

Original Article

Limb cooling with targeted arterial infusion of cold fluid alleviates scald injury: an experimental rabbit study

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Abstract: Background: To investigate the cooling and healing effect of different modalities: Hydrogel dressing[®] was compared with targeted artery injection of low temperature liquid as a coolant following application to a fresh deep partial thickness hot water scald in a rabbit hind limb model. Materials and methods: Fifty five rabbits were randomly divided into 5 groups. Treatment group received femoral artery injection of low temperature liquid and hydrogel dressing post burn 30 min or 1 hour. Control group were just scalded. Subcutaneous (T_{sc}) and deep muscle temperatures (T_m) were continually monitored in all wounds. After scald the rectal temperature were detected within 6 hours. The wounds were biopsied for histological assessment at 72 h and 3 weeks. Results: Effective cooling of the burn wound and an increased rate of wound healing was achieved by both treatment methods. The final temperature at 1h decreased to the preburn temperature. Compared with hydrogel dressing group (T_m decreased by $1.3 \pm 0.4^\circ\text{C}$), T_m decreased by $2.8 \pm 0.3^\circ\text{C}$ in femoral artery injection group, showing significant difference ($P < 0.05$). Artery injection of low temperature liquid and hydrogel dressing almost exert no influence on rabbit core temperature. Clinical and histological assessment at 21 days indicated more rapid healing in both the 30 min hydrogel dressing and artery injection burns compared with the controls and the 60 minutes intervention groups. Conclusion: This result indicates artery injection of low temperature liquid earlier to cooling limb is an effective means to reduce residual heat damage tissue without affect core temperature and increase wound healing.

Keywords: Scald, cooling temperature, artery injection

Introduction

Severe burns remain a significant public health problem in industrialized and developing countries, in terms of incidence, cost, and severity [1, 2]. The risks of burn during combat are also well recognized, and are unlikely to diminish because of the rapid proliferation of new and powerful thermobaric weapons that target the inherent vulnerability of the soldier to sustain burns [3, 4]. Prior studies documented that immediate cooling could improve the tissue response to thermal injury, reduce aerobic metabolism, increase epithelial cell growth [5-7]. Immersion into cold water also significantly reduced pain and discomfort, and reduced the mortality of experimental full thick-

ness burn from 64.5 to 2.58% [7]. So ordinary tap water was recommended by the British Burn Association as the treatment of choice for the first aid management of burns and scalds [8].

However, in clinic there a lot of patients are not suitable for this treatment, for example, burned area $> 20\%$ TBSA, cooling large areas of skin can lead to hypothermia, especially in children and elderly patients who could not endure the low temperature stimulation, because cooling may interfere their circulation system [9], which will result in serious consequence of shock patients. It was found that some large area burned patients lost the chance of cooling treatment because of above reasons, which

resulted in severe consequence. Targeted coolant delivery [10, 11], therefore, may limit the distribution of coolant and thus greatly reduce the amount used of coolant and potential interfere of circulation system. Burned patients often need artery blood gas examination [12]. Femoral artery always was selected. Femoral artery supplied low limbs blood. It can serve as a targeted deliver to be used for cooling the target site.

In current study, we compared targeted artery injection of low temperature liquid with hydrogel dressing as a coolant following application to a fresh deep partial thickness hot water scald in a rabbit hind limb model to test a hypothesis that targeted artery injection of low temperature liquid can cool the wound and deep tissue.

