BURNS 41 (2015) 882–889

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/burns

Review

Cooling of burns: Mechanisms and models

E.H. Wright [a,*], A.L. Harris [b], D. Furniss [c]

[a] Department of Plastic Surgery, Stoke Mandeville Hospital, and the Department of Oncology, University of Oxford, Green Templeton College, 43 Woodstock Road, Oxford OX2 6HG, United Kingdom
[b] Medical Oncology, Department of Oncology, University of Oxford, Green Templeton College, 43 Woodstock Road, Oxford OX2 6HG, United Kingdom
[c] Department of Plastic Surgery, Oxford University Hospitals, and the Botnar Research Centre, University of Oxford, Green Templeton College, 43 Woodstock Road, Oxford OX2 6HG, United Kingdom

ARTICLE INFO

Article history:
Accepted 10 January 2015

Keywords:
Burn
Cooling
Model
Animal
Human
Mechanism

ABSTRACT

The role of cooling in the acute management of burn is widely accepted in clinical practice, and is a cornerstone of basic first aid in burns. This has been underlined in a number of animal models. The mechanism by which it delivers its benefit is poorly understood, but there is a reduction in burns progression over the first 48 h, reduced healing time, and some subjective improvements in scarring when cooling is administered after burning.

Intradermal temperature normalises within a matter of seconds to a few minutes, yet the benefits of even delayed cooling persist, implying it is not simply the removal of thermal energy from the damaged tissues. Animal models have used oedema formation, preservation of dermal perfusion, healing time and hair retention as indicators of burns severity, and have shown cooling to improve these indices, but pharmacological or immunological blockade of humoural and cellular mediators of inflammation did not reproduce the benefit of cooling.

More recently, some studies of tissue from human and animal burns have shown consistent, reproducible, temporal changes in gene expression in burned tissues. Here, we review the experimental evidence of the role and mechanism of cooling in burns management, and suggest future research directions that may eventually lead to improved treatment outcomes.

Contents

* *Corresponding author*. Tel.: +44 07808933799.
E-mail address: edmund.wright@oncology.ox.ac.uk (E.H. Wright).
http://dx.doi.org/10.1016/j.burns.2015.01.004
0305-4179/

1. Introduction

1.1. *The basic model of a cutaneous thermal injury: three zones*

The classical model for a burn was proposed by Jackson in 1953 [1], and comprises three concentric zones of injury: a central zone of necrosis, a surrounding zone of stasis, and then a further zone of hyperaemia surrounding the zone of stasis.

The model was derived from observations of human burns and their progression to healing. The necrotic area was coagulated tissue that progressed to form an eschar. The hyperaemic zone always healed rapidly. The zone of stasis between these two zones derived its name from the lack of circulation within the dermal capillaries. This was shown pathologically by erythrocytes packed in the superficial plexus, whilst clinically the skin blanched on pressure. Furthermore, there was reduced tissue oxygen consumption, shown clinically by the lack of cyanosis of the tissue if the limb had a tourniquet applied. This area could progress to full thickness necrosis, or re-reepithelialise from the skin appendages.

In humans, this progression takes place over 24–48 h post-burn. Longitudinal histological studies have demonstrated progressive deepening of the level of the zone of stasis as evidenced by the deepest occluded capillary over the first 48 h following a burn [2]. As this is after the dermal temperature has returned to normal (*vide infra*), it indicates that the ongoing damage is mediated by other mechanisms that have been set in motion by the thermal injury, but continue long after the thermal energy has dissipated.

Salvage of the zone of stasis is an area of great interest in the management of burns as this represents an element of the burn where intervention by the Burn Surgeon may influence the outcome in terms of healing and scarring. A productive approach is to identify interventions that empirically improve survival of the zone of stasis within the burn, validate these improvements with objective, quantifiable assays, and then seek ways to mimic or augment these interventions' effects in the management of burns.

1.2. *Cooling of burns: traditional medicine, basic science and first principles*

Conventional first aid treatment of burns involves irrigation of the affected area with cool water, and this has been advocated since Galen (AD129-199), Rhazes (AD 852–923) and Earle (1799). As long as the temperature remains above 44 °C, the burning continues [3]. There are numerous anecdotal accounts of prompt cooling of the burn resulting in reduced scarring or reduced mortality [4]. Such accounts are cited in the introduction of one of the first published cooling experimental models [5]. A case is cited in which a scald to the upper limb in a young girl sustained by immersion in boiling milk was immediately cooled to the elbow in icy water in accordance with Scandinavian traditional medicine. The scarring was much reduced below the elbow compared to above. It was postulated that the cooling had a beneficial effect by reducing the amount of thermal energy imparted to the tissues and so reduced tissue damage to the cooled areas.

