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Red blood cell and tissue water content in experimental thermal injury

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Abstract

Oedema formation and changes in local blood flow are known phenomena in burns. The relationship between these two is not clearly described. The aim of this study was firstly to examine both the contents of red blood cells and tissue water in skin and subcutaneous fat after experimental burns of different depths in pigs, and secondly, to confirm our recent findings of the increased dielectric constant of skin and subcutaneous fat reflecting considerable oedema formation, especially in fat after thermal injury.

Methods: Superficial, partial and full thickness contact burns were created to pigs and followed for 24 h. Radioactive Cr-51 labelling of red cells was used to estimate the number of red cells in tissue, and the absolute amount of water was determined by lyophilisation.

Results: A decreased number of labelled red cells in skin and an increase in tissue water in subcutaneous fat were found regardless of burn depth. The highest water amount in fat was found in the partial thickness burns.

Conclusion: All burn depths resulted in a diminished number of labelled red blood cells in skin and a significant increase in the absolute water amount in subcutaneous fat at 24 h post injury. The findings in fat support our recent findings of highly elevated dielectric constants measured by the new in vivo method of dielectric measurements.

Keywords: Burn; Oedema; Red blood cell; Blood flow; Lyophilisation

1. Introduction

Severe burn induces oedema formation and changes in local blood volume and blood flow. The zone of stasis plays an integral role in the development of the final depth of the injury [1]. The deep dermal vascular plexus gives blood supply to the skin adnexes, which are the main source of keratinocytes needed for the re-epithelisation of superficial burns [2]. Hence, progressive reduction of dermal blood supply has an effect on the final depth of the burn wound. Diminished blood supply in the reticular dermis lowers the tendency of the burn wound to heal spontaneously leading to excision and grafting.

Oedema formation has a deleterious effect on the oxygen and nutrient delivery to the wound [3]. Oedema in burned tissue increases the risk of infection due to the lowered pO_2 values which might progress a partial thickness burn to a full thickness burn [3]. Leape found that 86% of the final increase in water content in full thickness burns in rat skin was accomplished within the first 5 min post injury [4]. Oedema formation consisted of a rapid phase during the first hour after burn and of a more gradual increase occurring during the next 12–24 h. Deep burns in sheep create less oedema than superficial ones [5,6]. The oedema in deep burns reabsorbs slowly, suggesting continued vascular or lymphatic occlusion leading to a smaller portion of vessels capable for fluid exchange [5].

The relationship between water and red cell contents in tissue after severe burn is not clearly described. The water content in different depths of tissue after experimental burns

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has been recently measured with dielectric measurements [7]. It was found that all burn depths induced a pronounced increase in the water content in the subcutaneous fat and that full thickness burns had lower tissue water content in whole dermis than partial thickness burns.

The aim of this study was to examine both red blood cell content and absolute tissue water in skin and subcutaneous fat after experimental burns of different depths in pigs, and to verify the findings of our previous study [7], where highly increased dielectric constant was found in fat relating to increased tissue water.

2. Materials and methods

2.1. Experimental protocol

The study was approved by the Institutional Animal Care and Use Committee of the University of Kuopio, Finland. Five female Finnish landrace pigs (28–38 kg) were premedicated, cannulated, anaesthetized and monitored as described previously [6]. Animals received 50% glucose infusion which was adjusted to maintain normoglycaemia (blood glucose 5–7 mmol/l). Normovolaemia was maintained with Ringer's acetate (Ringersteril, Baxter, Vantaa, Finland) according to CVP 4–7 mmHg. As the total surface area burned was very small (1.2–2.2%), no actual fluid resuscitation due to the burn was indicated.

Histologically confirmed superficial, partial thickness and full thickness contact burns were created with a brass plate heated up to 100 °C on both sides of the ventral body of each pig by using 1 s (1S), 3 s (3S) and 9 s (9S) contact times, respectively [6]. A total of 10 burns of each depth were thus created in the experiment. One control site (CS) next to the burned areas served as a reference site ($N = 5$) in each pig. The burns and control sites were 4 cm × 4 cm in size with a 3 cm distance between each site. The follow-up time was 24 h. The animals were kept sedated on the respirator throughout the study and killed with an overdose of saturated magnesium sulphate solution given in the heart.

2.2. Chromium-51 labelling of pig red blood cells

Two venous blood samples (8 ml each) were collected from each pig prior to inflicting the burns. Both specimens were anticoagulated with 1.5 ml of acid citrate dextrose-A (ACD-A) solution. The samples were centrifuged at 3000 rpm for 10 min. Supernatant plasmas and buffy coats were discarded taking care not to remove any red blood cells (RBC). 2 MBq of sodium chromate (Cr-51) solution (minimum 0.2 ml) was slowly added to each tube and the suspensions were incubated for 15 min at room temperature with continuous gentle mixing. Labelled cells were washed three times with isotonic saline and re-suspended in the original blood volume with isotonic saline [8]. Aliquot (0.5 ml) of the labelled RBC suspension was taken for a radioactivity measurement while the rest of the suspension was reinfused in the animal through a vein cannula at 23.5 h post burn. After 30 min (i.e. 24 h post burn), the animal was sacrificed with an intracardiac injection of magnesium sulphate. Burn ($N = 30$) and control ($N = 5$) sites were excised surgically and the skin was sharply separated from the subcutaneous fat horizontally with scissors. Each tissue sample ($N = 70$) was again cut vertically into two pieces, giving two tissue samples of skin and two samples of subcutaneous fat from each burn and control site for analysis. Each sample ($N = 140$) was placed inside a test tube. Each test tube and tissue sample was weighed separately.