Materials and methods

Animals and model implemented

The study was approved by the institutional committee for animal care in laboratory research. The animals were obtained from the Animal centre of experimental, the Fourth Military Medical University, China. Seventy New Zealand rabbits were used in the study. The animals were kept in separate cages under standard conditions and were fed with water ad libitum. Their average rabbit weight was 2.5 kg (range 2.5 ± 0.3 kg). Anesthesia was started with 3% sodium thiopentone (1 ml/kg) and maintained by intravenous doses (0.5 ml/h) administered through a catheter inserted into a marginal ear vein by micro pump (wzs-50 fz, China). The hind limbs of the rabbits were depilated before heated water was applied. The heated water was maintained at a temperature of 70°C thermostat-controlled water-bath (volume: 5 L) and applied for 10 s to create round deep partial thickness lesions of 3.3% TBSA at limb and superficial thickness lesions of 1% TBSA at footplate. All experiment work was carried out in a room at $24 \pm 2^\circ\text{C}$ maintained by air-condition (40~50% relative humidity). The deep of wound was identified by histological assessment. All animal experiments were approved by the Fourth Military Medical University on the Use and Care of Animals according to National Institutes of Health Guidelines.

Application of low temperature liquid and hydrogel dressing

There were six groups. Group A1 was scald control group. Group A2 was shame scald control group. Group B, C were treated with low temperature injection at 30 min or 1 h after scald. Group D, E were treated with HD-L type hydrogel dressing® (Changchun JA Biotech, China) 30 min or 1 h post-scald.

The wounded limb in Group B, C was cooled by 4°C balance salt solution (1.8 ml/kg TBSA) through the femoral artery. The low temperature liquid injection speed is 60 ml/min. The injection needle size is 26G. Group D, E were treated with hydrogel dressing at 30 min or 1 h. The hydrogel dressing uncovered after applied for 3 hours.

Temperature detecting

To detect the temperature of tissue, a standardized system was developed [13]. The thermocouple sensor was made like a needle (\varnothing 0.5 mm) which is convenience to punch into tissue. After anesthesia the small thermo detectors which were threaded into a sterile stainless steel trochar, were positioned two constant positions. One is under skin of intergroove between tibial muscle and gastrocnemius muscle (this temperature was named as T_u). The other is 1 cm lateral to prior intergroove and punch into the gastrocnemius muscle (this temperature named as T_m). All of them punctured through a small 0.1 mm incision, 1cm upper the edge of the burn mark line. The thermo detectors were maintained in place with a nylon non-absorbable suture threaded through a loop made in the thermo detectors. Then the temperature was detected throughout the experiment.

Histological evaluation

The wounds were biopsied within 72 hours after commencement of treatment. Thereafter all wounds were left exposed and allowed to heal spontaneously without any intervention. Biopsies were repeated at 3 weeks to assess the depth of each burn and measure the rate of wound healing. The biopsies were taken from standardized predetermined sites to limit histological sampling errors. The wounds were also assessed clinically by an independent surgeon.

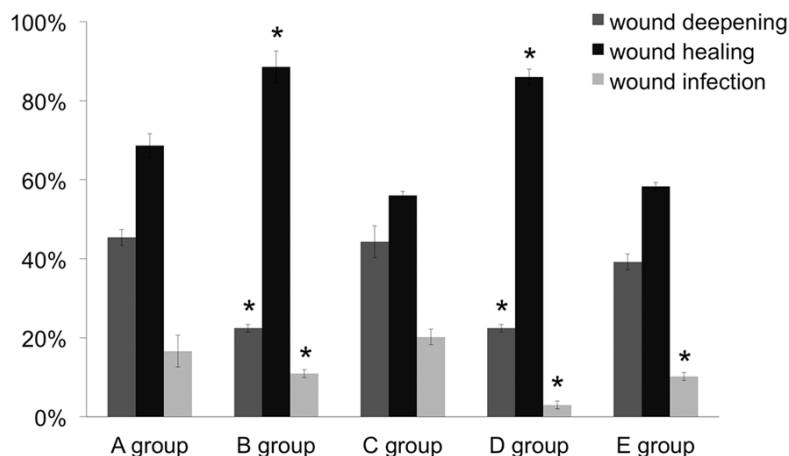


Figure 1. The assessment of the wounds after the scald for 21 days. There was significant difference between 30 min and 60 min groups in wound healing ratio ($P > 0.05$). Compared with groups A, C, E, there was significant difference in wound deep ratio in 30 min groups. Hydrogen treatment groups have a lower wound infection ratio compared with other groups. * $P < 0.05$.