Work with eggs found that cooling eggs previously immersed in boiling water in cool water resulted in less coagulation of the contents compared to those cooled in air, an effect exaggerated by the application of cloth wrappings to simulate clothing [5]. This supported the theory that the amount of heat delivered, and the rate of dissipation to a temperature below the critical threshold for tissue damage determined the severity of the burn. However, when the same study created scalds to the tails and backs of rats, cooling at low temperatures resulted in high mortality, even with tail scalds only representing a few percent total body surface area (TBSA), but better results in terms of histological necrosis and oedema. It was concluded that a balance was required between hypothermia and cooling the burn.

A retrospective study in humans has reflected this by demonstrating the tendency for scalds caused by more viscous liquids and with more delay to cooling – therefore with more prolonged contact and more sustained energy delivery – to be more likely to cause a burn requiring skin grafting, a marker of increased burn depth [6]. While an human model for CWT for burns showed only analgesic benefit [7], the choice of burning at 65 °C for 15 s would probably create full-thickness burns not responsive to cooling [8], and retrospective case series have shown that CWT reduces the need for grafting [4,9–11].

In this review, we will first summarise the evidence regarding the thermodynamic and molecular effects of cooling burns. Next, we will review the literature regarding cellular, humoral and gene expression changes in burns. Finally, we propose directions for future research that combines these two areas, and may produce novel treatments aimed at improving the outcome for patients with intermediate thickness burns.

2. Measurements of burn severity and the effects of cooling

2.1. Kinetics of dermal cooling

The effectiveness of cooling of a burn has been measured by the rate at which the dermal temperature returns to physiological or sub-physiological levels, indicating that the heat energy has been dissipated and is no longer damaging the tissues. This has been achieved by the placement of thermocouples within the dermis of the experimental animals' skin directly under the area where the burn is to be created allowing serial real-time temperature readings to be recorded.

In a rat model where a plasma-burner was used to create a full-thickness dorsal burn, dermal temperature returned to 40 °C from a peak of 100 °C within 4 min without cooling, while those burns cooled with ice until "at near-normal temperature" achieved the same temperature reduction within 2 min [12]. In porcine scald model that utilised water at 85 °C to produce a partial thickness burn, Jandera et al. found dermal temperature fell to below 40 °C in both non-cooled and in immediately water-cooled burns within 2 min of burning [13]. In another porcine scald model, again with water at 85 °C, all scalds had returned to below 40 °C within 10 min, whether cooled or not [14], while others reported intradermal temperatures of returning to below 40 °C within 2 min regardless of cooling, or the modality in which it was delivered [15–18]. A guinea pig model found the dermal temperature returned to normal at 30 min post-burn. A limited, single cohort study reported benefit in terms of analgesia and healing with CWT administered in hospital—by definition delayed [9].

Thermodynamic calculations of the reduction of Arrhenius tissue damage have shown in a standardised burn that the improved removal of thermal energy by water compared to air cannot account for the benefit of CWT [19], and CWT experiments listed above point towards a change in cell behaviour, and hence changes in gene transcription and translation. One- and two-dimensional modelling of a contact burn and subsequent water cooling support this finding [20], as do empirical models discussed previously.

Taken together, these data demonstrate that intradermal temperature returns rapidly to normal in a variety of experimentally produced burns. This period of time has elapsed before many patients can initiate cooling of their own burns, suggesting that the benefit of cooling is not purely heat dissipation. It also highlights the difficulty in producing a comparable burn model when using similar, and more so when using different animals.

2.2. Oedema and cooling

In animal models, oedema has become an accepted indicator of tissue damage from a thermal injury in the acute phase. Animal models have utilised either the hind paws for volume measurement using the mercury displacement technique [21], or the ears for wet-dry tissue mass ratio to calculate total water content of the tissues [22]. The former method had the advantage of allowing serial measurements over time, while the latter required sacrifice of the animal. These models also suffered the restriction that the burn had to be created in such a way as to avoid blistering, as this interferes with the measurement of oedema.

In 1979, it was demonstrated in a sheep model that immediate cooling reduced acute oedema, but cooling delayed by 2 min increased oedema compared to no cooling [23], supporting the thermal energy removal mechanism hypothesis, as temperatures rapidly return to normal after a thermal injury. Blomgren et al. found that application of water at 8 °C for 30 min immediately following the scald was the most effective in oedema reduction when compared to other durations [5]. A scalded rat paw model showed that best reduction in oedema was achieved with 0 °C Ringer's solution for 120 min, with 20 °C Ringer's solution worsening oedema compared to controls, possibly from a hyperaemic response [24].

Overall, these data, with improved results from the lowest temperatures of cooling, support the removal of heat from the tissues as the responsible mechanism for oedema reduction. However, this does not correlate to the findings of the studies that examined different modalities of and delays to cooling that found there was still benefit to healing with delayed cooling [18], and that ice was detrimental to healing compared to no cooling at all [14].

