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Tissue substance P levels in acute experimental burns

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Abstract

Purpose of study: To determine the tissue concentrations of substance P (SP) in burns of different depths and to see whether the concentrations of SP relate to the previously reported late increase in tissue histamine concentrations.
Materials and methods: Experimental animal study with pigs. Superficial, partial thickness and full thickness burns were created and the microdialysis method used to collect samples for substance P analysis from burned and non-burned control tissue during a 24-h follow-up.
Results: Substance P concentrations increase after 4 h in the partial and full thickness burns reaching the peak at 18 and 12 h, respectively. The increase was later and more modest in the superficial and control sites. At 24 h the SP median concentrations in the superficial, partial and full thickness burns were 28%, 85% and 140% higher than in the control site, respectively. There was a peak in the SP concentration in serum at 4 h followed by a decrease and stabilization at a level about 15 pg/ml.
Conclusions: The release of substance P in tissue is a possible cause of the late increase in tissue histamine in burns. Medical inhibition of SP is of clinical interest in preventing late histamine liberation in burns.

Keywords: Burns; Microdialysis; Substance P; Experimental; Pig

1. Introduction

Severe burn induces liberation of different mediators in skin. The potential release of neuropeptides, like substance P (SP), from the damaged nerve endings are known factors involved in the delayed histamine deliberation in burns. This neuropeptide induces vasodilation and vascular permeability by stimulating endothelial cells to round up, vascular smooth cells to relax, and mast cells to release histamine [1]. SP levels after burn have been determined from plasma [2], lymph [3,4], jejunum [5] and burn eschar [6]. Recently, we reported the use of microdialysis in collecting samples from burned tissue for analysis [7]. A burn depth-related increase in tissue histamine concentrations was found at 1 and 2 h post burn. Also, there was a late secondary increase in histamine concentrations at 24 h post injury.

The aim of this study was firstly to determine the concentrations of SP in burns of different depths and secondly to see whether the concentrations of SP relate to the previously found late increase in histamine concentrations.

2. Materials and methods

Three-month-old Finnish female landrace pigs ($N = 8$, weight 28–38 kg) were used for the burn experiments and additional two pigs served as non-burned controls. The animals were fasted for 12 h with free access to water prior to the experiment. After premedication with atropine 0.05 mg/kg and azaperone 8 mg/kg intramuscularly an ear vein was cannulated for administration of thiopental sodium (5–15 mg/kg i.v.) as induction of general anesthesia. Anesthesia was maintained with infusion of thiopentone (5 mg/kg/h). Pancuronium (2–4 mg boluses i.v.) was administered prior to creation of burns and for shivering when needed and fentanyl 30 μg/kg/h during the creation of burn wounds and 5 μg/kg/h thereafter for pain relief.

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Animals underwent tracheostomy and were mechanically ventilated with a volume-controlled ventilator (Servo 900E, Siemens Elema AB, Solna, Sweden) with a tidal volume of 10 ml/kg. Minute volume was adjusted to achieve normocapnia ($paCO_2$ 4.4–5.5 kPa). Fraction of oxygen in the inspiratory gas (FiO_2) was adjusted to keep arterial partial pressure of $O_2 > 13.3$ kPa. Positive end expiratory pressure of 5 cmH_2O was maintained throughout the study. Right carotid artery and internal jugular vein were cannulated for blood pressure and central venous pressure monitoring and blood sampling. Systemic and central venous pressures were recorded with quartz pressure transducers and displayed on a multimodular monitor and recorder (AS3, Datex-Ohmeda, Helsinki, Finland). Continuous information was collected automatically in 2-min intervals (Clinisoft, Datex-Ohmeda, Espoo, Finland). Heart rate was continuously monitored with electrocardiogram. Haemodynamics were recorded at 15-min intervals. A urinary catheter was placed for urinary output measurements. A thermostat-controlled operation table heater, warmed fluids and a heat reflector lamp were used to maintain normal body temperature (37–39 °C). 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, no actual fluid resuscitation due to burn was indicated. Arteriolar blood gases and hemoglobin levels were analyzed (ABL 520, Radiometer, Copenhagen, Denmark) in 2–3 h intervals. The lactate levels were determined with the YSI 2300 STAT plus (YSI Incorporated (Life Sciences), Yellow Springs, OH, USA) analysator and the blood sugar levels with the Precision Xtra meter (MediSense, Abbott Laboratories, USA).

