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Interactive effects of acupuncture on pain and distress in major burns: An experiment with rats

Ayse Ebru Abali MD[a], Tugrul Cabioglu MD[b], Handan Ozdemir MD[c], Mehmet Haberal MD, FACS[a,d,*]

[a] Burn Center, Baskent University Burn and Fire Disasters Institute, Ankara, Turkey
[b] Department of Physiology, Baskent University, Faculty of Medicine, Ankara, Turkey
[c] Department of Pathology, Baskent University, Faculty of Medicine, Ankara, Turkey
[d] Department of General Surgery, Baskent University, Faculty of Medicine, Ankara, Turkey

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ABSTRACT

This study sought to investigate the interactive effects of acupuncture on pain and distress and the local progress in the burn wound in an experimental major burn model. Forty-eight male Sprague-Dawley rats were divided into six groups: S group (sham/observation during 7 days after injury); SA group (sham/acupuncture/observation during 7 days after injury); B1 group (burns/observation during 1 h after injury); BA1 group (burns/acupuncture/observation during 1 h after injury); B7 group (burns/observation during 7 days after injury); and BA7 group (burns/acupuncture/observation during 7 days after injury). Pain and distress scores were evaluated throughout the study. The amounts of neutrophils and mononuclear cells were evaluated semiquantitatively, and the number of microvessels was evaluated quantitatively. Our data indicated that the average pain score of BA7 group was significantly lower than the other study groups. Histopathologic investigations indicate that the amounts of neutrophil and mononuclear cell and numbers of microvessels in the unburned skin were higher in acupuncture-applied groups. The number of microvessels in burn wounds of BA7 group was significantly higher than that of the other groups. Our data suggest that acupuncture provides low pain and distress scores in experimental rat model, and it contributes to wound healing with an enhanced angiogenesis during the acute phase of burns. Future clinical and experimental studies should be conducted to discern the benefits from acupuncture in pain management of burn patients.

1. Introduction

Burn trauma induces mechanical and thermal hyperalgesia in human skin, resulting in a hypersensitive state that accompanies inflammation [1–3]. Clinical observations and research suggest that burn patients do not become habituated to pain, and strong correlations are found among pain, psychological distress, and physiological outcomes in children and adults with burns [4]. In modern burn care, managing pain and anxiety is an indispensable component of burn treatment. Types of burn pain and anxiety are defined as *background pain*, *procedural pain*, and *breakthrough pain* [5].

All types of burn pain and anxiety result in psychological distress that causes a substantial delay in wound healing [6]. Stress-impaired wound healing is mediated primarily through

* *Corresponding author at*: Taşkent Cad. No: 77, Bahçelievler, 06490, Çankaya, Ankara, Turkey. Tel.: +90 312 212 8016; fax: +90 312 212 3133.
E-mail address: rektorluk@baskent-ank.edu.tr (M. Haberal).

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the hypothalamic–pituitary–adrenal, sympathetic–adrenal–medullary axes [7,8].

For standard pain management, burn teams prefer to administer pharmacological agents such as opioids, ketamine, nonsteroidal anti-inflammatory agents, and sedatives. In addition to pharmacological therapies, nonpharmacologic analgesics (e.g., virtual reality analgesia) contribute to pain management during both the acute phase and the period following [9–11].

Acupuncture is a popular nonpharmacological therapy used for pain control. It also is widely used for various diseases. Numerous studies indicate that acupuncture is safe and effective in treating a range of disorders such as obesity, osteoarthritis, fibromyositis, trigeminal neuralgia, postoperative pain, and headache [12–17].

Current knowledge regarding the interactive effects of acupuncture on burn trauma is limited. Few studies have investigated the role of acupuncture on burn care. Some concern themselves with the effects of acupuncture on pain and stress control, while others concerned themselves with local wound healing [12,18,19]. Recently, the effects of acupuncture on burn wounds were investigated in an experimental rat burn model, but the study was concerned with wound healing rather than burn pain and distress. In previous experiments, acupuncture has been applied around the burn wounds (Ashi points), and the classic acupuncture points (e.g., Back-Shu points) have not been considered [18].

The effects of acupuncture application to Ashi points and Back-Shu points for different types of burn pain and distress in major burn trauma are unknown. Therefore, more-detailed and progressive studies are required that investigate the interactive effects of acupuncture for pain and anxiety control in major burns. The present experiment on rats investigates the interactive effects of acupuncture on pain and distress control and the local progress in the burn wound in an experimental major burn model.

2. Materials and methods

Forty-eight male Sprague-Dawley rats weighing 300 ± 15 g were obtained and housed in the Başkent University Laboratory Animal Center (temperature 20 ± 2 °C, humidity 50 ± 10%, and 12-h light/dark cycle). The animals were kept at standard conditions and fed standard rat chow and water ad libitum for at least 1 week before the experiments began. All animals received human care in compliance with the National Institute of Health Guide for the care and use of laboratory animals, and the study was approved by Başkent University Animal Care and Ethics Committee (DA: 13/56).

