Journal of Plastic, Reconstructive & Aesthetic Surgery (2013) 66, 260—266

JPRAS
An International Journal of Surgical Reconstruction
www.JPRASurg.com

First-aid with warm water delays burn progression and increases skin survival☆

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Received 22 March 2012; accepted 13 September 2012

KEYWORDS
Burn wound conversion;
Cooling;
Ischemia;
Vasodilation;
Heat removal

Summary *Introduction:* First aid treatment for thermal injuries with cold water removes heat and decreases inflammation. However, perfusion in the ischemic zone surrounding the coagulated core can be compromised by cold-induced vasoconstriction and favor burn progression. The aim of this study is to evaluate the effect of local warming on burn progression in the rat comb burn model.
Methods: 24 male Wistar rats were randomly assigned to either no treatment (control) or application of cold (17 °C) or warm (37 °C) water applied for 20 min. Evolution of burn depth, interspace necrosis, and microcirculatory perfusion were assessed with histology, planimetry, respectively with Laser Doppler flowmetry after 1 h, as well as 1, 4, and 7 days.
Results: Consistent conversion from a superficial to a deep dermal burn within 24 h was obtained in control animals. Warm and cold water significantly delayed burn depth progression, however after 4 days the burn depth was similar in all groups. Interspace necrosis was significantly reduced by warm water treatment (62 ± 4% vs. 69 ± 5% (cold water) and 82 ± 3% (control); $p < 0.05$). This was attributed to the significantly improved perfusion after warming, which was present 1 h after burn induction and was maintained thereafter (103 ± 4% of baseline vs. 91 ± 3% for cold water and 80 ± 2% for control, $p < 0.05$).
Conclusion: In order to limit damage after burn injury, burn progression has to be prevented. Besides delaying burn progression, the application of warm water provided an

☆ This work has been partly presented at the last year's joint meeting of the "49. Jahrestagung der Österreichischen Gesellschaft für Plastische, Ästhetische und Rekonstruktive Chirurgie (ÖGPÄRC), 42. Jahrestagung der Deutschen Gesellschaft der Plastischen, Rekonstruktiven und Ästhetischen Chirurgen (DGPRÄC), und 16. Jahrestagung der Vereinigung der Deutschen Ästhetisch-Plastischen Chirurgen (VDÄPC)" in September 2011 in Innsbruck, Austria.

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http://dx.doi.org/10.1016/j.bjps.2012.09.014

additional benefit by improving the microcirculatory perfusion, which translated into increased tissue survival.

Introduction

Burn progression is a process by which initially unburned tissue deep to the burn wound becomes necrotic after the actual insult has ceased. Halt this phenomenon limits burn severity since the depth of the wound is a significant determinant for the healing capacity of the skin, which is directly related to the quality and quantity of the skin appendages left in the dermis. The conversion of a partial-thickness burn wound, that heals without or with only minor sequelae, to a full-thickness lesion that requires surgical therapy and results in permanent scarring or wound contracture represents the most dramatic negative evolution of the burn wound and may lead to severe morbidity requiring complex reconstructive procedures.[1–4]

The concept of burn progression in the zone of stasis surrounding the immediately coagulated central core of the thermal injury has been outlined more than 50 years ago.[5]

This jeopardized tissue is characterized by stagnant blood flow comparable to the ischemic penumbra in stroke.[6] In this setting of progressive ischemia a multitude of interdependent mechanisms are activated leading to increased capillary permeability, edema formation, metabolic derangements and finally to tissue necrosis.

Generally accepted first-aid for thermal injuries consists in cooling with cold tap water for 20 min[7] Admittedly counterintuitive, the application of warm water as a first-aid measure to prevent burn progression has not undergone scientific evaluation so far but deserves some attention. Obviously, running tap water of around 17 °C leads to faster heat dissipation than at 37 °C, but it will hardly be tolerated for 20 min and most of all it will inevitably induce vasoconstriction and thereby negatively influence blood perfusion in the critical zone of stasis. In contrast, it is well-known that local warming can increase skin blood flow.[8]

The beneficial effect of vasodilation in ischemic skin tissue has previously been shown in different experimental settings.[9–11] The aim of the present study is to compare the effects of local application of cold (17 °C) and warm water (37 °C) on burn progression in the rat comb burn model, where unburned interspaces, i.e. the ischemic zone surrounding the thermally injured tissue, represent the critical zone that is prone to progression if left untreated. We hypothesize that local application of warm water, by inducing vasodilatation and consequently improving perfusion of the critically perfused zone can prevent burn progression and thereby improve wound healing.