After inducing general anesthesia the ventral side of the body of the pig was shaved and washed with chlorhexidine solution (5 mg/ml). The burns were created with a custom made 4 × 4 cm brass block (weight 530 g) heated to 100 °C in boiling water. Only the weight of the block was used to create the burns and no pressure was applied. Contact times of 1, 3, and 9 s were used to create histologically confirmed superficial, partial thickness and full thickness burns [8]. There was one burn of each contact time on each side of the ventral body and one control area. Accordingly, in eight burned animals there were a total of 16 sites of each burn depth and eight control sites in this study.

After creating the burns, microdialysis was performed by using a CMA/Microdialysis Apparatus (Stockholm, Sweden) with a CMA/20 probe (14/10 PES, cut off 100 kDa, membrane length 10 mm, Carnegie, Sweden) with the cannula inserted in the dermo-adipose plane of each burn and control area [7]. Three CMA/Microdialysis Apparatuses and seven probes were used simultaneously for each burned animal: one probe was inserted into each burned area (six/animal) and one probe identically to the non-burned control site. The probe was perfused at a rate of 2 μl/min with Ringersteril (Baxter Oy, Finland). The dialysate was collected in 0.5 ml eppendorff tubes at 2, 4, 6, 12, 18 and 24 h post injury and placed on the ice bath. Dialysate samples were stored at −70 °C until analysis. In vitro recovery for the probe was previously determined by perfusing standard solution in room temperature mimicking the conditions of in vivo experiments. The in vitro recovery for substance P was 20%. All reagents used were of analytical grade or higher purity. Substance P standards and were purchased from Sigma (St. Louis, MO, USA).

Substance P peptide was analyzed by Cayman Chemical's Substance P EIA Assay Kit (Ann Arbor, MI, USA) with a working range of 3.9–500 pg/ml. A volume of 50 μl microdialysis and serum samples were analyzed according to the instructions of the manufacturer. The absorbance at 405 nm was measured using a microplate reader (Tecan SPECTRAFluor, Tecan Group Ltd., Maennedorf, Switzerland). All samples and calibrators (supplied by the manufacturer) were analyzed in duplicate. Serum samples were purified by solid phase extraction cartridges Varian Bond Elut C-18, 100 mg/1 cc (Harbor City, CA, USA) before EIA analysis.

The values are presented as median (±quartiles). Mann–Whitney test was used for statistical analysis between groups. All SP values below the detection limit (3.9 pg/ml) were given a value 1.95 pg/ml (=mean of 0 and 3.9 pg/ml) for statistical analysis purposes.

3. Results

In the non-burned control animals the SP concentrations in tissue were undetectable. The tissue concentrations of SP in the burned animals are presented in Fig. 1 and the concentrations in serum in Fig. 2. There were no changes in the tissue concentrations during the first 4 h post injury. The concentrations began to increase after 4 h in the partial and full thickness burns reaching the peak at 18 and 12 h,

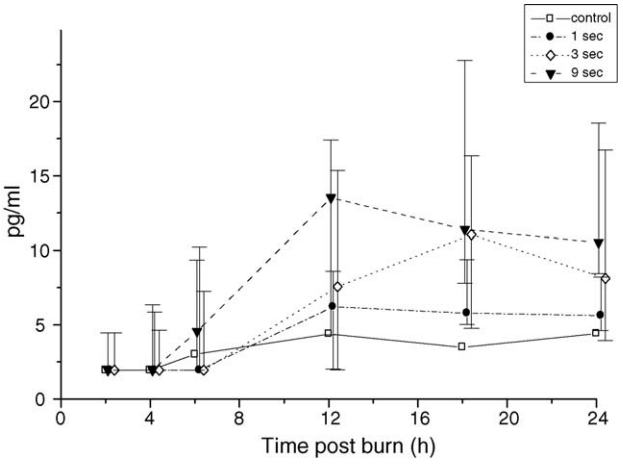

Fig. 1. Tissue concentrations (median + quartiles) of substance P in different burn and control sites.

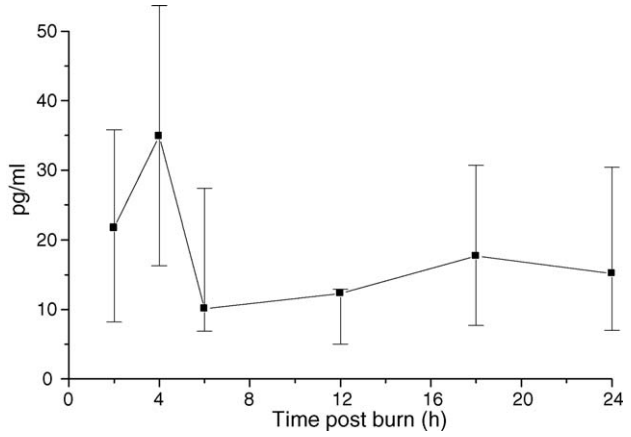

Fig. 2. Substance P concentrations in serum (median + quartiles).