3. Burn model

3.1. Burn model creation

A preliminary study was performed on two male Sprague-Dawley rats before beginning research. The preliminary study sought to observe the appropriate brass plate temperature and contact time that would create deep partial-thickness burns on the lower quadrants of the dorsa in the Sprague-Dawley rats. A 4-cm × 4-cm brass plate was used to induce burns. Temperature of the plate was monitored with a thermocouple device of a multimeter (Fluke 116 HVAC, Fluke South East Asia Pte Ltd, Singapore).

Rats were anesthetized with an intramuscular injection of 70 mg/kg ketamine hydrochloride (Alfamine 10%) and 7 mg/kg xylazine hydrochloride (Rompun 2%). The dorsa of the animals were shaved; burns were induced on the two lower quadrants of dorsa. The chosen temperature of brass plate for animal No. 1 was 200 °C, and it was 250 °C for animal No. 2. The contact periods were 5 s for the right lower quadrants and 10 s for the left lower quadrant. On histopathologic observation, the appropriate time for a deep partial-thickness burn wound was determined to be 5 s with a brass plate temperature of 250 °C.

3.2. Burn induction and wound dressings in the present study

In this study, rats were anesthetized with an intramuscular injection of 70 mg/kg ketamine hydrochloride (Alfamine 10%) and 7 mg/kg xylazine hydrochloride (Rompun 2%). The dorsa of the animals were shaved; burns were induced on the right lower quadrant of dorsum with a 4-cm × 4-cm brass plate with a burn size covering approximately 30% of the total body surface area. For deep partial-thickness burn induction, the 4-cm × 4-cm brass plate was held in the flame of a Bunsen burner with full oxygen supply until the thermometer measured 255 °C. The brass plate lost 5 °C of heat until it was put on to the right lower quadrants. The plate was put against the prepared skin for 5 s. All animals were promptly resuscitated with lactated Ringer's solution (2 mL/100 g) intraperitoneally.

The thickness of each cutaneous burn wound was confirmed by histopathologic examination. Skin samples were burned down to the middle third zones of the dermis in the B1, BA1, B7, and BA7 groups.

For wound-dressing changes during the study period, the rats were anesthetized with an intramuscular injection of 70 mg/kg ketamine hydrochloride (Alfamine 10%) and 7 mg/kg xylazine hydrochloride (Rompun 2%). After application of silver sulfodiazine (Silverdin, Deva, Turkey), wounds were covered with superior film with acrylic adhesive (Opsites, Smith and Nephew, USA).

4. Groups

4.1. S (sham group)

In the S group, eight healthy rats were anesthetized with an intramuscular injection of 70 mg/kg ketamine hydrochloride (Alfamine 10%; Alfasan Inc, Woerden, The Netherlands) and 7 mg/kg xylazine hydrochloride (Rompun 2%, Bayer Kimya San. Ltd. Sti., Istanbul, Turkey). The dorsa of the animals were shaved. Twenty minutes after shaving, wound dressings were put onto the lower dorsum and initial blood samples were obtained. Lactated Ringer's solution (2 mL/100 g) was injected intraperitoneally. Wound dressings were changed every other

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day; pain and stress scores were checked daily throughout the study (7 days). After obtaining blood samples and skin biopsies on the 7th day, the animals were euthanized.

4.2. SA (sham/acupuncture group)

In the SA group, eight healthy rats were anesthetized as described above. The dorsa of the animals were shaved. An imaginary wound was drawn by the help of a 4-cm × 4-cm brass plate onto the right side of the lower dorsum. Lactated Ringer's solution (2 mL/100 g) was injected intraperitoneally. Transient acupuncture needles were placed around the imaginary wound (Ashi points) and onto the relevant dermatomes (Back-Shu points) as described below. Twenty minutes after we had applied acupuncture, transient acupuncture needles were removed, and long-acting acupuncture needles were placed onto the Ashi points and the Back-Shu points. Wound dressings were put onto the lower dorsum, and blood samples were obtained. Acupuncture administration and wound dressings were repeated every other day; pain and stress scores were checked daily throughout the study. Blood samples and skin biopsies were obtained, and the animals were euthanized on the 7th day.

4.3. B1 group (burn group in which animals were observed for 1 h after injury)

In the B1 group, eight healthy rats were anesthetized as described above. The dorsa of the animals were shaved. Deep partial-thickness burns were induced on the right side of the lower dorsa. Lactated Ringer's solution (2 mL/100 g) was injected intraperitoneally. After 20 min, wound dressings were put onto the right side of the lower dorsum. Blood samples were obtained, skin biopsies were taken, and the animals were euthanized 1 h after burn induction.

4.4. BA1 (burn/acupuncture groups in which the animals were observed for 1 h after burn induction)

In this group, eight healthy rats were anesthetized as described above. The dorsa of the animals were shaved. Deep partial-thickness burns were induced on the right side of the lower dorsum. Lactated Ringer's solution (2 mL/100 g) was injected intraperitoneally. Transient acupuncture needles were placed onto the Ashi points and onto the Back-Shu points. Twenty minutes after transient acupuncture application, transient acupuncture needles were removed, and long-acting acupuncture needles were placed onto the Ashi points and the Back-Shu points. Wound dressings were placed onto the lower dorsum. Blood samples were obtained, skin biopsies were taken, and the animals were euthanized 1 h after burn induction.

