (5 mg/kg) on day 0. Next, the hairs of the dorsal region were shaved and the skin cleaned with 70% alcohol and povidone iodine. Under sterile conditions the skin was raised, folded at midline and maintained under the highest pressure grade with a no.25 Halsted mosquito forceps for a period of 2 hours (Fig.1), after which the skin was released and allowed to relax for 30 minutes.

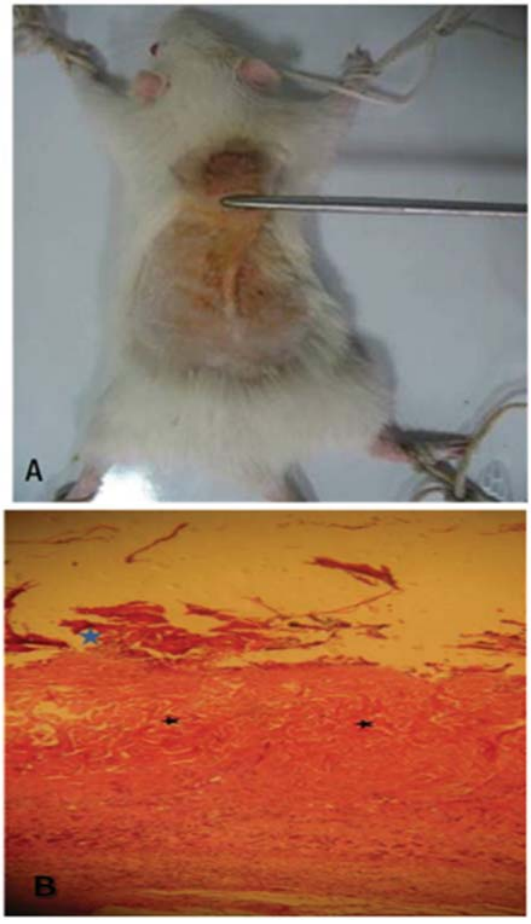

Figure 1. A) Creation of experimental pressure sore in the rat's skin using no. 25 Halsted mosquito forceps. B) H&E-stained slide showing the location of the wound. *: Degenerated epidermis. **: Degenerated dermis. Magnification: 100×.

A thin sheet of aluminum (3×5mm) was placed between each of the external (epidermal) skin sections and forceps clamps in order to provide an equal pressure distribution for all areas of the clamped skin. The courses of ischemia (2 hours) and perfusion (thirty minutes) were applied for 12 times over three consecutive working days (four periods per day). During the process of creating the pressure sore, the rats were given 1/2 dose injections of the anesthetic drugs from the onset of pressure sore induction (the first day) until day 4, when the pressure exertion was completed. Histological examination on day seven confirmed that the pressure sores were created [3] (Fig. 1).

Pentoxifylline (PTX) administration

We randomly divided the rats into three control and three experimental groups. The experimental groups received intraperitoneal injections of PTX (50 mg/kg) for 14, 21, and 28 days after the pressure sore was created on day 7 . Control groups received a similar volume of saline solution (without PTX).

Histomorphometrical examination

The rats were sacrificed by inhalation of chloroform at the end of the designated study periods and skin samples were taken from the wound and surrounding normal, undamaged skin. Samples were fixed in 10% formalin-saline, after which they were prepared for tissue processing by dehydration, clearing, and paraffin molding. Tissues were sectioned into 5µm thicknesses. From the serial sections, we examined one from each of the five sections. Totally, we examined five sections which had been stained by hematoxylin and eosin (H&E). In each section, ten microscopic fields at a magnification of 400× were morphometrically evaluated and analyzed by a calibrated light microscopy. The total number of cells that included macrophages, neutrophils, fibroblasts, and blood vessel sections (Fig.2) were examined in each 0.06 mm^2 field from the ten fields. We reported the mean number from five sections of

each of the rat specimens from each of the control and experimental groups. The eyepiece of the microscope contained 20×20 grids of 2×2 mm^2 dimensions. We used the same eyepiece to calculate the thicknesses of the newly formed epidermis and dermis.

Statistical analysis

Data were analyzed by the student's t-test for independent samples and presented as mean±SD. $P<0.05$ was considered statistically significant.

Results

Histological examination of the experimentally-induced pressure sores showed an inflammatory reaction that included numerous neutrophils and some macrophages in the wound bed. All rats remained alive after induction of pressure sore with no secretions or symptoms of infection in both control and experimental groups during the investigation. Figures 3-5 show the histological analyses of the rat's skin in the process of healing on days 14, 21, and 28 after induction of the pressure sore in the control and experimental groups. Tables 1-3 show the results of the histomorphometrical examination.

