Heat delays skin wound healing in mice

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Abstract

In vivo studies have shown that the combination of infrared radiation (IR) and visible light (VIS) is responsible for the activation of metalloproteinases, causing matrix degradation and damage to healthy skin. However, the role of heat originating from the VIS spectrum on wound healing remains poorly understood. Our objective was to investigate the macroscopic, microscopic and biochemical effects of heat induced by visible light on cutaneous wound healing in mice. Male mice were anesthetized, subjected to a cutaneous excisional wound and divided into two groups ($n = 10/\text{group}$) exposed to 23°C or 43°C in a thermal chamber for 30 min every other day, for 13 days. On day 14, the animals were sacrificed, and their lesions were processed for histochemistry, immunohistochemistry and protein expression analysis. The wound area was 42% greater 11 days ($p < 0.01$) and 29% greater 14 days ($p < 0.001$) after wounding in the 43°C group than in the 23°C group. The 43°C group presented a lower (17%) percentage of reepithelialized wounds ($p < 0.001$) 14 days after wounding. The length of the epidermal gap was greater in the 43°C group ($p < 0.01$). The volume density of myofibroblasts and the number of F4/80-positive macrophages was greater in the 43°C group ($p < 0.05$). The 43°C group showed increased protein expression of type III collagen ($p < 0.001$), decreased protein expression of type I collagen ($p < 0.05$), increased MMP-1 expression ($p < 0.05$), and decreased MMP-2 activity ($p < 0.001$). The protein expression of fibrillin-1 ($p < 0.001$), MMP-12 ($p < 0.05$), TGF- β 1/2/3 ($p < 0.01$) and ERK activation ($p < 0.05$) was increased in the 43°C group. Our results suggest that heat delays the stages of wound healing in mice.

Keywords: Heat, skin, wound healing, mice, extracellular matrix

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Introduction

The skin acts primarily as a protective barrier against the environment. The loss of the integrity of large skin portions as a result of a lesion can lead to severe disability or even death.¹ Cutaneous wound healing is a dynamic process that includes inflammation, granulation, tissue formation, and tissue remodelling.^{2,3} After injury, fibroblasts proliferate and migrate to deposit a rich matrix in the lesion area. A proportion of these fibroblasts differentiate into myofibroblasts, which are responsible for wound contraction.⁴ Thus, the process of wound healing can be affected when the activities of dermal fibroblasts and myofibroblasts are compromised by systemic and environmental factors. Although studies indicate that local and systemic factors may impair wound healing,⁵ the effects of heat on cutaneous wound healing are not well understood.

Solar radiation (SR) is essential for life on Earth; however, it is a major environmental risk factor for living organisms. The involvement of the ozone layer exposes South Australia, New Zealand and South America to high concentrations of

SR.⁶ The sunlight spectrum on the Earth's surface is 290–3000 nm and is 6.8% ultraviolet radiation (UV) (UVB: 290–320 nm and UVA: 320–400 nm), 38.9% visible light (VIS: 400–760 nm) and 54.3% near-infrared radiation (IRA: 760–1440 nm, IRB: 1440–3000 nm and IRC: 3000 nm to 1 mm).⁷ UV radiation-induced effects on the skin include solar elastosis, keratosis, skin cancer, and photo ageing.⁸

Although most studies about the adverse effects on the skin involve SR and UV radiation,^{9,10} the skin can be exposed to a dose of 75 J/cm²/h of IR in the summer in Munich, Germany.¹¹ Skin temperature varies between 27.6°C and 33.1°C; while IRA penetrates into the subcutaneous tissue without raising the temperature of the skin, IRB and IRC are absorbed into the epidermal layers and increase the temperature up to 45°C.^{12–14} Studies have shown that high doses of IR can damage human skin,^{15–18} whereas lower doses are widely used for the treatment of inflammatory processes.