4.5. B7 (burn group in which animals were observed for 7 days after burn induction)

In this group, eight healthy rats were anesthetized as described above. The dorsa of the animals were shaved. Deep partial-thickness burns were induced on the right side of the lower dorsum. Lactated Ringer's solution (2 mL/100 g) was injected intraperitoneally. Twenty minutes after burn induction, wound dressings were put onto the right side of the lower dorsum. Wound dressings were changed every other day. On the 7th day, blood samples and skin biopsies were obtained, and the animals were euthanized.

4.6. BA7 (burn/acupuncture group, in which the animals were observed for 7 days after burn induction)

In this group, eight healthy rats were anesthetized as described above. The dorsa of the animals were shaved. Deep partial-thickness burns were induced on the right side of the lower dorsum. Lactated Ringer's solution (2 mL/100 g) was injected intraperitoneally. Transient acupuncture needles were placed around the burn wound and on the relevant dermatomes. Twenty minutes after transient acupuncture application, transient acupuncture needles were removed and long-acting acupuncture needles were placed around the burn wound and on the relevant dermatomes. Acupuncture administration and wound dressings were repeated every other day. Blood samples and skin biopsies were taken and the animals were euthanized on the 7th day.

4.7. Acupuncture application

Ashi points were selected around the burn wounds, and the Back-Shu points on the related dermatome were used. As the related dermatome was placed at the levels of T10, T11, T12, L1, L2, and L3, the acupuncture points of the Back-Shu points were placed on these dermatomes and were preferred for application (i.e., UB 19, UB 20, UB 21, UB 22, UB 23, and UB 24). Location of the Back-Shu points is specified below:

UB 19 (Danshu): Lateral to Du7 (Zhongshu), at the level of the lower border of the spinous process of the 10th thoracic vertebra.
UB 20 (Pishu): Lateral to Du6 (Jizhong), at the level of the lower border of the spinous process of the 11th thoracic vertebra.
UB 21 (Wei Shou): Lateral to Du Meridian, at the level of the lower border of the spinous process of the 11th and 12th vertebrae.
UB 22 (San Jiao Shu): Lateral to Du 5 (Xuanshu), at the level of the lower border of the spinous process of the first lumbar vertebra.
UB 23 (Shenshu): Lateral to Du 4 (Mingmen), at the level of the lower border of the spinous process of the second lumbar vertebra.
UB 24 (1.5 cm lateral to the Du meridian at L3), at the level of the lower border of the spinous process of the third lumbar vertebra.

The steel acupuncture needles (KINGLI 0.16 mm × 7 mm, tiger Balm, Oral Liquid, Acupuncture Needle, moxa, massage product, Jiangsu, China) were placed onto these points (Fig. 1A). Transient acupuncture needles were kept on these points for 20 min. After the transient acupuncture application, long-acting steel needles were placed onto the same points (Fig. 1B). Wound dressings were put on the dorsa, and acupuncture application was repeated every other day when changing dressings (Fig. 2).

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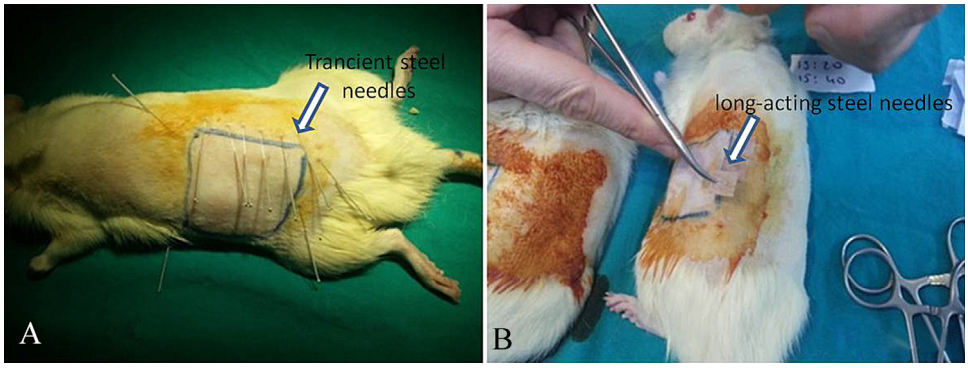

Fig. 1 – (A) Acupuncture needles were placed onto selected Ashi points and Back Shu points. (B) Following 20 min of transient acupuncture application, long-acting steel needles were placed onto the same points.

4.8. *Pain and distress measurement and management*

A method commonly used to assess pain is to score parameters that demonstrate changes in a particular animal's normal behavior and appearance that are associated with pain. Each protocol and species typically requires its own pain scoring system, and the frequency of observations and training of the persons making evaluations are specified for each protocol. A reliable method of pain assessment allows an appropriate analgesic regimen to be used and effectively evaluated.