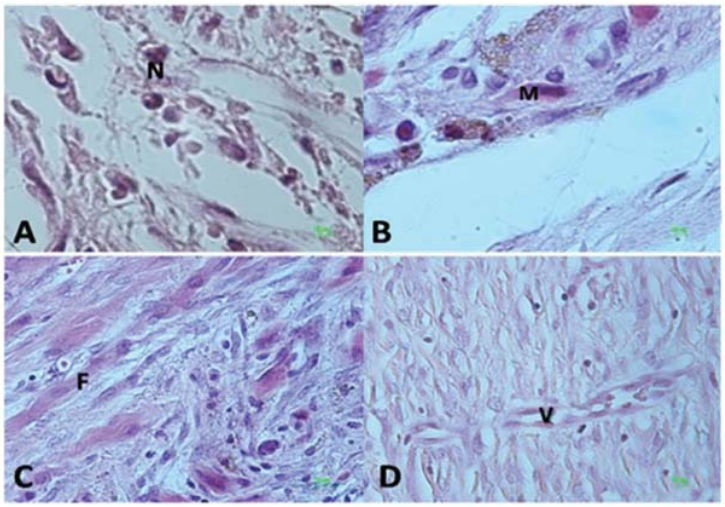

Figure 2. High resolution photomicrographs of the cells and a blood vessel from the repaired skin tissue in an experimentally-induced pressure sore at day 14 post-injury. A) N: Neutrophil; B) M: Macrophage; C) F: Fibroblast; D) V: Vessel. . (H&E stain. Magnification: 1000 ×)

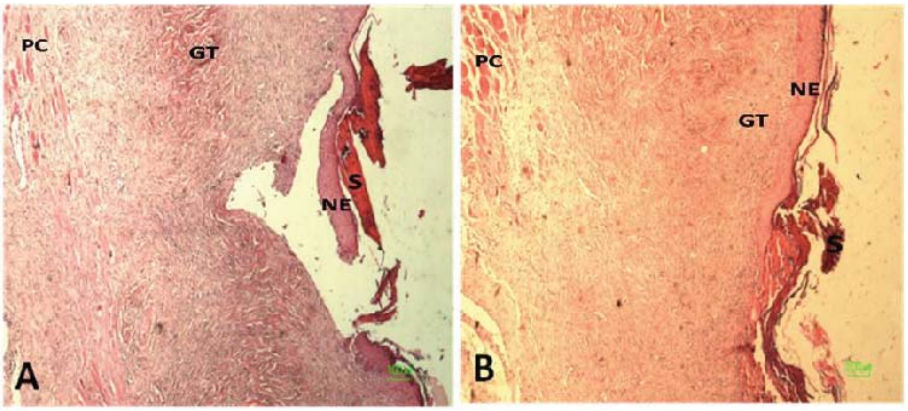

Figure 3. Photomicrograph of repaired rat skin tissue on day 14 after induction of experimental pressure sore. A) Control group. B) Experimental group.. NE: New epidermis; S: Scab; GT: Granulation tissue; PC: Panniculus carnosus . (H&E stain. Magnification: 100 ×)