Materials and methods

Animals

All experiments were carried out in accordance with the Swiss guidelines for animal experimentation and were approved by the Geneva Cantonal Veterinary authority.

A total of 24 male Wistar rats weighting 471 ± 10 g (Charles River Laboratories, L'Arbresle Cedex, France) were used in this study. Animals were housed in singles cages, at a room temperature of 22–24 °C and at relative humidity of 60–65% with a 12 h day–night cycle. Chow and water were available ad libitum.

Anesthesia and animal preparation

The rats were anesthetized for preparation and all measurements with an inhalation of a 2% isoflurane – air mixture. Animals received subcutaneous buprenorphine at a dose of 0.05 mg/kg bodyweight, 15 min before burn induction, and thereafter, every 12 h for the first 3 days. The animals were prepared 24 h prior to the experiments. The dorsal skin was shaved and depilated with a depilatory cream (Veet®. Reckitt Benckiser, Wallisellen, Switzerland), and the areas to be burned were outlined with a marking pen.

Burn induction

The comb burn model was used to create the burn wounds.[12,13] A chromium–nickel steel (V2A) template (Ornaplast Kunststofftechnik, Dagmersellen, Switzerland) weighting 136 g was immersed in boiling water for 15 min. With the animal in the prone position, the template was then applied to the previously shaved and marked area on the back, perpendicular to the skin surface and parallel to the spine. No pressure was applied and the template was removed after 60 s. Template temperature at the beginning of the burn injury was 87 ± 1 °C and 60 ± 1 °C at the end of the burn induction. Application of the template resulted in four 20 × 10 mm burn areas separated by three 20 × 5 mm unburned interspaces as defined by the notches of the template. Both sides of the back were burned. No dressing was applied.

Experimental groups and protocol

Animals were randomly assigned to three experimental groups of 8 animals each: a control group (CO) without any treatment, a group treated with cold water (17 °C) for 20 min (CW) and a group treated with warm water (37 °C) for 20 min (WW). Water treatment was performed immediately after burn induction with soaked gauzes (10 × 10 cm), directly applied to the burn area and changed every minute. Soaked gauzes were used for purpose of experimental control.

Animal preparation and baseline measurements were performed 24 h before burn induction. After burn induction the parameters were assessed after 1 and 24 h, as well as after 4 and 7 days. Wound healing and contracture were assessed once a week. The wound was considered as completely healed if the entire burned zone was epithelialized and free of eschar.

Endpoints

Planimetry

Digital photographs (DP, Panasonic DMC-TZ1, Osaka, Japan) were made at a fixed distance with constant focus for planimetric analysis. A computer-assisted image analysis system (Cap Image®, Zeintl Software; Heidelberg, Germany) was used to determine the total amount of interspace necrosis (% of the interspace surface) and the contracture rate (% of the total length of the unburned area). Time of total wound healing was reported in weeks.

Microcirculatory perfusion

Microcirculatory blood perfusion within the interspaces was performed using a PIM II Laser Doppler Perfusion Imager (PIM II Laser Doppler Perfusion Imager, LDPIwin 2.0.6 software, Lisca AB Berzelius Science Park, Linköping, Sweden). The surface probe measured microcirculatory blood flow to a depth of approximately 1 mm. The laser Doppler unit was calibrated according to the guidelines of the manufacturer. Because of the high intersite variability, the data are given in percentages of baseline.[14]

Histology

For each time point in all groups, 6 directly burned areas were harvested, fixed in 10% formalin for 24 h and stored in 70% alcohol until staining with Hematoxylin and Eosin. Each sample was then analyzed for burn depth by a pathologist who was blinded to the treatment applied. A scale from 1 to 5 ranging from epidermis (1), superficial dermis (2), intermediate dermis (3), deep dermis (4) to muscle (5) was used.[15]

Core temperature

The rectal temperature was measured prior to burn, immediately after burn induction and water treatment (ONBO Electronic Co. Ltd, Shen Zhen, China).