In order to validate the role of increased capillary leak, leading to oedema and burn progression, studies have attempted to pharmacologically mimic cooling. Boykin et al., utilising the hairless mouse ear model, showed reduced burn oedema at 2 h with cold water treatment (CWT), and that CWT reduced histamine loss from the burned tissues, with a subsequent reduction of peripheral oedema. This effect was recapitulated by administration of cimetidine, and to some extent indomethacin. There was no histological difference between CWT and control ears at 2 h. Interestingly, a mouse ear is only 7% TBSA and yet a scald to this area had an appreciable effect on oedema distant to the burn [25], and the systemic effects of burns need to be carefully considered when selecting control areas.

A study of much larger TBSA burns (40–50%) in guinea pigs found a reduced level of circulating histamine and improved physiological parameters in CWT animals compared to controls, in a manner similar to pre- and post-burn treatment with cimetidine [26]. Burn oedema in the rat paw was shown to be reduced by cimetidine, superoxide dismutase (SOD), catalase (CAT), and hydrocortisone, but worsened by albumen administration, indicating the involvement of reactive oxygen species as well as histamine in the pathophysiology of burns, and demonstrating a role for increased capillary leak in the generation of burn wound oedema [27]. However, another study found no benefit to cimetidine treatment in terms of oedema formation in all tissues, and that high doses were harmful [28]. The harmful effects of high-dose cimetidine were attributed to its cardio-depressive effects, and the larger TBSA compared to the other studies. A human study found no benefit from antihistamines in the healing of experimental burns [29].

In all, these findings show that histamine release from burned tissue has a role in the pathophysiology of burn wound oedema, but do not support a pivotal role for histamine suppression in mediating the effects of CWT, as the use of cimetidine did not reproduce the effect of cooling.

2.3. Cooling, dermal perfusion and microvasculature

Oedema can be thought of as a surrogate marker for tissue injury, but in fact oedema has been shown to have a poor correlation to histological signs of tissue damage [30], and reduction in oedema does not explain the benefit of cooling, as discussed above. Thus, further studies have assessed the effects of burning and cooling on the histology of the burnt tissue. Histological changes can be seen in the tissues: capillary damage as evidenced by the intercellular junctions forming gaps in the capillary walls; distinct spaces in the extracellular matrix; and neural destruction as evidenced by necrosis can be seen at 15 min following a deep burn. Furthermore, cytoplasmic damage with evidence of the venular endothelial cytoplasm becoming increasingly granular, organelle disruption and absence of micropinocytotic vesicles can be seen within 4 h of a deep burn [31].

Video microscopy of bat-wing microvasculature demonstrated vascular dilatation and stasis within the zone of thermal injury and leukocyte extravasation with platelet and erythrocyte clumping. CWT reduced stasis, oedema, and haemorrhage seen at 24 h post injury when compared to controls [32]. In a mouse ear model, the three zones of the burn described by Jackson were demonstrated using real-time video microscopy and the progressive occlusion of 10–12 μm vessels leading to a 10-fold increase in the zone of necrosis [33]. Erythrocyte aggregation was seen immediately, followed at 8–12 h by leukocyte adhesion, with platelets forming occlusive plugs between 90 min and 48 h [34]. In a mesenteric microvascular model for systemic burn oedema in rats, leukocyte adhesion was found to be significantly increased at 30–60 min after a 20% TBSA cutaneous burn and still above baseline at 6 h, underlining the difficulty in obtaining within-animal controls with large TBSA burns. Furthermore, an increase in vascular resistance combined with increased capillary permeability, mediated by circulating chemokines such as histamine, increased capillary leak [35]. A study in guinea pigs identified immediate cooling with ice water as improving hair follicle retention and dermal perfusion at 16 h as measured by India ink perfusion [36].

A variable delay prior to cooling thermal injuries in guinea pigs has also been investigated. Raine et al. found that the benefit of cooling to the preservation of dermal perfusion, as measured with India ink perfusion and Xenon washout, was significant with delays of up to 30 min before CWT commenced. Guinea pigs with up to 30 min delay to cooling healed with less scarring and more hairs than either untreated controls, or those waiting 60 min for cooling [37]. At 30 min the dermal temperature has returned to normal (*vide supra*), and the benefit of cooling cannot be explained by the normalisation of tissue temperature. While the benefit of faster cooling, as would be achieved by a lower-temperature coolant holds for a model of heat energy removal from tissues, given that delaying cooling until tissues are normothermic is still beneficial, and cooling with ice has an detrimental effect on burn healing, the empirical evidence is that heat removal is not responsible for the beneficial effect of cooling a burn.

