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Suction Blister Formation in Skin after Acute and Repeated Mast Cell Degranulation

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Mast cells and their proteases are thought to participate in the development of skin blisters in various pathological conditions. In this study, suction blistering was used as an experimental model to evaluate the significance of mast cells in blister formation after pre-treatment of normal skin with intradermal injections of 100 µg/ml compound 48/80 (a mast cell degranulator) or with 0.1% capsaicin cream. Tryptic and chymotryptic enzyme activities in blister fluids were measured with sensitive *p*-nitroanilide substrates. Repeated injections of compound 48/80 once a day on 3 or 5 consecutive days or capsaicin applications 3 times a day for 7 or 10 days were used to induce mast cell degranulation and inflammation in normal skin. Both treatments ultimately led to decreased wheal and erythema reactions before suction blistering, but neither treatment affected the size or formation rate of suction blisters. No suction blister fluids had detectable levels of chymotryptic activity, but blister fluids from bullous pemphigoid, herpes zoster and insect bullous eruption, used as the control, revealed clear chymotryptic activity. In addition, tryptic activity in suction blister fluids was not significantly altered after compound 48/80 and capsaicin pre-treatments. However, if the wheal reaction was induced immediately before suction blistering, a significantly increased rate in blister formation together with increased tryptic activity was found, but, unexpectedly, no chymotryptic activity could be detected in blister fluids. The results show that repeated mast cell degranulation in normal skin has no effect on the formation rate of suction blisters, which developed more rapidly on acutely whealing skin. This is probably due to skin oedema rather than mast cell proteases, since no chymotryptic activity was detected in suction blisters where tryptic activity exhibited high individual variation. *Key words: mast cell; protease; suction blister.*

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Mast cells and their serine proteinases, tryptase and chymase, have been assumed to be important regulatory elements in cutaneous inflammatory and blistering diseases (1–3). Bullous pemphigoid is characterized by urticarial lesions, which appear prior to blister formation. The participation of mast cells has been suggested to be a factor in the development of skin lesions in pemphigoid, since the early lesion mast cells are hypogranulated and their granules are spread extracellularly (3). In addition, high histamine and tryptase levels detected in pemphigoid blister fluids indicate mast cell activation (2, 4). In the healthy-looking skin of patients with dermatitis herpetiformis, injection of compound 48/80 has in some cases been found to cause a dermatitis herpetiformis-like bullous lesion, which suggests mast cell involvement (5).

Since suction blisters arise in the lamina lucida of the basement membrane (6), a suction blister device was used in this study to clarify whether (a) the matrix and basement membrane structures in normal skin can be affected by preceding compound 48/80 treatment leading to more rapid suction blister formation, or whether (b) depletion of mast cells from mediators by repeated administration of compound 48/80 could result in slower suction blister formation. Since sensory nerves and mast cells form a close anatomical and functional unit in skin and neuropeptides can induce mast cell degranulation (1), capsaicin pre-treatment (7) was used to clarify the possibility that disturbances in sensory-nerve and mast cell interactions could modulate suction blister formation. The levels of trypsin- and chymotrypsin-like enzyme activities were measured in the blister fluid in order to evaluate the influence of these compounds.

MATERIAL AND METHODS

Chemicals

Aprotinin, Tris(hydroxymethyl)aminomethane, bovine serum albumin (BSA), compound 48/80, 8-methyl-*n*-vanillyl-6-nonenamide (capsaicin), Z-Gly-Pro-Arg-*p*-nitroanilide, soybean trypsin inhibitor (SBTI) and heparin sodium salt from porcine intestinal mucosa were obtained from Sigma (St Louis, MO, USA). N-Succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide was purchased from Vega (Tucson, Alabama, USA).