The wounds were assessed clinically and histologically at 21 days. Factors assessed were: ratio of wound deepening, ratio of wound healing and ratio of wound infection.

Experimental procedure

An artery approach for the low temperature liquid we selected the femoral artery. After anesthesia, skin incision was made and the femoral artery was exposed. After infliction of the scald lesion and the cooling procedure, the burn wounds were treated with exposure. The lesions were excised at above time point and fixed in buffered formaldehyde.

Statistical analysis

Statistical analyses were completed using SPSS11. In all studies, a 2-way analysis of variance (ANOVA) for repeated measures with 2 within subject variables (time elapsed since application of cooling treatment; type of cooling) was used to determine statistical significance among groups for respective differences. For all analyses, P -values were calculated using the Greenhouse-Geisser epsilon correction for repeated measures. When statistical significance was determined for main or interaction effects, a Neumann-Keuls post hoc analysis was performed to locate significant differences when $P < 0.05$.

Results

Sixty five rabbits were available for assessment. Five rabbits died in the post-burn period from respiratory depression.

Assessment of skin-cooling properties

Hot water scald resulted in a mark temperature increase in group A. The T_u increasing rate was $6.1 \pm 0.4^\circ\text{C}/\text{second}$ and the top temperature reached $61.2 \pm 3.1^\circ\text{C}$. A sharp temperature decreasing followed scald stop. When the decreasing tendency became slowly, both T_u and T_m remained relative

higher than prior-burns. This higher lever temperature lasted 65.1 ± 4.6 min and then T_u and T_m decreased lower than prior-burn. T_m change delayed 3 ± 1 seconds compared with T_u .

There was significant temperature decreasing after cooling in both treatment groups. While there were no significant inter-group difference in ΔT_m or ΔT_u after application of the treatments. T_m was unexpectedly significantly lower ($2.8 \pm 0.3^\circ\text{C}$) in artery injection group compared with hydrogel dressing ($P < 0.05$). In hydrogel group T_m had a slight decrease after treatment ($1.1 \pm 0.2^\circ\text{C}$). There are significant difference of T_m between hydrogel groups and low temperature injection groups ($P < 0.01$). While no difference ($P > 0.05$) of T_u decrease between hydrogel groups ($2.6 \pm 0.2^\circ\text{C}$) and low temperature injection groups ($2.6 \pm 0.2^\circ\text{C}$). After cooling, the T_m was higher than T_u in both treatment groups, while the T_m was lower than T_u in A group.

After injection of low temperature liquid, there are significantly decreased T_u and T_m . In hydrogel dressing application groups, there was significant decrease in T_u , while a little changes in T_m .

Assessment of wound healing properties

While the rabbits survived for the duration of the experiment, wound sites were evaluated until post-scald 21 days. The control group and

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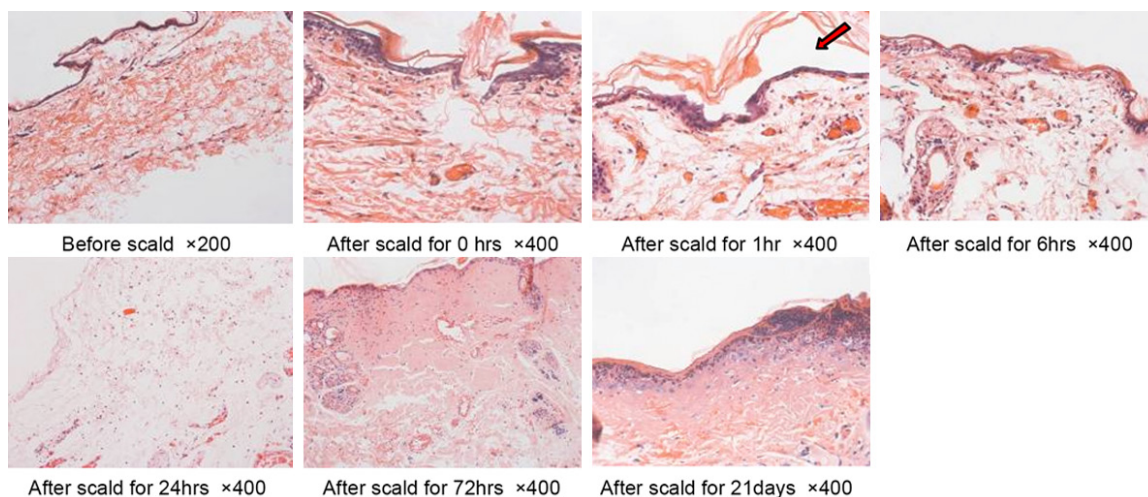


