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ORIGINAL ARTICLE

Effect of piracetam and nimodipine on full-thickness skin burns in rabbits

Elif Sari[1] & Gungor C Dincel[2]

1 Department of Plastic, Reconstructive and Aesthetic Surgery, Kirikkale University Faculty of Medicine, Kirikkale, Turkey
2 Gumushane University, Siran Mustafa Beyaz Vocational School, Laboratory and Veterinary Health Program, Gumushane, Turkey

Key words
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Correspondence to
E Sari, MD
Department of Plastic, Reconstructive and Aesthetic Surgery
Faculty of Medicine, Kirikkale University
Ankara-Kirikkale Road, 7th km Yahsihan
Kirikkale 71450
Turkey
E-mail: drelifsanli@hotmail.com

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Abstract

The potential of several drugs for full-thickness skin burns has been investigated, but the treatment of such burns remains a challenge in plastic surgery. The present study was designed to determine the effect of systemic and topical administration of piracetam and nimodipine on full-thickness skin burn wound healing. A total of 36 New Zealand male rabbits were divided into six groups. Full-thickness skin burns were produced in all the groups, except the control group. Piracetam was administered systemically (piracetam-IV) and topically (piracetam-C) for 14 days, and nimodipine was administered systemically (nimodipine-IV) and topically (nimodipine-C) over the burn wounds for 14 days. The sham group underwent burn injury but was not administered any drug. After 21 days, gross examination and histopathological analysis were performed and the results were compared statistically. Nimodipine-C and nimodipine-IV had no effect on burn wound healing. However, both piracetam-IV and piracetam-C significantly enhanced the healing of the full-thickness skin burn wounds, although the latter was more effective, useful and practical in burn wound healing. The histopathological features of the wounds in the piracetam-C group were closer to those of the control group than those of the other groups. Piracetam-C rather than piracetam-IV may promote full-thickness burn wound healing in rabbits.

Introduction

Full-thickness burns involve the epidermis and dermis of the skin (1). In addition to skin damage, they may impair fluid and nutritional metabolism, as well as the haematological status and immune system, and cause sepsis (1). Full-thickness burn wound management involves long-term hospitalisation, expensive medications, several surgical approaches and a long rehabilitation period.

Several herbal products and medicines have been used to accelerate the healing period of burn wounds. These include olive oil (2), topical aqueous oxygen (3), topical zinc oxide (4), topical aloe vera extract (5) and silver sulfadiazine (6). Among these, silver sulfadiazine is the most widely used agent worldwide (6). However, recent studies reported delayed healing of full-thickness burns and scarring associated with the use of silver sulfadiazine (7,8). Studies have also reported that using silver sulfadiazine for more than 3 weeks caused renal toxicity, leukopenia and resistance to the drug (9–11). Therefore, physicians are looking for a new drug for full-thickness burns (7,12).

Key Messages

- several factors are responsible for a successful wound healing
- the aim of this study was to investigate the possible beneficial effects of piracetam and nimodipine on burn wound healing process
- nimodipine administered both cutaneously or systemically had no effect on burn wound healing
- piracetam had beneficial effect on burn wound healing
- however, cutaneous piracetam were more effective on burn wound healing rather than systemic piracetam

Piracetam is a pharmacological substance, which is widely used for cerebral vascular insufficiency (13). It decreases the time of recovery from hypoxia (13) and enhances both phospholipid and protein synthesis (14). Piracetam also increases membrane fluidity and stabilises lipid membranes in stressful situations, which induces increased lipid peroxidation (15,16). Piracetam also favours the normalisation of immunity

by effecting lipid peroxidation (17). In addition, it reduces peripheral inflammation and decreases neuropathic pain and thermal hyperalgesia (18–20).

Nimodipine is an L-type calcium channel blocker. It has been used clinically and has been shown to have a dilatator effect in cerebral arterioles, thereby increasing the cerebral blood flow (21). It is a neuroprotective drug, which enhances the supply of oxygen and nutrients to injured regions (22).

In the present study, we aimed to investigate the possible beneficial effects of piracetam and nimodipine on full-thickness burn wound healing in rabbits.

Materials and methods

This experimental study was performed in accordance with the guidelines for the use of laboratory animal subjects in research set by the Ethical Committee of Ankara Training and Research Hospital, Ankara, Turkey (Number: 0019/313).

Piracetam (Nootropil 1 g/5 ml, UCB Pharma AŞ., Istanbul, Turkey) was administered intravenously (IV) in a dose of 2 g/day for 14 days and cutaneously in a dose of 2 g/day 1 ml/day to each wound after a burn injury (23). Nimodipine (Nimotop 0.2 mg, Bayer HealthCare AG, Leverkusen, Germany) was administered in a dose of 2·5 mg/kg/day for 14 days after the burn injury (24).

Anaesthesia was performed with intraperitoneal administration of 40 mg/kg of ketamine HCl (Ketalar®, Pfizer Inc., New York, USA) and 5 mg/kg of xylazine HCl (Rompun® 2%; Bayer HealthCare AG, Leverkusen, Germany).