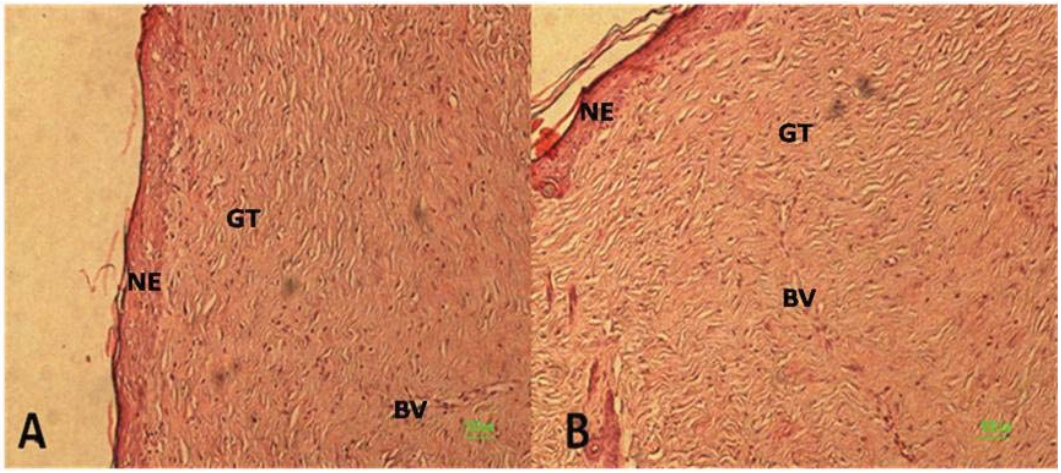

Figure 4. Photomicrograph of repaired rat skin tissue on day 21 after induction of an experimental pressure sore.
A) Control group; B) Experimental group. NE: New epidermis; S: Scab; GT: Granulation tissue; BV: Blood vessels. (H&E stain. Magnification: 400 ×)

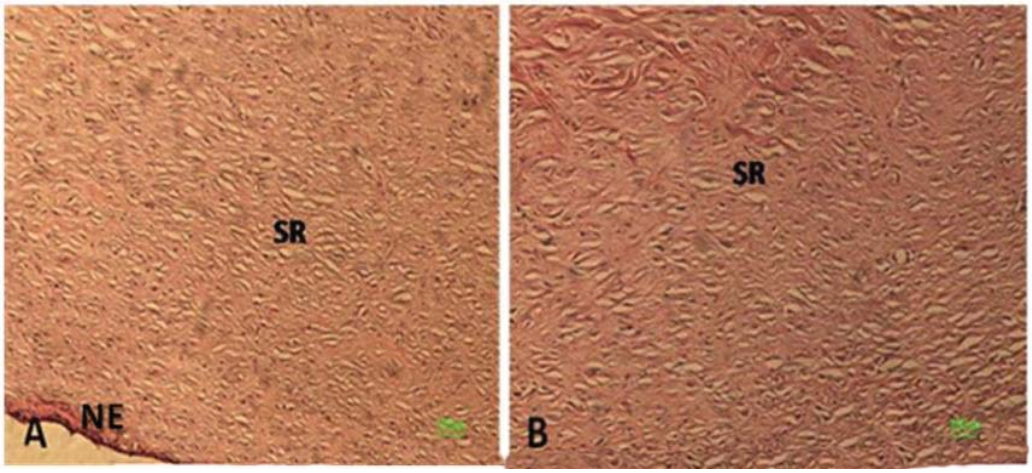

Figure 5. Photomicrograph of repaired rat skin tissue on day 28 after induction of the experimental pressure sore. A) Control group. B) Experimental group. ,
NE: New epidermis, SR: Scar. (H&E stain. Magnification: 400 ×)

Table 1. Day 14 histological parameters (mean±SD) for control and experimental groups

Parameters / Groups	Thickness of the new dermis (mm)	Thickness of the new epidermis (mm)	Blood vessels (n)	Neutrophils (n)	Macrophages (n)	Fibroblasts (n)
C*	2.167±0 75	0.012.5±0.0125	17.33±7.39	55.00±35.16	16.67±3.20	17.17±7.08
E**	1.600±0.63	0.012.5±0.0125	33.83±38.22	56.33±27.47	16.33±6.05	20.50±11.64
P-value	0.1884	1.0000	0.3237	0.9431	0.9075	0.5624

*C: Control; **E: Experimental

Table 2. Day 21 histological parameters (mean±SD) for control and experimental groups

Parameters / Groups	Thickness of the new dermis (mm)	Thickness of the new epidermis (mm)	Blood vessels (n)	Neutrophils (n)	Macrophages (n)	Fibroblasts (n)
C*	1.167±0.4	0.07287±0.0175	11.67±4.30	13.50±14.06	11.50±3.93	14.83±6.30
E**	1.167±0.4	0.052±0.015	11.17±8.38	9.000±5.79	12.50±11.02	13.00±6.87
P-value	1.00	0.3910	0.8992	0.4854	0.8384	0.6405

*C: Control; **E: Experimental

Table 3. Day 28 histological parameters (mean±SD) for control and experimental groups

Parameters / Groups	Thickness of the new dermis (mm)	Thickness of the new epidermis (mm)	Blood vessels (n)	Neutrophils (n)	Macrophages (n)	Fibroblasts (n)
C*	1.083±0.54	0.0475±0.025	4.800±2.77	4.80±3.34	9.6±4.72	10.4±1.101
E**	1.33±0.447	0.04±0.0375	3.0±1.22	3.40±2.30	8.00±4.85	11.6±1.14
P-value	0.2959	0.1950	0.2211	0.4631	0.8733	0.146

*C: Control; **E: Experimental

Despite the increased numbers of fibroblasts in the experimental groups compared with their relative controls, there were no significant differences noted.