This evidence of microvascular occlusion has lead researchers to investigate the effects of anti-thrombotic agents. The effect of heparin on this progressive dermal ischaemia was investigated by Robson et al. using a guinea pig model and India ink perfusion, with no improvement of dermal perfusion between treatment and control groups [38]. Similar work using guinea pigs and the antithromboxane agents imidazole, dipyradamole, and methimazole found improved dermal perfusion in vivo and with India ink injection studies when compared to controls, and suggested a beneficial role in the reduction of Thromboxane A2 activity. This was confirmed with immunohistochemistry in the same specimens [39]. However, the effect of a highly selective thromboxane synthase inhibitor Dazmegrel did not improve dermal perfusion when administered either systemically or topically in guinea pigs, but in low doses did improve healing at 22 days [40]. This could indicate that Dazmegrel is acting directly on the burned tissues, rather than improving healing by maintaining microvascular patency, by reducing progressive necrosis, and preserving epidermal appendages from which re-epithelialisation can begin.

2.4. Cooling and healing

Ultimately, the key outcome in treatment of burns is healing of the burn with minimal scarring. Therefore, the effects of timing, temperature, and modality of cooling have been investigated with healing as an endpoint. In a rat model previously discussed, immediate cooling using ice reduced the area of unhealed wound at 43 days compared to untreated controls [12].

A porcine model compared immediate cooling to cooling initiated after 30 min. Cooling was administered with abdominal swabs soaked in water at 14–16 °C or gel treatment each applied for 1 h. Here, it was found that the temperature of all burns, including the untreated control, were below 40 °C within 2 min, yet cooling after a 30 min delay was still beneficial. Burns treated with CWT healed better than those subjected to cooling by gel treatment [13]. Another porcine study of different modalities of cooling found running water at 22 °C was superior to water sprayed onto the burn every 30 s, or wet towels changed every 3 min in terms of burn propagation and healing [15].

When different temperatures of CWT, delivered as wet towels at predetermined temperatures changed every 3 min were compared in a porcine model, it was found that iced water at 1–8 °C was more detrimental than non-treatment, CWT at 12–18 °C lead to intermediate healing results, and the best healing was demonstrated when there was a 30 min delay before 3 h CWT at 16–18 °C, again not in keeping with simple removal of thermal energy [14]. A further porcine study of optimal duration of CWT at 22.4 °C found 20 min most beneficial in terms of histological burn depth, with 5, 10 and 30 min less effective. There was no difference in healing time, possibly because the burns were all superficial partial thickness [16]. In another porcine study of temperature of CWT, burns treated for 20 min with CWT running across the wound at 1.6 l/min at 15 °C healed with less scar contracture than burns treated with either ice granules applied directly to the skin, or CWT with water running at 1.6 l/min at 2 °C, although there were problems creating a consistent, reproducible burn [17]. Another porcine model found no difference in histological appearance of the tissue at 9 days between

burns cooled immediately, and at 5, 20 and 60 min delay for 20 min with tap-water at 22.4 °C [18].

Taken together, these studies demonstrate the importance of cooling when using an outcome relevant to patients. Furthermore, they emphasise that normalisation of dermal temperature is not the sole mechanism mediating the effects of CWT, and further investigation of this mechanism might yield therapeutic targets for novel burns treatments. Finally, the studies demonstrate that developing a model of consistent burn depth, consistent cooling temperature, delay and duration, will be important in future experimental work. The burn depth in such a model should be mid-dermal, representing the depth of burn most likely to benefit from intervention in humans.

3. Mechanisms of burn pathophysiology

The above literature point to mechanisms not related to thermal energy dissipation mediating the effects of CWT. It is therefore important to understand the cellular pathophysiology of burns at the gene, protein and cellular level, in order to facilitate the identification of potential therapeutic targets.

3.1. *Humoural and cellular mediators*

The role of leukocytes in the pathophysiology of burn has already been alluded to above [34,35]. These cell populations respond to inflammatory mediators and chemotactic factors that have been investigated in animal models. When the hind paws of irradiated rats were scalded there was no reduction in the volume of oedema compared to rats with normal numbers of neutrophils, implying the neutrophils do not have pivotal role in burn oedema [41]. In a rabbit model, anti-ICAM and anti-CDw18 monoclonal antibodies were used to block neutrophil adhesion to the vascular endothelium, and resulted in improved perfusion of the zone of stasis, and less progression of the zone of stasis to necrosis [42]. Taken together, these results again dissociated burn oedema from tissue damage, but implicated neutrophil adhesion to vascular endothelium in the progression of the zone of stasis.

One of the main modes of action of the neutrophil is to produce reactive oxygen species via the NADPH pathway, and this was investigated in guinea-pigs treated with allopurinol, DMSO, and SOD prior to creation of a 5% TBSA burn. No difference was found in the rates of healing, hair retention, or de-differentiation of skin appendages, suggesting that the reactive oxygen species do not play a major role in burn [43].

