Acta Dermatovener (Stockholm) 56: 217–221, 1976

THE CUTANEOUS REACTIONS TO KALLIKREIN, PROSTAGLANDIN AND THURFYL NICOTINATE IN CHRONIC URTICARIA AND THE EFFECT OF POLYPHLORETIN PHOSPHATE

P. Thune, T. J. Ryan, S. M. Powell and J. R. Ellis

From the Department of Dermatology, National Hospital of Norway, University Hospital, Oslo, and the Department of Dermatology, Slade Hospital, Oxford, England

Abstract. The erythema and wealing resulting from the application of thurfyl nicotinate ointment (Trafuril) and from the inoculation of kallikrein has been studied in patients with chronic urticaria and normal controls. Polyphloretin phosphate (PPP) suppressed the reaction in controls but in patients with urticaria it increased the reactions to Trafuril and had little effect on the kallikrein reaction. PPP also suppressed the PGE_2-induced erythema in normal controls but not in urticaria patients. In a separate study using fibrinolysis autography, prostaglandin (PG) E_2 and $PGF_2\alpha$ depressed fibrinolysis in the skin of two pigs and both kallikrein and Trafuril suppressed fibrinolysis in human skin. It is suggested that the inflammatory reaction induced by thurfyl nicotinate and kallikrein is mediated in part by a prostaglandin-like action. Several anomalies in the action of Trafuril in skin diseases can be explained if such prostaglandin-like activity is mediated in part through inhibition of fibrinolysis.

Key words: Prostaglandins; Kallikrein; Thurfyl nicotinate; Fibrinolysis; Polyphloretin; Chronic urticaria

The underlying mechanism in chronic urticaria is unknown although it has long been known that it is not based primarily on allergy. Accordingly a biochemical basis with acquired or genetically determined abnormalities has yet to be worked out. Such causes have been suggested by the observed atypical, delayed reactions to histamine, and the stronger than normal reactions to thurfyl nicotinate, PGE and kallikrein (9, 10). Furthermore, several PG antagonists (i.e. antiphlogistica) and even colour additives and preservatives, can provoke or exacerbate the symptoms (7, 12). These facts suggest the operation of certain enzymatic systems related to a secondary inflammatory reaction (21).

Of particular interest in this connection is the reaction evoked by thurfyl nicotinate. The abnormal dermographism caused by this substance can be suppressed by aspirin but not by antihistaminica or by pretreatment with 48/80, a histamine liberator (22). Similarly, the symptoms of chronic urticaria are not or are only partly influenced by antihistaminica. Consequently, the question is raised as to whether or not PG or PG-like receptor mechanisms are involved in chronic urticaria and the abnormal reactions to thurfyl nicotinate, PGE and kallikrein.

In order to provide more information pertinent to this question we have investigated the influence of a specific PG-antagonist, polyphloretin phosphate (PPP) (3, 5), on these reactions. Furthermore, the part played by the fibrinolysis system is of interest in this connection, as in urticaria, and particularly in vasculitis, there is a shift in the balance towards excessive fibrin deposition or excessive fibrinolysis (14). Accordingly, it also seemed of interest to measure the fibrinolytic activity following the application of thurfyl nicotinate and PG. For convenience, the measurement of fibrinolytic activity following PG treatment was undertaken in pig skin.

MATERIALS AND METHODS

Examination of fibrinolysis. Approximately 30 mg of thurfyl nicotinate ointment (Trafuril®—Ciba, Switzerland) was rubbed gently into 10 cm^2 normal looking skin over the greater trochanter in 7 normal subjects and in 11 subjects with chronic urticaria.

Biopsies were taken at a time of maximum response which varied from 1–2 hours, and from a control site over the opposite greater trochanter. The tissue was frozen at −20°C and subsequently 8–10 cryostat sections were cut.

Table I. *Percentage of total area of sections of skin showing fibrinolysis before and after application of thurfyl nicotinate in normal skin and urticaria*

Normal skin ($n=7$)		Urticaria ($n=11$)	
Pre[a] (%)	Post[b] (%)	Pre[a] (%)	Post[b] (%)
49	19	38	7
57	18	43	8
55	16	31	10
35	1	47	14
33	7	39	41
42	8	41	15
48	9	46	17
62	30	49	1
		22	7
		20	5
		52	2

[a] Before application.
[b] After application.

