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PROSTAGLANDIN E_2 IN BLISTER FLUID OF BULLOUS DISEASES AND EXPERIMENTAL SUCTION BLISTERS

Kjell Strandberg and Östen Hägermark

From the Department of Pharmacology, Karolinska institutet, and Departments of Clinical Pharmacology and Dermatology, Karolinska sjukhuset, Stockholm, Sweden

Abstract. Prostaglandin(PG)-like activity in the fluid of spontaneous and suction blisters was measured by bioassay on isolated guinea-pig colon after acidic lipid extraction. The fluid from spontaneous blisters in 15 patients with various bullous dermatoses, such as pemphigoid, porphyria cutanea tarda, erythema multiforme, contact dermatitis, X-ray dermatitis, all contained measurable amounts of activity, varying from 0.4 to 54 ng/ml, expressed as PGE_2-activity. From 4 patients with pemphigoid, samples of fluid were collected, adequate to permit of analysis regarding the identity of the spasmogenic material. In silicic acid column chromatography, thin-layer chromatography, and in reversed phase partition chromatography, the major part of the biological activity co-chromatographed with ^3H-PGE_2. In one patient part of the activity coincided with $PGF_{2\alpha}$. The PG-like activity of experimental suction blisters was found to be significantly higher in patients with dermatitis herpetiformis than in control and psoriasis patients. The appearance of PGs in blister fluid is compatible with a role as chemical mediator involved in blister formation.

Key words: Prostaglandin, Blister fluid; Bullous dermatoses; Suction blisters

The biochemical events leading to blister formation in bullous diseases are still mainly unknown (3, 6). It has been assumed that proteolytic enzymes are activated (7, 8, 18, 22), possibly via complement-mediated reactions, as complement factors are bound to the skin in pemphigus and bullous pemphigoid (9, 11). In these two diseases, immunofluorescence studies have demonstrated the presence of both circulating and tissue-bound auto-antibodies directed to the primary site of pathology in the epidermis, i.e. to the intercellular substance in pemphigus and to the basement membrane in pemphigoid (5, 17).

Recently, prostaglandin E_2 (PGE_2) was identified in human burn blister fluid (2). In skin, PGE_2 is a potent vasodilator (21), possibly acting in part by releasing histamine (15, 23). These properties suggest that PGE_2 might be a chemical mediator involved in blister formation, since vasodilation and increased capillary permeability are believed to be of importance for this phenomenon (7). In the present investigation we have analysed the PG content of blister fluid of varying origin, including suction blisters.

MATERIALS AND METHODS

Patients and sampling technique

Spontaneously occurring blisters were anlysed in 6 patients with various bullous diseases and 9 patients with bullous pemphigoid (Table I). In 10 patients with dermatitis herpetiformis (DH) and 4 of the patients with spontaneous blisters, suction blisters were produced in clinically uninvolved, abdominal skin using the "Dermovac" device (Instrumentarium, Helsinki, Finland) described by Kiistala (20). Two suction cups were applied. Inside each cup was an adapter plate with 5 holes, each with a diameter of 8 mm. Suction pressure was 200–260 mmHg below atmospheric pressure. Eleven patients with minor skin disorders served as controls. Suction blisters were also produced in 5 patients with psoriasis. Suction time (blistering time) varied between 30 and 150 min (Table II).

Both types of blister were punctured with injection needles and the fluid collected in plastic syringes. The fluid was then added to an equal volume of saline and 2 vol of ethanol containing 0.2% *d,l*α-tocopherol as antioxidant (2). The samples were stored at −20° until analysis. The blister fluid from spontaneous bullae was withdrawn before any treatment with corticosteroids was started. The patients with DH were undergoing treatment with dapsone (Avlosulfon; ICI, Macclesfield, England) and had no or virtually no skin lesions at the time of blister suctioning. None of the patients received analgesics on the 2 days preceding the sampling.

Table I. *Spontaneous blisters*

Patient	Age	Sex	Diagnosis	Blister fluid volume (ml)	PG-activity (ng/ml)
K. D.	70	♂	X-ray dermatitis (mycosis fungoides)	2.0 0.8	2.0 3.8
E. L.	66	♂	Porphyria cut.tarda	0.5	6.6
G. S.	36	♀	Herpes zoster (+Hodgkin's dis.)	0.2	11.1
M. N.[a]	25	♀	Contact dermatitis	1.5	3.1
S. L.	11	♀	Erythema multiforme	1.5	1.5
A. B.	51	♀	Traumatic blister	0.2 2.0	8.0 1.6
S. B.	88	♀	Bullous pemphigoid	8.6	54.0
E. H.[b]	88	♀	Bullous pemphigoid	12.0 0.2	3.6 9.5
S. L.	85	♂	Bullous pemphigoid	0.2	7.5
H. O.	84	♂	Bullous pemphigoid	4.0	3.8
E. W.[a]	79	♀	Bullous pemphigoid	2.4	0.4
V. R.[a]	71	♂	Bullous pemphigoid	6.2	15.3
K. G.	70	♂	Bullous pemphigoid	1.0	5.3
G. E.	91	♀	Bullous pemphigoid	0.5	5.7
G. B.[a]	58	♂	Bullous pemphigoid	1.1	3.8