Recent studies indicate that exposure of human skin to IR stimulates the expression of matrix metalloproteinase

(MMP-1), decreases type I procollagen, increases neoangiogenesis, and induces the infiltration of inflammatory cells and DNA oxidative stress.^{12,19} A study comparing the effect of heat with and without IRA on the healing of skin wounds in rats showed that isolated heat delayed the closure of skin wounds.²⁰ Heat increases the expression of MMP-1, MMP-3, MMP-12 and modulates the synthesis of elastin and fibrillin.^{21,22}

In contrast to UV radiation and IR, which are known to facilitate the cutaneous photo ageing and formation of free radicals and reactive oxygen species (ROS), the possible similar effects of VIS on human skin are poorly characterized. Vandersee and colleagues showed that VIS can produce free radicals and ROS in human skin through radiation with blue-violet light.²³ *In vivo* studies have shown that the combination of IR and VIS is responsible for the activation of MMPs, causing matrix degradation and damage to healthy skin.²³ However, the role of heat from the VIS spectrum remains poorly understood in wound healing. However, some studies have shown that a slight increase in the room temperature may improve wound healing in diabetic patients (associated with electrical stimulation)²⁴ and *in vitro* studies suggest that an increase in temperature may accelerate the wound healing process, by accelerating fibroblast migration.²⁵ Therefore, due to this controversy in the literature and to the lack of clear evidence from animal models, the aim of this study was to investigate the macroscopic, microscopic, and biochemical effects of heat induced by visible light on the healing of excisional skin lesions in mice.

Materials and methods

Animals

Male Swiss mice aged 8–10 weeks (25–35 g) had free access to food and water and were maintained in a room at 23°C and with a 12-h light/dark cycle. All procedures were performed in accordance with the Brazilian legislation regarding animal experimentation (no. 11.794, from October 8, 2008). This study was approved by the Ethical Committee for Animal Use of the State University of Rio de Janeiro (CEA/022/2014).

Heat exposure chamber

Heat exposure was conducted in a wooden box that was 15 mm thick²⁶ with the indicated external (52 cm long, 24 cm wide and 21 cm high) and internal (42.5 cm long, 21.5 cm wide and 18.8 cm high) dimensions. The temperature was controlled by a rotary dimmer (Pratis - Pial Legrand, São Paulo, Brazil) coupled to two incandescent bulbs (100 W) (Philips Standard, 100 W, 127 v, Barueri, Brazil). The temperature of 43°C and the time of exposure were chosen based on the study of Shin et al.²⁷ In a pilot study (data not shown), we performed daily exposure to heat during the first three days only. In the pilot study as well as in the present study, heat exposure delayed closure of skin wounds.

The measurement of the intensity of visible light

The intensity of visible light was measured with a spectrometer (Ocean Optics Red Tide 650, average of 50 scans, USA). This lamp has a colour temperature of 2700 K. The luminous flux of 1350 lm and average lux were assessed every 5 min for 30 min and were 35.5 ± 0.4 lux (Digital light meter – Minipa MLM-1332). To measure the temperature and humidity inside and outside of the box, a digital thermo-hygrometer was employed (INCOTERM, Porto Alegre, Brazil).

Temperature measurement

The skin surface temperature was measured using a non-contact thermometer²⁸ (OEM, Mainland, China) before the heating process and at 5-min intervals thereafter.

Experimental design

Mice ($n = 20$) were intraperitoneally anesthetized with ketamine (150 mg/kg b. wt.) and xylazine (15 mg/kg b. wt.). The hair on the back of the mice was shaved. Using a template, a square (1 cm²) was drawn on the animal's back and a full-thickness excisional wound was created, using scissors, with the excision of the epidermis, dermis and hypodermis, exposing the panniculus carnosus.²⁹ The wound was not sutured or covered and healed by second intention (does not have its margins approximated by sutures). After the injury, the animals were divided as follows: the 23°C group ($n = 10$) – the mice were placed in the exposure chamber with the lamps turned off for 30 min; and the 43°C group ($n = 10$) – the mice were exposed to 43°C in the heat exposure chamber for 30 min. For exposure to heat, the two lamps were lit to full power until an internal temperature of 43°C was reached, and then the power was reduced to stabilize the temperature. When the temperature was stable, the animals were placed in the heat exposure chamber for 30 min. Exposure to heat started on the first day after the injury and lasted for 13 days, with exposure occurring every other day.²⁷