Statistical analysis

Data were analyzed with Stata software, version 11.0. All values are expressed as mean ± standard error of the mean (SEM). Check for parametric assumptions i.e. normality of distribution and homogeneity of variance were performed with the use of the Shapiro–Wilk test respectively the robust equal variance test. Comparison between the 3 groups included ANOVA and the post hoc Bonferroni test when parametric assumptions were satisfied, otherwise the equivalent non-parametric Kruskall–Wallis test followed by the measures to correct the α-error according to Bonferroni probabilities. Differences were considered significant at $p < 0.05$ respectively $p < 0.016$ (0.05/3).

Results

Burn depth

Histologic evaluation of burn depth 1 h after burn induction showed consistent and uniform damage to the epidermis and superficial dermis in all animals (score: 2 ± 0) (Figure 1). Within 24 h, tissue damage significantly

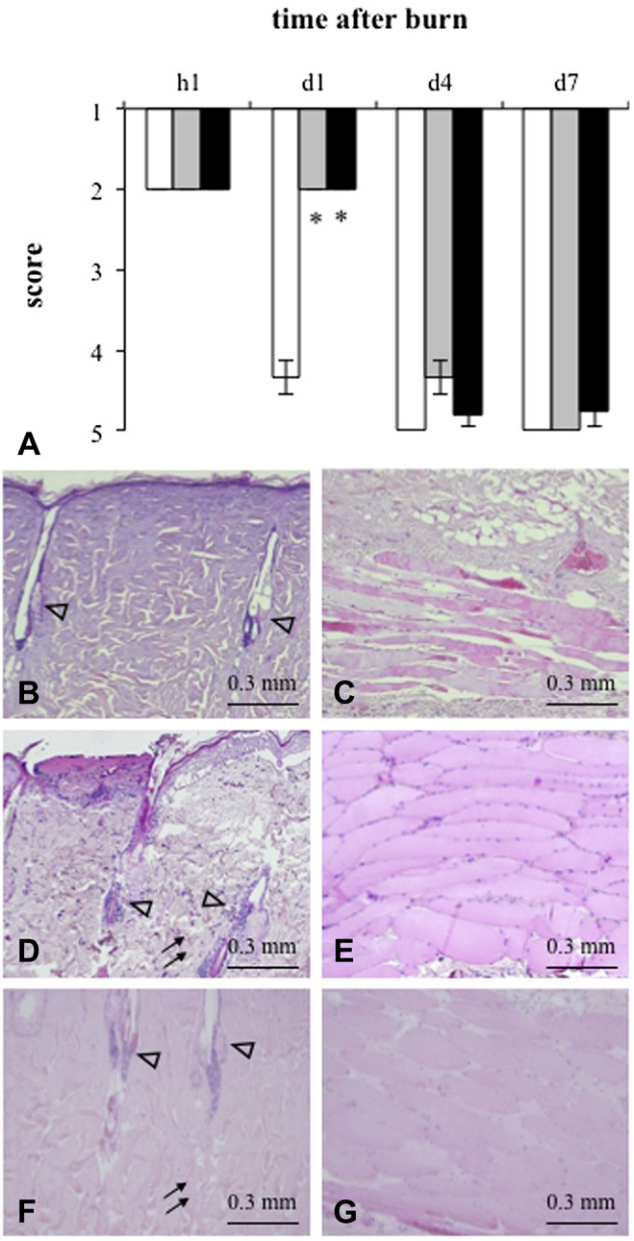

Figure 1 Burn depth progression Time course of burn depth progression in untreated animals (CO; white bars) as well as in animals receiving cold water treatment (CW; gray bars) respectively warm water treatment (WW; black bars) (A). Local treatment of either temperature delays secondary burn depth progression without preventing final burn depth at day 7. Burn depth progression score: 1. epidermis; 2. superficial dermis; 3. intermediate dermis; 4. deep dermis; 5. muscle. Mean ± SEM; *$p < 0.05$ vs. control; $n = 6$/group. Morphology of dermis respectively muscle after H&E staining at day 1 after burn. Controls develop complete architectural destruction of dermis, skin appendixes (arrow heads); (B) and muscle (C), whereas local cold water (D, E) and warm water (F, G) treatment were able to transiently maintain viability of deep dermis (arrows), appendixes (arrow heads; D, F) and muscle (E, G). Scale bar: 0.3 mm.

progressed to the deep dermis in control animals, whereas in animals treated with both cold and warm water no progression occurred at this time point (4.3 ± 0.2 for CO vs. 2.0 ± 0.0 for CW and WW; $p < 0.05$). Over the next three days however, burn depth increased in the two treatment groups to the deep dermis (5 ± 0 for CO vs. 4.3 ± 0.2 for CW and 4.8 ± 0.1 for WW, ns). On day 7, all animals showed damage to all analyzed layers.