For evaluating pain and distress in rodents, a number of scoring systems are created by animal research laboratories [20]. In the present study, evaluating pain and distress was based on the "*Pain & distress rating scale and long-term post surgery monitoring for non-USDA regulated rodent study*," which had been arranged by Bloomington Institutional Animal Care and Use Community [21]. The scale was modified by changing the assessment scores of "surgical site" to the assessment scores of "burn wound" (Table 1). Once-a-day fentanyl hydrochloride injections (0.02 mg/kg IM) were routine for all animals. Two veterinary technicians who were charged with care of the animals also were responsible for observing the subjects' behaviors and then scoring the animals. They noted the scores and the other signs and symptoms of pain and distress every 24 h. If the score was higher than 2 in any category, and/or if the total score was higher than 3, they consulted the veterinarian for additional analgesia, warming, or hydration of the affected subjects. The veterinary technicians also noted the frequency and dosage of analgesia, lactated Ringer's solution injections, and warming ordered by the veterinarian. Each animal was placed in a separate cage after the procedure.

Fig. 2 – Wound dressings were put on the dorsa after wound care and acupuncture application.

4.9. *Tissue processing*

Skin biopsies were obtained from both burned and adjacent nonburned areas in each animal, and were fixed in formalin and embedded in paraffin blocks. Several 4-μm-thick sections were obtained from each paraffin-embedded block, and these were processed with hematoxylin and eosin. All representative sections of skin biopsies were examined histologically under a light microscope in a blinded fashion for the degree of burn depth, neovascularization, and inflammation by a grid at a magnification of ×200, using an eyepiece screen with an examination area of 0.25 mm^2. In addition, the presence or absence of neutrophil infiltration throughout the biopsy was determined.

Grade 0: There was no inflammatory cell infiltration.
Grade 1: Neutrophils comprised <10% of the examination area of 0.25 mm^2.
Grade 2: Neutrophils comprised ≥10% of the examination area of 0.25 mm^2.

In evaluating the inflammation, the density of mononuclear inflammatory cells (e.g., lymphocytes, macrophages, and plasma cells) was screened. The density of mononuclear inflammatory cells and neutrophil leukocytes in the skin biopsies was graded in a semiquantitative manner using a 0–2+ grading scale.

Grade 0: There was no inflammatory cell infiltration.
Grade 1: Inflammatory cells comprised <30% of the examination area of 0.25 mm^2.
Grade 2: Inflammatory cells comprised ≥30% of the examination area of 0.25 mm^2.

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Table 1 – Pain and distress scale which is used in the present study[a]

Assessment score	0	1	2	3
Attitude and posture	Alert and not hunched	Not alert or hunched	Not alert and hunched	Not responsive to stimuli
Gait and movement	Active	Somewhat inactive	Completely inactive	Lying on side
Burn wound site	Clean and not swollen	Exudates or swelling	Exudates and swelling	Increased wound depth in examination
Appetite	Eating and drinking	Reduced eating or drinking	Not eating or drinking	Not eating or drinking for >3 days
Elimination	Normal	Softer than normal	Diarrhea	Diarrhea >3 days

Treatment plan (in consultation with attending veterinarian).
- Scores = 3 in any category: euthanize immediately.
- Scores = 2 in any category: evaluate and consult with veterinarian.
- Total score > 3: consult with veterinarian.

Score > 1 in appetite: provide easy access to food and treats.

[a] Ref. [21] was modified for the present experimental rat model of burn.

The entire area of sections, both in burned and nonburned areas in each animal, was evaluated with a grid, and calculated for evaluation of microvessel density. Microvessel density was determined by counting all vessels at a total magnification of ×200 using an eyepiece screen with an examination area of 0.25 mm^2. Results are presented as the number of microvessels per unit of area.

Statistical analyses were performed using SPSS software (Statistical Package for the Social Sciences, version 11.0, SSPS Inc, Chicago, IL, USA). Results for pain and distress scores are given as medians ± interquartile range (IQR), and quantitative variables are given as means ± SD. One-way analysis of variance (ANOVA) and the Bonferroni correction were used when variables had normal distributions, and the Kruskal–Wallis test was used for variables with non-normal distributions. When statistically significant results were found with the Kruskal–Wallis test, the Mann–Whitney *U* test with the Bonferroni correction was used. For evaluation of categorical data, the chi-square test was performed. Values for $P < 0.05$ were considered statistically significant.

5. Results

No burn wound infections and no unburned skin infections were observed in the study group. All of the long-acting acupuncture needles stayed safely on the Ashi points and the Back-Shu points until they were taken away for the next acupuncture application and wound dressing. Daily fentanyl injections were routine for all of the animals in the study group. The veterinary technicians noted additional fentanyl injections only in the B7 group, because of the high pain and distress scores. Animals in the B7 group also required additional injections of lactated Ringer's solution and warming.

