Acute microcirculatory changes after scalding of the rat paw

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ABSTRACT

A scalding model in the anaesthetized rat was used to measure acute circulatory reactions after heat exposure. Local blood flow of both hindpaws was recorded simultaneously and continuously by laser Doppler flowmetry before, during and for 2 hours following scalding. The scalding injury was inflicted by dipping the right hindpaw into hot water at 60 °C for 20 s. Concomitantly, the mean arterial blood pressure (MAP) was displayed on a chart recorder. MAP was obtained by cannulation of the common carotid artery. Oedema formation was calculated by measuring the volume changes of the hindpaws in a plethysmometer before and 30, 60 and 120 min after scalding. Scalding was followed by a biphasic increase of cutaneous circulation. During the first minute after heat provocation, an immediate increase in blood perfusion of about 400% was recorded, followed by a slow decrease of circulation. At 30 min after scalding, there was a secondary phase of increased microcirculation of approximately 230%. A slow decline of cutaneous circulation then followed, and after about 60 min the value was stabilized at ~100% above pre-burn level throughout the observation time. Almost no change of perfusion was observed on the contralateral unscalded paw. The scalding injury was followed by a progressive oedema formation on the scalded paw, measured by a volume increase of ~72% during the observation period, whereas the non-scalded paw showed no change. MAP remained at a stable level throughout the experiment except for a short-lasting transient increase of ~10% at the same time as the first peak of blood perfusion. We could thus confirm that scalding in the present model is accompanied by an immediate and marked increase in the peripheral circulation of the scalded paw followed by a later propagation of oedema, and that these inflammatory changes do not appear to be related to central haemodynamic alterations.

Keywords: laser Doppler flowmetry, mean arterial blood pressure, microcirculation, neurogenic inflammation, oedema, scalding.

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Scalding is the most usual course of burns (Thomsen & Sørensen 1967). A common burn like a restricted scalding injury is followed by obvious clinical signs such as localized oedema and pain. The severity of the injury is determined by the interdependency of temperature and duration of heat exposure (Arturson & Jakobsson 1985). Burning heat has a direct destructive effect upon living tissues, causing denaturation of macromolecules, cell membrane lysis and alterations of vital homeostasis of cells (Lee et al. 1994). As a consequence of physical damage and the release of different chemical mediators, the burn instantly initiates inflammatory reactions. Histamine, serotonin, bradykinin, arachidonic acid metabolites, cytokines and the release of oxygen free radicals have all been proposed as mediators influencing the microvascular reactions following thermal injury (Arturson 1985). Pharmacological intervention using different anti-inflammatory drugs to counteract the oedema reactions has been attempted but is only partially successful (Arturson 1985). Recent research has suggested that the release of sensory neuropeptides as a result of C-fibre activation promotes the oedema formation in scalding injuries (Jonsson et al. 1986, Hagerstrand et al. 1987). The pathogenesis of oedema formation after the burn injury is due to vasodilatation, increased microvascular permeability and enhanced extravascular osmotic activity (Arturson 1985). Recently, it has been suggested that the transcapillary fluid loss is further promoted by increased negative interstitial hydrostatic fluid pressure in the thermally injured...
skin as a result of denaturation of the collagen structure (Lund et al. 1992). However, the pathogenesis of the reaction following the thermal injury with subsequent oedema formation and fluid losses is not completely understood. The present method was developed to allow the continuous observation in real time of cutaneous microcirculation before, during and after scalding, in combination with simultaneous monitoring of the mean arterial blood pressure and intermittent measurement of oedema formation. It was concluded that local scalding causes an immediate and significant increase in peripheral circulation, followed by a delayed oedema reaction unrelated to central haemodynamic changes.

