

ENZYME ACTIVATION AND INHIBITION INDUCED BY COLD PROVOCATION IN A PATIENT WITH COLD URTICARIA

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Abstract. A 33-year-old female patient with acquired cold urticaria, together with her 8-year-old healthy daughter, was subjected to a brief period of cold exposure. The effect of this treatment upon a number of key factors of the plasma coagulation, kallikrein and complement systems was investigated. Cold air provocation caused increased fibrinolysis, together with a measurable consumption of the protease inhibitors α 1-antitrypsin (α 1AT), α 2-macroglobulin (α 2M) and C1-inactivator (C1INA). Kaolin activation of the patient's plasma elaborated exceptionally high levels of esterolytic activity, both before and after cold exposure, indicating pre-enzyme lability. Both subjects had abnormally high serum ratios α 2M/ α 1AT. Impressive leucocytosis was observed in the symptomless child.

Key words: Enzymes; Inhibitors; Urticaria

Little is known of the serum factor(s) possibly involved in eliciting the symptoms of cold urticaria (19). Though increased plasma histamine levels were found to be associated with cold urticaria (1, 10), no increased response to intradermal histamine was detected (11); no elevated levels of histamine were found in the cooled arm of normal or cold-sensitive individuals (8).

Participation of the kallikrein-kinin system in the formation of cold-induced wheals has been suggested (3, 12, 14). Evidence has also been cited for the involvement of the fibrinolytic system in the urticarial reaction. Low levels of blood fibrinogen were reported in 20 out of 27 patients with chronic urticaria (15), while increased fibrinolytic activity was demonstrated in the cantharidin blister fluids of patients with urticaria (9). It is known that, through plasmin, the complement system may become activated (16).

If these complex systems are in some way involved in urticarial reactions, the ease of activation of the key factors by various stimuli may constitute an important parameter in the disease process. An

equally fundamental factor may be the inhibitory power of the blood or tissue fluids for depressing the enzymatic action of kallikrein, plasmin, C1-esterase, lysozomal proteases, etc. The wellknown case of C1-esterase inactivator deficiency in patients with hereditary angio-oedema represents an almost classic example (6). Duck et al. (7) detected significantly depressed antitryptic and antichymotryptic activity in the serum of patients with acquired cold urticaria, as was recently confirmed by Doeglas & Bleumink (5). Upon cold exposure, the inhibitory capacity of trypsin and chymotrypsin became even more deficient.

We recently had occasion to study a number of key factors of the above-mentioned effector systems in a patient with cold urticaria and in her (clinically normal) 8-year-old daughter.

MATERIALS AND METHODS

1. Clinical investigations

The patient was a 33-year-old woman, suffering from cold urticaria of 18 years' duration; a 3-year period of hay fever preceded the first attack, which was provoked by a shower of hail. Since the onset of the cold urticaria, the hay fever symptoms have totally disappeared. Numerous attacks of cold urticaria followed in the ensuing years, usually provoked by moderately cool air or by lukewarm water. Cold food or drinks caused dysphagia. The immediate urticarial response was manifested by a general swelling with minor purpura of the fingers and toes, rigidity of the arms and legs, and by itching urticaria on the exposed parts of the body. The symptoms usually abated within 3 hours. Because the ice-cube provocation test was positive, but a repeated search for cryoglobulins or -haemagglutinins remained unsuccessful, the patient's condition was classified as essential cold urticaria of the acquired type. In the previous 18 years, she had been clinically evaluated several times and had received polypragmatic therapy, mainly unsuccessful. Physical and routine laboratory examination revealed no abnormalities. Two