No observations were noted for the B1 and BA1 groups, because they were euthanized 1 h after burn induction. The S, SA, B7, and BA7 groups were observed for 7 days. During the initial 24 h, six animals (with pain and distress scores higher than 3) in the B7 group needed consultation by the veterinarian. In the second 24 h, the number of animals that needed consultation by veterinarian was four in the B7 group. However, according to pain and distress scoring, no animals needed consultation by the veterinarian. In the S, SA, and BA7 groups (Table 2). The animals that were consulted by veterinarian had additional analgesics, fluid resuscitation, and warming according to their circumstances. Three rats in the B7 group were found dead on the 3rd day after injury. Postmortem examinations revealed that one of the animals had died owing to gastric stress ulcer bleeding, and the other two animals died because of undefined causes, with high pain and distress scores.

6. Pain and distress scores

6.1. *Medians for the study of pain and distress scores*

Pain and distress measurements revealed that animals in the S, SA, B7, and BA7 groups had pain or distress experience to

Table 2 – The distribution of numbers of the animals whose pain and distress scores were higher than 3.

Group	Number of animals whose pain and distress scores are >3 (veterinarian consultation) (day 1)	Number of animals whose pain and distress scores are >3 (veterinarian consultation) (day 2)
S group	0	0
SA group	0	0
B7 group	6	4
BA7 group	0	0

Animals with pain and distress scores higher than 3 in the B7 group needed consultation by veterinarian in the initial 48 h, while animals in S, SA, and BA7 groups did not.

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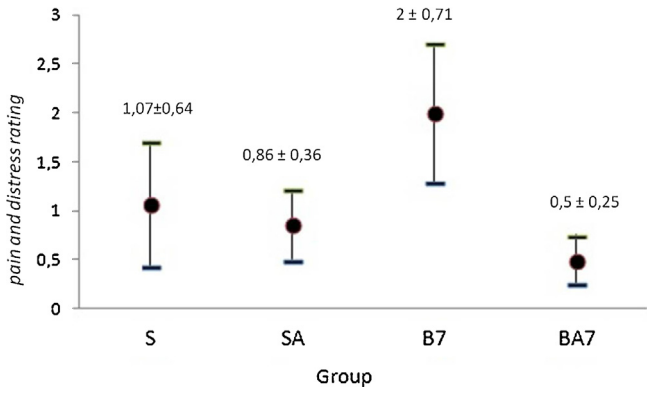


Fig. 3 – *B7 group had the highest median pain and distress level (median ± IQR, P = 0.00). **BA7 had the lowest median pain and distress levels (median ± IQR, P < 0.05).

some degree. However, animals in the B7 group had the highest median pain and distress levels (2.0 ± 0.71; P = 0.00), and animals in the BA7 group had the lowest median pain and distress levels (0.5 ± 0.25; P < 0.05). These findings indicate that acupuncture administered to healthy animals produced pain and distress to some degree, but acupuncture application to animals with major burns lowered the hypersensitive state produced by these major burns (Fig. 3).

6.2. *Day-by-day observations*

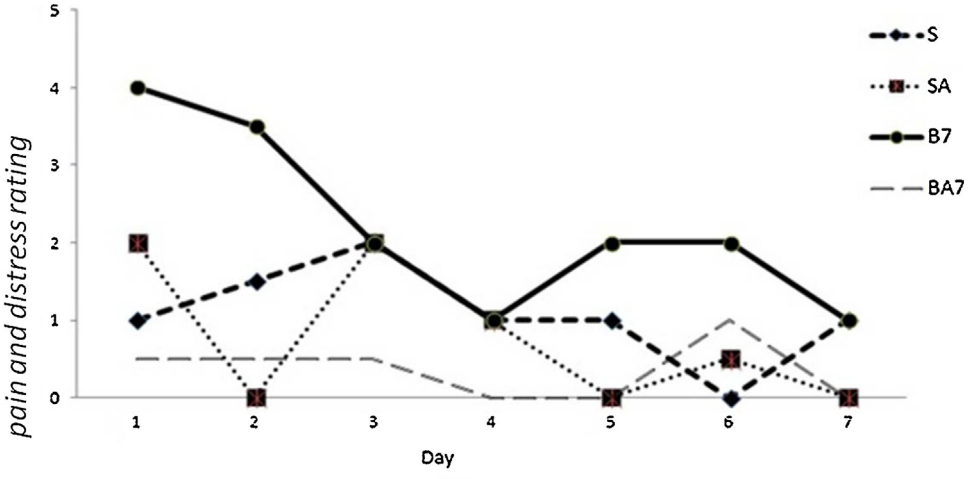
On day 1, animals in the SA group had significantly higher pain and distress levels than did animals in the S and BA7 groups (P < 0.05). On days 1 and 2, pain and distress scores were significantly higher for animals in the B7 group (4.0 ± 3.75 and 3.5 ± 2.75) than they were for animals in the S (1.0 ± 1.75 and 1.5 ± 2.0), SA (2.0 ± 1.75 and 0.0 ± 0.75), and BA7 groups (0.5 ± 1.75 and 0.5 ± 1.75).