[a] Suction blisters were also analysed (cf. Table II).
[b] Two occasions, 2 months apart.

Extraction and solvent partition

The blister fluid samples were evaporated free of ethanol under reduced pressure at room temperature. If the fluid volume was less than 2 ml it was complemented with water. After acidification to pH 3 by the addition of 3 M citric acid the solution was extracted twice with 2 vol of light petroleum ether, which was discarded. PGs were then extracted twice with 2 vol of ether. The ether extract (acidic lipid extract) was washed with water until neutral and evaporated to dryness *in vacuo* or shaken twice with 2 vol of 0.2 M Sörensen phosphate buffer, pH 8. The combined buffer portions were adjusted to pH 3 with citric acid and extracted with ether and evaporated as described. The more extensive extraction procedure was employed for material to be subjected to chromatography. The recovery of PGs averaged 95%, as determined by the recovery of tracer amounts of ^{3}H-labelled PGE_2 with a specific activity of 4.5 Ci/mmole added to samples of blister fluids carried through the extraction procedure.

Chromatographic procedures

1. Silic acid chromatography. Columns containing 1 g of silic acid (Mallinckrodt, 100 mesh) activated at 115°C were used. The acidic lipid extract was applied to the column in ethyl acetate/benzene (1 : 9). Elution was performed by employing a linear gradient of ethyl acetate in benzene starting with 1 : 9 and finishing with ethyl acetate. Remaining lipid material was eluted by 25 ml of methanol. Fractions of 1–2 ml were collected.

2. Reversed phase chromatography. This was performed on 4.5 g hydrophobic Supercel using 4 ml of isooctanol/chloroform (1 : 1) as the stationary phase and 300 ml methanol/water (1 : 1) as the moving phase (4). Fractions of 2–3 ml were collected.

3. Thin-layer chromatography (TLC). Preparative TLC was carried out as described in detail elsewhere (1). Chromatoplates were prepared from a mixture of 30 g Silica gel H (Merck) and 60 ml water with or without 1 g silver nitrate. The following solvent systems were used: benzene/dioxane/acetic acid (20 : 20 : 1) (A I) and ethyl acetate/acetic acid/methanol/2,2,4-trimethylpentane/water (110 : 30 : 35 : 10 : 100) (A II). The former solvent system allows group separation of PGEs and PGFs, whereas the latter system separates PGs according to degree of saturation (14).

Bioassay

PG-like activity in extracts and eluates was measured by bioassay on isolated guinea pig colon suspended in Tyrode's solution at 37°C, gassed with 5% CO_2 in O_2 (19). Biological activity is expressed in terms of PGE_2.

RESULTS

Spontaneous bullae

The acidic lipid extracts prepared from blister fluids of 15 patients with spontaneous bullae all contained measurable amounts of smooth-muscle stimulating activity. The concentrations varied from 0.4 to 54 ng/ml of PGE_2 (Table I). In 9 patients with pemphigoid the average content was 10.8 ng/ml of PGE_2. The exact age of the bullae was not known. While some had developed overnight before the collection, others were older.

In 4 patients with pemphigoid, amounts of blister fluid were collected sufficient to permit of analysis