Interspace necrosis

One hour after burn induction, the interspaces were intact in all groups (Figure 2A, B). In contrast to the histologically determined burn depth, surface extension of the burn lesion was similar in control and treatment groups after 24 h. Interspace tissue necrosis occurred between day 1 and 4 in all animals. Warm water significantly reduced the extension of necrosis when compared to controls after 4 days ($p < 0.05$, Figure 2) and when compared to cold water at day 7 (65 ± 4% for WW vs. 81 ± 4% for CW and 94 ± 2% for CO; $p < 0.05$).

Interspace perfusion

1 h after burn induction, all subjects showed a decreased perfusion in the interspaces (Figure 3). Whereas application of cold water did not influence perfusion compared to control animals, warm water led to a significant improvement (81 ± 2% for WW vs. 62 ± 2% (CW) and 63 ± 1% (CO); $p < 0.05$). Over the remaining observation period, perfusion steadily increased. Of interest, only warm water treatment did re-establish baseline values (103 ± 4% (WW) vs. 91 ± 3% (CW) and 80 ± 2% (CO), at day 4; $p < 0.05$).

Core temperature

Warm water significantly increased core temperature in these animals that were under general anesthesia, where no attempt was made to maintain body temperature during the experiments (34.7 ± 0.1 (WW) vs. 33.1 ± 0.2 °C (CO) and 32.3 ± 0.3 °C (CW); $p < 0.05$) (Table 1).

Healing time and wound contracture

No significant difference, neither in final scarring time nor in contracture rate, was noted among the different groups (Figure 4). In all animals, burn wounds healed after a median time of approximately 11 weeks. The contracture rate was between 25 and 29%.