Sections were covered with a film of fibrin as described by Todd (19) and modified by Turner et al. (20). Sections were left overnight at 4°C and then incubated for 10–30 min. After fixing and staining with haemotoxylin, areas of lysis were estimated using the method of Dodman et al. (4). Paired sections were examined by immunofluorescence technique for the presence of fibrin-related antigen. The fibrinolytic activity following intradermal (i.d.) injections with PGE_2 and $PGF_2\alpha$ into unprepared skin of two Large White pigs was also examined with the same technique as described above. The prostaglandins were obtained in stock solutions containing 50 μg PG/ml. 10 μg and 30 μg of PGE_2 and 10 μg and 50 μg of $PGF_2\alpha$ were injected into various sites in the pig's skin. Biopsies were taken 30 min after the injections, including biopsies for control.

Examinations with PPP. Forty patients suffering from chronic urticaria were investigated. Twenty-seven healthy persons of the same age group selected from the hospital staff served as controls. Pretreatment with polyphloretin phosphate (PPP) was performed as described by Søndergaard & Jørgensen (16). Each person received three i.d. injections with 100 μg of PPP in 0.1 ml saline at hourly intervals. Kallikrein (Padutin powder) was dissolved in saline to 20 units per ml and 2 units were injected i.d. at a volume of 0.1 ml. Thurfyl nicotinate ointment (Trafuril®) was applied to a 1 cm^2 area and was left on for 10 min before being gently removed with a cotton swab. The area was then cleansed with a 60% alcohol solution. For tests with PGE_2 on normal controls and subjects with chronic urticaria the stock solution was diluted to 5 μg/ml and 0.1 ml, i.e. 0.5 μg was injected i.d. Thurfyl nicotinate, kallikrein, and PGE_2 were tested with and without pretreatment of the area with PPP. Uninvolved skin of the flexor aspect of the right and left forearm served as test areas. The pretreatment with PPP was performed on only one side. The diameters of the erythematous and oedematous reactions were measured after 20 min, 1 hr and 2 hr.

RESULTS

Fibrinolysis. It will be seen from Table I that in all cases the skin treated with thurfyl nicotinate showed depression of fibrinolysis. The values represent the percentages of section surface showing lysis. With immunofluorescence microscopy, no fibrinogen was detected in the sections. Table II shows the results of the pig studies. A depression in fibrinolysis following PGE_2 and PGF_2 versus controls was found in all test sites.

Effect of PPP. The mean erythema and weal reactions and the standard errors of mean (S.E.) are illustrated in Table III.

Kallikrein. PPP caused a marked reduction of the erythema in the control group, but it had no apparent influence on the erythema in the urticaria group. This difference in reaction between normal persons and patients with chronic urticaria was most distinct at 1 hour and 2 hours and was statistically significant for 6 out of 13 patients with urticaria ($P<0.001$). The erythema produced by kallikrein without pretreatment was obvious greater at 2 hours in the urticaria group than in the normal controls, but the difference was not statistically significant ($P>0.1$) (Fig. 1). The erythema also had a tendency to last longer in the patient group, in some cases up to 3 days.

The weal induced by kallikrein was larger in the patients than in the normal controls. In 2 patients it increased during the first 24 hours and lasted for about 96 hours. The difference between the two

Table II. *Fibrinolytic activity following i.d. injections of PGE_2 and PGF_2 into unprepared skin of pigs*

Control sites were not injected

Substances	*n*	Skin reaction	Fibrinolysis (%)
PGE_2, 10 μg	2	Erythema Slight induration	14–33
Controls	2	–	29–50
PGE_2, 30 μg	1	Erythema Slight induration	23
Control	1	–	32–35
PGF_2, 10 μg	2	Erythema No induration	15–23
Controls	2	–	29–50
PGF_2, 50 μg	1	Erythema No induration	13
Control	1	–	32–35

Table III. *Effect of polyphloretin phosphate (PPP) i.d. on skin reactions induced by kallikrein, thurfyl nicotinate and PGE_2 (mean area in $mm^2 \pm S.E.$)*