A total 36 New Zealand male rabbits weighing 2500–3000 g were handled according to ethical guidelines. The animals were randomly divided into six groups:

- Group 1: Control, $n = 6$ (only skin biopsies were performed; neither burn injury nor drug administration was performed in this group).
- Group 2: Sham, $n = 6$ (only burn injury and skin biopsies were performed; drug administration was not performed in this group).
- Group 3: Nimodipine-IV, $n = 6$ (burn injury was performed, and nimodipine IV was administrated daily for 14 days).
- Group 4: Nimodipine-C, $n = 6$ (burn injury was performed, and nimodipine was applied topically over the burn wound daily for 14 days).
- Group 5: Piracetam-IV, $n = 6$ (burn injury was performed, and piracetam was administrated IV daily for 14 days).
- Group 6: Piracetam-C, $n = 6$ (burn injury was performed, and piracetam was applied topically over the burn wound daily for 14 days).

Full-thickness burn wound design

A copper cylinder with a diameter of 2 cm and a weight of 500 g was placed in hot water for at least 30 min before the beginning of the test. The rabbits were anaesthetised with ketamine HCl (40 mg/kg) and xylazine HCl (5 mg/kg). The dorsal region of the rabbits was shaved and cleaned with betadine solution. The cylinder was taken out of the hot water (90–93°C) and placed immediately on the skin for 10 seconds with no pressure. In a series of preliminary experiments, biopsies have shown that this method results in a uniform full-thickness burn wound (25) (Figure 1). Immediately after the burn injury, wound management was performed according to the experimental group designs described above.

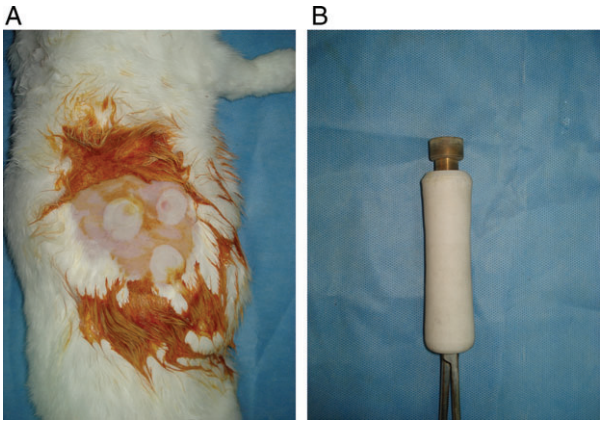

Figure 1 Full-thickness burn wound design; (A) Macroscopic view of the burn wounds, (B) copper cylinder with a diameter of 2 × 2 cm used in the burn wound design.

Table 1 Burn wound scoring used in the clinical assessment, modified from the index of Keast *et al.* (26)

Numerical score	Measure of the wound (cm)	Exudative volume	Exudate quality
0	0	None	Serous*
1	0·1–0·5	Small†	Serosanguineous‡
2	0·6–1	Moderate§	Sanguineous¶
3	1·1–1·5	Large||	Seropurulent**
4	1·6–2·0		Purulent††

*Thin, watery, clear to yellow, usually odourless.
†Exudate fully controlled, non-absorptive dressing may be used, wear time up to 7 days.
‡Thin, watery, pink to light red, usually odourless.
§Exudate controlled, absorptive dressings may be required, wear time 2–3 days.
¶Frank blood, bright red.
||Exudate uncontrolled, absorptive dressings required, dressing may be overwhelmed in less than 1 day.
**Thin, watery, white to cream, possibly foul odour.
††Thick, translucent to opaque, white to cream, possibly foul odour.

Gross examination

A physician who was blinded to the study assessed the burn wounds on day 21 using a modified version of a previous burn wound scoring table (26) (Table 1). This assessment method is quick, sensitive and simple to apply and can be used to assess wound diameters, exudate volumes and the exudate quality of the burn wound.

Histopathological analysis

Histopathological examination was performed as described earlier (27). Briefly, the burn wound tissues were collected

Table 2 Histopathological scoring (HPF = high-power field)

	Scores					
Variables	1–2·4	2·5–3	3–3·4	3·5–4	4–4·4	4·5–5
Epithelialisation quality	None	Preliminary	Partial	Completed but not mature	Completed partial mature	Completed mature
Neocollagenisation quality	None	Slight	Partial	Completed irregular	Completed partial regular	Completed regular
Inflammation	None	Too little	Slight	Moderate	Severe	Very severe
Vascularity	None	5 HPF	6–8 HPF	9–12 HPF	13–16 HPF	≥16 HPF
Necrosis	None	Too little	Focal	Multifocal	Severe intensive and extensive	Very severe intensive and extensive

for histopathology and fixed in 4% paraformaldehyde in phosphate-buffered saline at a pH of 7·4 for 48 hours. They were then washed under tap water and left overnight. The tissues were routinely prepared by dehydration through a graded alcohol and xylene series, and the tissue samples were then embedded in paraffin blocks. Paraffin serial sections were cut at a thickness of 4–5 μm, placed onto poly-L-lysine-coated glass slides and mounted on glass slides. Haematoyxlin-eosin (H&E) staining was performed, and the sections were histopathologically analysed using a binocular light microscope (Olympus BX51; Olympus, Tokyo, Japan) according to the histopathological scoring table (Table 2). The quality of epithelialisation and collagenisation was assessed, and inflammation, vascularisation and necrosis scores were obtained. Additionally, the epidermal and dermal thicknesses, number of vessels and ulcer diameters were measured with a Leica CCD camera DFC420 (Leica Microsystems Imaging Solutions, Ltd., Cambridge, UK), connected to a Lecia DM4000 B microscope (Leica Microsystems Imaging Solutions, Ltd.).

Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Sciences (PC 17.0, SPSS Inc, Chicago) package program. As the diameters of the ulcers were normally distributed, and the variations were homogenous between all the groups, the data were statistically analysed using a one-way analysis of variance (ANOVA). $P < 0{\cdot}05$ was defined as a significant difference.

The other variables were not normally distributed, and the between-group variation was not homogenous. Therefore, Kruskal–Wallis multiple variant analysis was performed, and a Mann–Whitney *U* test with Bonferroni correction was used to determine the statistically significant differences (post hoc evaluation) between the groups. Significance was defined at $P < 0{\cdot}0083$.

Results

Macroscopic results

Wound sizes

The results of Mann–Whitney *U* test showed a statistically significant difference between the wound sizes in the piracetam-C group and those of the other groups ($P < 0{\cdot}0083$) (Table 3 and 4). The wound sizes were smaller in the piracetam-C group than in the other groups according to the gross views of the wounds in photographs taken on day 21 (Figure 2). The wound sizes in the other groups were very similar (Table 3).

Exudate volumes

The exudate volumes were statistically significantly higher in the nimodipine-IV and sham groups than in the other groups ($P < 0{\cdot}0083$) (Tables 3 and 4). The lowest exudate volume was observed in the piracetam-C group. However, there were no statistical differences between the piracetam-C group versus the nimodipine-C group or between the piracetam-C group vs. the piracetam-IV group ($P > 0{\cdot}0083$) (Tables 3 and 4).

Quality of the exudate

The exudate quality of the sham and nimodipine-IV groups was statistically significantly lower than that of the other groups ($P < 0{\cdot}0083$) (Tables 3 and 4). There were no statistically significant differences between the nimodipine-C, piracetam-IV and piracetam-C groups ($P > 0{\cdot}0083$) (Tables 3 and 4).

Histopathological results

The histopathological data are shown in Figure 3.

Epidermal thickness

The thickest epidermis was observed in the sham and nimodipine-C groups. The burns in these groups seemed to have healed, with hypertrophic scarring (Figure 3). The epidermis was thinnest in the control group and piracetam-C group, and there was no statistically significant difference between these two groups ($P > 0{\cdot}0083$) (Tables 3 and 5).

Dermal thickness

The dermal thickness was lowest in the control group. The thickness of the dermis of the piracetam-C group was almost similar to that of the control group, and there was a statistically significant difference in the dermal thickness of the piracetam-C group compared with that of the other groups ($P < 0{\cdot}0083$) (Tables 3 and 5). There were no statistically significant differences between the sham and nimodipine-IV, sham and nimodipine-C groups or between the two nimodipine groups ($P > 0{\cdot}0083$) (Tables 3 and 5).

Table 3 Descriptive results (*N*: number of animals)

Group name	Variable	*N*	Minimum	Maximum	Mean	SD
Control	Epidermal thickness	6	25·33	41·14	31·80	6·50
	Dermal thickness		238·31	252·65	245·80	6·09
	Vessel count		4·00	7·00	5·83	1·16
	Epithelialisation quality		5·00	5·00	5·00	0·00
	Collagenisation quality		1·50	2·30	1·86	0·34
	Inflammation score		1·00	1·00	1·00	0·00
	Vascularity		1·00	1·30	1·15	0·10
	Necrosis		1·00	1·00	1·00	0·00
Sham	Epidermal thickness	6	190·32	345·71	259·13	65·84
	Dermal thickness		2389·81	2609·42	2515·94	97·61
	Vessel count		14·00	25·00	18·33	4·54
	Epithelialisation quality		1·00	1·00	1·00	0·00
	Collagenisation quality		3·30	3·80	3·53	0·19
	Inflammation score		4·20	4·60	4·36	0·16
	Vascularity		4·80	5·00	4·93	0·08
	Necrosis		4·90	5·00	4·96	0·05
	Wound size		2·00	4·00	3·50	0·83
	Exudate volume		1·00	2·00	1·66	0·51
	Exudate quality		0·00	1·00	0·66	0·51
	Ulcer diameter		360·83	382·75	374·38	9·28
Nimodipine-IV	Epidermal thickness	6	190·20	262·51	223·92	31·42
	Dermal thickness		2225·65	2785·87	2445·21	223·45
	Vessel count		13·00	19·00	15·50	2·34
	Epithelialisation quality		3·40	4·10	3·70	0·26
	Collagenisation quality		3·30	4·40	3·85	0·44
	Inflammation score		3·10	3·90	3·40	0·32
	Vascularity		4·20	4·90	4·55	0·25
	Necrosis		2·60	3·50	3·10	0·38
	Wound size		2·00	4·00	2·83	0·75
	Exudate volume		1·00	3·00	1·83	0·75
	Exudate quality		0·00	3·00	1·16	0·98
	Ulcer diameter		330·34	463·73	369·63	48·02
Nimodipine-C	Epidermal thickness	6	234·24	298·60	278·06	22·41
	Dermal thickness		2332·07	2882·74	2628·61	190·16
	Vessel count		21·00	30·00	25·50	3·88
	Epithelialisation quality		1·00	1·00	1·00	0·00
	Collagenisation quality		2·60	3·50	3·05	0·37
	Inflammation score		5·00	5·00	5·00	0·00
	Vascularity		4·10	4·90	4·56	0·33
	Necrosis		5·00	5·00	5·00	0·00
	Wound size		2·00	4·00	3·33	1·03
	Exudate volume		0·00	0·00	0·00	0·00
	Exudate quality		0·00	0·00	0·00	0·00
	Ulcer diameter		445·37	584·12	518·69	47·49
Piracetam-IV	Epidermal thickness	6	69·74	99·05	88·7	9·85
	Dermal thickness		1405·68	1686·76	1520·30	123·56
	Vessel count		11·00	15·00	12·33	1·75
	Epithelialisation quality		4·20	4·80	4·35	0·23
	Collagenisation quality		4·20	4·60	4·40	0·16
	Inflammation score		1·90	2·60	2·25	0·28
	Vascularity		3·10	3·60	3·36	0·17
	Necrosis		1·80	2·50	2·15	0·24
	Wound size		2·00	4·00	3·66	0·81
	Exudate volume		0·00	1·00	0·33	0·51
	Exudate quality		0·00	1·00	0·33	0·51
	Ulcer diameter		270·48	323·96	303·67	25·80
Piracetam-C	Epidermal thickness	6	36·36	52·87	47·80	5·99
	Dermal thickness		1030·56	1415·87	1204·21	137·80
	Vessel count		8·00	13·00	10·33	1·96
	Epithelialisation quality		4·20	4·80	4·50	0·20
	Collagenisation quality		4·20	4·60	4·40	0·16
	Inflammation score		1·10	1·90	1·48	0·28
	Vascularity		2·50	3·40	3·01	0·39
	Necrosis		1·10	1·80	1·53	0·26
	Wound size		1·00	1·00	1·00	0·00
	Exudate volume		0·00	0·00	0·00	0·00
	Exudate quality		0·00	0·00	0·00	0·00
	Ulcer diameter		143·80	192·76	171·39	16·17