Figure 2. Histopathology of skin biopsy specimens of group A with HE staining at different time. Red arrow show there is local tissue exudation and big blister after scald 1 hour. Subcutaneous tissue loosened. Inflammatory cells infiltrated organization 24 hours after injury. When the wounds healing after the scald for 21 days, there are many inflammatory cells infiltrated organization. The wounds were covered by slough and granulation tissue and partial to no re-epithelialization had occurred in wounds. Viable adnexal structures were also absent in wounds.

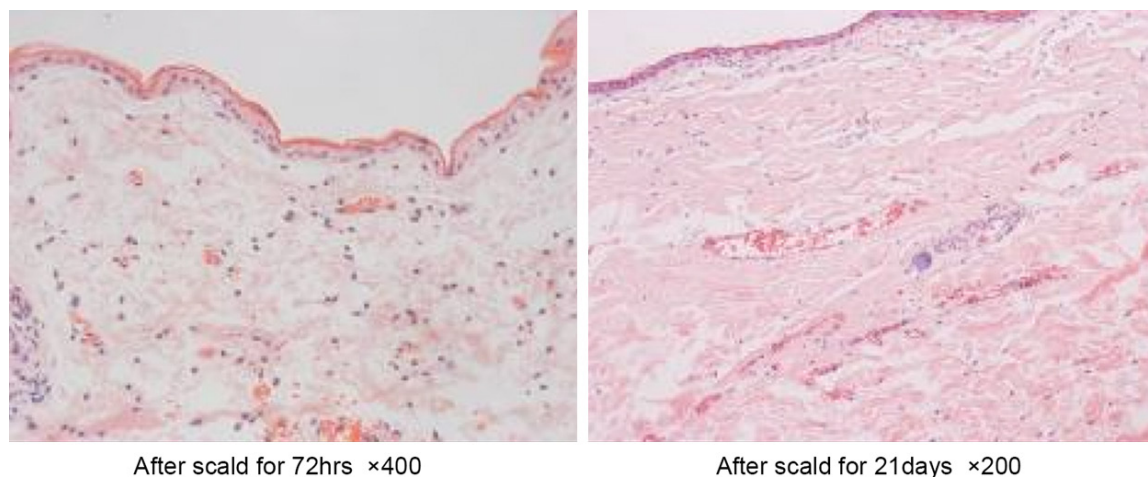


Figure 3. Histopathology of skin biopsy specimens of group B with HE staining at 72 hrs and 21 days. The wounds showed almost complete healing after the scald for 21 days.

60 min treatment groups have significantly less wound healing ratio and resulted bad outcome of wound healing after 21 days. In contrast, 72~89% of the wounds treated with the low temperature liquid and hydrogel dressing in 30 min had healed after 21 days (**Figure 1**). This healing rate was significantly different with control wounds and 60 min groups. Furthermore, 21 days later, wounds treated with the low temperature and hydrogel dressing in 30 min showed a lower wound deepening and infection rate than other groups ($P < 0.05$), suggesting a benefit resulting from the presence of the cool-

ing treatment within 30 min. The rate of wound infection in the hydrogel dressing groups was significantly lower in than A group and the artery injection group, $P < 0.05$. It is the reason that hydrogel dressing-covered wounds remained unchanged 6 hour post-scald.

