Thermal Skin Injury: Effect of Fluid Therapy on the Transcapillary Colloid Osmotic Gradient

HENNING ONARHEIM, M.D., AND ROLF K. REED, M.D.
Department of Physiology, University of Bergen, Norway

Submitted for publication February 2, 1990

INTRODUCTION

Thermal injury rapidly increases vascular permeability and leads to marked edema formation [1-3]. Alterations in capillary permeability have been determined from accumulation of radiolabeled tracer proteins as well as changes in water content [1, 4, 5]. Changes in lymph composition and flow have also been used to determine alterations in the composition of interstitial fluid [2, 3]. Under steady-state conditions lymph may be representative of interstitial fluid composition. However, a burn injury followed by fluid resuscitation will probably not represent a steady state; in that case lymph may well be different from interstitial fluid.

The effects of fluid therapy on interstitial colloid osmotic and hydrostatic pressures in thermally injured skin were investigated in anesthetized rats subjected to full-thickness scald burns to 40% of the body surface area and resuscitation for 3 hr by either lactated Ringer’s or plasma. Interstitial fluid hydrostatic pressure (Pif) was reduced from -2 mm Hg to -20 to -40 mm Hg after injury, which will profoundly increase transcapillary filtration. Following the onset of fluid therapy, Pif increased to slightly positive values. In control, colloid osmotic pressure in plasma (COPp) was 20.6 ± 0.4 mm Hg and in interstitial fluid (COPif) 13.7 ± 0.3 mm Hg (means ± SEM). The transcapillary oncotic pressure gradient (COPgrad = COPp - COPif) was 6.9 ± 0.4 mm Hg. Following nonresuscitated thermal injury, COPp declined to 18–19 mm Hg (P < 0.05) and COPif was reduced to 10.4 ± 0.5 mm Hg (P < 0.05). Fluid therapy by lactated Ringer’s markedly reduced COPp (12.3 ± 0.3 mm Hg; P < 0.05), and COPgrad was almost abolished (2.6 ± 0.7 mm Hg; P < 0.05). In contrast, plasma infusion maintained COPp, whereas COPgrad increased significantly (11.1 ± 1.2 mm Hg; P < 0.05). Noncolloid saline solutions have been preferred for the initial fluid therapy for burns. The present study provides evidence that this will reduce both COPp and COPgrad, a situation in which edema formation will be favored.

The wick method was developed to collect interstitial fluid for measurements of protein concentration and colloid osmotic pressure (COP) [6]. The wick technique has also been used to investigate alterations in colloid osmotic pressure in interstitial fluid (COPif) under pathological conditions like congestive heart failure and nephrotic syndrome and following extracorporeal circulation [7-9].

After thermal injury colloid osmotic pressure has been determined directly in interstitial fluid in a few studies: analyses were performed on fluid collected by subcutaneous wicks [4, 10] or by a wick catheter technique [11]. Lund and Reed [4] determined COPif following moderate-size and extensive burns in rats, but with no fluid resuscitation. In patients with extensive burns COPif was determined using wick fluid [10] or a wick catheter [11], but not until 6 hr after the injury. In the present study alterations in COPif in the early period after thermal injury were determined. The effects of different resuscitation fluids on the transcapillary oncotic gradient were studied by direct measurements in interstitial fluid, thereby circumventing the problem of possible lack of interstitial-lymphatic steady-state.

Recently a marked negativity of the interstitial hydrostatic pressure (Pif) was observed following thermal injury [12, 13]. In order to fully outline the postburn changes in the interstitial pressures, we further addressed how infusion of lactated Ringer’s and plasma affected Pif.

MATERIALS AND METHODS

Nonfasted female Wistar rats (weight range 205–270 g) were anesthetized with pentobarbital (50 mg/kg, ip), with supplementary doses as needed to maintain anesthesia throughout the study period. Rectal temperature was maintained at 37°C by a servocontrolled heating pad. PE50 catheters were placed in the jugular vein and the carotid artery for fluid infusion and collection of blood samples.