Substances	Subjects	20 min		1 hour		2 hours	
		Weal	Erythema	Weal	Erythema	Weal	Erythema
Kallikrein	Normal ($n=8$)	185±40	460 ±493	174 ±90	262 ±158	282 ±176	113 ± 94
Kallikrein+PPP	Normal ($n=8$)	148±25	257 ± 83	157 ±42	26[a]± 19	148 ± 33	7[a]± 7
Kallikrein	Urticaria ($n=13$)	298±46	420 ± 72	342 ±66	288 ± 64	221 ± 63	250 ±116
Kallikrein+PPP	Urticaria ($n=13$)	278±69	395 ± 94	293 ±82	285 ± 80	189 ± 72	258 ±103
Thurfyl nic.	Normal ($n=10$)	156±61	891 ±263	143 ±43	1 163±457	85 ± 42	285 ± 92
Thurfyl nic.+PPP	Normal ($n=10$)	116±29	348 ±124	93 ±33	285 ± 98	31 ± 13	141 ± 94
Thurfyl nic.	Urticaria ($n=13$)	174±72	649 ± 53	162 ±71	1 342±502	49 ± 21	259 ±124
Thurfyl nic.+PPP	Urticaria ($n=13$)	218±80	875 ±563	346[b]±84	1 854[b]±763	295[b]±103	1 082 ±847
PGE_2	Urticaria ($n=14$)	110±29	560 ± 57	113 ±35	456 ± 73	61 ± 25	195 ± 55
PGE_2+PPP	Urticaria ($n=9$)	139±45	657 ±178	64 ±13	351 ± 42	40 ± 12	241 ± 51
PGE_2	Normal ($n=9$)	60±14	432[c]± 43	36[c]±17	456 ± 96	20 ± 12	159 ± 71
PGE_2	Normal ($n=6$)	71±16	327[c]± 64	59 ±20	275 ± 37	49 ± 21	160 ± 31

[a] Significantly different from urticaria in 6 subjects ($P<0.001$).
[b] Significantly different from normal in 6 subjects ($P<0.001$).
[c] Significantly different from urticaria ($P<0.1$).

groups was, however, not statistically significant ($P>0.1$). Pretreatment with PPP caused only a slight reduction in the response.

Thurfyl nicotinate. Following pretreatment with PPP a marked decrease in erythematous response was observed in the controls, whereas a reverse reaction, i.e. an increase, occurred in the patient group. This difference in response was most distinct at 1 hour. However, despite the large arithmetic difference it was statistically significant ($P<0.001$) only for 6/13 patients (cf. kallikrein). As far as is known, these 6 patients did not differ clinically from the others within the same group, but their reactions were definitely more pronounced. Without pretreatment no clear difference in erythematous response could be observed between the two groups.

The weal induced by thurfyl nicotinate showed the same pattern of reaction. After PPP pretreatment it was reduced in the control group whereas a marked increase was observed in the patients. The difference in response between the two groups was significant for 6 out of the 13 patients with chronic urticaria ($P<0.001$) at 1 hour and 2 hours. Three of these patients were the same who also had an abnormal erythematous reaction to thurfyl nicotinate after the pretreatment. The wealing response was markedly increased and lasted for 96 hours.

PGE_2. The reactions to this prostaglandin were generally greater in patients with chronic urticaria than in normal controls. This difference was statistically significant at 20 min as a significant inhibition ($P<0.1$) of the erythema was observed only in the control group, and then at 20 min. In the urticaria group the erythematous reaction was not significantly suppressed by PPP, and neither was the oedematous reaction in either of the two groups.

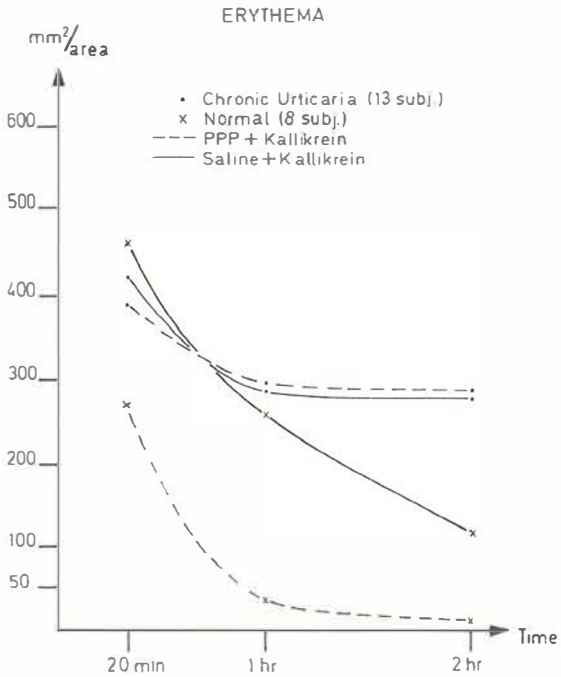

Fig. 1. The effect of PPP on the erythema produced by kallikrein in normal subjects (x) and in patients with chronic urticaria (●). ---, PPP+kallikrein; —, Saline+kallikrein.