Macroscopic analyses

To evaluate wound contraction and reepithelialization (macroscopically), immediately after wounding and 3, 7, 11 and 14 days later, a transparent plastic sheet was placed over the wound, and the lesion's margins were traced.³⁰ The wound area was measured using Image J software (National Institutes of Health, Bethesda, MD). The results were expressed as a percentage of the original wound area (mean $\pm$ standard mean error). The percentage of reepithelialized wounds was estimated by the number of closed wounds per group 14 days after wounding.

Tissue harvesting and microscopic analyses

Fourteen days after wounding, the mice were killed by CO₂ exposure. Five lesions and adjacent normal skin per group were collected, fixed in formalin (pH 7.2) and embedded in paraffin. The paraffin-embedded sections were stained with haematoxylin-eosin to analyze the wound area and the

length of the epidermal gap. Lesions from five animals per group were collected and frozen at -70°C for each group. The frozen lesions were macerated in lysis buffer, and the total protein concentration was determined using a bicinchoninic acid protein assay (Thermo Fisher Scientific, Rockwood, TN, USA). The lysate was used to perform immunoblotting.

Sections (5 μm thick) were stained with haematoxylin-eosin to measure the epidermal gap, which is defined as the distance in micrometres (μm) between the edges of the lesion. For this, the slides were digitized using a Panoramic Digital Slide Scanner (3DHistech Ltd., Budapest, Hungary). The length of the epidermal gap was measured using Panoramic Viewer software (3DHistech Ltd., Budapest, Hungary).

To analyze cell density, scanned images were used. A stereological tool (point counting) was employed as previously described by our group.³¹

Immunohistochemistry and quantification

Immunohistochemistry was used to investigate the numbers of neutrophils (myeloperoxidase) and macrophages (F4/80). The following antibodies were used: rat monoclonal to myeloperoxidase (#71674; Santa Cruz Biotechnology, Santa Cruz, CA, USA; 1:500) and rat monoclonal to F4/80 (#497; Serotec Inc., Raleigh, NC, USA; 1:500), as previously described.^{32,33} To quantify the number of immunostained cells, five random fields per animal (14,978 μm^2) were analyzed as previously described.^{32,33} The results were presented as cells per mm^2 .

Quantification of myofibroblasts was performed using sections immunolabelled with mouse monoclonal antibody to α -smooth muscle actin (#0851; DAKO, Carpinteria, CA, USA; 1:100) and the anti-mouse EnVision System (#4001; DAKO; 1:20), as previously described.³⁴ The volume density of myofibroblasts was evaluated using point counting and a video microscopic system, as previously described.^{32,35,36} The results were presented as the volume density of myofibroblasts ($V_{V(31)}$).

Immunoblotting

Five lesions per group were collected and frozen at -80°C . Frozen lesions were macerated in lysis buffer (20 mM Tris-HCl pH 7.5, 138 mM sodium chloride, 10% glycerol, 1% Triton X-100, 2 mM ethylenediaminetetraacetic acid, 10 $\mu\text{g}/\text{mL}$ leupeptin, 0.025% phenylmethylsulfonyl fluoride; Sigma-Aldrich, St Louis, MO, USA). After centrifugation, the total protein concentration was determined using a bicinchoninic acid protein assay (Thermo Fisher Scientific, Rockwood, TN, USA). Proteins (30 or 50 μg) were resolved by 8 or 10% sodium dodecylsulfate-polyacrylamide gels (SDS) and were transferred to polyvinylidene fluoride (PVDF) membranes. Protein molecular weight standards (Bio-Rad, Hercules, CA, USA) were included. The membranes were blocked with 5% non-fat milk in powder form (Nestlé, São Paulo, Brazil) dissolved in phosphate buffer containing 0.05% Tween-20 and were probed overnight at 4°C with rabbit anti-type I collagen precursor (Col-1 p) (Millipore, Temecula, CA, USA,