The back and abdomen were closely clipped. Scald burns were inflicted on 40% of the body surface area (BSA) (20% BSA on the back plus 20% BSA on the
abdomen) [14]. Exposure of back skin to boiling hot water for 10 sec and abdominal skin for 4 sec results in uniform, full-thickness injuries [15]. Sham control animals were treated similarly to the animals in the burn groups except for immersion in water.

Citrated rat plasma was produced by exsangui nation of 500-g Wistar rats; one part of sodium citrate (3.13%) was added per seven parts of whole blood. The colloid osmotic pressure of this plasma was 13–14 mm Hg.

**Measurements**

**Wick method.** Interstitial fluid was collected with multifilamentous nylon wicks (Enkalon 3 × 3, Product- groep Industrielle Garens, Arnhem, Holland). The wicks, each consisting of three strands made up of 300 filaments of about 25 μm in diameter, were rinsed in acetone, ethanol, and distilled water [16] and soaked in 0.9% saline prior to implantation [17]. Pairs of double-stranded wicks were placed subcutaneously on the back in lengths of 4–6 cm by means of a straight suture needle [17]. To prevent evaporation, implantation of wicks and all handling of wicks in open air were performed inside an infant incubator, which served as a humidified chamber (100% relative humidity at room temperature).

For each sampling of wick fluid, two double-stranded wicks were implanted. Wicks were separated by at least 1 cm. The larger part of the rat's back was exposed to thermal injury; the small area of normal skin available precluded collection of wick fluid from noninjured areas. After an implantation time of 60 min, the wicks were swiftly pulled out and their central part transferred to 2-ml centrifuge tubes supplied with a funnel [16]. Wicks that were visibly bloodstained were discarded [6]. After centrifugation, 5–10 μl of wick fluid could be pipetted up from the bottom of the centrifuge tubes.

**Colloid osmotic pressure.** COP was measured in serum (COPs) and in wick fluid (COPw) with a colloid osmometer designed for sample volumes as low as 5 μl [18]. Diaflo YM10 ultrafiltration membranes (Amicon, Danvers, MA) were used on the osmometer. This membrane will reject 90% of globular solutes of mol wt 10,000 and >98% of albumin (manufacturer's specifications). COP was expressed as the absolute value of the recorded pressure drop (see Fig. 2). COP measurements were performed on the day of the experiment and are expressed as the mean values of duplicate analyses. Parallel COP measurements usually deviated by less than 0.5 mm Hg. A house standard of rat serum (COP around 22 mm Hg) was analyzed daily as a control (day-to-day coefficient of variation: 5%).

The wick technique was tested in vitro by soaking wicks in rat serum or rat serum diluted with saline and thereafter processed as described above. COP of the fluid obtained after centrifugation was identical to that of the control serum. Wick fluid from wicks soaked in normal saline consistently gave readings of zero pressure. In separate in vivo experiments COP in wick fluid obtained from eight parallel wicks gave a coefficient of variation of 9–13%.

**Interstitial fluid hydrostatic pressure.** $P_d$ was measured by micropipets [19, 20]. Sharpened glass micropipets (2- to 4-μm tip diameter) filled with 0.5 M NaCl were inserted into dermis. The pipets were connected to a servocontrolled counterpressure unit ("servonull") designed to keep the electric conductance within the tip of the pipet constant. Any external hydrostatic pressure acting on the micropipet either by forcing "isotonic" fluid into the pipet or by sucking hypertonic pipet fluid out will alter the electric conductance within the pipet tip. A two-way membrane pump connected to the pipet will generate a counterpressure to readjust the electrical conductance within the pipet tip. This counterpressure generated by the membrane pump is identical to the external pressure [20]. Only pipet positions with no visible deformation of the skin surface, as judged through a stereomicroscope, were accepted for registration of $P_d$.