The mean numbers of macrophages, neutrophils, and blood vessels in the wound sites and the mean thicknesses of the demis and epidermis in the experimental groups (14, 21 and 28 day groups) were almost equal or slightly less or more compared with their relative control groups, which was not statistically significant.

Discussion

In a previously published study of our research, we have shown that PTX significantly increased the parameters of biomechanical tests that included maximal stress and work up to maximum force in rats with pressure sores, compared with the control groups [3]. These results indicated that administration of PTX accelerated the healing process of pressure sores. In the present study we decided to study the role of PTX on histological parameters in rats with pressure sores compared to their control groups. We observed higher numbers of the some histological parameters, in the experimental groups compared to their relative controls, however these results were not statistically significant. PTX did not increase the histological parameters, thus its mechanism of action on healing pressure sores appeared to be the result of a different mechanism.

In our previous study, we found that administration of PTX positively affected the strength and maturity of the repairing tissue [3], however this was not verified by increased numbers of histological findings of current study. Similar results were obtained by Karasoy et al. and Tireli et al. who researched skin and intestinal sores [16, 19]. Karasoy et al. observed that administration of PTX significantly increased the tensile strength of skin wounds in healthy compared to control mice, however there were no significant differences in histological parameters between the control and experimental mice. Their justification for increasing the tensile strength of wounds treated by PTX was that tissue perfusion increased in these wounds [16].

Tireli et al. showed that administration of PTX

during the healing process of intestinal grafts, lead to increased numbers of fibroblast cells, increased tensile strength and the amount of the amino acid, proline, under conditions such as ischemia and reperfusion wounds [19].

Our former study [3] in addition to studies by Karasoy et al. [16] and Tireli et al. [19] have shown significantly increased tensile strength in wounds in the experimental groups which indicated that fibroblasts might be more active in collagen synthesis. However, the findings by Dans and Isseroff differ, of which the difference might possibly be related to the culture medium conditions in their studies [39].

Isaac and colleagues investigated the effects of PTX on human fibroblasts derived from hypertrophied scars after burning, where they reported decreased synthesis of type III collagen fibers in these cells [40].Dans and Isserof investigated the combined effects of PTX and interferon on fibroblasts and wound contraction in vitro. They have concluded that PTX might delay wound contraction under in vivo conditions and cause decreased production of scar tissue in sores associated with severe scar tissue [39,40]. However, it seems that additional cellular and molecular research is warranted for clarification.

Tireli and colleagues have investigated the positive effects of PTX on healing a small intestine anastomosis after induction of ischemia. They reported that the dose of PTX (50 mg/kg) used in their study [19] caused significant healing in the experimental group. This dose was chosen since it was an effective dose for the reduction of tissue neutrophils. Neutrophils affect tissue by secreting proteolytic enzymes and free radicals in the wound bed of pressure sores during healing process [19,41]. In our pilot study we observed a positive effect of PTX (50 mg/kg) on the pressure sore. Thus in the current study, we used 50 mg/kg PTX for the experimental groups.

Numerous studies have used PTX in experimental models of skin flaps [9-11]. In those studies PTX was injected for a defined period of time into the animals, and skin flaps were created followed by administration of PTX for a longer duration. In studies by Bayat et al. and Pratt et al. [10,11], PTX was administered seven days before surgery in the first stage, but did not lead to any significant increase in flap survival. Thus in the second series, PTX was administered 14 days before flap surgery which lead to a significant increase in skin flap survival. According to these results administration of PTX prior to surgery was necessary in order to achieve positive results. However, in that research the flap model was used under ischemia conditions, which differed from the pressure sore model. In the present study, we administered PTX after the pressure sores were created, and observed a positive effect.

In those studies [9-11] the dose of PTX was 20 mg/kg, which was lower than the dose used in the present study. Possibly, if higher doses of PTX were used in those studies a positive effect of PTX on flap survival could have been observed without the preoperative administration of PTX. Additional research is necessary to confirm this comment. In other studies The researchers have suggested that PTX should not be routinely used in cases of acute ischemic stroke [42].

The present study has shown that administration of PTX at a dose of 50 mg/kg to an experimental model of pressure sores in rats did not significantly affect the histological parameters such as numbers of cells, blood vessels and thicknesses of the repaired tissue compared with the control groups. As the histological parameters in the current study did not increase, thus there seems to be another mechanism of action for PTX on pressure sores.

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