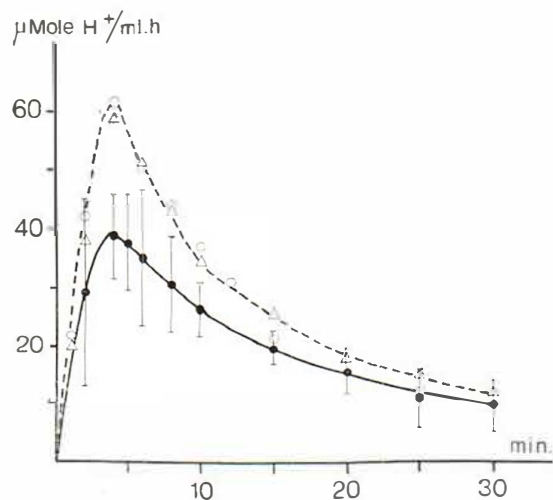


Fig. 1. Generation and inhibition of esterase activity in the plasma of the cold urticaria patient (---) before (Δ) and 10 min after a 15 min episode of cold exposure ($\circ$). —, Mean of identical in vitro experiments with the plasma of 8 control subjects; normal area indicated by mean $\pm$ 2 S.D. of the group. Enzyme activity calculated from the slope of reaction velocity tracings recorded on a Radiometer pH-stat Titrigraph. Experimental conditions: 3 mg enzyme substrate (TAMe) and 0.3 ml plasma in a final volume of 0.8 ml 0.9% NaCl, pH 7.4, in a double-walled polythene vessel accommodating a glass/calomel combination electrode; temp. 37°C, automatic titration with 0.01 N NaOH under a stream of nitrogen. Washed kaolin suspension (100 μ g in 100 μ l 0.9% NaCl) added at time zero.

weeks prior to the present investigation, all medication was stopped and ice cube provocation was checked; this test elicited within 3 minutes a large wheal with pseudopods surrounded by an area of intense erythema. The cutaneous response to intradermal histamine was quite normal.

Because the family history revealed cold urticaria in the patient's father and grandfather, permission was requested to include the patient's 8-year-old daughter in the investigation. The child was in good health and had no history of urticaria or allergic disease; the ice cube provocation test was negative.

Venous blood samples were obtained 6 weeks prior to cold air provocation, at a time when both subjects were clinically normal. Blood was also obtained 15 min before exposure to cold. Both subjects were then submitted to cold air exposure in a climate room for 15 min; initial air temperature and relative humidity were 10°C and 75% R.H., which had changed to 4°C and 90% R.H. by the end of the experiment. Both subjects wore warm clothing and a pair of trousers; the contralateral arm and hand were completely uncovered, the ipsilateral remained completely covered.

Eight minutes after entering the climate room, the mother's fingertips turned intensely red, spreading rapidly, proximally. She indicated itching and pain when 2 min later diffuse general swelling of all fingers became apparent; the oedema likewise extended proximally. Hand- and finger function was

Table 1. Plasma variation of some fibrinolytic parameters before and after cold air provocation

FDP = fibrinogen degradation products

	Patient		Child		Normal controls
	Pre	Post	Pre	Post	
Fibrinogen, mg/100 ml	410	360	265	200	230–400
FDP, mg/100 ml	<1	<1	<1	N.D.	<1
Thrombin time, sec	18.9	19.4	24.1	28.7	18.4 $\pm$ 3.0
Reptilase time, sec	21.5	22.0	24.2	28.0	20.7 $\pm$ 1.5

severely hampered after 15 min of cold exposure. The covered hand and arm showed no visible or subjective reaction, nor were there any respiratory, gastrointestinal or general symptoms. All symptoms disappeared within 3 hours after exposure; by that time, no delayed reaction at the site of the venous punctures, as discussed by Michaëlsson (14), had been observed.

Aside from some shivering, the child displayed no clinical or subjective symptoms during the 15 min period of cold exposure. Ten minutes after termination of cold exposure, blood samples were again obtained for investigation.

2. Laboratory investigations

Blood samples for the obtaining of serum were allowed to clot in glass tubes for 2 h at room temperature or, separately, at 37°C in a water bath. Citrated plasma samples were immediately centrifuged at 3 500 r.p.m. for 10 min. Serum aliquots not immediately used were stored at -90°C.