1:1000), mouse anti-type III collagen (Col-3) (Millipore, 1:600), mouse anti-MMP-1 (Santa Cruz Biotechnology, 1:200), goat anti-fibrillin-1 (Santa Cruz Biotechnology, 1:200), rabbit anti-MMP-12 (Santa Cruz Biotechnology, 1:200), rabbit anti-precursor transforming growth factor (TGF)- β 1/2/3 antibody (Santa Cruz Biotechnology, 1:200), rabbit anti-pERK (Santa Cruz Biotechnology, 1:200), and mouse anti-ERK (Santa Cruz Biotechnology, 1:200). The membranes were then washed and incubated with peroxidase-conjugated anti-mouse (1:100) (DAKO), anti-goat (1:200) or anti-rabbit (1:500) secondary antibodies, all from Santa Cruz Biotechnology. Bound antibodies were detected by enhanced chemiluminescence (Santa Cruz Biotechnology). For chemiluminescence detection, the ChemiDoc MP system (Bio-Rad Laboratories Inc., Hercules, CA, USA) was used. Subsequently, the membranes were stripped and reprobed with a mouse anti- β -actin antibody (42 kDa, Sigma-Aldrich). The bands were quantified by densitometry analysis, which was performed using Adobe Photoshop version 7.01 (Adobe Systems, San Jose, CA, USA), and the results were expressed as arbitrary units.

Zymography

The gelatinase activity of matrix metalloproteinase-2 (MMP-2) was analyzed by zymography in five samples of frozen scar tissue per group, as described previously.³⁷ Frozen fragments of each wound were placed in 1 mL of lysis buffer (20 mM Tris-HCl, pH 7.5, 138 mM NaCl, 10% glycerol, 1% Triton X-100, 2 mM EDTA, 10 mg/mL leupeptin, and 1 mM phenylmethylsulfonyl fluoride), macerated and centrifuged at $3.864g$ for 30 min at 4°C . The lysates were collected and stored at -80°C . Twenty micrograms of protein from each lysate was resolved on an 8% SDS-polyacrylamide resolving gel containing 1 mg/mL gelatin (Sigma-Aldrich) layered with a 4% SDS-polyacrylamide stacking gel. Lysate from a human placenta was used as a positive control for gelatinolytic activity.³⁸ Protein molecular weight standards (Amersham Pharmacia Biotech, Buckinghamshire, UK) were included for molecular weight estimation. Following protein separation, the gels were washed in 2.5% Triton X-100 and incubated in development buffer, pH 8.4 (50 mM Tris-HCl, 5 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, and 2 mM ZnCl_2), for 12 h at 37°C without agitation. The gels were then stained with 0.25% Coomassie Brilliant Blue R solution for 30 min and then destained (50% methanol, 10% glacial acetic acid, and 40% distilled water) to obtain a contrast between the gelatinolytic bands and the gel background. Images captured with the ChemiDoc MP system (Bio-Rad Laboratories) were used for detection. Densitometry was performed on the scanned images using Adobe Photoshop software version 7.01 (Adobe Systems, San Jose, CA, USA), and the results were expressed as arbitrary units.

Statistical analysis

The values for all measurements are expressed as the mean $\pm$ the standard error of the mean (SEM). Analysis of the data was performed using repeated measures ANOVA (Geisser-Greenhouse) with Dunn's post test for wound

closure and unpaired *t*-test for the other parameters, with $p < 0.05$ as the least significant level. InStat GraphPad software was used to perform the statistical analysis (GraphPad InStat version 6.00, GraphPad Software, San Diego, CA, USA).

Results

Heat exposure delays wound healing and reepithelialization

During the exposure period, the temperature and humidity of the chamber were constant (online Supplementary Figure 1). The light used to heat the chamber was visible light with a maximum spectral emission between 614–628 and 661–677 nm. The emission spectrum can be seen in online Supplementary Figure 2.