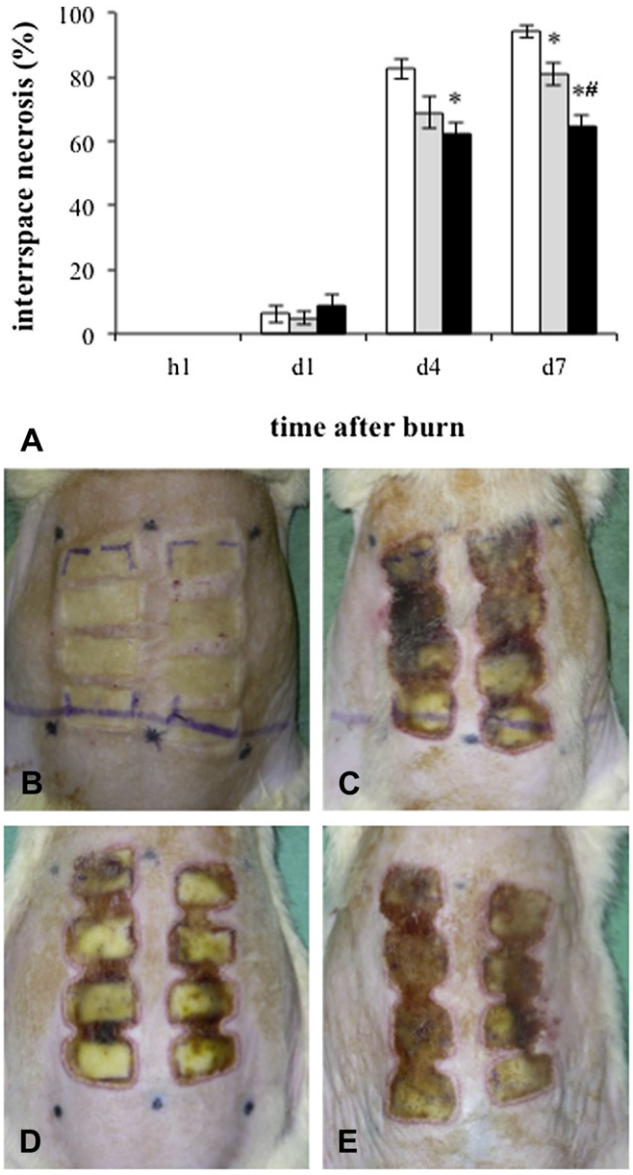

Figure 2 Interspace necrosis Time course of secondary burn surface progression given in percent of the total interspace area in untreated animals (CO; white bars) as well as in animals receiving cold water treatment (CW; gray bars) respectively warm water treatment (WW; black bars) (A). Mean ± SEM; *$p < 0.05$ vs. controls; #$p < 0.05$ vs. cold water treatment; $n = 8$/group. Tissue morphology 1 h (B) and 7 days after burn in an untreated animal (C) as well as after cold water (D) and warm water (E) treatment. Note the decreased width of interspace necrosis after cold water and particularly warm water treatment.

Discussion

Interruption of the intercalated vicious circles of ischemia, inflammation, coagulation, and pain to control damage and limit progression of tissue destruction should be the goal in emergency management of burn lesions. Currently, cooling of thermal burns with cold water or tap-water as soon as possible for 20 min is recommended.[7] After cooling in the emergency setting, the wound is cleansed and a protective dressing is applied. Depending on local preferences, the dressing may include antimicrobial, fibrinolytic or wound regenerative agents, however it is free of any substances intended to actively interfere with burn progression.

With the present study we were able to show that the use of warm water (37 °C) for first aid in burn trauma was more effective than 17 °C cold water. It not only similarly delayed burn progression compared to cold water, but

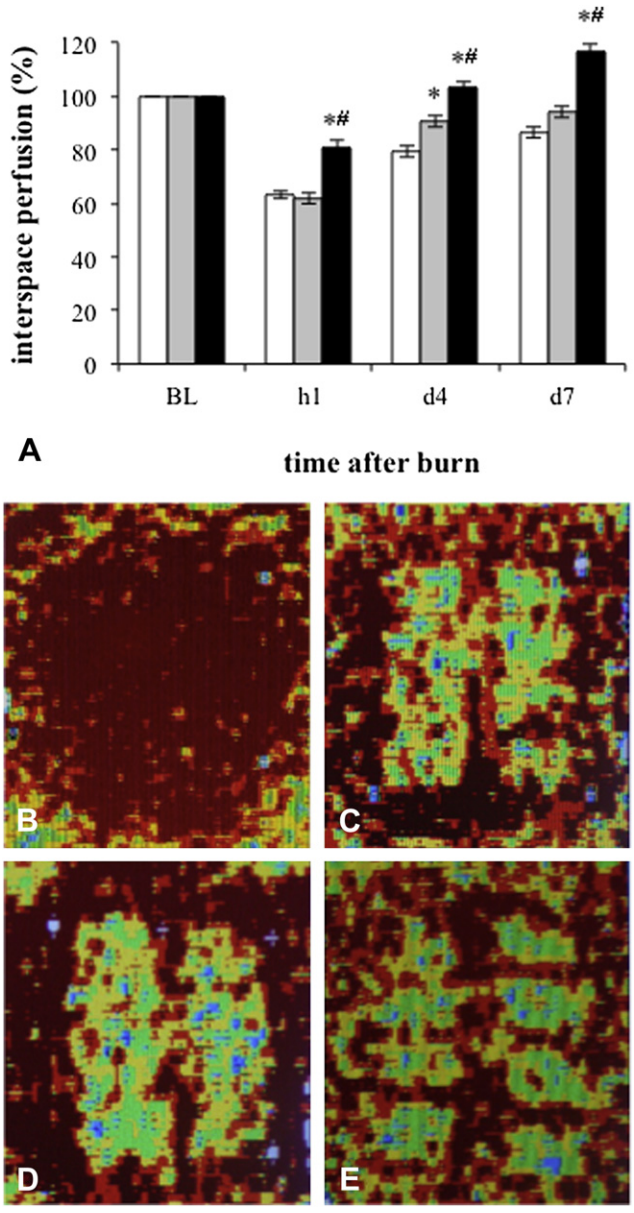

Figure 3 Interspace perfusion Time course of interspace perfusion in untreated animals (CO; white bars) as well as in animals receiving cold water (CW; gray bars) respectively warm water (WW; black bars) (A). Mean ± SEM; *$p < 0.05$ vs. control; #$p < 0.05$ vs. cold water; n = 8/group. Flow pattern before (B) and 1 h after burn in an untreated animal (C) as well as after cold water (D) and warm water (E). Note the maintained interspace perfusion after warm water application (E) indicated by yellow and red pixels.