Pressure readings were accepted when the following criteria were met [20]: (A) recorded pressure was constant when increasing the feedback gain; (B) fluid communication between pipet and interstitial fluid was free (a suction pressure applied by the pump resulted in increased electrical resistance in the pipet tip); and (C) zero pressure reading before and after measurement of $P_d$ was reproducible.

**Experimental Protocol**

**Wick study.** The experimental procedure was divided into three phases:

A. Control period: Following insertion of vascular lines, wicks were inserted subcutaneously on the back and removed after a 60-min equilibration period. The animals were then allocated to one of five groups:

<table>
<thead>
<tr>
<th>Group</th>
<th>Thermal injury</th>
<th>Fluid therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>2. No fluid group</td>
<td>40% BSA</td>
<td>None</td>
</tr>
<tr>
<td>3. Lactated Ringer’s</td>
<td>40% BSA</td>
<td>Lactated Ringer’s 5 ml/hr</td>
</tr>
<tr>
<td>4. Plasma group</td>
<td>40% BSA</td>
<td>Plasma 2.5 ml/hr</td>
</tr>
<tr>
<td>5. Infusion only</td>
<td>None</td>
<td>Lactated Ringer’s 5 ml/hr</td>
</tr>
</tbody>
</table>

B. Induction of thermal injury: This procedure required 5–7 min, whereafter the animal was returned to the incubator.

C. Postburn observation period: A second pair of subcutaneous wicks were implanted for a 60-min period immediately after thermal injury. Fluid infusion was started 15 min after thermal injury, correspondingly 15 min after sham burn. A third pair of wicks was inserted from 120 to 180 min postinjury. Carotid blood samples
were collected prebum and 1 and 3 hr postburn for determination of hematocrit (HCT) and COP\textsubscript{p}.

**Measurement of \( P_d \) by micropuncture.** \( P_d \) in dermis was measured in control, whereafter a 40% BSA thermal injury was inflicted. Animals were resuscitated by lactated Ringer's at 5 ml/hr iv (lactated Ringer's group, \( n = 6 \)) or by plasma at 2.5 ml/hr iv (plasma group, \( n = 6 \)). Infusions were started 15 min after injury and continued for a 3-hr study period.

For practical reasons acceptable measurements of \( P_d \) could usually not be obtained for the first 5–10 min postburn. During the recording of \( P_d \) liquid mineral oil was applied to the skin to avoid evaporation from the skin surface. Measurements were continued up to 180 min postinjury.

**Statistical Methods**

Results are presented as means ± SEM. Analysis of variance (ANOVA) with repeated measures over time, one-way ANOVA, and \( t \) tests (paired and unpaired) were employed to assess differences between groups or treatments. Differences were assumed significant if \( P < 0.05 \). Biomedical datapack (BMDP, version 1985) was used for statistical analysis.

**RESULTS**

Results are presented in Figs. 1–3 and in Table 1. Results from the group which received lactated Ringer's but no burn are only presented in the table.

Preburn COP\textsubscript{p} was 20.6 ± 0.4 mm Hg (grand mean). In the control group COP\textsubscript{p} remained stable for the entire study period. Within 1 hr after thermal injury COP\textsubscript{p} was reduced to 16.3 ± 0.8 mm Hg in animals that were not resuscitated (\( P < 0.05 \)). Following resuscitation by lactated Ringer’s, COP\textsubscript{p} decreased markedly: within 3 hr COP\textsubscript{p} was reduced to 18.3 ± 0.8 mm Hg in animals that were not infused by around 3% 1 hr postinjury in both the lactated Ringer's and the plasma groups, but returned toward control values 3 hr postinjury in both groups. When COP was measured in sera or in wick fluid from noninjured skin, a stable equilibrium pressure was rapidly obtained (see Fig. 2). No “overshoot” (transient positive pressure peak) was observed after the membrane was washed with saline, indicating that normally, larger molecules did not leak through the membrane. Samples of wick fluid collected after thermal injury consistently gave an initial, negative pressure deflection before a flat equilibrium pressure was obtained (Fig. 2). Further, an “overshoot” was observed after the membrane was washed with saline (Fig. 2). Such changes were not observed in the plasma samples.