The average mouse body temperature before exposure was $31.8 \pm 0.31^\circ\text{C}$ and during the exposure to 43°C peaked at $33.5 \pm 0.19^\circ\text{C}$ in 30 min. Five minutes after exposure to 43°C for 30 min, the mouse body temperature was $32.3 \pm 0.09^\circ\text{C}$ (online Supplementary Figure 3).

To investigate the effects of heat on wound healing, we analyzed the area of the wound, reepithelialization and epidermal gap length. The 43°C group lesions showed 42% and 29% increases in area 11 ($p < 0.01$) and 14 ($p < 0.001$) days after wounding, respectively, compared with the 23°C group (Figure 1(a) and (b)).

The percentage of reepithelialized wounds was reduced in the 43°C group (Figure 1(c)). When reepithelialization was measured, we verified that the percentage of reepithelialized wounds in the 43°C group was decreased by 17% ($p < 0.001$) compared with the 23°C group, indicating incomplete reepithelialization 14 days after wounding (Figure 1(d)). Furthermore, the 43°C group showed an increased epidermal gap length compared with the 23°C group ($p < 0.01$) 14 days after wounding (Figure 1(e)).

Heat exposure increases the number of myofibroblasts and macrophages

The number of cells was evaluated 14 days after the injury to analyze the granulation of tissue; both groups showed similar numbers of inflammatory cells (Figure 2(a)). The 43°C group had a higher volume density of myofibroblasts ($p < 0.01$) (Figure 2(b)), a similar number of MPO-positive neutrophils (Figure 2(c)), and a greater number of F4/80-positive macrophages ($p < 0.05$) compared with the 23°C group (Figure 2(d)).

Effect of heat exposure on the components of the extracellular matrix in the wound healing area

To investigate the influence of heat on the expression of extracellular matrix proteins during wound healing, the protein expression of TGF- β 1/2/3, type I and III collagen, and MMP-1, and MMP-2 gelatinolytic activity was investigated. The protein expression of TGF- β 1/2/3 was greater in the 43°C group ($p < 0.01$) than in the 23°C group 14 days after injury (Figure 3(a)). Fourteen days after wounding, the 43°C group presented increased expression of type III collagen ($p < 0.001$) and decreased expression of type I

collagen ($p < 0.05$) compared with the 23°C group (Figure 3(b)). This result corresponds with the increase in MMP-1 expression ($p < 0.05$) and the reduction of MMP-2 activity ($p < 0.001$) observed in the 43°C group compared with the 23°C group (Figure 3(c) and (d)), indicating an imbalance between degradation and collagen deposition 14 days after wounding.

To investigate the effect of heat exposure on the elastic components of the extracellular matrix, we analyzed the expression of fibrillin-1, a component of elastic fibres, and MMP-12, an elastase. The expression of fibrillin-1 ($p < 0.001$) and MMP-12 ($p < 0.05$) increased in the 43°C group compared with the 23°C group (Figure 3(e) and (f)). Our results suggest that the increased production of fibrillin-1 induced by heat may have contributed to the accumulation of elastic material in the extracellular matrix of the wound area.

Heat induces the activation of ERK mitogen-activated protein kinase pathways

The expression of MMPs is associated with the coordinated activation of mitogen-activated protein kinases (MAPKs). Thus, we investigated ERK activation by exposure to heat. Fourteen days after wounding, the 43°C group showed increased activation of pERK compared with the 23°C group ($p < 0.05$) (Figure 4).

Discussion

The role of heat produced by the visible light spectra during wound healing is not clear. In 2012, the production of ROS, pro-inflammatory cytokines, and MMP-1 expression in human skin after visible light exposure was reported.³⁹ Another study showed that exposure to approximately 27°C delayed the healing of cutaneous wounds in rats compared with the rats exposed to 27°C added to the infrared radiation.²⁰ Our study showed that exposure to 43°C , achieved by exposure to visible light, delayed the closure of wounds 11 and 14 days after injury.