2.3. Determination of the number of labelled RBCs in tissue samples

Simultaneous with the collection of the blood samples for labelling, one blood sample (5 ml) was collected from each pig for the determination of the RBC count. The RBC count per milliliter of blood was determined by Sysmex K-4500 analyser (Sysmex, Kobe, Japan). Since, a known volume of blood was drawn for labelling, total number of the labelled and reinfused RBCs could be estimated. The radioactivity of the tissues and the labelled RBC suspensions were counted with a 1480 Wizard gamma counter (Perkin-Elmer, Turku, Finland) using a counting time of 30 min. The RBC suspensions were diluted 1:10000, with isotonic saline prior the measurement. Background-corrected counts for the diluted RBC suspensions were used to calculate counts of a single RBC. The number of the labelled RBCs per gram of tissue was determined by using background-corrected total counts for tissue samples and calculated counts of a single RBC.

2.4. Determination of tissue water content

Wet-weighed tissue samples were frozen at −70 °C and lyophilisised with a Hetosicc CD52 freeze drying apparatus (Heto, Birkerød, Denmark). Lyophilisation was continued until the difference between two successive weightings was smaller than 0.002 g. The water content (g) was calculated as the difference between the wet and dry weight.

2.5. Statistics

The results are presented as mean ± 1 S.D. The non-parametric Wilcoxon-signed rank test (two-tailed) was used for statistical analysis. A p-value <0.05 was considered statistically significant.

3. Results

3.1. Number of radioactively labelled red cells

The changes in the numbers of labelled red cells (LRC) per gram of wet tissue at different burn sites compared to the

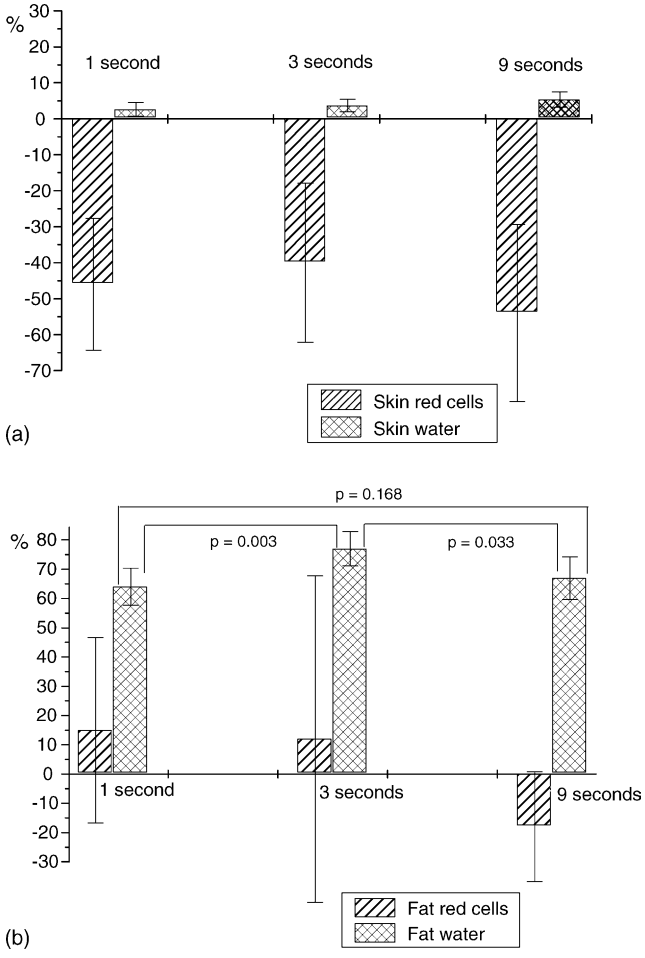

Fig. 1. The changes (mean ± S.D.) in the amount of labelled red cells and tissue water (a) in skin, and (b) in subcutaneous fat at different burn sites. Values express the percentual changes compared to the non-burned control site.

non-burned control sites are presented in Fig. 1. The 1S, 3S and 9S burns caused a 46%, 40% and 54% decrease, respectively, in LRC in skin (Fig. 1a) at 24 h post injury ($p = 0.028$ in all). The 1S and 3S burns caused a 15% and 12% increase, respectively, in LRC in subcutaneous fat (Fig. 1b) compared to the control site, but the changes were not statistically significant. The 9S burns caused a decrease of 19% in LRC compared to the control site (CS) ($p = 0.028$) and was significantly lower than the 1S ($p = 0.01$). The difference between the 3S and 9S burn sites was non-significant ($p = 0.091$). At 1S and 9S burn sites, the concentration of red cells in fat was higher than in skin ($p = 0.003$ and $p = 0.010$, respectively).