Table 1 Time course of core temperature (°C).

Time-point	Baseline	Post burn	Post treatment
Control (CO)	35.2 ± 0.4	34.9 ± 0.3	33.1 ± 0.15
Cold water (CW)	35.2 ± 0.2	34.6 ± 0.1	32.3 ± 0.3
Warm water (WW)	34.7 ± 0.2	34.6 ± 0.5	34.7 ± 0.1*,#

Values are mean ± SEM. *$p < 0.05$ vs. CO, #$p < 0.05$ vs. CW.

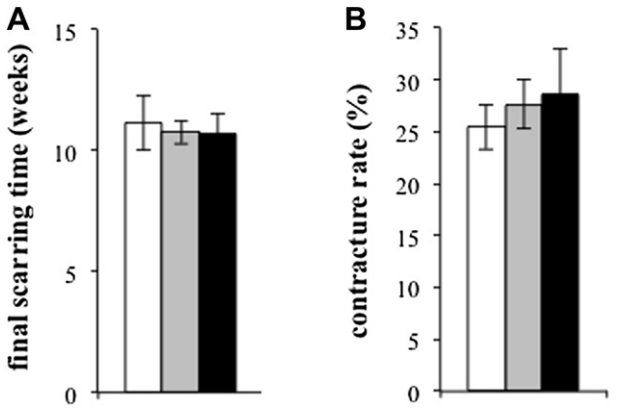

Figure 4 Final scarring time and contracture rate Final scarring time (A) and contracture rate (B) in untreated animals (CO; white bars) as well as in animals receiving cold water (CW; gray bars) respectively warm water (WW; black bars). $n = 8$/group.

further reduced surface extension of tissue necrosis. Application of warm water induces vasodilation and increases capillary recruitment and consequently increases microcirculatory blood flow.[16] Normalization of microhaemodynamics led to a significantly reduced final interspace surface necrosis when compared to cooling with 17 °C cold water in the present study.

The comb burn model was originally designed to imitate the clinical relevant progression in burn depth with the extension of burn surface, i.e. necrosis of the initially unburned, spared zones in between the four burn squares that are supposed to represent the zone of stasis at risk for necrosis if left untreated.[12] The previously not described discrepancy between the progression in burn depth developing within 24 h and the extension of surface necrosis evolving over 4—7 days may be explained as follows: The relatively thin skin of rats and the severity of the burn injury lead to a rapid progression of the actual burn depth (histologically assessed) in untreated animals. The burn injury impairs the dermal perfusion from the cranial and caudal sides of the interspaces sparing only the perfusion from the medial and lateral sides, similar to a bipedicled flap. Therefore, necrosis of the interspaces (planimetrically assessed) is a consequence of the burn injury and progression of the directly injured tissue. Complete dermal destruction and thereby blocking of the perfusion from the burned areas within 24 h in the control animals renders the previously unburned tissue rapidly dependent from the lateral perfusion, whereas the delay in burn depth progression by water application maintains a certain level of perfusion from underneath the burned areas. This may be sufficient to save part of the interspace tissue from necrosis as surgical delay can increase flap survival.[17] Vasodilation induced by application of warm water further improves microvascular perfusion in the interspaces and additionally protects the tissue from necrosis.

In control animals, burn depth consistently progressed within 24 h from a superficial to a deep dermal lesion. Both treatments delayed this by at least 24 h. This delay in burn depth progression can be considered a respectable success for a treatment that lasted only 20 min but did not alter the

ultimate fate of the dermis underlying the directly burned zone. The fact that the application of the hot chromium—nickel steel template for 60 s — a relatively long time with respect to the thickness of rat skin — resulted in full thickness burn injuries is not surprising. In the clinical setting, this delay could open the window for therapeutic intervention that aims to maintain and improve microcirculatory perfusion.