Treatment of skin for suction blistering

Experiment I. This experiment included 7 healthy volunteers (5 females and 2 males, age range 23–48 years, mean 38 years). Before blister induction, 3 adjacent skin areas of each subject were selected either on the medial forearm (4 subjects) or on the thigh (3 subjects). Of the 3 skin areas, the first was treated with 0.1% capsaicin cream (the drug was dissolved in Novalan® base cream, Orion Corporation, Helsinki, Finland) 3 times a day for 7 days and with 100 µl 0.9% NaCl intradermal injection per day on 3 consecutive days immediately before inducing suction blisters. The second skin area, imitating the first, was treated topically with Novalan cream and with an injection of 100 µl (100 µg/ml) compound 48/80. The last injection of the compound 48/80 was given 1–2 h before starting the blister induction. By then, the wheal and flare had subsided entirely. The third skin area, which served as the control, was treated with both Novalan cream and 0.9% NaCl injections. In each skin area, 2 adjacent 100 µl injections were given matching the adjacent caps in the suction blister device. All injections were given by the same person. Before starting the experiment, each 25 cm^2 skin area was marked with skin tape. The suction blister device (Dermovac, Ventipress OY, Finland) was applied on each treated skin area using a vacuum pressure of 300 mmHg. Full-size blisters were obtained within approximately 2.5 h. Blister fluid samples were collected immediately from 2–3 intact blisters on each skin area.

Experiment II. This suction blister experiment included 3 healthy female subjects (age range 31–48 years, mean 42 years). Suction blis-

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Table I. *Tryptic and chymotryptic activities in suction blisters induced immediately after compound 48/80 injection into normal skin and in blister fluids of different bullous diseases*

Blister fluid	Tryptic activity (U/l)		Chymotryptic activity (U/l)	
	Control	SBTI	Control	Aprotinin
Suction blisters:				
Subject 1				
Control I	12.1	9.9	ND	ND
Control II	11.4	10.7	ND	ND
Compound 48/80	32.8	12.9	ND	ND
Subject 2				
Control I	24.0	19.7	ND	ND
Control II	33.0	19.7	ND	ND
Compound 48/80	45.2	14.8	ND	ND
Subject 3				
Control I	5.8	4.8	ND	ND
Control II	4.2	3.8	ND	ND
Compound 48/80	23.8	18.6	ND	ND
Bullous diseases:				
Herpes zoster				
Patient 1	7.2	5.4	1.4	0.70
Patient 2	8.3	6.1	0.94	0.32
Patient 3	8.3	7.1	0.25	0.07
Bullous pemphigoid				
Patient 1	23.2	19.6	0.17	0.16
Patient 2	40.9	34.8	0.28	0.24
Insect bullous eruption				
Patient 1	20.8	17.9	0.30	0.05
Erythema multiforme				
Patient 1	18.4	17.2	ND	ND
Epidermolysis bullosa simplex				
Patient 1	20.2	15.9	ND	ND

Suction blisters: Control I = control area without any treatment; Control II = single 100-µl injection of 0.9% NaCl; and Compound 48/80 = single 100-µl injection of 100 µg/ml compound 48/80 immediately before suction blister.
SBTI, soybean trypsin inhibitor. ND, not detected.

tering was performed as described in experiment I, but 0.1% capsaicin/base cream was applied for 10 days and 100 µg/ml compound 48/80 was injected for 5 days immediately before inducing suction blisters.

Experiment III. This experiment included 3 subjects (2 females and 1 male, age range 21–51 years, mean 32 years), and the suction blister device was placed immediately on areas treated with two adjacent injections of 100 µl (100 µg/ml) compound 48/80 or 100 µl 0.9% NaCl. Control blister fluid was obtained from an untreated area not exposed to any injection. Capsaicin was not used in this experiment.

In the second and third experiments suction blistering was performed on the lateral aspect of the thigh. The area of flare and wheal was measured after 15 min and 30 min of compound 48/80 injection. The longest and shortest diameter of the wheal was measured and the area was calculated using the ellipse formula. The duration of the flare caused by 0.1% capsaicin was observed by the subject. All subjects belonged to the nursing or research personnel of the Department of Dermatology, Kuopio University Hospital.

As controls for comparing the enzyme activity levels, blister fluid samples were collected from spontaneous blisters of patients with bullous pemphigoid (2 subjects), herpes zoster (3 subjects), erythema multiforme (1 subject), epidermolysis bullosa simplex (1 subject) and insect bullous eruption (1 subject).