Histological assessment

Histological samples taken 72 h post scald showed deep partial thickness were made in control groups. In A, C, E groups, tissue destruction was marked with coagulative necrosis of

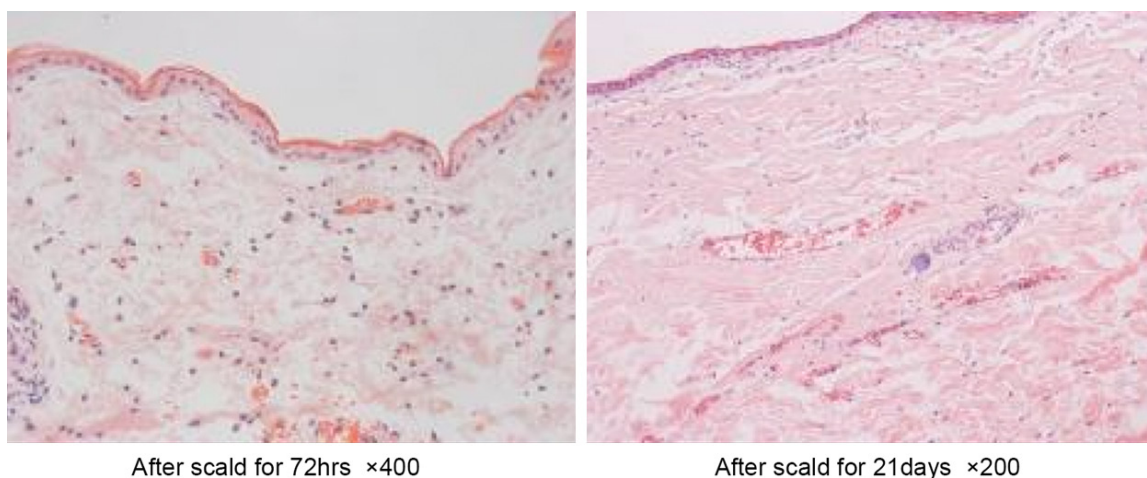


Figure 4. Histopathology of skin biopsy specimens of group D with HE staining at 72 hrs and 21 days. The wounds showed almost complete healing after the scald for 21 days.

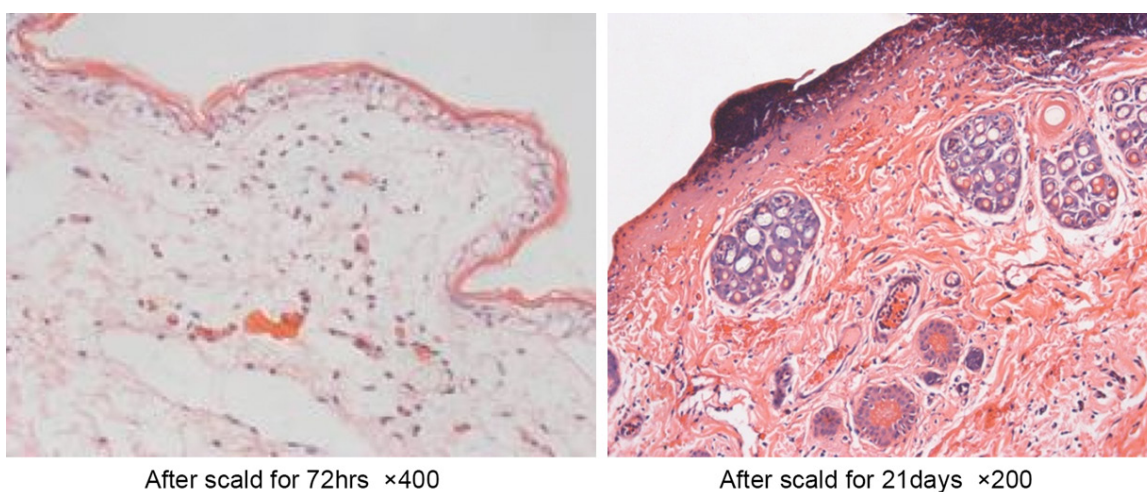


Figure 5. Histopathology of skin biopsy specimens of group C with HE staining at 72 hrs and 21 days. There are many inflammatory cells infiltrated organization. The wounds were covered by slough and granulation tissue and partial to no re-epithelialization had occurred in wounds. Viable adnexal structures were also absent in wounds.