DISCUSSION

Inflammatory reactions can be divided into two stages—early and late; the pattern of reaction in chronic urticaria is probably of the second type (21). The role of fibrinolysis in inflammation is still debated, but in general it is thought to be activated in early inflammatory events and depressed in delayed inflammation, possibly through exhaustion of activator production by endothelium (8, 14). A total loss of fibrinolysis following kallikrein injections has previously been demonstrated by Ryan et al. (14). This in itself may be an important factor in perpetuating the inflammation. Histamine, on the other hand, promotes local fibrinolysis, at least initially.

Aspirin is considered to activate fibrinolysis and it enhances the wealing in some urticarial subjects in whom blood fibrinolysis may be initially more active (1). The rather prolonged redness and oedema seen with thurfyl nicotinate in patients with chronic urticaria resembles in some way the sunburn reaction in which fibrinolysis is also depressed (6). This reaction can be inhibited by non-steroidal anti-inflammatory substances (aspirin, etc.), i.e. well-known inhibitors of prostaglandin synthesis (15).

Although the thurfyl nicotinate reactions have been used in several studies, the underlying mechanism is still unknown. Michaëlsson et al. (13) demonstrated that thurfyl nicotinate prepared the skin for an enhanced purpuric response in patients known to develop purpura after ingestion of tartrazin. The effect was apparently prevented by phenformin and ethyl oestranol, known promotors of fibrinolytic activity. The idea is entertained that such provocation by thurfyl nicotinate is somewhat similar to the vascular reaction to bacterial endotoxin, immune complexes and other agents in skin prepared by previous injury as observed in the Shwartzman's phenomenon, and for the hyperreactivity of the skin in disorders such as Behcet's disease in which alteration or exhaustion of fibrinolysis is one of the suggested mechanisms (2, 11).

PPP has a specific PG-blocking activity and acts by competitive antagonism (5) which reduces the erythematous reaction of PG. The weal reaction is probably due to endogenous histamine release and is normally not influenced by PPP (16). In normal persons the action of endogenous histamine is mainly balanced by the adrenergic system but it has been suggested (18) that in chronic urticaria and asthma, histamine acts like PGF and is balanced mainly by PGE. All antagonists of prostaglandin leave histamine unopposed in urticarial subjects, while in normal subjects such antagonists merely reduce the prolonged mild inflammatory effect of prostaglandin itself.

Søndergaard & Greaves (17) suggested that the inflammatory reaction to thurfyl nicotinate in normal subjects was not mediated by histamine, bradykinin, or serotonin, but they observed that the skin of patients with chronic urticaria showed more histamine-like activity than that of normal subjects. The normal depression of fibrinolysis by thurfyl nicotinate and the effect of a specific prostaglandin antagonist observed in this study, offer a possible explanation for the recorded unusual responses of the skin to this substance.

The paradoxical effect of PPP on the reactions to thurfyl nicotinate and PGE_2 and the lack of effect on the kallikrein-induced erythema in patients with chronic urticaria strongly suggest an abnormality of the prostaglandin or prostaglandin-like receptors in these patients or an abnormal metabolism of PGE to

substances with opposite or antagonistic action. One consequence of the action of thurfyl nicotinate and kallikrein on these receptors is an ultimate depression in fibrinolytic activity.

ACKNOWLEDGEMENTS

Polyphloretin phosphate (PPP) was kindly supplied by Dr B. Fredholm, AB Leo, Helsingborg, Sweden. Kallikrein was purchased from Bayer AG, BRD, as Padutin powder in ampoules of 40 units. The prostaglandins E_2 and F_2 were kindly supplied by Astra, Södertälje, Sweden, and Upjohn Ltd, Crawley, Sussex, England.

This study was supported by the Norwegian Research Council for Science and the Humanities, and by the Wellcome Trust.