Short term cooling is considered a panacea in a progress that probably lasts 24—48 h. Besides pain killing properties, it is alleged with anti-inflammatory and anti-edematous properties,[18—23] supposed to improve microcirculatory function[23] and last but not least to improve wound healing.[14,24—28] Cooling did confer some benefit, however the mechanisms by which cold water did delay burn depth progression are less obvious since perfusion was not different from control animals. As previously reported, cooling may directly inhibit the inflammatory cascade and edema formation.[29] Reduction of edema may be beneficial in the short term, but if obtained by vasoconstriction and reduced blood flow it may further jeopardize the critically perfused tissue and simply postpone the normalization of perfusion. Cooling may also increase ischemic tolerance to the tissues.[30] Hypothermia has been shown to be among the most effective treatment modalities for limiting ischemia-associated cellular injury.[31] Also, any potential positive interference with pain perception and interaction with healing cannot be excluded. However, a recent study did question its benefit on hyperalgesia following burn injury in humans.[32]

One of the major arguments to use cold water is to remove heat. However, the amount of cooling necessary to remove heat and prevent burn progression is not known. Recent work showed only mild if any improvement in burn wound outcome with different protocols of cooling.[14,25—28,33,34] Interestingly, many of the protocols used yielded very low, non-physiological intradermal temperatures rapidly after cooling. In addition, it was shown that "no treatment" resulted in temperatures below a critical point where protein denaturation occurs after approximately 2 min[25] This implies that removal of heat as a criterion to apply cold water only holds validity only for a relatively short interval. Also, it raises the question of potential local damage secondary to cooling (not considering the risk of "systemic" hypothermia), even with tap water, as has been demonstrated with ice water.[34,35]

It might be that future refinements in emergency treatment of burn injuries include both cooling (heat removal and anti-inflammatory action) and warming (improved perfusion). Substantial benefit however will hardly be obtained by first aid measures alone and novel therapeutic strategies targeting burn progression should be elaborated.

The healing time and wound contracture were not significantly influenced by water treatment, regardless of the temperature. This is in contrast to the postulations of most recent studies that favor cooling in spite of only minimal differences in outcome observed.[14,25—29,33,34] First of all, it should be stressed that burn progression and wound healing are two different processes with opposite directions. However, prevention of burn progression leads to a more superficial burn wound with increased healing potential resulting in more rapid recovery with less scarring and contracture. The absence of any beneficial effect on wound healing in the present experiments might be due to the severity of inflicted burn lesion on the one hand and the thin rodent skin on the other hand and the short duration of treatment.

Clinically, the conversion of a burn wound from a superficial to a deep lesion is dramatic as it changes management strategies, the healing time, but above all the quality of the final result. Restitutio ad integrum can be expected in superficial lesions whereas deep dermal lesions require tangential excision and skin grafting or heal with scarring and contracture. In large surface burn areas this conversion can actually influence mortality. In these cases the concomitant burn shock, fluid resuscitation as well as the potential for development of hypothermia are issues that were not in the scope of the present study but have to be taken into consideration.

A limitation of the present model, that yielded consistent burn lesions and progression, is that rodent skin differs in many aspects from human skin. Whereas the ischemic zone surrounding the thermally injured tissue on the surface is representing the zone of interest prone to progression (interspaces), the measurement of burn depth gives additional information. Burn deepening is more rapid since the burn injury is relatively severe and the skin in contrast to human skin relatively thin, non-adherent and contains a panniculus carnosus.

In conclusion, damage control in burn injury by prevention of burn progression has to be improved and should target the different mechanisms implied, mainly ischemia. Whereas the currently suggested first aid measure, i.e. application of cold water, delayed burn progression, warm water provided additional benefit with significantly improved interspace survival secondary to improved perfusion. The obtained delay in burn progression creates a therapeutic window for targeted intervention to prevent burn progression.

Conflict of interest statement

None.

Acknowledgment

The authors would like to thank Joanne Stalder (Division of Clinical Pathology, University Hospitals of Geneva, Geneva, Switzerland) for her excellent technical assistance, and Dr. Xavier Tenorio and Dr. Thierry Bezzola for their instructions.

The study was supported by the grant SNF 32-108408.05 entitled "pathophysiology, prevention and treatment of critical muscle and skin ischemia" granted to Y. Harder & R. Wettstein.

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