the surface epidermis and superficial portion of the dermal appendages. Deep dermal structures and all fibroadipose tissue were viable after 21 days (**Figure 2**). In group B and D, wounds were all partially thickening. These histological changes reflect the influence of cooling therapy decreased the thermally induced tissue destruction. After 21 days, wounds showed almost complete healing in group B, D (**Figures 3, 4**). There was no histological difference between the injection cooling and hydrogel cooling groups, $P > 0.05$. In A, C and E groups, healing was noted to be poor. The wounds were covered by slough and granulation tissue and partial to no re-epithelialization

had occurred in wounds. Viable adnexal structures were also absent in wounds (**Figures 2, 5, 6**).

Scald treatment immediately induced small arteries and capillaries obvious vasodilatation. Mass of red blood cells filled with the vessel lumen. There was local tissue exudation and big blister after scald 1 hour. Subcutaneous tissue loosened. Inflammatory cells infiltrated organization 24 hours after injury. Artery injection of low temperature liquid and hydrogel dressing after 30 min showed the number of dilation of blood vessels significantly reduced. Both could significantly reduce local tissue

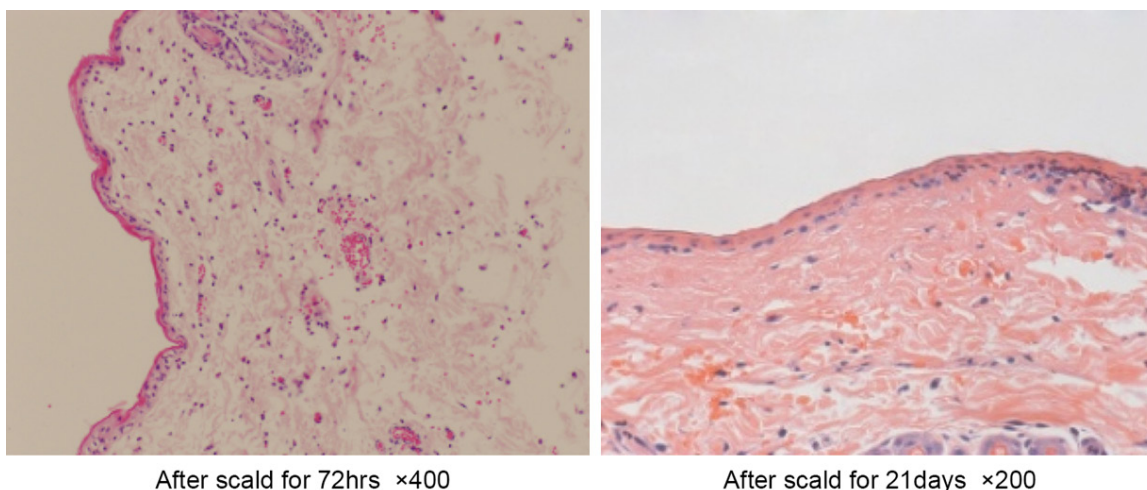


Figure 6. Histopathology of skin biopsy specimens of group E with HE staining at 72 hrs and 21 days. There are many inflammatory cells infiltrated organization. The wounds were covered by slough and granulation tissue and partial to no re-epithelialization had occurred in wounds. Viable adnexal structures were also absent in wounds.

inflammatory cell infiltration. After 21 days all the cooled wounds showed almost complete healing. There was no histological difference between the water cooled and Hydrogel 30 minutes cooled wounds. In group A, C and E wounds healing were poor. Seven out of 8 of the wounds were covered by slough and granulation tissue and partial to no re-epithelialization had occurred in 6/8 wounds. Viable adnexal structures were also absent in 5 wounds.