In a rat model, complement activation, the formation of C5a, and neutrophil chemotaxis following a burn to the hindquarters was significantly reduced by SOD and CAT, DMSO and DMTU, allopurinol, lodoxamine and deferoxamine, but neutrophil depletion had no effect on C5a levels, indicating that reactive oxygen species originating from the xanthine oxidase pathway are responsible for the activation of C5 to C5a [44]. In a rat model, NK1 receptor antagonist SR140333 inhibited thermal oedema formation, but had no effect on neutrophil accumulation in the tissues [45].

Overall, it would seem that free radicals play an important role in burn pathology, and are responsible for complement activation, but do not originate from neutrophils, which in turn play an important role in burn progression, implying the parallel actions of different humoural and cellular pathways, which synergise in burn pathology. Simply blocking one pathway does not halt the progression of burns. While none of the studies above compared the various interventions to CWT, the fact that they did not replicate the benefits of CWT in different studies, but using similar animals would indicate that CWT does not effect its benefits by blocking the single cellular or humoural event in question.

3.2. *Burns and gene expression*

There exist in the literature some pilot studies of gene expression in burns in both humans and animal models. A rat model has shown that modest systemic hypothermia of 31–33 °C core temperature reduced the progression of ''second degree'' burns as measured histologically, even when delayed by 2 h, and had a consistent and reproducible down-regulatory effect on the expression of matrix metalloproteinase 9 (MMP9) mRNA, while up-regulating the expression of CXCL13, lipopolysaccharide binding protein, CCL6 and CCL24. These molecules have important functions in B-cell maturation, reduction of endotoxin load and improved bacterial opsonisation; keratinocyte proliferation; and collagen synthesis and deposition by fibroblasts [46]. This model used within-animal control areas, and it is worth noting that previous studies found small %TBSA burns had a systemic effect on tissue oedema, which could confound their conclusions [25].

As we are concerned with the management of human burns, human burn tissue is of the greatest interest in terms of genetic expression. In a comparison between normal skin excised from unburned patients in the course of cosmetic procedures, and debrided deep partial and full thickness burns at up to 17 days post injury, burned tissues showed expression of genes related to apoptosis, differentiation, and proliferation and healing were up-regulated, with temporal differences when the burns were grouped in 0–3, 4–7 and >7 days [47]. This study pooled patients' samples to neutralise individual responses, and the burn group was extremely heterogeneous with burns of 2–80%, and variable thickness. It is also unclear which parts of the burn were sampled. Nonetheless, it demonstrated proof of concept that it is feasible to perform genomic analysis on burned human skin, and that there are changes in gene expression over time. A diagrammatic summary of upregulated genes is given in Fig. 1. A further study of microRNA in three human burns showed significant differences in microRNA in denatured dermis from the extremities and normal skin left over from split skin grafts taken from the trunk, with possible effects on Wnt, VEGF and MAPK. These burns were traumatic, and the samples were collected at 4 days post injury, with possible confounding variables in the site of the burns and the control skin, mechanism of injury, and anatomical sites of the burn, but may nonetheless point to the role of gene expression and post-transcription modification in burns pathophysiology [48].

A human study of skin excised from burn patients in the course of their normal surgical treatment examined protein expression with mass-spectrometry and 2D-difference gel electrophoresis. Normal skin from breast reduction surgery

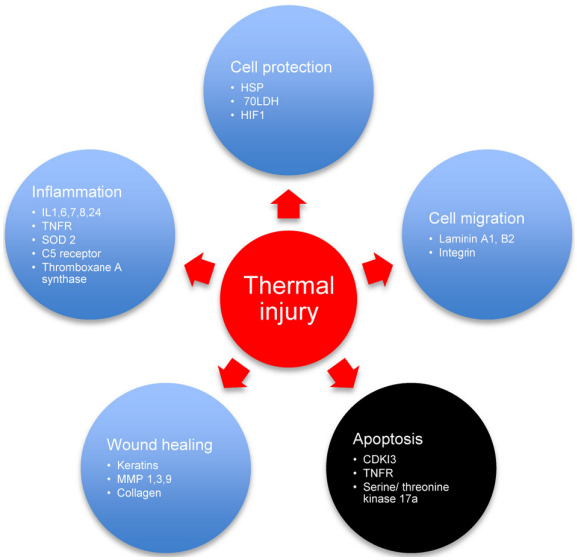

Fig. 1 – Gene expression significantly upregulated in burned human skin.