REFERENCES

1. Braun, H., Nitzschner, H. & Peschel, H.: Fibrinolyse Untersuchungen bei chronische rezidivierende Urtikaria mit niedrigem Fibrinogen Spiegel. Z Hautkr *45*: 761, 1970.
2. Chajek, T., Aronowski, E. & Izak, G.: Decreased fibrinolysis in Behcet's disease. Tromb Diath Haemorrh *29*: 610, 1973.
3. Diczfalusy, E., Fernø, O., Fex, H., Høgberg, B., Lindrot, T. & Rosenberg, T.: Synthetic high molecular weight enzyme inhibitors. I. Polymeric phosphates of phloretin and related compounds. Acta Chem Scand *7*: 913, 1953.
4. Dodman, B. A., Cunliffe, W. J., Dodman, E. A. & Webster, J. A.: A method of measuring well defined areas within sections applied to assessment of tissue fibrinolysis. *In* Methods in Microcirculation Studies (ed. T. J. Ryan, B. Jolles & G. Holti). H. K. Lewis & Co., 1972.
5. Eakins, K. E., Karim, S. M. M. & Miller, J. D.: Antagonism of some smooth muscle actions of prostaglandins by polyphloretin phosphate. Br J Pharmacol *39*: 556, 1970.
6. Grice, K., Ryan, T. J. & Magnus, I. A.: Fibrinolytic activity in lesions produced by monochromator ultraviolet irradiation in various photodermatoses. Br J Dermatol *83*: 637, 1968.
7. Granholt, A. & Thune, P.: Urticaria and angioedema induced by antiphlogistica, preservatives and dye additives in food and drug. (In Norwegian with summary in English.) Tidsskr Nor Lægeforen *95*: 20, 1975.
8. Innes, D. & Sevitt, S.: Coagulation and fibrinolysis in injured patients. J Clin Pathol *17*: 1, 1964.
9. Juhlin, L. & Michaëlsson, G.: Cutaneous reactions to kallikrein, bradykinin and histamine in healthy subjects and in patients with urticaria. Acta Dermatovener (Stockholm) *49*: 26, 1969.
10. — Cutaneous vascular reactions to prostaglandins in healthy subjects and in patients with urticaria and atopic dermatitis. Acta Dermatovener (Stockholm) *49*: 251, 1969.
11. McKay, D. G.: Vessel wall and thrombogenesis endotoxin. Trom Diath Haemorrh *29*: 11, 1973.
12. Michaëlsson, G. & Juhlin, L.: Urticaria induced by preservatives and dye additives in food and drugs. Br J Dermatol *88*: 525, 1973.
13. Michaëlsson, G., Petterson, L. & Juhlin, L.: Purpura caused by food and drug additives. Arch Dermatol *109*: 49, 1974.
14. Ryan, T. J., Nishioka, K. & Dawber, R. P. R.: Epithelial–endothelial interaction in the control of inflammation through fibrinolysis. Br J Dermatol *84*: 501, 1971.
15. Snyder, D. S. & Eaglstein, W. H.: Intradermal antiprostaglandin agents and sunburn. J Invest Deramtol *62*: 47, 1974.
16. Søndergaard, J. & Jørgensen, H. P.: Blockade by polyphloretin phosphate of the prostaglandin E_1-induced human cutaneous reaction. Br J Dermatol *88*: 51, 1973.
17. Søndergaard, J. & Greaves, M. W.: The responses of skin to tetrahydrofurfuryl nicotinate (Trafuril) studied by continuous skin perfusion. Br J Dermatol *82*: 14, 1970.
18. Szczeklik, A., Gryglewski, R. J. & Czerniawska-Mysik, G.: Relationship of inhibition of prostaglandin biosynthesis by analgesics to asthma attacks in aspirin-sensitive patients. Br Med J *1*: 67, 1975.
19. Todd, A. S.: The histological localisation of fibrinolysin activator. J Pathol *78*: 281, 1959.
20. Turner, R. H., Kurban, A. K. & Ryan, T. J.: Fibrinolytic activity in human skin following epidermal injury. J Invest Dermatol *53*: 458, 1959.
21. Winkelmann, R. K.: Molecular inflammation of the skin. J Invest Dermatol *57*: 197, 1971.
22. Winkelmann, R. K., Wilhelmj, C. M. & Horner, F. A.: Experimental studies on dermographism. Arch Dermatol *92*: 436, 1965.

Received July 2, 1975

P. Thune, M.D.
Department of Dermatology
Rikshospitalet
Oslo
Norway