Table II. *Suction blisters*

Patient	Age	Sex	Diagnosis	Suction time (min)	Blister fluid volume (ml)	PG-activity (ng/ml)
Control group						
M. N.[a]	25	♀	Contact dermatitis	Not measured	0.30	0
K. T.	48	♂	Hand eczema	120	0.36	1.4
L. H.	34	♂	Hand eczema	110	0.41	1.3
T. H.	31	♂	Eczema	125	0.31	3.9
M. B.	22	♀	Hand eczema	135	0.25	3.0
L. Ö.	60	♀	Leg ulcer	135	0.11	0
K. B.	48	♂	Atopic dermatitis	100	0.46	4.1
B. T.	53	♂	Tinea pedis	120	0.40	5.5
L. L.	30	♂	Tinea pedis	140	0.45	4.9
K.-E. L.	22	♂	Recurrent aphthosis	125	0.28	0
E. S.	70	♂	Intertrigo	110	0.65	3.4
	Mean ±S.D.			122±13	0.36±0.14	2.5±2.0
N. S.	69	♂	Psoriasis	110	0.40	4.0
K. A.	28	♀	Psoriasis	120	0.40	1.9
M. S.	28	♂	Psoriasis	110	0.42	6.2
C. K.	20	♀	Psoriasis	90	0.58	2.6
H. S.	27		Psoriasis	85	0.45	0
	Mean±S.D.			103±15	0.45±0.08	2.9±2.3
E. W.[a]	79	♀	Bullous pemphigoid	30	0.40	5.7
V. R.[a]	71	♂	Bullous pemphigoid	Not measured	0.30	23.3
G. B.[a]	58	♂	Bullous pemphigoid	90	0.32	6.9
	Mean±S.D.					12.0±9.8
M. H.	65	♀	Dermatitis herpetiformis	90	0.35	11.9
B. S.	26	♀	Dermatitis herpetiformis	125	0.50	17.6
U. B.	42	♀	Dermatitis herpetiformis	150	0.30	74.7
E. E.	51	♂	Dermatitis herpetiformis	120	0.56	45.0
K.-E. K.	45	♂	Dermatitis herpetiformis	90	0.45	37.8
G. N.	60	♂	Dermatitis herpetiformis	70	0.41	36.6
J. S.	53	♂	Dermatitis herpetiformis	130	0.36	19.4
M. S.	33	♀	Dermatitis herpetiformis	130	0.32	26.6
R. E.	53	♂	Dermatitis herpetiformis	105	0.41	12.1
H. B.	48	♂	Dermatitis herpetiformis	130	0.23	16.3
	Mean±S.D.			114±24	0.39±0.10	28.8±17.1

[a] Spontaneous blisters were also analysed (cf. Table I).

as to the identity of the spasmogenic material. To these samples, tracer amounts of ^{3}H-PGE$_2$ were added before starting the extraction procedure. In 2 patients most of the spasmogenic activity was recovered from the zone corresponding to ^{3}H-PGE$_2$ on TLC using silver nitrate impregnated plates and solvent system AII. In one of these patients a minor peak of activity was associated with the solvent front and in the other patient some activity co-chromatographed with PGF$_{2\alpha}$ as determined from a separate marker plate.

In the 2 other patients the acidic lipid extract was subjected to silicic acid chromatography using a linear gradient elution scheme. One such chromatography is shown in Fig. 1. It can be seen that the biological activity coincides with the radioactivity due to ^{3}H-PGE$_2$. The elution profile was similar in the other experiment. Reversed phase partition chromatography of the bioactive material from Fig. 1 showed that the biological activity appeared in a single peak which co-chromatographed with that of ^{3}H-PGE$_2$ (Fig. 2).

Suction blisters

In an attempt to investigate blister fluid obtained under more controlled conditions, suction blisters were produced in a number of patients with various skin disorders. Notably, suction blister fluid in patients with dermatitis herpetiformis (DH) contained higher concentrations of PG-like activity than was

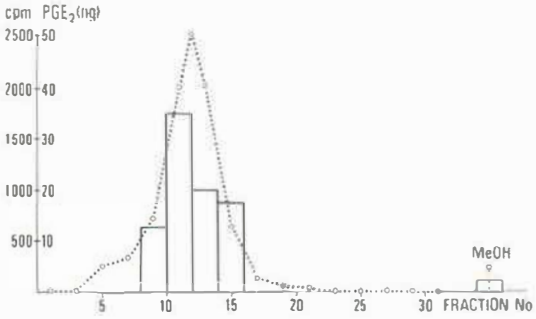

Fig. 1. Silicic acid chromatography of smooth muscle contracting lipid material from 6.3 ml blister fluid from a patient with pemphigoid. Silicic acid 1 g. Linear gradient starting with ethylacetate/benzene (1:9) and finishing with ethylacetate. Fractions 2.0 ml. Biological activity (guinea pig colon) is denoted by columns and radioactivity due to added ^{3}H-PGE_2 by a dotted line.

found in many of the other cases. For this reason the study of this patient category was extended. The average amount of PG-like activity measured in acidic lipid extracts prepared from suction blisters of 10 consecutive DH patients was greater than that formed in the control subjects ($p<0.001$) (Table II). Due to the poor clinical condition of most of the pemphigoid patients before corticosteroid treatment, suction blisters were produced in only 3 patients. In these, the average amount of PH-like activity found seemed to be greater than in the controls, whereas it was less than in the DH patients. The suction blister fluid in psoriasis patients contained PG-like activity levels similar to those of the controls.