The effect of osmometer membrane “pore size” (cut-off level) was evaluated for two different osmometer membranes. The Amicon YM10 membrane (used throughout the present study) was compared to the Amicon PM30 membrane (30,000 mol wt cutoff), which has been used in a number of previous studies [4, 16, 21]. The YM10 membrane gave 1-2 mm Hg higher values for COP around 20 mm Hg and 1 mm Hg higher values for COP around 10 mm Hg. Equilibration of small ions over the osmometer membrane was evaluated in separate experiments: 0.5 M NaCl gave a very transient pressure response when applied on the osmometer membrane. Sodium citrate (citric acid: mol wt 192) gave a more pronounced, still transient pressure response when applied on the osmometer membrane, and sodium bicarbonate (bicarbonate: mol wt 84) gave a more subatmospheric pressure (−2.0 ± 0.2 mm Hg) in control (Fig. 3). Following thermal injury \( P_d \) rapidly declined to −20 to −40 mm Hg (Fig. 3). The most negative values were observed in the first 10- to 20-min intervals. Following fluid infusion, \( P_d \) returned toward control values and later remained slightly positive. COP\textsubscript{p} became positive with plasma infusion sooner than it did following infusion of lactated Ringer's (\( P < 0.05 \))(Fig. 3).
TABLE 1
Colloid Osmotic Pressures in Plasma (COPₚ), Interstitial Fluid (COPᵢ), and Colloid Osmotic Gradient (COPₕ₉) in Controls and following Thermal Injury

<table>
<thead>
<tr>
<th>Burn</th>
<th>Control</th>
<th>No fluid</th>
<th>Lactated Ringer’s</th>
<th>Plasma</th>
<th>Infusion only</th>
</tr>
</thead>
<tbody>
<tr>
<td>COPₚ (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>21.1 ± 0.8</td>
<td>20.1 ± 0.7</td>
<td>19.7 ± 0.5</td>
<td>21.3 ± 1.0</td>
<td>21.0 ± 0.6</td>
</tr>
<tr>
<td>60 min</td>
<td>20.8 ± 0.9</td>
<td>18.3 ± 0.8*</td>
<td>15.0 ± 0.6**</td>
<td>20.7 ± 1.0</td>
<td>17.7 ± 0.3*</td>
</tr>
<tr>
<td>180 min</td>
<td>20.9 ± 0.9</td>
<td>19.0 ± 0.7</td>
<td>12.3 ± 0.3**</td>
<td>22.4 ± 0.9*</td>
<td>16.1 ± 0.8*</td>
</tr>
<tr>
<td>COPᵢ (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>12.7 ± 0.7</td>
<td>13.7 ± 0.4</td>
<td>13.6 ± 0.8</td>
<td>14.9 ± 0.5*</td>
<td>13.6 ± 1.7</td>
</tr>
<tr>
<td>60 min</td>
<td>11.5 ± 0.7*</td>
<td>12.5 ± 0.6</td>
<td>12.7 ± 0.7</td>
<td>13.4 ± 0.5*</td>
<td>11.5 ± 0.9</td>
</tr>
<tr>
<td>180 min</td>
<td>10.9 ± 0.9*</td>
<td>10.4 ± 0.8*</td>
<td>9.7 ± 0.7*</td>
<td>11.3 ± 0.8*</td>
<td>8.4 ± 0.3*</td>
</tr>
<tr>
<td>COPₕ₉ (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8.4 ± 0.6</td>
<td>6.4 ± 0.7</td>
<td>6.0 ± 0.8*</td>
<td>6.4 ± 0.9</td>
<td>7.4 ± 1.7</td>
</tr>
<tr>
<td>60 min</td>
<td>9.2 ± 0.4*</td>
<td>5.8 ± 0.6*</td>
<td>2.3 ± 0.9**</td>
<td>7.3 ± 0.8</td>
<td>6.1 ± 0.8*</td>
</tr>
<tr>
<td>180 min</td>
<td>10.0 ± 0.4*</td>
<td>8.5 ± 0.8</td>
<td>2.6 ± 0.7**</td>
<td>11.1 ± 1.2*</td>
<td>7.7 ± 0.7</td>
</tr>
</tbody>
</table>