Pain and distress scores were higher in the B7 group on days 3–7 when compared with animals in the BA7 group (P < 0.05). There were no statistically significant differences in pain and distress scores in animals of the S, SA, and BA7 groups on days 1, 2, 5, 6, and 7. On days 3 and 4, pain and distress levels of animals in the BA7 group were statistically significantly lower than those of the other groups in the study (P < 0.05; Fig. 4).

7. Histopathologic examination

7.1. *Normal skin*

Histopathologic examination of unburned skin covering the Ashi points and the Back-Shu points revealed that no neutrophils were observed in the S and the B1 groups. Minimal neutrophil accumulations were observed in 25% of the SA group, 37.5% of the BA1 group, 50% of the B7 group, and in 100%



	S	SA	B7	BA7
day1	1 ± 1,75	2 ± 1,75	4 ± 3,75	0,5 ± 1,75
day2	1,5 ± 2,0	0 ± 0,75	3,5 ± 2,75	0,5 ± 1,75
day3	2 ± 1,0	2 ± 1,0	2 ± 1,50	0,5 ± 1,0
day4	1 ± 1,0	1 ± 1,75	1 ± 3,00	0 ± 0,75
day5	1 ± 2,5	0 ± 1	2 ± 1,5	0 ± 1,0
day6	0 ± 1	0,5 ± 1	2 ± 1,5	1 ± 0,75
day7	1 ± 2	0 ± 1	1 ± 0,5	0 ± 0,75

Fig. 4 – Pain and distress scores were higher in the B7 group on days 3–7 when compared with animals in the BA7 group (P < 0.05). There were no differences in pain and distress scores in animals of the S, SA, and BA7 groups on days 1, 2, 5, 6, and 7. On days 3 and 4, pain and distress levels of animals in the BA7 group were lower than those of the other groups in the study (median ± IQR, P < 0.05).

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Table 3 – The distribution of the neutrophilleukocyte density in the unburned skin and burn wounds.

Group	Unburned skin			Burn wounds		
	Grade 0 n(%)[a]	Grade 1 n(%)[b]	Grade 2 n(%)[c]	Grade 0 n(%)[a]	Grade 1 n(%)[b]	Grade 2 n(%)[c]
S	8(100%)	0	0	–	–	–
SA	6(75%)	2(25%)	0	–	–	–
B1	8(100%)	0	0	3(37.5%)	5(67.5%)	0
BA1	5(62.5%)	3(37.5%)	0	1(12.5%)	7(87.5%)	0
B7	4(50%)	4(50%)	0	0	4(50%)	4(50%)
BA7	0	8(100%)	0	0	4(50%)	4(50%)

[a] Grade 0: there was no neutrophil infiltration.
[b] Grade 1: neutrophils comprised <10% of the examination area of 0.25 mm^2.
[c] Grade 2: neutrophils comprised ≥10% of the examination area of 0.25 mm^2.

of the BA7 group (Table 3, Fig. 5). No mononuclear inflammatory cells (e.g., lymphocytes, macrophages, and plasma cells) were observed in the S, SA, B1, BA1, and B7 groups. However, minimal mononuclear inflammatory cells (e.g., lymphocytes, macrophages, and plasma cells) were present in 62.5% of the BA7 group. These findings indicate that acupuncture application to the Ashi points and the Back-Shu points triggered a mild inflammatory process on the unburned Back-Shu skin at day 7 after burn.

Quantitative measurement of microvessels in the unburned skin samples showed that there were no significant differences in the numbers of neovascular structures of the S (5.5 ± 3.75), SA (8 ± 3.5), and B1 groups (6.5 ± 1.75); however, there were minimally increased numbers of microvessel density in the SA group, but this was not statistically significant ($P > 0.05$). The BA1 group had statistically significantly higher numbers of microvessels than did the S and the B1 groups. There were no statistically significant differences among the numbers of microvessels in the B1 (11.0 ± 3.5), the B7 (12 ± 3.75), and the BA7 groups (14.5 ± 7.50; $P > 0.05$; Fig. 6). These data indicate that the number of microvessels on the Ashi-points and Back-Shu points (which are located on the unburned skin) increased after the first acupuncture application and burns; the result was also similar in a slightly increased microvessel density on the unburned skin around the wound.

7.2. *Burned skin*

Histopathologic examination of burn wounds in the B1, BA1, B7, and BA7 groups showed that all of the burn wounds had inflammatory changes to some degree. In the B1 group, 67.5% of the animals had small amounts of neutrophils; in the BA1 group, 87.5% of the animals had small amounts of neutrophils in the burn wounds. All of the animals in the B7 and the BA7 groups showed an accumulation of neutrophils; in 50% of the animals of the B7 group and in 50% of the animals in the BA7 group, accumulation of neutrophils was dense (Table 3).