was used as a control, and the burns were again grouped as 0–3, 4–7 and >7 days. Heat shock protein (HSP) 90 and lactate dehydrogenase B were up-regulated in all groups. Haptoglobulins were also upregulated, including two associated with squamous cell carcinoma (SCCA 1 and 2) and a leukocyte elastase inhibitor (SLPI). A protein associated with migration and proliferation (Gelsolin) was down-regulated, while the cancer-associated protein 40S ribosomal SA (34/67-kDa laminin receptor) was increased in all burns compared to controls. GTPase activating protein IQGAP1 was upregulated in all burns. Keratin 1 decreased post-burn, while two isoforms of keratin 5 were increased, as were nine isoforms of keratin 6. Again, these data show that there is a definable change in protein expression in burns, with a temporal pattern fitting to the known pathophysiology of burns wounds. HSPs, those genes associated with anaerobic metabolism (LDH), and protease inhibitors – known to be essential in wound healing – are all altered in burns [49], yet novel pathways of potential biologic interest have also been discovered [50]. None of these studies investigated the effects of cooling. Burns that are excised are by definition deep, have not benefitted from CWT, even if it was administered, and conclusions as to gene-expression modification in human burns mediated by CWT cannot be reached from these studies alone.

4. Discussion

These studies have established that there is a clinical benefit in terms of healing [9] and reducing the need for surgery [10,51] from the administration of CWT to superficial burns, and that these benefits have been reproduced in some animal studies, when the correct burn depth and modality of CWT has been selected [13–15,46,52]. The mechanism by which the benefit is delivered is not thermal energy removal, or oedema reduction, and pharmacological antagonism of histamine release has been ineffective in both animals [25,27,28] and humans [29]. Many of the other interventions discussed above have not been compared directly to CWT, but have not reproduced the benefits of CWT in other studies of the same animal types. The small-animal models suffer from contamination of the control areas by the systemic effects of the burns that represent a significant %TBSA [22,30,35]. Human models have all used deep burns, either through the methodology of the experiment [7,29], or the fact that the patients were undergoing debridement of deep burns prior to skin grafting [48,50]. This limits what can be learned from the results in terms of how CWT improves outcomes for partial-thickness burns. However, these studies have demonstrated proof of concept that it is possible to extract RNA and proteins from burns, and that there are consistent temporal changes seen in these tissues. Further characterisation of these changes through the study of

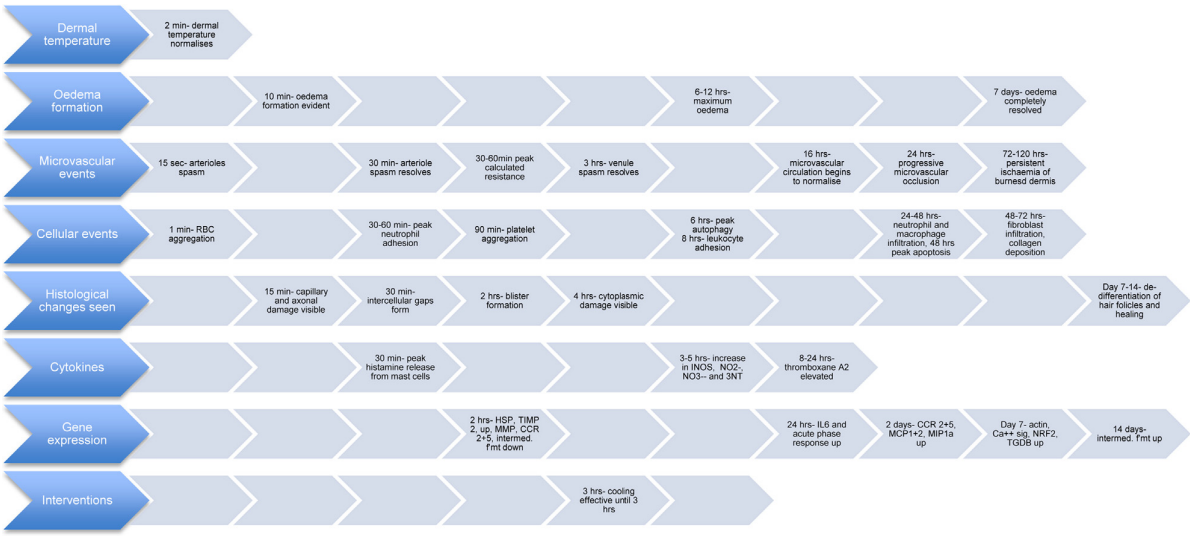

Fig. 2 – Summary of the temporal sequence of events in burns across the published models of thermal injury.

controlled burns in normal human skin may reveal therapeutic targets for the management of burns. This will need careful ethical consideration, study design and patient recruitment, but the scientific feasibility of such work has been demonstrated in previous works cited in this review.