METHODS

The experiment was carried out on 10 freely moving male albino Sprague–Dawely rats (250–300 g; ALAB, Stockholm, Sweden). The rats were housed in cages with free access to food and water, at 24 °C with a 12 h light/dark cycle. Anaesthesia was induced by an intraperitoneal injection of sodium pentobarbital (50 mg kg⁻¹) continuously repeated (10 mg kg⁻¹ h⁻¹). The animals were kept on a warm pad to avoid temperature loss and the core temperature was repeatedly controlled. Immediately after the experiments, the rats were killed by an overdose. The experiments were approved by the local ethical committee. A polyethylene catheter was inserted into the carotid artery and mean arterial blood pressure (MAP) was displayed continuously on a chart recorder (Grass Polygraph 79D). Volumes of both hindpaws were measured with a plethysmometer (Ugo Basil 7150) before scalding and then at 30, 60 and 90 min after scalding. The blood flows of the plantar surfaces of both hindpaws were simultaneously and continuously monitored with a dual-channel Periflux PF 4001 laser Doppler flowmeter (Perimed AB, Sweden) connected to an A/D converter box (PF 472, Perimed AB, Sweden). Blood flow changes were expressed in perfusion units (PU), and represent relative changes and not absolute values. After calibration, the probe (PF 408) on the left paw was adapted through the probe holder (PH 104) using a double-stick adhesive tape, whereas the probe on the right paw (PF 407) was firmly fixed by gluing (Loctite 406, Irland) a plastic probe holder (PH 07–5) of diameter 4.5 mm to the skin surface. The Periflux 4001 was equipped with two semiconductor laser units both transmitting laser light of 780 nm. The PeriSoft software (Perimed AB, Sweden) installed in an IBM-compatible computer was used to store, retrieve and analyse data.

Before scalding, the rats were kept in a firm position to achieve a stable baseline recording. Scalding was performed by immersing the right hindpaw, attached to the laser Doppler probe, for 20 s in water at 60 °C; the contralateral hindpaw served as control.

Data are presented as means ± SEM. Basal recordings were set to 100%. The difference between groups was determined by one-way analysis of variance (ANOVA), Friedman test and Dunn's multicomparison test. P < 0.5 was considered to be significant.

RESULTS

Unilateral scalding of the right hindpaw in 10 rats was followed by an immediate increase in blood perfusion of 390 ± 54.3% during the first initial minute (Fig. 1, point A). This immediate phase was followed by a slow decline of circulation, until after 30 min a secondary phase of increased perfusion was noted. This secondary phase of increased perfusion was 230 ± 66.9% above the pre-burn level (Fig. 1, point B). After the first hour,

![Figure 1](https://example.com/figure1.png)

**Figure 1** Percentage change of laser Doppler flow value as related to the baseline value (100%) of scalded paws (●) and non-scalded paws (○), n = 10.
perfusion stabilized at 113.5 ± 59.2% above the pre-scalding level during the rest of the observation. Almost no change in cutaneous circulation was observed on the contralateral unscalded paw (Fig. 1). The scalding injury initiated a progressive oedema reaction in the scalded paw, with an increased volume of 72 ± 12.6% after 120 min of observation (Fig. 2). There was no oedema reaction observed on the contralateral side. Except for a small short-lasting increase of mean arterial blood pressure immediately after scalding, the value remained at a stable level throughout the observation time (Fig. 3).

**DISCUSSION**

When the cutaneous resistance is exceeded above the burning threshold, a progressive oedema follows as a result of a direct heat effect on the tissues along with the onset of inflammatory reactions. The clinical signs of cutaneous redness and local swelling are due to vasodilatation and plasma extravasation. These circulatory events have been extensively investigated. The onset of reactions and their interrelationship in accordance with central hemodynamic influences of the acute phase after scalding are not completely known. In the present study, on-line recording of the microcirculation was achieved by a specially designed laser Doppler probe which was retained in the same position throughout the experiment.