respectively. The increase was more modest in the superficial and control sites. The full thickness burns had higher SP concentrations than the superficial burn sites ($p = 0.001$) and the control sites ($p = 0.001$). At 24 h the median concentrations in the superficial, partial and full thickness burns were 28%, 85% and 140% higher than in the control site, respectively. There was a peak in the concentration in serum at 4 h followed by a decrease and stabilization at a level around 15 pg/ml.

4. Discussion

Understanding the roles of different mediators in burns is crucial in the attempt of burn wound manipulation. The final aim of mediator research in burns is to find a way to inhibit burn depth progression. With targeted burn wound manipulation the development of burn-related tissue ischemia might be prevented or reversed. Heparin has been beneficial only when given pre burn [9] but ibuprofen and imidazole inhibited microvascular occlusion given as late as 6 h post injury by inhibiting prostacyclin deliberation to dermal vasculature [10]. The beneficial effects on dermal ischaemia of topical methylprednisolone acetate [11], prostaglandin synthesis inhibitors indomethacin and acetylsalicylic acid [12], thromboxane inhibitors [13], ibuprofen [14,15], C1-inhibitor [16] and lidocain and prilocain [17] have also been documented.

When the initial vasoconstriction in the zone of ischemia subsides [18], the increasing blood flow may bring other mediators to the wound site, like anaphylatoxins [19] triggering the release of histamine locally from the originally surviving mast cells. Partially damaged mast cells liberate histamine, but are able to survive and make new histamine granules. Furthermore, the potential release of neuropeptides, like substance P, from the damaged nerve endings are known factors involved in the delayed histamine deliberation in burns [1].

Substance P was discovered by von Euler and Gaddum 1931. The isolation was carried out in the 1970's [20]. SP is synthesized in the ribosome as a larger protein and then enzymatically converted into the active decapeptide. It is secreted by nerves and inflammatory cells such as macrophages, eosinophils, lymphocytes and acts by binding to the neurokinin-1 receptor. SP regulates smooth muscle contractility, vascular permeability and immune function in the GI-tract. SP-induced release of inflammatory mediators, such as cytokines, oxygen radicals, arachidonic acid derivatives and histamine potentiates tissue injury and stimulates leukocyte recruitment hence enhancing the inflammatory response [21]. It induces degranulation of mast cells and histamine and serotonin release by a receptor-independent mechanism [22]. It also contributes to the development of oedema in the rat hind paw after burn [23]. Löfgren et al. induced scald injuries to the rat hindpaw [24]. Blood flow was recorded by laser flowmetry. Pre-treatment with neuropeptide antagonists attenuated the first increase of blood flow and almost abolished the secondary increase in blood flow. Their conclusion was that sensory neuropeptides play a significant role in the blood flow increase following thermal injury.

In our previous study [7] we presented a marked initial burn depth-related increase in tissue histamine concentrations after burning. Full thickness burns induced higher histamine concentrations than partial thickness and superficial burns. A secondary increase was found at 24 h after injury. In the same study the histamine concentrations in plasma decreased until 12 h post burn and stabilized after that. Accordingly, no late increase in histamine levels in plasma was seen as was in tissue. Hence, the late rise in tissue histamine concentration was rather a local event in the burned areas than a systemic one.

One aim of this study was to see whether the possible changes in SP concentrations in burned tissue relate to the late rise in tissue histamine. Initially the SP concentrations were very low. They began to increase at 4 h in deep and partial thickness burns reaching a peak at 12 and 18 h post injury, respectively. The increase was more modest in superficial burns. Therefore, it is possible that the increase in substance P concentrations causes the late secondary increase in tissue histamine concentrations. The finding of the highest SP concentrations in deep burns is in accordance with the fact that deep burns destroy more nerve endings than superficial burns. Also, the more profound damage in deep burns explains the earlier peak in SP concentrations compared to partial thickness burns. There was also a small increase in the SP concentrations in the non-burned control sites. This might arise from the increased SP levels seen in serum.

In conclusion, this study demonstrates an increase in substance P levels in burned tissue. This precedes that of histamine demonstrated earlier with the microdialysis method. Hence, the release of substance P in tissue is a possible cause of the late increase in tissue histamine in burns. Medical inhibition of SP is of clinical interest in preventing late histamine liberation in burns.

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