5. Conclusions

In summary, the above work has established some important principles. Firstly, the benefit of CWT has been established in clinical experience, and proven in animal models. Secondly, the benefit of CWT cannot be explained purely on the basis of removal of thermal energy from the damaged tissues. Thirdly, there are important cellular and humoral mediators involved in burn pathophysiology, but modifying these individually does not affect burn progression in animal models. Finally, there are changes in gene and protein expression in response to thermal injury, and the pattern of expression changes over time from burn. The events in these systems that have been elucidated from various human and animal models are summarised in temporal relationship to one another in Fig. 2. Integration of these findings in further experimental work in healthy human volunteers could yield valuable therapeutic targets for future burn treatment.

REFERENCES

[1] Jackson DM. The diagnosis of the depth of burning. Br J Surg 1953;40:588–96.
[2] Watts AM, Tyler MP, Perry ME, Roberts AH, McGrouther DA. Burn depth and its histological measurement. Burns 2001;27:154–60.
[3] Moritz AR. Studies of thermal injury III. The pathology and pathogenesis of cutaneous burns an experimental study. Am J Pathol 1947;23:915–41.
[4] Hodson AH. Treating burns by initial cooling. J R Soc Med 1992;85:121.
[5] Ofeigsson OJ. Observations and experiments on the immediate cold-water treatment for burns and scalds. Br J Plast Surg 1959;12:104–19.
[6] Chiu TW, Ng DCK, Burd A. Properties of Matter matter in assessment of scald injuries. Burns 2007;33:185–8.
[7] Raghupati N. First-aid treatment of burns: efficacy of water cooling. Br J Plast Surg 1968;21:68–72.
[8] Bull JP. Burns. Postgrad Med J 1963;39:717–24.
[9] Shulman AG. Ice water as primary treatment of burns. JAMA 1960;173:1916–9.
[10] Nguyen NL, Gun RT, Sparnon AL, Ryan P. The importance of immediate cooling—a case series of childhood burns in Vietnam. Burns 2002;28:173–6.
[11] Cuttle L, Pearn J, McMillan JR, Kimble RM. A review of first aid treatments for burn injuries. Burns 2009;35:768–75.
[12] Moserov J, Behounkova E, Prouza Z. Subcutaneous temperature measurements in a thermal injury. Burns 1975;1:267–8.
[13] Jandera V, Hudson DA, de Wet PM, Innes PM, Rode H. Cooling the burn wound: evaluation of different modalities. Burns 2000;26:265–70.
[14] Venter THJ, Karpelowsky JS, Rode H. Cooling of the burn wound: the ideal temperature of the coolant. Burns 2007;33:917–22.
[15] Yuan J, Wu C, Holland AJ, Harvey JG, Martin HCO, La Hei ER, et al. Assessment of cooling on an acute scald burn injury in a porcine model. J Burn Care Res 2007; 28:514–20.
[16] Bartlett N, Yuan J, Holland AJ, Harvey JG, Martin HCO, La Hei ER, et al. Optimal duration of cooling for an acute scald contact burn injury in a porcine model. J Burn Care Res 2008;29:828–34.
[17] Cuttle L, Kempf M, Kravchuk O, Phillips GE, Mill J, Wang X-Q, et al. The optimal temperature of first aid treatment for partial thickness burn injuries. Wound Repair Regen 2008;16:626–34.
[18] Rajan V, Bartlett N, Harvey JG, Martin HCO, La Hei ER, Arbuckle S, et al. Delayed cooling of an acute scald contact burn injury in a porcine model: is it worthwhile? J Burn Care Res 2009;30:729–34.
[19] Van de Sompel D, Kong TY, Ventikos Y. Modelling of experimentally created partial-thickness human skin burns and subsequent therapeutic cooling: a new measure for cooling effectiveness. Med Eng Phys 2009;31:624–31.
[20] Ng EYK, Chua LT. Comparison of one- and two-dimensional programmes for predicting the state of skin burns. Burns 2002;28:27–34.
[21] Arturson G, Jakobsson OP. Oedema measurements in a standard burn model. Burns 1985;12:1–7.
[22] Blomgren I, Eriksson E, Bagge U. Effect of cold water immersion on oedema formation in the scalded mouse ear. Burns 1982;9:17–20.
[23] Demling RH, Mazess RB, Wolberg W. The effect of immediate and delayed cold immersion on burn edema formation and resorption. J Trauma 1979;19:56–60.
[24] Jakobsson OP, Arturson G. The effect of prompt local cooling on oedema formation in scalded rat paws. Burns 1985;12:8–15.
[25] Boykin JV, Eriksson E, Sholley M, Pittman RN, Scholley MM. Cold-water treatment of scald injury and inhibition of histamine-mediated burn edema. J Surg Res 1981; 31:111–23.
[26] Boykin JV, Crute SL. Mechanisms of burn shock prevention after severe scald injury by cold-water treatment. J Trauma 1982;22:859–66.
[27] Björk J, Arturson G. Effect of cimetidine, hydrocortisone superoxide dismutase and catalase on the development of oedema after thermal injury. Burns 1983;9:249–56.
[28] Searcy RM, Cone JB, Bowser BH, Caldwell FT. The deleterious effects of high dose cimetidine in acute thermal injury. Burns 1982;9:62–5.
[29] Sevitt S, Bull J, Cruickshank C, Jackson D, Lowbury E. Failure of an antihistamine drug to influence the course of experimental human burns. Br Med J 1952;12:57–62.
[30] Blomgren I, Eriksson E, Bagge U. The effect of different cooling temperatures and immersion fluids on post-burn oedema and survival of the partially scalded hairy mouse ear. Burns 1985;11:161–5.
[31] Nanney LB. Changes in the microvasculature of skin subjected to thermal injury. Burns 1982;8:321–7.
[32] Wiedeman MP, Prince Brigham M. The effects of cooling on the microvasculature after thermal injury. Microvasc Res 1971;3:154–61.
[33] Eriksson E, Boykin JV, Pittman RN. Method for in viva microscopy of the cutaneous microcirculation of the hairless mouse ear. Microvasc Res 1980;19:374–9.
[34] Boykin JV, Eriksson E, Pittman RN. Microcirculation of a scald burn: an in vivo experimental study of the hairless mouse ear. Burns 1981;7:335–8.
[35] Eriksson E, Robson MC. New pathophysiological mechanism explaining post-burn oedema. Burns 1977;4:153–6.