It is possible that heat plays a role during the inflammatory stage of cutaneous tissue repair. After skin injury, neutrophils are attracted to the wound site, followed by macrophages.⁴⁰ Neutrophils are more active in the early stages of inflammation, and in the absence of infection, the infiltration of neutrophils ceases within a few days.² The number of neutrophils in granulation tissue was similar in the mice exposed to 27°C and the mice exposed to 27°C associated with infrared radiation.²⁰ In our research, we observed a similar number of MPO-positive neutrophils, regardless of the temperature, indicating that the heat did not influence the action of neutrophils during wound healing.

Additionally, macrophages release TGF- β during the inflammatory phase, which attracts fibroblasts to the area of injury, initiating the formation of granulation tissue, thus playing an essential role in the transition between inflammation and fibroplasia⁴¹ and accelerating wound healing.⁴² However, the permanence of macrophages in the later stages of tissue repair indicates delayed healing. In our study, we observed an increase in the number of F4/80-positive macrophages and the extent of TGF- β 1/2/3

Figure 1 Macroscopic analysis of wound healing in heat-exposed mice. (a) Photographs of the wounded mice exposed to 23°C and 43°C at 3, 7, 11, and 14 days after wounding. (b) Percentage of original wound area at 3, 7, 11, and 14 days after wounding (graphical representation). (c) Percentage of reepithelialized wounds 14 days after wounding (graphical representation). (d) Representative images of the epidermal gap in the 23°C and 43°C groups 14 days after wounding. Left, macroscopic images; the dotted line represents the border of the non-reepithelialised region. Right, histological images of the epidermal gap. The area between the lines represents the epidermal gap. Hematoxylin-eosin, bar = 500 µm. (e) Epidermal gap 14 days after wounding (graphical representation). Data ($n = 10$ per group) are expressed as the mean $\pm$ SEM. ** $p < 0.01$, *** $p < 0.001$ compared between the 23°C vs 43°C groups (non-parametric repeated measures ANOVA with Dunn's post test for wound area, and unpaired t -test for percentage of reepithelialized wounds and epidermal gap). (A color version of this figure is available in the online journal.)

Figure 2 Microscopic analysis of wound healing on heat-exposed mice. (a) Volume density of inflammatory cells ($Vv \%$) in the wound area 14 days after wounding. (b) Stereological analysis showing the volume density of myofibroblasts ($Vv \%$) in the α -SMA-positive myofibroblasts in the wound area 14 days after wounding. (c) MPO-positive neutrophils and (d) F4/80-positive macrophages in the wound area 14 days after wounding. Data ($n = 6$ per group) were expressed as the mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$ compared between the 23°C vs 43°C groups (Unpaired t test). Bar = 50 μm . (A color version of this figure is available in the online journal.)

protein expression in the 43°C group compared with the 23°C group 14 days after injury.

The proliferative phase is characterized by the migration of fibroblasts to the site of injury, to synthesize and deposit collagen to form new tissue or scar tissue.^{43,44} Toyokawa et al. showed an increase in fibroblast infiltration in mice exposed to IR associated with 27°C exposure up to seven days post wounding.²⁰ An increase in the expression of TGF- β 1 and TGF- β 2 24 hours after heating the skin of the buttocks to 43°C for 90 min was observed in another study.⁴⁵ During the remodelling phase, extracellular matrix synthesis is reduced, and the majority of endothelial cells, myofibroblasts and macrophages die by apoptosis to reduce the cellularity of the granulation tissue.^{4,44} Fourteen days after injury, myofibroblasts of the 43°C

group showed a higher bulk density and a higher number of F4/80-positive macrophages than the 23°C group; this is a feature typical of a delayed wound healing process.