All of the samples in the B1, BA1, B7, and BA7 groups showed differing amounts of mononuclear inflammatory cells (e.g., lymphocytes, macrophages, and plasma cells). Dense amounts of mononuclear inflammatory cells were seen in 25% of the animals in the B1 and 37.5% of BA1 groups. No statistically significant differences were seen between the amounts of mononuclear cells of the B7 and the BA7 groups. These findings indicate that acupuncture did not cause a difference in neutrophil and mononuclear reactions in the burn wound at 7 days after burn.

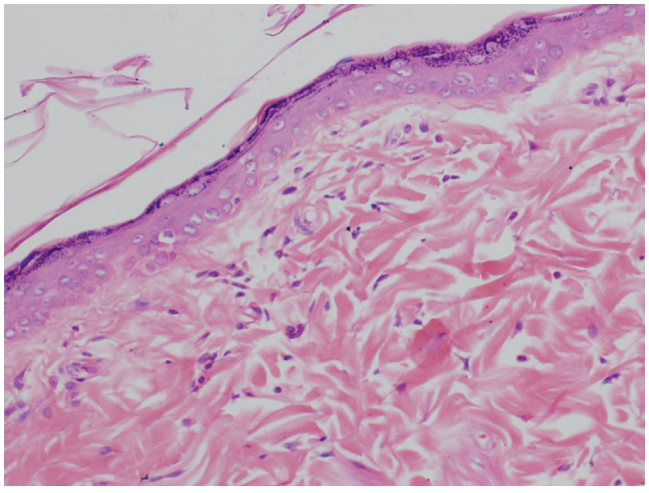

Fig. 5 – In the unburned skin: minimal neutrophil accumulations were observed in 25% of the SA group, 37.5% of the BA1 group, 50% of the B7 group, and in 100% of the BA7 group (H–E ×200).

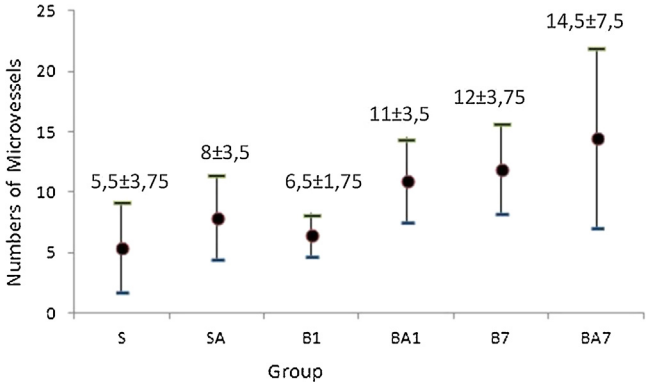

Fig. 6 – In the unburned skin: *BA1 group had significantly higher numbers of microvessels than the S group and the B1 group (mean ± SD, $P < 0.05$). **There were no significant differences between the numbers of neovascular structures of B7 group and of BA7 group (mean ± SD, $P > 0.05$).

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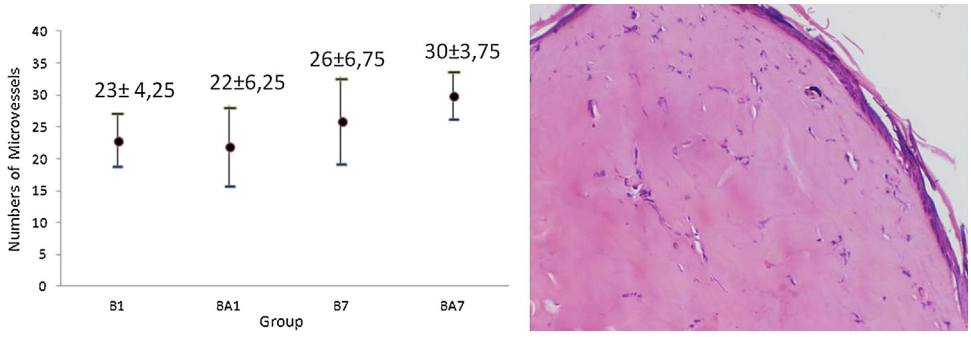

Fig. 7 – In the burn wounds: *number of microvessels in BA7 group was significantly higher than B1 group, BA1 group, and B7 group (mean ± SD, P < 0.05). Improved angiogenesis of a burn wound in the BA group is shown in the histopathological image (H–E, ×200).

Quantitative measurement of microvessel density showed that no statistically significant differences existed between animals in the B1 group (23 ± 4.25) and those in the BA1 group (22 ± 6.25; $P < 0.05$). The number of microvessels in the B7 group (26 ± 6.75) was significantly higher than they were in the B1 and BA1 groups ($P < 0.05$). However, the number of microvessels was significantly higher in the BA7 group (30 ± 3.75) when compared with the B1, the BA1, and the B7 groups ($P < 0.05$; Fig. 7). The significantly high numbers of microvessels in the BA7 group on day 7 showed that continuous and repeated acupuncture applications in this group resulted in increased angiogenesis, which is important in wound healing [14,15,18].