Table 4 Comparison of macroscopic findings of the groups (bold indicates $P < 0{\cdot}0083$, U = results of Mann–Whitney U-test)

Variables	Groups		U	P
Wound size score	Sham	Nimodipine-IV	9·500	0·147
		Nimodipine-C	17·000	0·937
		Piracetam-IV	15·500	0·598
		Piracetam-C	0·000	**0·002**
	Nimodipine-IV	Nimodipine-C	12·000	0·306
		Piracetam-IV	7·500	0·068
		Piracetam-C	0·000	**0·002**
	Nimodipine-C	Piracetam-IV	15·000	0·523
		Piracetam-C	0·000	**0·002**
	Piracetam-IV	Piracetam-C	0·000	**0·001**
Exudate volume score	Sham	Nimodipine –IV	16·000	0·715
		Nimodipine –C	0·000	**0·002**
		Piracetam –IV	2·000	**0·007**
		Piracetam –C	0·000	**0·002**
	Nimodipine-IV	Nimodipine –C	0·000	**0·002**
		Piracetam –IV	2·000	0·068
		Piracetam –C	0·000	**0·002**
	Nimodipine-C	Piracetam –IV	12·000	0·138
		Piracetam –C	18·000	1·000
	Piracetam-IV	Piracetam-C	12·000	0·138
Exudate quality score	Sham	Nimodipine –IV	13·000	0·336
		Nimodipine –C	6·000	0·019
		Piracetam –IV	12·000	0·269
		Piracetam –C	6000	0·019
	Nimodipine-IV	Nimodipine –C	3·000	**0·006**
		Piracetam –IV	8·000	0·075
		Piracetam –C	3·000	**0·006**
	Nimodipine-C	Piracetam –IV	12·000	0·138
		Piracetam –C	18·000	1·000
	Piracetam-IV	Piracetam –C	12·000	0·138

Number of vessels

There was a statistically significant difference in the number of vessels between the control group compared with that of the other groups ($P < 0{\cdot}0083$) (Tables 3 and 5). The vascularity count was lowest in the control group, and the vascularity count of the piracetam-C group was closest to that of the control group. There were no statistically significant differences between the sham and nimodipine-IV, sham and nimodipine-C groups or between the two piracetam groups ($P > 0{\cdot}0083$) (Tables 3 and 5).

Epithelialisation quality score

The epithelialisation quality score of the piracetam-C group was closest to that of the control group, although there was a statistically significant difference in the score of the two groups ($P < 0{\cdot}0083$) (Tables 3 and 5). There was no statistically significant difference in the epithelialisation quality score between the sham and nimodipine-C groups or between the two piracetam groups ($P > 0{\cdot}0083$) (Tables 3 and 5).

Collagenisation quality score

There were no statistically significant differences in the collagenisation quality score between the two piracetam groups or between the sham and nimodipine-IV groups ($P > 0{\cdot}0083$) (Tables 3 and 5). Although the collagenisation quality score of the nimodipine-C group was closest to that of the sham group histologically, there was a statistically significant between-group difference ($P < 0{\cdot}0083$) (Tables 3 and 5). New collagenisation areas were almost regular in both piracetam groups.

Inflammation score

Although the inflammation score was statistically significantly different between all the groups, the inflammation score of the piracetam-C group was closest to that of the control group ($P < 0{\cdot}0083$) (Tables 3 and 5). The inflammation score was significantly higher in both the nimodipine and sham groups (Tables 3 and 5).

Vascularity score

Although the vascularity scores of the two piracetam groups were closest to those of the control group, there were statistically significant differences between the scores ($P < 0{\cdot}0083$) (Tables 3 and 5).