3.2. Total water amount in skin and subcutaneous fat

In the unburned control site, the content of water in skin was 72.3% and in subcutaneous fat 36.6% (Table 1). While the water content in skin increased only slightly (Fig. 1a), the water content in fat (Fig. 1b) increased 64%, 77% and 67% in the 1S, 3S and 9S burn sites, respectively, compared to CS

Table 1
Water amount (%) in lyophilisised skin and subcutaneous fat for the control site (CS) and different burn sites (1S, 3S, 9S)

	Skin	Fat
CS	72.3	36.6
1S	74.2	59.9
3S	75.0	64.8
9S	76.2	61.1

($p = 0.028$ in all). The water content in the 3S burn site was higher than in the 1S ($p = 0.003$) and in the 9S ($p = 0.033$).

4. Discussion

The aim of this study was to determine the concentration of labelled red cells and the absolute amount of water in skin and subcutaneous fat, 24 h after creating burns of different depths in pigs and to verify our recent findings of burn-related elevation of dielectric constants in fat. The main findings were that burn causes a significant decrease in the number of labelled red blood cells in skin and a significant increase in the amount of water in subcutaneous fat at 24 h post injury regardless of the depth of the injury.

The pathologic changes in the burn wound circulation are progressive over the first 24 h [9]. Impairment of blood flow begins with events occurring in the microvasculature, including platelet microthrombus formation and vasoconstriction [10]. During the first minute after injury, scald burn in rat has caused a 400% increase in blood perfusion measured by laser Doppler [11]. At 60 min, the perfusion was about 100% above the normal level. On the other hand, deep burns have decreased blood flow compared to the non-burned sites [9,12,13]. Similar results were found in deep flame burns using cytometry in sheep, where skin blood flow was also decreased during a 72-h study [14]. However, in the same study, a biphasic reaction in blood flow was found in subcutaneous fat, where the initial decrease in blood flow was followed by a later hyperaemic phase on the third day post burn [14]. In the present study, the number of radioactive red cells in skin was lower at all depths of burns than in the non-burned control sites at 24 h post injury. This supports previous reports where in severely burned areas, the impairment of blood flow ensued within a couple of hours and was delayed for up to 16–24 h in less-severe regions [15]. Our study also demonstrated that the 9 s burn caused a significant decrease in the number of labelled red blood cells in fat, which is in accordance with the findings of Ferguson et al. in guinea pigs [13]. Histologically, these 9 s burns have previously been found to be deep, full thickness burns [6]. The decreased number of red cells in fat in these deep burns is in accordance with both the burn trauma, extending down to the fat causing vascular thrombosis and possibly the increased amount of oedema, which results in a decreased number of red cells per gram of tissue.

Deep scald burns in sheep have less swelling than superficial ones [5,7,16]. Even though the temporal development of the swelling process might be identical in both burns, the reabsorption of fluid is slower in deep burns, suggesting continued vascular or lymphatic occlusion [5]. In sheep, oedema has been found to be maximal between 12 h and 18 h post injury, and at 24 h reabsorption was already occurring [5]. In our study with pigs, we found the highest amount of water in skin in the 9S site. Although the finding was not statistically significant, it might reflect the previous finding of the slow reabsorption process described in deep burns [5].

The greatest changes in tissue water contents were found in subcutaneous fat. Compared to the non-burned control site, the superficial (1S), partial thickness (3S) and full thickness (9S) burns caused a 64%, 77% and 67% increase in the absolute amount of water, respectively. In experimental burns in pigs, the recent dielectric measurements have also indicated a marked increase in tissue water content in subcutaneous fat regardless of burn depth at all time points in a 72-h follow-up study [7]. When performed with 300 MHz, the dielectric measurement specifically gives information about the amount of both bound and free water (=total water) in tissue [17]. It was found that partial and full thickness burns could be differentiated during the first 24 h according to their oedema-formation characteristics [7]. A pronounced increase in the water amount in fat has also been described previously in 40% total body-surface area full thickness burns in sheep [14]. In superficial burns, only the papillary vascular plexus is destroyed. Most of the blood circulation in the skin is preserved making it the likely source of the oedema fluid. When the burn depth increases, the circulation of both the papillary and reticular dermal plexuses are increasingly deteriorated. Thus, the remaining patent blood vessels are in the subcutaneous fat, explaining the increased fat oedema, especially in partial thickness and deep burns.

In conclusion, all burn depths resulted in diminished number of labelled red blood cells in skin and a significant increase in the absolute water amount in subcutaneous fat at 24 h post injury. The findings in fat support the previous findings of highly elevated dielectric constants measured with the dielectric measurements [7].

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