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Microdialysis for detection of dynamic changes in tissue histamine levels in experimental thermal injury

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Abstract

Histamine is an important mediator contributing to oedema formation after thermal injury. Tissue histamine concentrations have been previously determined by analyzing tissue biopsies. The microdialysis method enables continuous collection of samples from the extracellular tissue fluid. In this experimental burn study on pigs samples from the extracellular fluid for histamine analysis were collected from superficial, partial thickness and full thickness burn sites during a 24-h period. There was a burn depth-related increase in histamine concentrations during the first 2 h post injury. Deep burns induced a more profound initial increase in tissue histamine concentration than the partial thickness and superficial burns. Histamine concentrations at all burn sites declined until 12 h post injury. There was a second rise in tissue histamine concentrations between 12 and 24 h post injury without a rise in plasma histamine concentrations. Histamine concentrations at all burn sites were higher than at the non-burned control sites. The microdialysis technique is an easily applicable method of collecting on-line samples from burned tissue. This method provides a useful tool in investigating the effects of different treatment modalities on the secretion of substances into interstitial fluid within burned tissue.
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1. Introduction

Oedema formation is characteristic of burn injury. Histamine is an important mediator contributing to the early phase of increased vascular permeability after burn trauma [1]. The secretion of histamine from mast cells leads to an enhancement of xanthine oxidase activity and increased production of free oxygen radicals, which damage microvascular endothelial cells leading to increased vascular permeability and oedema [2]. In rats, plasma concentration of histamine immediately after burn is related directly to the extent of surface injury while the time-related profile is related to the depth of the injury [3]. In cats, 30% full thickness burn causes up to a three-fold increase in plasma histamine levels, with the peak concentration occurring within 30 min post injury [4]. Thermal injury increased the blood histamine levels but decreased the skin histamine levels at 5 min post burn in dogs [5]. The early rise in blood histamine was suggested to be primarily due to the release of histamine from the skin [5]. This is supported by the finding that severe burn injury initiates a higher increase in early post burn tissue fluid histamine levels than in moderate injury in rat paws [6]. The release of histamine closely parallels the development of oedema and once oedema is fully developed, no further release of histamine occurs [6]. A delayed (7–14 days post burn) rise in histamine content in rat and mouse skin after a superficial burn has also been described [7].

In practise, biopsies have been obtained in order to determine tissue histamine concentrations after thermal injury [5–7]. Recently, an alternative method for measurement of water soluble substances in the extracellular tissue fluid has been developed. With the microdialysis technique it is possible to continuously collect samples from the extracellular fluid [8]. The technique is based upon the

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principle, that water soluble molecules of the extracellular space will passively move through the pores of the semipermeable membrane of the microdialysis probe. To our knowledge, this technique has not been previously described in burn wound research.

The aim of this study was to test the microdialysis technique in experimental burns by collecting extracellular fluid from burned tissue for analysis of locally secreted substances using histamine as the mediator analysed.

2. Materials and methods

2.1. Animal handling and anaesthesia

This study was approved by the Institutional Animal Care Committee of the University of Kuopio. Female Finnish landrace pigs ($N = 5$, age 3 months, weight 28–38 kg) were fasted for 48 h with free access to water prior to the experiments. After premedication with atropine (0.05 mg/kg i.m.) and azaperone (8 mg/kg i.m.), an ear vein was cannulated for administration of thiopenthal sodium (5–15 mg/kg i.v.) as induction of general anaesthesia. Animals were tracheostomised and ventilated with a volume-controlled ventilator (Servo 900E, Siemens, Elema, Sweden) at a tidal volume of 10 ml/kg. Minute volume was adjusted to achieve normocapnia ($p\mathrm{aCO_2}$ 4.4–5.5 kPa, 34–41 mmHg). Fraction of oxygen in the inspiratory gas ($\mathrm{FiO_2}$) was adjusted to keep arterial partial pressure of $O_2 > 13.3$ kPa (100 mmHg). Positive end expiratory pressure of 5 $\mathrm{cmH_2O}$ was maintained throughout the study. Anaesthesia was maintained with infusion of thiopentone (5 mg/kgh). Fentanyl was administered 30 μg/kg/h during the creation of burn wounds and 5 μg/kg/h thereafter. Pancurone was admistered in 2–4 mg boluses i.v., when needed for shivering. Body temperature was monitored via the Swan-Ganz catheder. The animals were kept sedated on the respirator throughout the study, after which they were sacrificed with an intracardial injection of magnesium sulphate solution.