The highest vascularity scores were detected in the sham and the two nimodipine groups. There was no statistically

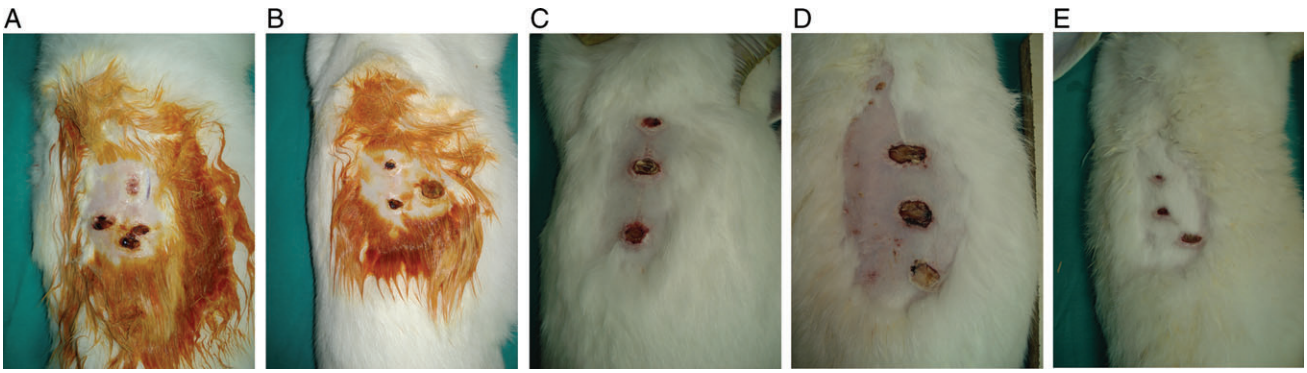

Figure 2 Macroscopic view of the full-thickness burn wounds of the groups on day 21; (A) sham, (B) nimodipine-IV, (C) nimodipine-C, (D) piracetam-IV, and (E) piracetam-C groups. The wound diameter was smallest in the piracetam-C group, and no exudate was detected in this group (E).