There are many methods described in studies of cutaneous circulation and oedema formation. Post-mortem observations of vascular changes in different histological preparations have been carried out (Jackson 1953) as well as various methods of intravital microscopy (Boykin et al. 1980). There are several possible methods of oedema measurement, such as the use of different dyes (Sevitt 1958) as well as weight measurements (Blomgren 1984).
Due to various heat provocation methods used in different burn models, local oedema reactions of varied magnitude and duration are observed. In the present study of acute circulatory events following scalding, there was a biphasic reaction, showing an immediate increase of cutaneous circulation above 400% within the first minute, followed by a secondary phase of increased perfusion of 230% after 30 min. The initial peak of blood flow is in accordance with an immediate pronounced vasodilatation (Fig. 4) due to the heat provocation (Fig. 1, point A). The secondary phase of increased perfusion (Fig. 5) is in line with the onset of inflammatory reactions (Fig. 2, point A), suggested to be mediated by neurohumoral factors. An inflammatory mediated hyperaemia probably explains the increased level of perfusion as measured by laser Doppler flowmetry during the rest of the observation time (Fig. 1). This is in contrast with the results of Blomgren et al. (1984) using a scalding model of the mouse ear in which a progressive decrease in the circulation was recorded. However, one must notice that the mouse ear has a thickness of only 0.3 mm, consisting of two layers of skin separated by a thin layer of cartilage and fat, and muscle deposits are very scanty (Boykin et al. 1980).

The oedema formation that followed in the present study revealed a somewhat later onset and progression with a mean increase in volume of 28% after 30 min, 47% after 60 min and 72% at 120 min (Fig. 2).

Earlier investigations have shown that the inflammatory oedema reaction may be divided into early and delayed phases. Both usually occur in the same burn, the latter merging into and supplementing the former (Sevitt 1958). This may be of clinical importance, since the later phase might be mediated by humoral as well as neurochemical factors and therefore pharmacological intervention could be possible. Recent studies have proposed the contribution of sensory neuropeptides to these scalding-induced delayed oedema reactions. Pretreatment with capsaicin, known to cause a depletion of sensory neuropeptides (Jansco et al. 1967), has been shown to inhibit late oedema formation in rats (Hägerstrand et al. 1987). Using real-time laser Doppler measurement as in the present study, a secondary phase of significantly increased cutaneous circulation was observed (Fig. 1, point B). It is possible that this delayed increase of perfusion is partly due to the activation of sensory primary afferents, which leads to cutaneous vasodilatation associated with increased vascular permeability, referred to as neurogenic in-

Figure 4 Original recording on-line from one rat showing laser Doppler flow in absolute values in the left non-scalded paw (line a) and the right scalded paw (line b). MAP is also shown (line c). The x-axis (time) is compressed eight times.
flammation (Jansco et al. 1967). Several vasoactive neuropeptides have been proposed as mediators of these reactions and two polypeptides in the tachykinin family, substance P (SP) and neurokinin A (NKA), are of special interest. These two neuropeptides are known to cause vasodilatation and plasma extravasation (Foreman et al. 1983). Recent studies in our laboratory using the microperfusion technique of scalded paws followed by radioimmunoassay of perfusates revealed significantly increased concentrations of NKA immunoreactivity during the first hour after scalding. SP showed no change in immunoreactivity during the same time course. The absence of SP immunoreactivity may be due to the increase of endopeptidase activity and breakdown of SP. Thus, NKA seems to be one important factor in the scalding-induced inflammatory reaction. This suggestion gains support from studies by Duggan et al. (1990) in which nociceptive heat stimulation of the cat hindpaw was followed by a widespread and persisting release of NKA as compared with SP from the dorsal horn.

Also of great importance in experimental burn studies is the control of central haemodynamics and the maintenance of a normal blood pressure. In the present investigation, mean arterial blood pressure remained at a stable level throughout the experiment and there was no sign of influence on haemodynamic or respiratory parameters. This means that the vascular changes observed are not a secondary phenomenon correlated to alterations in the systemic blood pressure.

We can thus conclude that the non-invasive method presented allows on-line monitoring of the peripheral circulation in combination with concomitant measurement of central blood pressure and intermittent estimation of oedema formation. The investigations are to be continued to give further understanding of the delayed phase of increased perfusion and the possible contribution of sensory afferents.

REFERENCES
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