8. Discussion

Our data show that acupuncture provides low pain and distress scores during 7 days after the burn, and improved angiogenesis in an experimental rat model of major burns. In the major burn group without acupuncture application (B7), high pain-distress scores and early deaths were seen during 7 days after the burns. As a major burn is one of the most severe and complicated inflammatory and hyperalgesic states, it begs the question: how does acupuncture bring about lower pain and distress levels, and how does improved angiogenesis occur in burn wounds?

The effects of acupuncture can be explained by viscerocutaneous, cutaneo-visceral, cutaneo-muscular, and viscero-muscular reflexes. Segmental dispersion of the sympathetic and parasympathetic systems, which is produced by acupuncture, is related to the location of acupuncture points, such as the Back-Shu point [22,23]. Acupuncture application of the Back-Shu points stimulates pain control mechanisms by increasing the levels of neurotransmitters such as endorphins, endomorphins, enkephalins, serotonin, and norepinephrine in the brain and in the blood [21]. These neurotransmitters are also well-known modulators of inflammation, the hypothalamic–pituitary–adrenal axes, and the sympathetic–adrenal–medullary axes [24–29]. Stimulation of these pain-control reflexes and inflammation-related pathways triggers the way acupuncture affects pain and distress control, as well as inflammation [22–24].

Ashi points – which also were used in the present study – are different from classic acupuncture points. They are located at any site on the body and are chosen by the practitioner because of their responsive nature to the disease or trauma. Recent studies show that applying acupuncture for postoperative orthopedic pain and bone fracture disease onto Ashi points is as effective as applying Back-Shu points [14]. Therefore, the lower observed algesic state in our study can be explained by the production of strong viscero-cutaneous, cutaneo-visceral, cutaneo-muscular, and viscero-muscular reflexes, and a strong stimulation of ''pain control system'' by simultaneous use of both Ashi and Back-Shu points [22–24]. As our experimental study is the first experimental study where these acupuncture points were used simultaneously in a burn pain model, it is essential that further studies are used to determine the exact effects of simultaneous application of Back-Shu acupuncture points and Ashi points in major burns.

Our data show that the BA1 group had a significantly higher number of microvessels in the unburned skin than did the S and B1 groups. In the unburned skin samples of the SA group, the number of microvessels was higher in the S and B1 groups, but it was not statistically significant. The number of microvessels in the burn wounds of the BA7 group was significantly higher than that of entire study group. According to these findings, it appears that acupuncture also has modulated the inflammatory process in the affected areas [13,31].

Acupuncture is known to increase the local blood circulation, with subsequent increase of local neuropeptides, cytokines, and other vasoactive substances. Previous studies have shown that acupuncture could have effects on wound healing process via inhibition of inflammatory cytokines and improvement of proliferative cells and basic-fibroblast growth factor expression [14,15,18,32]. We think that the dense histopathologic changes in our study in unburned skin and burn wounds of acupuncture-applied groups may be related to modulation of these inflammatory cytokines by acupuncture.

One theory suggests that placement and manipulation of acupuncture needles stimulate a signaling in the body along connective tissue planes that triggers a biomechanical and biochemical reaction, because when minute stretching of the applied connective tissue (as what occurred in acupuncture needling) occurs, fibroblasts in the tissue react in the series of biochemical and biomechanical responses [33,34]. In the

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current study, increased numbers of microvessels in unburned skin samples of all acupuncture applied groups as well as the dense amounts of neutrophils and mononuclear cells in the unburned skin samples of the BA7 group seem to support this theory. These inflammatory cells are the initial inflammatory cells that cause fibroblast proliferation in the following phases of wound healing. Dense amount of neutrophils and mononuclear cells on the acupuncture-applied unburned skin and increased angiogenesis in burn wounds may predict fibroblast activity in the following phases of burn wound healing. Findings of a recent study by Lee et al. [18] support this: they revealed that leukocyte numbers and macrophage inflammatory protein-1α (MIP-1α) expression on the burn wounds were significantly reduced, and that basic-fibroblast growth factor expression was increased in the burn wounds by acupuncture treatment at 14 days after burn induction.

Postburn inflammatory responses in burned and unburned skin are thought to underscore the pain experienced by burn patients [30,35–38], and proinflammatory cytokines (e.g., interleukin-6) also are important mediators of burn pain [38]. Therefore, modulating postburn inflammatory response by acupuncture may hold beneficial outcomes for both pain management and wound healing [4,35–39]. It seems that acupuncture modulates both pain and distress scores and local inflammatory responses in a rat model; however, it is unclear whether acupuncture acts via its effects on postburn inflammatory responses or successful pain and distress control in the acute phase of burns have influenced the whole scene. Further studies are required.

In conclusion, our findings suggest that applied acupuncture on both Back-Shu and Ashi points results in a less-algesic state in an experimental burn model, and its contribution to pain and distress control is associated with signs of improved angiogenesis during the acute phase. Acupuncture appears to become a benefit to burn care, and future clinical and experimental studies must be conducted to discern the appropriate use of acupuncture in pain management of burn patients.

Conflict of interest

We confirm that there are no conflict of interests and source of funding for the present manuscript.

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