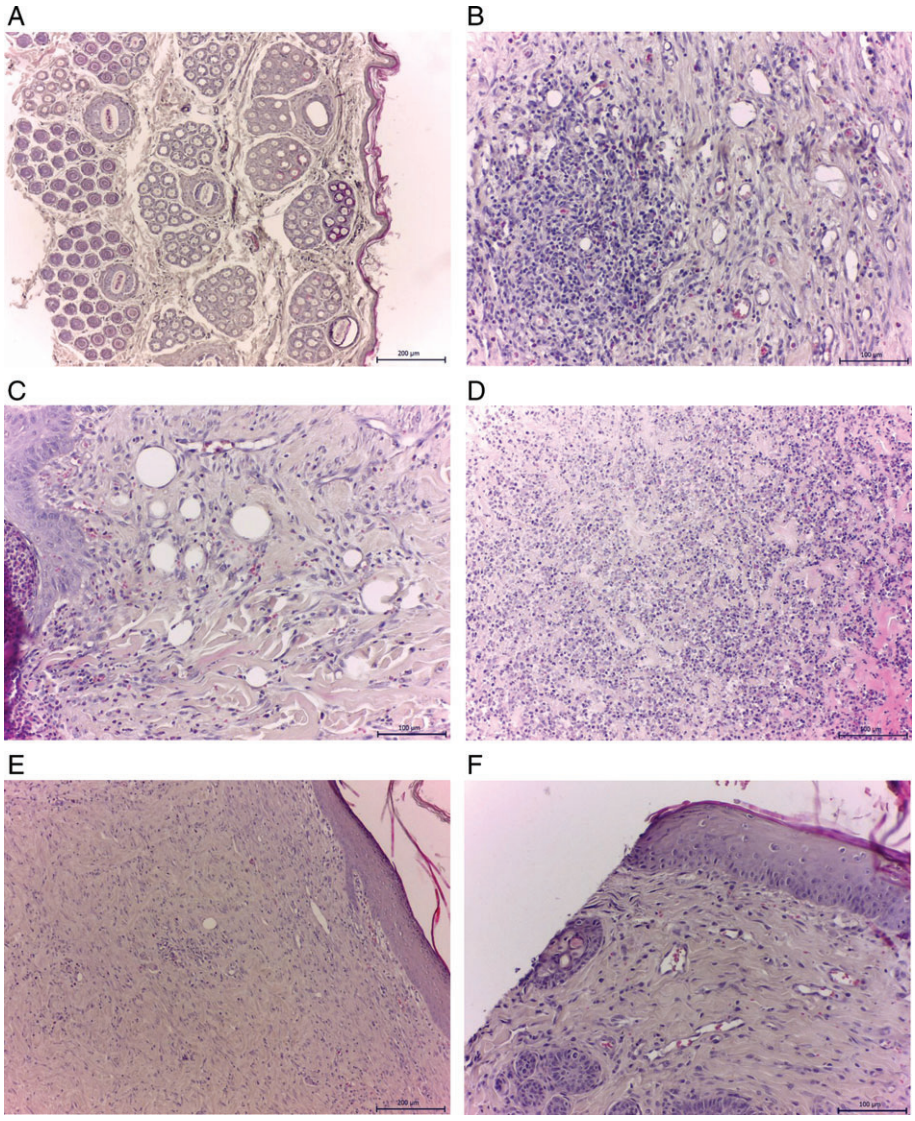

Figure 3 (A) Histopathological appearance of normal skin in the control group, H&E, bar = 200 μm. (B) Histopathological appearance of the dermis of a rabbit in the sham group. Severe inflammatory cell infiltration and irregular connective tissue were seen. H&E, bar = 100 μm. (C) Histopathological appearance of the epidermis and dermis of a rabbit in the nimodipine-IV group. Partial reepithelialisation and moderate inflammatory cell infiltration were seen. H&E, bar = 100 μm. (D) Histopathological appearance of the dermis of a rabbit in the nimodipine-C group. Severe inflammatory cell infiltration and many degenerating inflammatory cells, in addition to irregular connective tissue and necrosis, were observed. H&E, bar = 100 μm. (E) Histopathological appearance of the epidermis and dermis of a rabbit in the piracetam-IV group, showing partial reepithelialisation and a near-complete underlying dermis. Regular connective tissue was also observed in this group. H&E, bar = 200 μm. (F) Histopathological appearance of the epidermis of a rabbit in the piracetam-C group, showing full reepithelialisation and a complete dermis. Regular connective tissue was also observed in this group. H&E, bar = 100 μm.

significant difference in the scores of the two nimodipine groups ($P > 0{\cdot}0083$) (Tables 3 and 5). The vascularity scores were highest in the sham group, and the difference was statistically significant compared with that of the other groups ($P < 0{\cdot}0083$) (Tables 3 and 5).

Necrosis score

The necrosis scores were significantly different between the groups ($P < 0{\cdot}0083$) (Tables 3 and 5), except between the sham and nimodipine-IV groups ($P > 0{\cdot}0083$) (Tables 3 and 5). Necrosis was intense in the nimodipine and sham groups (Table 3). Necrosis was lowest in the piracetam-C group and closest to that of the control group.

Ulcer diameter

There were statistically significant between-group differences in the ulcer diameter in all the groups ($P < 0{\cdot}05$) (Tables 3 and 6), except between the sham and nimodipine-IV groups according to the ANOVA test results ($P > 0{\cdot}05$) (Tables 3 and 6). The ulcer diameters were smallest in the piracetam-C group.

Discussion

Wound healing requires increased metabolic activity of various types of cells (28). In full-thickness burn wounds, injured blood vessels result in hypoxia. Although hypoxia causes neovascularisation in the beginning of the wound healing process (29), long-term hypoxia impedes wound healing. Moreover, hypoxic stress causes the deposition of free oxygen radicals, which initiate peroxidative decomposition of the phospholipids of cellular membranes and damage the inner mitochondrial membrane (30). Mitochondrial de-energisation initiates a sequence of events that leads to the loss of cell viability, with subsequent adverse reactions reoccurring in a vicious circle (30).

In the present study, we administered nimodipine and piracetam both systemically and topically to investigate their systemic and local effects.

Table 5 Comparisons of histological parameters of the groups (bold indicates $P < 0{\cdot}0083$, U = results of Mann–Whitney U-test)