2.2. Hemodynamic monitoring

Right carotid artery and internal jugular vein were cannulated for blood pressure and central venous pressure (CVP) monitoring and blood sampling. Systemic arterial and central venous pressures were recorded with quartz pressure transducers and displayed on a multimodular monitor and recorder (AS3, Datex-Ohmeda, Helsinki, Finland). The data was registered automatically in 2-min intervals (Clinisoft, Datex-Ohmeda, Helsinki, Finland). All pressure transducers were zeroed to the level of the heart. Heart rate was continuously monitored with electrocardiogram. Hemodynamics was recorded at 15-min intervals. A urinary catheter was placed through a small incision in the lower part of the abdomen for urinary output measurements.

Animals received 50% glucose infusion, which was adjusted to maintain normoglycemia (5–7 mmol/L). Normovolemia was maintained with Ringer's acetate (Ringersteril, Baxter, Vantaa, Finland) according to CVP 4–7 mmHg.

3. Experimental protocol

The ventral side of the body of the pig was shaved and washed with chlorhexidine solution (5 mg/mL). The burns were inflicted by using a 40 mm × 40 mm × 25 mm brass block, weight 530 g, heated to 100 °C in boiling water as described earlier [9]. Contact times of 1, 3 and 9 s were used between the block and the animal skin to create superficial, partial thickness and full thickness burns, respectively [9]. A total of six burns were created per animal, one of each depth on both sides of the ventral body. There was a non-burned control site in the middle of the body (Fig. 1). Burns were covered with 0.9% saline dressings to prevent them from drying.

4. Microdialysis and histamine assay

After creating the burns, microdialysis was performed by using a CMA/Microdialysis apparatus (Stockholm, Sweden) with a CMA100 probe (shaft 25 mm, membrane length

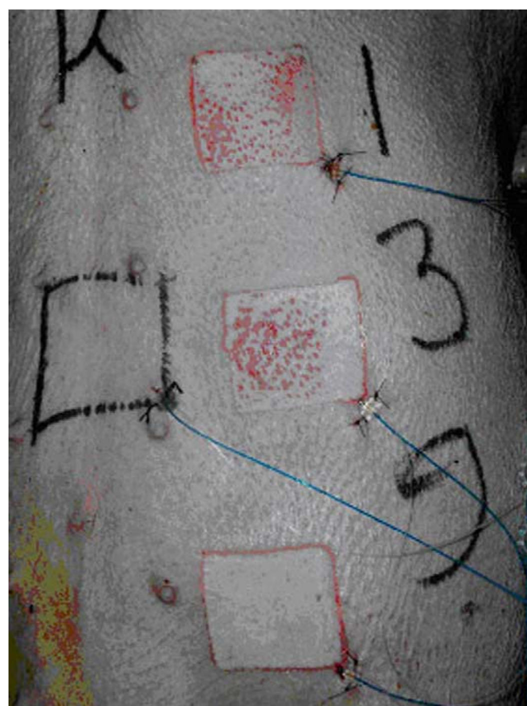

Fig. 1. The three different burn sites (1, 3 and 9 s contact burns) and the non-burned control site in the ventral body of the pig. Each area has the microdialysis probe (blue) inserted in the dermis-subcutis interface from the corner of each area.

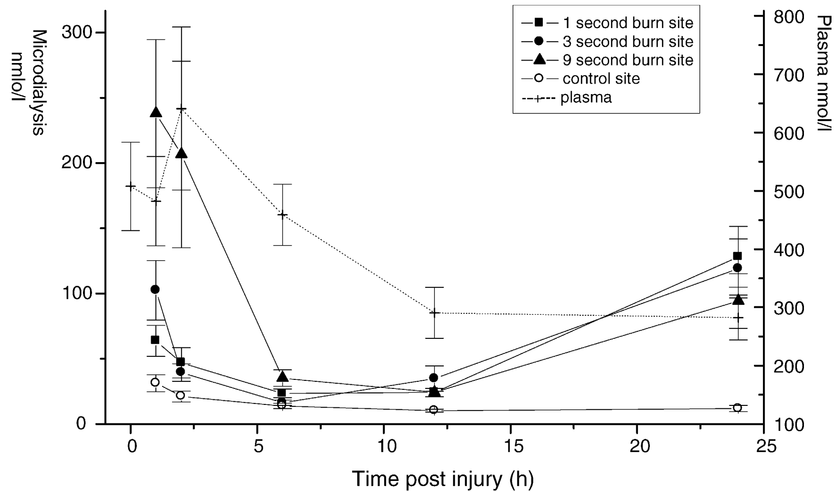

Fig. 2. Histamine levels (median ± iq-range) in the microdialysis eluate and plasma at different time points.