Note: Infusion only group: no thermal injury, lactated Ringer’s infused at the same rate as thermal injury, resuscitated by lactated Ringer’s.

Means ± 1 SEM, n = 8.
* P < 0.05 vs preburn.
+ P < 0.05 vs control group (by ANOVA and t test).

DISCUSSION

The wick method was developed to collect interstitial fluid for measurements of protein concentration and colloid osmotic pressure [6, 17]. Wick fluid was found to give a correct estimate of COPᵢ in subcutaneous tissue provided that sufficient proteins were added to the wick before or after insertion [17].

Two recent modifications of the wick technique have suggested that saline-soaked wicks might give a certain underestimate of COPᵢ [21, 22]. However, “the crossover method” [22] requires insertion of at least three wicks for each determination of COPᵢ, which will limit its application for repeated measurements in a small animal. “Dry wicks” [21, 22] could have been used; still, saline-soaked wicks were preferred to allow for repeated measures and for comparisons to previous studies [4, 10].

Fluid replacement seems to have been sufficient since HCT was only transiently elevated in the resuscitated cases. On the basis of the alterations in HCT (corrected for blood samples), we predict that 3 hr postinjury plasma volume was unchanged from control in plasma-resuscitated animals and reduced by 5% in animals that received infusion of lactated Ringer’s.

The Amicon YM10 membrane was used on the osmometer; this membrane has a pore diameter which should safely exclude all plasma proteins. Wick fluid from thermally injured skin gave an initial, negative pressure deflection on the osmometer prior to a stable equilibrium pressure (Fig. 2). A transient positive pressure peak occurred following washing of the membrane. Analogue observations were made following application of hypertonic saline, citrated rat plasma, or sodium citrate on the osmometer (Fig. 2), while no such phenomena were seen in wick fluid from noninjured skin. These observations suggest that after thermal injury molecules of molecular weights below the cutoff level for the membrane leaked through the intact osmometer membrane. This is in accordance with previous suggestions that
There was a certain reduction in \( \text{COP}_p \) with time even in the control experiments (12.7 vs 10.9 mm Hg; \( P < 0.05 \)). When wick techniques are used to collect interstitial fluid it may therefore be relevant to specify the timing of wick fluid samples in relation to the induction of anesthesia.

In the present study \( \text{COP}_p \) was reduced by 3–4 mm Hg after thermal injury (\( P < 0.05 \)). In extensively burned patients, resuscitated mainly by acetated Ringer’s, \( \text{COP}_p \) in injured skin was reduced compared to that in normals [10]. A significant reduction of \( \text{COP}_p \) in severely burned patients was also found 4–8 hr after thermal injury, using a wick catheter technique [11]. In contrast, in rats \( \text{COP}_p \) was not significantly altered following larger (40% BSA), nonresuscitated burns, whereas following smaller (10% BSA) burns even a certain increase of \( \text{COP}_p \) was observed [4].