Blister fluid samples were cleared immediately by centrifugation at 10,000 rpm in an Eppendorf table-top centrifuge and stored at –20°C for the measurement of tryptic and chymotryptic activities. The study protocol was approved by the Ethics Committee of Kuopio University Hospital, Kuopio, Finland.

Measurement of tryptic and chymotryptic activities in blister fluids

The assay mixture (200 µl) contained 10 µl of blister fluid, 50 µg/ml heparin and 85 mM Tris-HCl, pH 7.6. Tryptic activity was measured with 0.2 mM Z-Gly-Pro-Arg-pNA in the presence or absence of 100 µg/ml soybean trypsin inhibitor and chymotryptic activity with 0.2 mM Suc-Ala-Ala-Pro-Phe-pNA at 1.7 M NaCl in the presence or absence of 100 µg/ml aprotinin. The linear hydrolysis rate of both substrates was measured by monitoring the absorbance at the wavelength of 405 nm, using 96-well assay plates. These substrates and assay conditions are sensitive for mast cell tryptase and chymase (8, 9). Further, soybean trypsin inhibitor is not able to inhibit tryptase but it inhibits to varying extent several tryptic enzymes from plasma (9, 10). On the other hand, aprotinin can not inhibit chymase but it inhibits another chymotryptic enzyme from mast cells, cathepsin G (8).

Statistical analysis

Student's *t*-test was used to test statistical significance ($p < 0.05$).

RESULTS

Suction blister formation

Capsaicin induced an erythematous reaction, which was maximal about 1 h after topical application, but attenuated gradually after repeated treatments (3 times a day for 7 or 10 days), as is expected during capsaicin treatment (7). A single injection of compound 48/80 induced wheal and erythema within 15 min, but repeated intradermal injections for 2 or 4 days (on 3 or 5 consecutive days) produced a diminished area of wheal and flare (wheal area from 18.5 ± 11 cm^2 seen on the first day to 5.8 ± 3 cm^2 on the third day, $p < 0.02$, $n = 7$), which suggests depletion of mast cells from granules (11, 12). In addition, the duration of wheal decreased from 2.0 ± 0.6 h to 0.7 ± 0.3 h ($p < 0.00001$, $n = 7$). No spontaneous blister formation was observed during the course of injections.

In the first and second experiments, the fully developed blisters appeared within 2.5 h and at about the same time on each treated area. No apparent differences could be observed in the rate or size of blister formation. In the third experiment, the fully developed blisters appeared within 2.47 ± 0.1 h on the NaCl-treated control area, within 2.61 ± 0.2 h on the area without any treatment and within 1.67 ± 0.09 h on the area treated with compound 48/80 ($p < 0.001$, $n = 3$).

Tryptic and chymotryptic activities in suction blister fluids

In all experiments, tryptic activity, but not chymotryptic activity, could be detected in blister fluids. In the first experiment, tryptic activity in suction blister fluids from control, capsaicin- and compound 48/80-treated sites was 9.8 ± 9.2, 6.4 ± 3.5 and 6.4 ± 3.2 U/l ($n = 7$), respectively, but the activity was reduced to 7.3 ± 4.4, 4.9 ± 2.4 and 6.5 ± 3.7 U/l, respectively, when soybean trypsin inhibitor was present in the assay mixture. In the second experiment, the corresponding values in control, capsaicin- and compound 48/80-treated sites were 18.5 ± 7.4, 20.2 ± 7.2 and 17.7 ± 11.7 U/l ($n = 3$), respectively, but only 9.8 ± 5.0, 11.3 ± 3.0 and 8.9 ± 1.8 U/l, respectively, in the presence of soybean trypsin inhibitor. Both capsaicin and

compound 48/80 could not significantly alter tryptic activity in either experiment. However, variation between subjects was high, and increased, decreased and unchanged tryptic activity following both pre-treatments was observed.

The third experiment showed that the injection of compound 48/80 immediately before inducing suction blisters could increase tryptic activity in all three subjects to 1.4 to 5.7-fold compared with the controls. However, only one subject displayed clearly elevated tryptic activity in the presence of soybean trypsin inhibitor (Table I). Despite the extensive wheal reaction no chymotryptic activity could be detected in any of the suction blister fluids (Table I).