10 mm, diameter 0.5 mm, molecular weight cut-off limit of 20 kDa) as in histamine studies in humans [10]. A total of three probes can be connected to each microdialysis pump. A thick needle (Intraflon 2, 16G, ∅ 1.6 mm, Vycon, Ecquen, France) was inserted from one corner of the burned area towards the opposite corner, where the tip of the needle was pushed through the skin. The needle was removed and probe was inserted inside the lumen of the cannula, which was then pulled out from the insertion site leaving the tip of the probe in the middle of the burned area at the dermis-subcutis interface. Three CMA/Microdialysis apparatuses and seven probes were used simultaneously for each burned animal: one probe was inserted into each burned area (six per animal) and one probe identically to the non-burned control site (Fig. 1). The positioning of each probe takes about 1 min. The flow rate was set at 3.3 μL/min which gives a relative recovery of about 35% for histamine in vitro [11]. Eluate fluid for histamine analyses were collected from burned animals at 1, 2, 6, 12 and 24 h post injury using a collection time of 30 min for each sample. Hence, the first sample collection started at 30 min post burn. Samples for the determination of plasma histamine concentrations were collected from a venous cannula before creating the burn and at the same time as the microdialysis samples. The microdialysis probe was not inserted prior to initiating the burn trauma due to the risk of damaging the probe with heat. Histamine assay was performed by the radio enzyme method using tritium-labelled *S*-adenosylmethione (15 Ci/mmol, Amersham International, Amersham, UK) and liquid scintillation counting for measurement of the radioactivity [12]. At the end of the experiment all the burned sites were excised with the microdialysis probe still in place. In order to verify the correct location of the probe, the skin and subcutaneous fat were separated sharply with scissors.

5. Statistics

The histamine concentrations are presented as median ± interquartile (iq)-range. The correlation co-efficient (R^2) was calculated using the Excel for Windows programme. A non-parametric Wilcoxon test was used for statistical analysis. All analyses were performed using SPSS for Windows (SPSS Inc., Chicago, IL). A p-value < 0.05 was considered statistically significant.

6. Results

All animals survived the experimental period. Hemodynamics and temperature remained stable during the study (data not presented). There were no signs of wound infections or technical problems concerning the sample collection. The mean surface area burned per animal was 1.2% of the total surface area of the animal. The mean temperature of the brass plate after creating the 1, 3 and 9 s burns were 96.2, 94.5 and 91.6 °C, respectively. When dissecting the burned areas, every microdialysis probe was located in the dermis-subcutis interface.

6.1. Microdialysis

The histamine concentrations in the microdialysis eluate and plasma are presented in Fig. 2. There was a good correlation between the contact time and the tissue histamine concentration at 1 and 2 h post burn with correlation coefficients (R^2) of 0.999 and 0.927, respectively (Fig. 3). At 1 h post injury the histamine levels in the eluate in the 9 s (9S) burns were significantly higher than in the 1 s (1S) and 3 s (3S) burn sites ($p = 0.003$ and 0.019, respectively). At 2 h the 9S site had higher histamine levels than other burn sites (9S versus 1S: $p = 0.01$, 9S versus 3S: $p = 0.003$). At

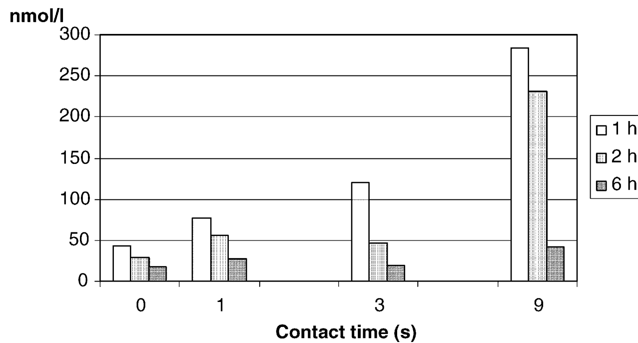

Fig. 3. Burn duration-related histamine concentration in tissue presented as a function of burn duration at 1, 2 and 6 h post injury. The values at 0 s represent the non-burned control site.