\( \text{COP}_{\text{grad}} \) was 6–8 mm Hg in the control situation, which compares well to previous studies in the rat [4, 16]. \( \text{COP}_{\text{grad}} \) was not significantly altered after thermal injury in the nonresuscitated cases (Table 1). This contrasts previous observations that \( \text{COP}_{\text{grad}} \) was nullified or even reversed after nonresuscitated thermal injury [4]. However, in that study experiments were not performed within a humidified chamber [4]. Evaporative fluid losses from wicks handled in the open will rapidly increase \( \text{COP}_p \) [16] and may lead to erroneously low values for \( \text{COP}_{\text{grad}} \). In the present study handling of wicks solely within an incubator eliminated that problem: in recovery studies wick fluid from wicks soaked in serum gave the same cap as the primer.

Following volume replacement by lactated Ringer’s, \( \text{COP}_{\text{grad}} \) declined to 2.6 \( \pm \) 0.7 mm Hg (\( P < 0.05 \)), which still is significantly different from a zero gradient (\( P \)}
Fluid transport across the capillary wall is described by the Starling equation,

\[ J = CFC \cdot (P_e - P_d - \sigma \cdot (COP_p - COP_d)) \]

where \( J \) is the volume of fluid filtered across the capillary wall, \( CFC \) is the capillary filtration coefficient, \( P_e \) is the capillary hydrostatic pressure, \( P_d \) is the interstitial fluid hydrostatic pressure, and \( \sigma \) is the capillary reflection coefficient for plasma proteins. Normally the net filtration pressure (the net pressure imbalance across the capillary wall) is on the order of 0.5 mm Hg.

For methodological reasons \( P_{d} \) could only be measured in dermis; \( P_{d} \) in dermis may not necessarily be representative for whole skin (including both dermis and subcutis) [12]. Still, following thermal injury \( P_{d} \) decreased from a normal value of ~2 mm Hg to values around -20 to -40 mm Hg (Fig. 3). The markedly negative \( P_{d} \) in injured skin may increase the net filtration pressure up to two orders of magnitude and will be the main driving force for edema formation in the initial stages. After the first 30-min period postinjury, the negative \( P_{d} \) subsided (Fig. 3). From 1 hr postinjury \( P_{d} \) was around ambient pressure or became slightly positive, as is seen in other edematous states [20]. When compared to data from Lund et al. [12] of \( P_{d} \) following nonresuscitated thermal injury (Fig. 3), normal \( P_{d} \) in the present study was reached sooner with resuscitation, and more promptly following plasma infusion (\( P < 0.05 \) vs lactated Ringer’s).

The gradient in colloid osmotic pressure between plasma and the interstitium will influence the net transcapillary fluid transport: a reduced effective \( COP_{\text{grad}} (\sigma \cdot COP_{p}) \) will favor edema formation. After thermal injury, \( \sigma \) has been estimated to be reduced from 0.87 to 0.45 [23]; this may further contribute to a reduced effective \( COP_{\text{grad}} \).

When \( P_{d} \) is positive, \( P_{e} \) (capillary hydrostatic pressure) and \( COP_{\text{grad}} \) will be the driving forces for fluid filtration. A reduced \( COP_{\text{grad}} \) may facilitate edema formation also in noninjured tissue. Actually, fluid replacement by infusion of lactated Ringer’s after thermal injury was found to increase fluid accumulation in noninjured tissue [3, 4].

This is to our knowledge the first study where colloid osmotic pressure has been determined directly in interstitial fluid following different forms of parenteral fluid substitution. Plasma and lactated Ringer’s had opposite effects on \( COP_{\text{grad}} \) after a standardized thermal injury. Plasma maintained, or actually increased, \( COP_{\text{grad}} \) whereas lactated Ringer’s almost annihilated \( COP_{\text{grad}} \). Saline solutions (without colloid) have been preferred for the initial fluid therapy in burns. The present study underlines that this will reduce both \( COP_{p} \) and \( COP_{\text{grad}} \) and may therefore favor edema formation.

ACKNOWLEDGMENTS

We are grateful to Eli Gunn Kjerlaug and Malvin Gismervik for technical assistance. The study received financial support from The Norwegian Research Council for Science and the Humanities and The Norwegian Council on Cardiovascular Diseases.

REFERENCES