[36] Saranto JR, Rubayi S, Zawacki BE. Blisters, cooling, antithromboxanes, and healing in experimental zone-of-stasis burns. J Trauma 1983;23:927–33.
[37] Raine TJ, Heggers JP, Robson MC, London MD, Johns L. Cooling the burn wound to maintain microcirculation. J Trauma 1981;21:394–7.
[38] Robson MC, Kucan J, Paik K, Heggers JP. The effect of heparin on dermal ischaemia after burning. Burns 1977;5:260–4.
[39] Robson MC, DelBeccaro EJ, Heggers JP, Loy GL. Increasing dermal perfusion after burning by decreasing thromboxane production. J Trauma 1980;20:722–5.
[40] Wang SL, Silberstein EB, Lukes S, Robb E, Zou WZ, Bruno L, et al. The effect of the thromboxane synthetase inhibitor Dazmegrel (UK-38,485) on wound healing, dermal ink perfusion and skin blood flow measurements in deep partial thickness burns. Burns 1986;12:312–7.
[41] Jakobsson OP, Benediktsson G, Arturson G. Early post-burn oedema in leucocyte-free rats. Burns 1985;12:18–21.
[42] Mileski W, Borgstrom D, Lightfoot E, Rothlein R, Faanes R, Lipsky P, et al. Inhibition of leukocyte-endothelial adherence following thermal injury. J Surg Res 1992; 52:334–9.
[43] Melikian V, Laverson S, Zawacki B. Oxygen-derived free radical inhibition in the healing of experimental zone-of-stasis burns. J Trauma 1987;27:151–4.
[44] Oldham KT, Guice KS, Till GO, Ward PA. Activation of complement by hydroxyl radical in thermal injury. Surgery 1988;104:272–9.
[45] Pintér E, Brown B, Hoult JRS, Brain SD. Lack of evidence for tachykinin NK1 receptor-mediated neutrophil accumulation in the rat cutaneous microvasculature by thermal injury. Eur J Pharmacol 1999;91–8.
[46] Rizzo JA, Burgess P, Cartie RJ, Prasad BM. Moderate systemic hypothermia decreases burn depth progression. Burns 2013;39:436–44.
[47] Greco JA, Pollins AC, Boone BE, Levy SE, Nanney LB. A microarray analysis of temporal gene expression profiles in thermally injured human skin. Burns 2010;36:192–204.
[48] Liang P, Lv C, Jiang B, Long X, Zhang P, Zhang M, et al. MicroRNA profiling in denatured dermis of deep burn patients. Burns 2012;38:534–40.
[49] Ashcroft GS, Lei K, Jin W, Longenecker G, Kulkarni AB, Greenwell-Wild T<ET AL>. Secretory leukocyte protease inhibitor mediates non-redundant functions necessary for normal wound healing. Nat Med 2000;6:1147–53.
[50] Pollins AC, Friedman DB, Nanney LB. Proteomic investigation of human burn wounds by 2D-difference gel electrophoresis and mass spectrometry. J Surg Res 2007;142:143–52.
[51] Cuttle L, Kravchuk O, Wallis B, Kimble RM. An audit of first-aid treatment of pediatric burns patients and their clinical outcome. J Burn Care Res 2009; 30:1028–34.
[52] Cuttle L, Kempf M, Liu P-Y, Kravchuk O, Kimble RM. The optimal duration and delay of first aid treatment for deep partial thickness burn injuries. Burns 2010; 36:673–9.