Variable	Compare groups (*I*/*J*)	*U*	*P*
Epidermal thickness	Control/Sham	0·000	**0·004**
	Control/Nimodipine-IV	0·000	**0·004**
	Control/Nimodipine-C	0·000	**0·004**
	Control/Piracetam-IV	0·000	**0·004**
	Control/Piracetam-C	2·000	0·010
	Sham/Nimodipine-IV	13·000	0·423
	Sham/Nimodipine-C	17·000	0·873
	Sham/Piracetam-IV	0·000	**0·004**
	Sham/Piracetam-C	0·000	**0·004**
	Nimodipine-IV/Nimodipine-C	3·000	0·016
	Nimodipine-IV/Piracetam-IV	0·000	**0·004**
	Nimodipine-IV/Piracetam-C	0·000	**0·004**
	Nimodipine-C/Piracetam-IV	0·000	**0·004**
	Nimodipine-C/Piracetam-C	0·000	**0·004**
	Piracetam-IV/Piracetam-C	0·000	**0·004**
Dermal thickness	Control/Sham	0·000	**0·004**
	Control/Nimodipine-IV	0·000	**0·004**
	Control/Nimodipine-C	0·000	**0·004**
	Control/Piracetam-IV	0·000	**0·004**
	Control/Piracetam-C	0·000	**0·004**
	Sham/Nimodipine-IV	12·000	0·337
	Sham/Nimodipine-C	10·000	0·200
	Sham/Piracetam-IV	0·000	**0·004**
	Sham/Piracetam-C	0·000	**0·004**
	Nimodipine-IV/Nimodipine-C	9·000	0·150
	Nimodipine-IV/Piracetam-IV	0·000	**0·004**
	Nimodipine-IV/Piracetam-C	0·000	**0·004**
	Nimodipine-C/Piracetam-IV	0·000	**0·004**
	Nimodipine-C/Piracetam-C	0·000	**0·004**
	Piracetam-IV/Piracetam-C	2·000	**0·010**
Vessel count	Control/Sham	0·000	**0·004**
	Control/Nimodipine-IV	0·000	**0·004**
	Control/Nimodipine-C	0·000	**0·004**
	Control/Piracetam-IV	0·000	**0·004**
	Control/Piracetam-C	0·000	**0·004**
	Sham/Nimodipine-IV	11·500	0·295
	Sham/Nimodipine-C	4·500	0·300
	Sham/Piracetam-IV	2·000	**0·010**
	Sham/Piracetam-C	0·000	**0·004**
	Nimodipine-IV/Nimodipine-C	0·000	**0·004**
	Nimodipine-IV/Piracetam-IV	4·500	0·029
	Nimodipine-IV/Piracetam-C	1·000	**0·006**
	Nimodipine-C/Piracetam-IV	0·000	**0·004**
	Nimodipine-C/Piracetam-C	0·000	**0·004**
	Piracetam-IV/Piracetam-C	9·000	0·141
Epithelialisation quality score	Control/Sham	0·000	**0·001**
	Control/Nimodipine-IV	0·000	**0·002**
	Control/Nimodipine-C	0·000	**0·001**
	Control/Piracetam-IV	0·000	**0·002**
	Control/Piracetam-C	0·000	**0·002**
	Sham/Nimodipine-IV	0·000	**0·002**
	Sham/Nimodipine-C	18·000	1·000
	Sham/Piracetam-IV	0·000	**0·002**
	Sham/Piracetam-C	0·000	**0·004**
	Nimodipine-IV/Nimodipine-C	0·000	**0·002**
	Nimodipine-IV/Piracetam-IV	0·000	**0·004**
	Nimodipine-IV/Piracetam-C	0·000	**0·004**
	Nimodipine-C/Piracetam-IV	0·000	**0·002**
	Nimodipine-C/Piracetam-C	0·000	**0·002**
	Piracetam-IV/Piracetam-C	10·000	0·187
Collagenisation quality score	Control/Sham	0·000	**0·004**
	Control/Nimodipine-IV	0·000	**0·004**
	Control/Nimodipine-C	0·000	**0·004**
	Control/Piracetam-IV	0·000	**0·004**
	Control/Piracetam-C	0·000	**0·004**
	Sham/Nimodipine-IV	11·000	0·260
	Sham/Nimodipine-C	5·000	0·036
	Sham/Piracetam-IV	0·000	**0·004**
	Sham/Piracetam-C	0·000	**0·004**
	Nimodipine-IV/Nimodipine-C	2·500	0·013
	Nimodipine-IV/Piracetam-IV	4·000	0·024
	Nimodipine-IV/Piracetam-C	3·500	0·019
	Nimodipine-C/Piracetam-IV	0·000	**0·004**
	Nimodipine-C/Piracetam-C	0·000	**0·004**
	Piracetam-IV/Piracetam-C	17·500	0·935
Inflammation score	Control/Sham	0·000	**0·002**
	Control/Nimodipine-IV	0·000	**0·002**
	Control/Nimodipine-C	0·000	**0·001**
	Control/Piracetam-IV	0·000	**0·002**
	Control/Piracetam-C	0·000	**0·002**
	Sham/Nimodipine-IV	0·000	**0·004**
	Sham/Nimodipine-C	0·000	**0·002**
	Sham/Piracetam-IV	0·000	**0·004**
	Sham/Piracetam-C	0·000	**0·004**
	Nimodipine-IV/Nimodipine-C	0·000	**0·002**
	Nimodipine-IV/Piracetam-IV	0·000	**0·004**
	Nimodipine-IV/Piracetam-C	0·000	**0·004**
	Nimodipine-C/Piracetam-IV	0·000	**0·002**
	Nimodipine-C/Piracetam-C	0·000	**0·002**
	Piracetam-IV/Piracetam-C	0·500	**0·005**
Vascularity	Control/Sham	0·000	**0·004**
	Control/Nimodipine-IV	0·000	**0·004**
	Control/Nimodipine-C	0·000	**0·004**
	Control/Piracetam-IV	0·000	**0·004**
	Control/Piracetam-C	0·000	**0·004**
	Sham/Nimodipine-IV	2·000	**0·009**
	Sham/Nimodipine-C	2·500	0·012
	Sham/Piracetam-IV	0·000	**0·004**
	Sham/Piracetam-C	0·000	**0·004**
	Nimodipine-IV/Nimodipine-C	16·000	0·746
	Nimodipine-IV/Piracetam-IV	0·000	**0·004**
	Nimodipine-IV/Piracetam-C	0·000	**0·004**
	Nimodipine-C/Piracetam-IV	0·000	**0·004**
	Nimodipine-C/Piracetam-C	0·000	**0·004**
	Piracetam-IV/Piracetam-C	8·000	0·106
Necrosis	Control/Sham	0·000	**0·002**
	Control/Nimodipine-IV	0·000	**0·002**
	Control/Nimodipine-C	0·000	**0·001**
	Control/Piracetam-IV	0·000	**0·002**
	Control/Piracetam-C	0·000	**0·002**
	Sham/Nimodipine-IV	0·000	**0·002**
	Sham/Nimodipine-C	12·000	0·394
	Sham/Piracetam-IV	0·000	**0·003**
	Sham/Piracetam-C	0·000	**0·003**
	Nimodipine-IV/Nimodipine-C	0·000	**0·002**
	Nimodipine-IV/Piracetam-IV	0·000	**0·004**
	Nimodipine-IV/Piracetam-C	0·000	**0·004**
	Nimodipine-C/Piracetam-IV	0·000	**0·002**
	Nimodipine-C/Piracetam-C	0·000	**0·002**
	Piracetam-IV/Piracetam-C	1·000	**0·006**