Throughout the remodelling phase, type III collagen, a major component of granulation tissue, is gradually replaced by type I collagen, the main dermal protein.⁴³ Exposure to temperatures of 41°C for 1 h twice a week increases collagen production in healthy skin,⁴⁶ whereas pulse exposure to 45°C and 60°C increases the regulation of procollagen type I and III in human dermal fibroblasts.⁴⁷ This process is dependent on MMPs secreted by macrophages, endothelial cells and fibroblasts, whose activity is controlled by tissue inhibitors of metalloproteinases (TIMPs).^{1,4} Normal tissue repair requires a balance between

Figure 3 Collagen and MMP expression and activity during wound healing in heat-exposed mice. (a) Protein expression of TGF- β 1/2/3. (b) Protein expression of type III collagen and type I collagen. (c) Protein expression of MMP-1. (d) Activity of MMP-2 by a zymographical assay of matrix metalloproteinase (MMP-2). (e) Protein expression of fibrillin-1 and (f) MMP-12. Densitometry expressed as arbitrary units (a.u.) for immunoblotting of TGF- β 1/2/3 (47 kDa), type I collagen (70–90 kDa), type III collagen (138 kDa), MMP-1 (52 kDa), fibrillin-1 (330–350 kDa) and (f) MMP-12 (59 kDa) and the zymographical assay of MMP-2. β -Actin (42 kDa) was used as a constitutive protein for normalization. Data ($n = 5$ per group) were expressed as the mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ compared between the 23°C vs 43°C groups (unpaired t -test)

the activity of MMPs and TIMPs. Our study showed that 14 days after injury, exposure to 43°C increased the expression of type III collagen and decreased the expression of type I collagen.

There is no consensus on whether IR or the heat generated by IR can induce the activation of MMPs. Shin et al. demonstrated that healthy hairless mice exposed to 43°C for 15 or 30 min for 6 weeks showed increased

Figure 4 ERK during wound healing in heat-exposed mice. Protein expression of pERK (42–44 kDa). β -Actin (42 kDa) was used as a constitutive protein for normalization. Data ($n = 5$ per group) were expressed as the mean $\pm$ SEM. * $p < 0.05$ compared between the 23°C vs 43°C groups (unpaired t -test)

MMP-13 expression but unchanged MMP-2 and MMP-9 expression.²⁷ We observed that heat increased MMP-1 expression and reduced MMP-2 activity. These findings suggest that the heat activated MMP-1, causing degradation of type I collagen. Type I collagen degradation products were consumed by the gelatinase activity of MMP-2.

Elastin, which contributes to the elasticity of the skin, reappears at the end of the remodelling phase; however, scar tissue does not have the same elastic properties as healthy tissue.^{4,43} Heat from sunlight modulates the synthesis of tropoelastin, elastin, and fibrillin-1, resulting in the development of solar elastosis. Fibrillin-1 expression increases in the dermis of aged skin. While the gene expression of fibrillin-1 decreases with acute heat exposure, it increases with chronic exposure in cultured fibroblasts of human skin. Heat increases the expression of the fibrillin-1 gene in the epidermis while reducing its expression in the dermis, which leads to a set of abnormal elastic fibres.²¹ In our study, we observed increased protein expression of fibrillin-1 and MMP-12 in the mice exposed to 43°C 14 days after wounding.

Previous studies have shown the relationship between the expression of MMP-1 and MAPK activation. Park et al. observed that heat increases the gene and protein expression of MMP-1 and MMP-3, but not MMP-2, by ERK pathway activation via an IL-6 dependent autocrine mechanism.²² We observed that the mice exposed to 43°C heat showed increased protein expression of phosphorylated ERK 14 days after wounding.

In conclusion, we have shown that heat delayed the healing of skin wounds in mice. This delay was characterized by an increase in the wound area and the gap associated with a reduction of the reepithelialized area. Our findings suggest a possible role of heat in the imbalance between the deposition and the degradation of collagen and elastic fibres in the extracellular matrix.

Authors' contribution: MASS designed the experiments, conducted the research, analyzed the data and wrote the manuscript; ETLT and FSS conducted the research and analyzed the data; AMAC designed the experiments, analyzed the data, critically revised and reviewed the manuscript for important intellectual content.

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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