The amounts of spasmogenic material obtained in the suction blister fluids were insufficient to allow further analysis as to its identity.

DISCUSSION

The present experiments showed the presence of smooth muscle contracting material in acidic lipid extracts prepared from blister fluid from patients with spontaneous bullae or suction blisters. The solvent partition features of the material suggested its possible PG nature. From 4 patients with pemphigoid, sufficient material was available to allow further characterization of the bioactive material. In silic acid column chromatography and TLC as well as in reversed phase partition chromatography, the major part of the biological activity followed the radioactivity due to added ^{3}H-labelled PGE_2. In one experiment the presence of $PGF_{2\alpha}$ was also indicated. Since the assay organ, i.e. the guinea pig colon, is less sensitive to PGFs, than to PGEs (19) and the overall biological activity found was low, it is quite possible that $PGF_{2\alpha}$ activity present in the other experiments might have escaped detection. This uncertainty could possibly be resolved by using a specific radioimmunoassay procedure. In fact preliminary experiments, in which such a technique was employed, have demonstrated the presence of immunoreactive PGE and PGF-activity and PG-metabolites in blister fluid of patients with eczema (Hägermark & Strandberg, unpublished). It is of further interest in this context that the concentrations of PGs measured were of a similar order of magnitude to those found in the present study in which a bioassay procedure was employed.

Thus, in all probability, pemphigoid blister fluid contains PGE_2. Human epidermis can both synthesize and metabolize PGE_2 (16, 25) and the appearance of PGE_2 in human burn blister fluid has been attributed to membrane damage stimulating the PG biosynthesis (2). A similar origin of pemphigoid PGE_2 is conceivable considering the gross morphological changes occurring in this disease, i.e. PGE_2 is produced in the skin secondary to its damage. On the other hand, once PGE_2 is generated it might contribute to the formation of blister fluid, since PGE_2 is a potent vasodilator, and since it possibly enhances the vascular permeability increasing effects of other factors operating by releas-

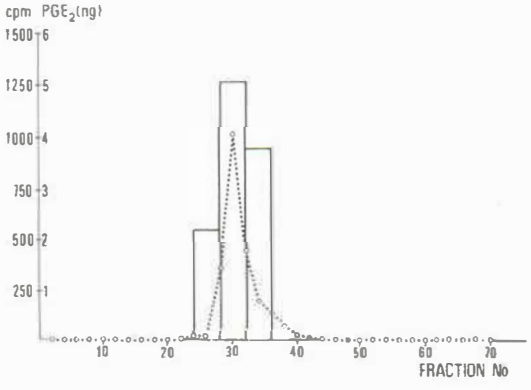

Fig. 2. Reversed phase partition chromatography of spasmogenic material (Fig. 1) purified by extraction and silicic acid chromatography. Fractions 2.6 ml. Biological activity is denoted by columns and radioactivity due to added ^{3}H-PGE_2 by a dotted line.

ing skin histamine (12, 15, 23). Conversely, the possibility cannot be excluded that PG generation serves the useful purpose of inhibiting further release of enzymes from polymorphonuclear leukocytes by increasing the intracellular cyclic AMP concentration (24).

PG-like activity was also demonstrated in suction blister fluid from four different groups of patients. The concentration of PG-like activity was significantly higher in patients with dermatitis herpetiformis than in patients with psoriasis or eczema. This was not due to a longer blistering time in the DH patients, since there was no significant difference between the time required to produce blisters in DH and control patients. Comaish & McVittie (10) found increased blistering time in DH patients and decreased blistering time in pemphigoid. The shortest time observed by us, 30 min, was in a patient with pemphigoid; the PG-activity in this blister was fairly high, 5.7 ng/ml.

Using a radioimmunoassay capable of detecting 10 picograms of PGE_2 per ml, Goldyne et al. (13) found that cantharidin and contact-dermatitis blisters contained significant PG-activity, whereas suction blister fluid was devoid of activity. However, using another technique for blister formation, these investigators have recently demonstrated the presence of PGE_2 in suction blister fluid too (Goldyne, personal communication). It is therefore conceivable that the PG-like activity detected in the suction blister fluid from the DH patients is attributable to the presence of PGE_2. Clearly, however, further analysis of the spasmogenic material produced in DH is warranted before any speculations as to its appearance in this disease can be justified.

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Ö. Hägermark, M.D.
Department of Dermatology
Karolinska sjukhuset
S-104 01 Stockholm 60
Sweden