Tryptic and chymotryptic activities in blister fluids of different bullous diseases

Except for herpes zoster, high tryptic activity levels were measured in all blister fluids, and they are close to those measured in suction blister fluids immediately after the injection of compound 48/80 (Table I). However, in contrast to suction blister fluids, clear chymotryptic activity was detected in both pemphigoid patients and in 1 patient with insect bullous eruption (Table I). Blister fluids from 3 patients with herpes zoster exhibited relatively low tryptic activity but high chymotryptic activity (Table I). Therefore, if a suction blister fluid contains chymotryptic activity the employed suction blister and enzyme assay methods in this study are strongly supposed to be competent to detect released chymotryptic activity (Table I).

DISCUSSION

Previous studies have suggested that mast cells are involved in different bullous eruptions (2–5, 13). The degranulation of mast cells could be a result of complement activation and formation of anaphylatoxin C3a and C5a, or due to the action of neuropeptides and cytokines (1). Although mast cells are not in direct morphological contact with the basement membrane, released granules could reach this zone. Subsequently, tryptase and chymase could cleave the basement membrane directly (14, 15) or indirectly by first activating collagenolytic proteinases (14–17) and stimulating fibroblasts (18). Thus, mast cell activation in the urticarial wheal could result in weakening of the connective tissue and, consequently, increased sensitivity to blister formation during suction blister induction.

A total treatment duration of 2 days with compound 48/80 was assumed to be long enough to cause any mast cell-dependent alterations to the normal dermal connective tissue, but a 4-day treatment was also used for confirmation. Although repeated compound 48/80 injections resulted in significantly diminished wheal, probably due to depletion of mast cells from granules (11, 12), this pre-treatment had no apparent effect on the rate or size of suction blister formation. Similarly, the pre-treatment with capsaicin for 7 or 10 days led to attenuation of the erythematous reaction, suggesting depletion of sensory nerves from neuropeptides (7), but no alterations in suction blister formation were observed. Furthermore, no significant alterations in tryptic activity were detected although the variation between subjects was high. This suggests that in some cases tryptase was present in the basement membrane zone in increased quantities, and in other cases tryptase was mostly depleted from that zone. The reason for the high variation in tryptic activity, despite the clearly diminished wheal area, could be that large tryptase-heparin proteoglycan complexes diffuse in and are cleared from the extracellular matrix slowly. At the moment, no physiological inhibitor is known for tryptase suggesting prolonged action time in the extracellular environment (1). However, other tryptic enzymes from plasma may also account for the variation in activity levels (10). The unresponsiveness to compound 48/80 and capsaicin pre-treatments and high variation in tryptic activity suggest that mast cells, tryptase and sensory nerves are not the key factor in suction blister formation on normal skin.

The single compound 48/80 injection immediately prior to suction blistering increased both the rate of suction blister formation and tryptic activity. However, only 1 subject out of 3 showed clearly elevated tryptic activity in the presence of soybean trypsin inhibitor. This suggests that other tryptic enzymes, e.g. those from plasma (10), may be more significant than tryptase in the suction blister formation. In addition, the dermal oedema itself could explain, in part, this blistering sensitivity. However, since high tryptic activity levels were measured in blister fluids of bullous diseases, paralleling the previous study (2), tryptase could maintain the developed blisters in these diseases by continuously degrading fibronectin (14) and activating collagenolytic metalloproteinases (14, 16).

One marked finding was that chymotryptic activity was not detected in suction blister fluids, indicating no role in the development of suction blisters. This may be due to slow diffusion of even larger chymase-heparin proteoglycan complexes than tryptase-heparin proteoglycan complexes (19) or rapid inactivation of chymase and cathepsin G by protease inhibitors (20, 21) in an acute wheal reaction on normal skin. In blister fluids from bullous eruptions, chymotryptic activity was detected, suggesting a role in their pathomechanism (15). This could be due to inactivation of α_1-proteinase inhibitor and α_1-antichymotrypsin by chymase (20), and a balance may have been reached between chymase inactivation and protease inhibitor cleavage in more chronic conditions. Therefore, chymase may well be inducing blisters (15) in circumstances where its controlling mechanisms fail.

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