Table 6 Results of one-way ANOVA of the ulcer diameters of the groups (bold and * indicates $P < 0{\cdot}05$)

Group (*I*)	Group (*J*)	Mean differences (*I*–*J*)	SD	*P*
Sham	Nimodipine-IV	4·75	19·28	1·000
	Nimodipine-C	−144·30*		**0·000**
	Piracetam-IV	70·71*		**0·025**
	Piracetam-C	202·99*		**0·000**
Nimodipine-IV	Sham	−4·75	19·28	1·000
	Nimodipine-C	−149·05*		**0·000**
	Piracetam-IV	65·95*		**0·041**
	Piracetam-C	198·24*		**0·000**
Nimodipine-C	Sham	144·30*	19·28	**0·000**
	Nimodipine-IV	149·05*		**0·000**
	Piracetam-IV	215·01*		**0·000**
	Piracetam-C	347·30*		**0·000**
Piracetam-IV	Sham	−70·71*	19·28	**0·025**
	Nimodipine-IV	−65·95*		**0·041**
	Nimodipine-C	−215·01*		**0·000**
	Piracetam-C	132·28*		**0·000**
Piracetam-C	Sham	−202·99*	19·28	**0·000**
	Nimodipine-IV	−198·24*		**0·000**
	Nimodipine-C	−347·30*		**0·000**
	Piracetam-IV	−132·28*		**0·000**

Macroscopic findings

In the macroscopic evaluation, the smallest wounds were in the piracetam-C group at the end of the study. This could be due to the protein synthesis–enhancing effect of the drug. The exudate volume was lowest in the two piracetam groups and in the nimodipine-C group, and the exudate quality was similar in these three groups. The wound size and exudates of the piracetam-IV and nimodipine-IV groups were closest to those of the sham group. This could be due to the reduced accumulation of the drugs around the burn wounds when they were administered systemically.

Histopathological findings

Other criteria of qualified burn wound healing are the epithelialisation quality and epidermal and dermal thicknesses. The epidermal thickness and epithelialisation quality of the control and piracetam groups were similar. However, the thickness was higher in the nimodipine-C group, which was closest to that of the sham group. The epithelialisation quality was lowest in the sham and the two nimodipine groups. According to these findings, it appears that nimodipine was unable to decrease inflammation and that it caused hypertrophic scarring. The dermal thickness findings and collagenisation quality support this idea, with the dermal thicknesses highest in the sham and the two nimodipine groups. The dermal thickness in the piracetam groups was almost the same as that of the control group. Therefore, piracetam appears to heal burn wounds without hypertrophic scarring when administered both systemically and locally. Although neovascularisation has a positive effect on wound healing, increased neovascularisation results in hypertrophic wound healing. In the present study, the vascularity levels of the control and piracetam group were very similar. The vascularity levels were two times higher in the sham and nimodipine groups compared with the control and piracetam groups. Although we observed increased vascularisation in the nimodipine and sham groups, this did not appear to prevent necrosis of the wound tissue. The ulcer diameters were greatest in the sham and nimodipine groups and smallest in the piracetam-C group.

Several drugs have been reported to be beneficial in burn wound healing (31–36). However, full-thickness burn wound healing is still a challenge in plastic surgery. Expected changes leading to wound healing are neovascularisation, decreased inflammation in the burn area and enhanced rapid epithelialisation. In this context, piracetam seemed to be effective in full-thickness burn wound healing, but nimodipine was not.

Piracetam was used with hyperbaric oxygen in only one study in the literature (37), and it was reported that systemic piracetam decreased inflammation in the early stages of burn wound healing. However, the study did not include topical administration of the drug nor examined the acute stages of burn wound healing.

The pharmacokinetic profile of piracetam has been well documented in the literature. It is absorbed and disseminated well systemically, with a bioavailability of almost 100%, and excreted in the urine and eliminated after 30 hours (38). Piracetam exerts a membrane-stabilising effect by decreasing scopolamine-induced stress following lethal cell injury, which increases lipid peroxidation (16). The increase in lipid peroxidation is accompanied by T-cell inhibition, with the latter inducing the normalisation of immunity by decreasing lipid peroxidation (39). Piracetam also has analgesic effects (18), reducing thermal hyperalgesia induced by tumour necrosis factor-α (TNF-α) (20). This property of the drug could be helpful in decreasing painful stimuli in skin burns.

Few studies have reported that piracetam has some transient side effects (40), including anxiety, insomnia, irritability, headaches, agitation, nervousness, tremor and hyperkinesia, when administrated systemically (41). Although the systemic effects of piracetam have been well documented, the topical effects of this drug are unclear. Therefore, further studies on topically administered piracetam should be performed in the future.

Most studies of topical agents have compared their effects on burn wound healing with those of silver sulfadiazine cream (4,5,34). We did not compare topical piracetam with silver sulfadiazine cream because our aim was to investigate only the beneficial effects of nimodipine and piracetam on full-thickness burn wound healing. Further comparative studies could be carried out between topical piracetam and topical silver sulfadiazine.

To conclude, neither topically nor systemically administered nimodipine significantly facilitated burn wound healing, whereas piracetam was effective in full-thickness burn wound healing when administered either topically or systemically. Although the present study suggests that topical piracetam is a safe, practical and effective treatment for full-thickness burn wounds, further clinical studies are needed to confirm these findings.

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Conflict of interests

None.

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