Discussion

Thermal injury is caused by heating tissues above a critical temperature at which the tissues are damaged [14]. The extent of a thermal injury is determined by temperature and mass of the burning object etc. Tissues of which water is the main component, like the skin, are characterized by high specific heat and low thermal conductivity. As a result, such tissues become overheated slowly, but their cooling is also slow [15]. The duration of overheating is thus longer than the action of the external burning agent. Our Lord study also testified the point that the temperature of T_u and T_m lasted higher lever 65.1 ± 4.6 min after scald and then decreased lower than prior-scald. So cooling the burn tissue as sooner as possible is regarded as a very important treatment of choice for burn care [5, 6, 16, 17].

Cooling the burn surface is one of the oldest methods of treatment [18]. Study shows imme-

diate cooling burn surface cannot only stop the heat continually damage, but also improve the tissue response to thermal injury. Immersion into cold water also significantly reduces pain and discomfort and reduced the mortality of experimental full thickness burn from 64.5 to 2.58% [7, 15, 18, 19].

There are many ways to cooling the burn wound such as shower, immerse, tap-water, cooling dressing and so on. But all of them are surface cooling. Nowadays, artery injection technique was used in many fields. In our research field there is an important example that is intra-arterial infusion calcium solutions to the extremities for hydrofluoric acid burns treatment [20]. The benefit of this technique is treatment can quickly and directly be applied to target tissue. Apart from above it is a kind of targeted delivery of treatment agent to the site of burn and can greatly reduce drug used amount [21]. Enlighten by this, we designed artery coolant delivery system.

The optimum temperature at which to achieve maximum benefit remains undetermined. Experiments have been conducted with temperatures ranging between -6 and $+12.6^{\circ}\text{C}$ [7]. These temperatures refer to the surface temperatures of the coolant and the effect on intra-dermal temperatures is not recorded.

However, cold water compresses at $1-8^{\circ}\text{C}$ were shown to confer no benefit to burned skin as

the corresponding intradermal temperatures of between 13.5°C and 15.5°C cause extensive cryodestruction leading to more tissue damage and delayed healing in comparison to wounds not cooled or cooled with water temperature between 12 and 15°C [22]. Though our study used 4°C liquid, but in our preliminary experiment we find the minimum temperature of abdominal aorta blood and 4°C cool water mixture is $6.2 \pm 0.5^\circ\text{C}$ (injection speed is 60 ml/min). And the effect of artery coolant infusion is short time. Thus in the conducted experiment we chose liquid at approximately 4°C as the most practical and useful coolant.

Our study showed that after scald the temperature of under skin and deep tissue kept a higher level temperature about 65.1 ± 4.6 min. So the cooling treatment may take effect for scald within 1 hour.

The early treated wounds consistently demonstrated more advanced healing. At 21 days almost complete healing was evident in most treatment group wounds. Less wound deepening and infection in treatment groups than control group. In 30 min cooling groups, the wounds both clinically and histologically showed limited damage and advanced healing. These findings have obvious clinical implications. However, due to the dynamic and progressive nature of local tissue destruction, we believe that therapy must be instituted as soon as possible to be of any value. There are many questions must to be faced that low temperature injection liquid is readily available as a compact, easily transportable, available in different sizes, time-consuming to perform femoral artery catheterization. Anyway, this technique is expected to be a good alternative to cool the limb scald patients. Although our study clearly demonstrated the beneficial effects of cooling the partial thickness burned wound for at least 30 min by means of either cold liquid injection or with hydrogel dressing, but the optimal rate and volume of artery injection are not researched.

In summary, these data show that artery injection of low temperature liquid is a new effective modality of cooling scald limb in promoting initially faster wound healing, and providing a transient, cooling effect to the limb skin without the adverse effect of inducing whole-body hypothermia like ordinary cooling method. But further studies are necessary to evaluate the

side effect such as heart rate, blood pressure and oncotic pressure change. Further clinical studies are required to confirm the efficiency of low temperature liquid as an emergency topical antithermal agent.

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Disclosure of conflict of interest

None.

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