6 h the histamine concentration of the 9S site was higher compared to the 1S and 3S sites ($p = 0.03$ and 0.002, respectively). At 12 and 24 h, there were no differences between any burn sites. At all time points all burn sites had higher histamine concentrations than the control sites ($p = 0.012$–0.034) except the 3S at 6 h. There was a significant decrease in histamine concentrations between 2 and 6 h post burn in all burn sites (1S and 3S, $p = 0.007$; 9S, $p = 0.008$). No changes were found between 6 and 12 h. However, between 12 and 24 h post injury there was a significant increase in tissue histamine concentrations in all burn sites (1S and 3S, $p = 0.007$; 9S, $p = 0.028$). The highest concentration of histamine in the end of the study was found in the 1S and 3S sites and the lowest concentration in the 9S, although the differences between different burn sites at this time were not statistically significant.

6.2. *Plasma histamine concentrations*

Thermal injury caused an increase in histamine concentration until 2 h post injury (Fig. 2). Thereafter, the histamine concentrations decreased reaching a plateau at 12 h post injury. There was no late increase in plasma histamine level as was seen in the burn sites.

7. Discussion

Histamine has been considered an important local mediator in burn related oedema formation [1]. The suggested pathways to increased local permeability are through nitric oxide release and/or stimulation of oxygen free radical formation [13]. Previously, tissue histamine concentrations have been mainly determined by analysing tissue biopsies [5–7]. With the microdialysis method, it is possible to collect fluid dialysed from the extracellular fluid without biopsies. This method has been documented in other fields of research both in pig [14] and human skin [8,10,11,15–19]. It has also been seen to be a useful tool for investigating the activation of mast cells and systemic release of histamine by muscle relaxants in humans [17].

The main finding of this study was that the microdialysis technique is applicable in burn wound research. We were able to detect burn depth-related changes in tissue histamine levels. Thermal injury resulted in high initial tissue histamine concentrations, which decreased until 6 h post burn. At 1 h post burn the histamine concentrations in tissue were directly related to the heat exposure time (Fig. 2). A second rise in tissue histamine levels was inversely related to the burn depth without a rise either in the control site or in plasma.

Mast cells are widely distributed in the connective tissue of skin and in mucous membranes of the digestive and respiratory tract, especially near small blood vessels [20]. In normal skin, mast cells occur in the greatest density in the superficial dermis [15]. Thermal trauma causes degranulation of mast cells and histamine liberation [20–22]. In our study, the highest concentration of histamine in the microdialysis eluate was seen in the one hour samples post injury. At that time the histamine levels in the full thickness (9S) burns were significantly higher than in the non-burned control site and the superficial (1S) burns (Fig. 2), which is in accordance with previous studies where tissue histamine levels increased more in severe thermal injury compared to moderate ones [6]. The tissue histamine levels were elevated also in the non-burned control site indicating either a systemic effect caused by the burns or a reaction caused by the insertion of the microdialysis probe. Previously, an up to 47% rise in non-burned skin histamine levels has been reported after a 20% full thickness burn in rats [23].

The early rise in plasma histamine in cutaneous burns has been considered to be primarily due to the release of histamine from the skin [4,5]. Also, interstitial histamine in the dermis has been found to represent specifically the local tissue concentration [16]. In the present study, the highest concentration of plasma histamine was seen later than the highest concentration in tissue. This supports the previous suggestion of the early rise in blood histamine being primarily due to the release of histamine from the skin [5]. Deep burns induce a higher initial tissue concentration of histamine than superficial burns [6]. It is likely that deep burns destroy more mast cells than superficial ones, leading thus to an enhanced liberation of histamine. However, this seems to be in conflict with the fact that the vast majority of skin mast cells are located in the upper dermis [15]. Accordingly, if the upper dermis of the burned site would be the only source of histamine in skin, a full thickness burn would yield the same tissue histamine concentration than a burn that is limited to the superficial third of the dermis. Therefore, it is possible that histamine is released from the middle and deep dermis also. Another explanation is a more profound permeability disorder of deep burns allowing enhanced histamine leakage from the damaged blood vessels. Hence, the origin of the high tissue concentration of histamine in deep burns could be partly due to the

histamine from plasma instead of local mast cells, since the blood vessels in this depth of tissue are not totally obliterated at 24 h post burn due to the progression of burn depth until 48 h [9]. Basophils in blood can also a source of histamine. In addition, as the depth of the burn injury is known to progress in time [9,24], the fact that no further increase in tissue or plasma histamine levels are found during the first 12 h, does not support the role of dermal mast cells, as the sole source of histamine release in deep burns.

There was a late increase in tissue histamine concentrations in all burn sites beginning after 12 h post injury. The median histamine levels were 20, 19 and 8 times greater at 24 h in the superficial, partial and full thickness burns, respectively, compared to the control site (Fig. 2). However, the differences between burn sites were not statistically significant. The fact that there was no simultaneous increase in plasma histamine concentrations, suggests a local event rather than a systemic one responsible for the late increase in tissue histamine concentrations. The delayed increase in tissue histamine levels might be due to the local progressive inflammatory response, which is known to occur in burns [25]. It is also possible, that the late histamine secretion from the originally surviving mast cells is triggered by other mediators, like anaphylatoxins [26]. Partially damaged mast cells liberate histamine, but are able to survive and make new histamine granules. Furthermore, when the initial vasoconstriction subsides [24], the increasing blood flow might bring other mediators to the wound site triggering the release of histamine locally. The possible release of neuropeptides, like substance P, from the damaged nerve endings, may also be involved in delayed histamine deliberation [27]. This neuropeptide induces vasodilatation and vascular permeability by stimulating endothelial cells to round up, vascular smooth cells to relax, and mast cells to release histamine. Also, the subsiding oedema at burn sites might lead to increased histamine concentrations in tissue. A late increase in tissue histamine levels has been reported earlier at 2 days [22] in rats and at 14–21 days [7] in mice post injury. The follow-up times of different histamine studies are inconsistent varying from 120 min to 21 days [2–7,23,28]. In a similar follow-up time with the present investigation with rat paws, no late increase in tissue histamine levels after thermal injury was noted [6].

Plasma histamine concentrations increase within 1 min after thermal injury in rats [4]. In the present study, plasma concentrations were elevated already in the pre burn samples. This is likely due to the initiation of general anaesthesia and cannulation of the animal before creating the burns [4]. Hence, the present pre burn plasma histamine concentration does not give the true baseline level of plasma histamine concentration in these animals. The highest concentration was measured at 2 h, which is in accordance with the peak concentration of plasma histamine in rats 2 h post injury after a 30% partial thickness burn [4]. However, earlier peak concentrations have also been reported [2,3,5,6,28]. We found no late increase in plasma histamine levels, which may be due to stable general anaesthesia during the study period and no further manipulation of the animal or the wound.

With the microdialysis method we were unable to collect very early samples due to both the collection time of 30 min of the samples and the possibility to insert the probe only post injury. With the microdialysis technique the collection time of samples is usually 15–120 min, depending both on the volume of eluate needed for the laboratory analysis and the flow rate of the perfusion fluid limiting the collection of very early samples. Due to the risk of destroying the microdialysis probe due to excessive heat, when contact burns are created the probes were inserted post burn. Hence, no pre burn tissue histamine concentrations were measured.

As the microdialysis method has been shown to collect about 35% of the histamine in a standardised solution in vitro [11], it can be postulated that the concentrations of histamine in tissue in this study are actually higher than the measured values, hence, being closer to those in plasma. Furthermore, the histamine liberation caused by the insertion of the microdialysis probe has been found to last up to about 40 min [29]. This, in addition to the manipulation of the animal prior to creating the burns, explains at least partly the increase in histamine values in the eluate from the non-burned control sites in the beginning of the study. Since burns of three different depths were created to each animal, the plasma histamine levels in superficial, partial thickness of full thickness burns separately could not be determined. Also, because tissue histamine concentrations were not additionally analysed from tissue biopsies, blister fluid or local lymph, the histamine concentrations from the dialysate in this study, could not be compared to concentrations analysed by other methods.

To our knowledge, this is the first time the microdialysis technique has been reported in burn wound research. It is an easily applicable method of collecting on-line samples from burned tissue. This method provides a useful tool in investigating the effects of different treatment modalities on the secretion of substances into interstitial fluid within burned tissue.

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