Fibrinogen, fibrinogen degradation products (FDP), thrombin time and Reptilase time were established by routine haematological procedure. Single radial immunodiffusion was employed for the immunochemical determination of the enzyme inhibitors α 1-antitrypsin (α 1AT), α 2-macroglobulin (α 2M) and the complement factors C4 (β 1E) and C3-proactivator (C3PA), using Partigen® plates from Behringwerke, Germany; standard protein serum and plasma references for calibration were obtained from the same firm. The plasma enzyme inhibitor C1-inactivator (C1INA) was determined by one-dimensional rocket electrophoresis according to Laurell (13), using rabbit anti-human C1INA purchased from Behringwerke, Germany. Plasma inhibitors of bovine trypsin were also determined functionally according to Dietz et al. (4), using α -N-benzoyl-DL-arginine-p-nitroanilide (BAPNA) as a substrate.

Spontaneous serum and plasma esterase ("kallikrein") activity, kallikreinogen, and kallikrein inhibitors, were assessed essentially according to the kaolin activation method of Colman et al. (2); *p*-Tosyl-L-arginine methylester (TAMe) was used as a substrate, but instead of the discontinuous assay method with chromotropic acid, continuous pH-stat recording of the liberation of titratable H^+ ions was used. Enzyme activity was evaluated from reaction velocities of TAMe-consumption, which were read from the slopes of the recorder tracings on a Radiometer Titrigraph at any given time after the addition of the washed kaolin activator (100 μ g of kaolin in 0.3 ml of plasma, containing 3 mg of TAMe substrate at a constant pH of 7.4).

Table II. Influence of short-term cold air provocation on serum levels of α 1-antitrypsin (α 1AT), α 2-macroglobulin (α 2M) and C1-inactivator (C1INA), determined by immunodiffusion

Total antitryptic activity of the serum expressed as mg (bovine) trypsin inhibited per ml of serum per hour; C1INA expressed in mm peak height in rocket electroimmunodiffusion and compared with controls under internal laboratory standard conditions

	Patient			Child			Controls	
	6 weeks previous	Exposure		6 weeks previous	Exposure		Adults	Children (7-9 yrs)
		Pre	Post		Pre	Post		
C1INA, mm	19	19.4	18.9	23.5	17.7	16.8	22.6 $\pm$ 3.1	22.6 $\pm$ 3.1
AT, mg/ml h	N.D.	2.75	2.63	N.D.	2.25	1.75	4.50 $\pm$ 1.38	3.33 $\pm$ 0.60
α 1AT, mg/100 ml	155	225	190	150	130	120	289 $\pm$ 67	310 $\pm$ 103
α 2M, mg/100 ml	424	464	408	596	628	628	240 $\pm$ 60	614 $\pm$ 118
Ratio α 2M/ α 1AT	2.74	2.06	2.14	3.97	4.83	5.23	0.86 $\pm$ 0.33	2.02 $\pm$ 0.45

RESULTS AND DISCUSSION

Several of the known key enzymes in the coagulation, kinin-generating, and complement systems, are capable of hydrolysing TAME, i.e. Factor XIIa, plasmin, thrombin, kallikrein, and C1-esterase. An impression of the balance between generation and inhibition of total esterolytic activity for TAME in plasma or serum, collectively termed "kallikrein" by Colman et al. (2), can be gained by kaolin activation *in vitro*. Fig. 1 shows that the patient's plasma behaved differently in this respect than that of a number of control plasma samples from healthy volunteers and patients with chronic or solar urticaria. The kallikrein level of the patient's plasma after contact activation with kaolin had a maximum value of 150% of the value observed with the controls, indicating that the effector systems were intrinsically more labile, or that the circulating prekallikrein level was considerably above normal. Though the onset and extent of subsequent enzyme inhibition was quite efficient, depression of enzyme activity lagged behind normal when time is taken into consideration (Fig. 1). The pattern of enzyme activation and inhibition of the child's plasma was well within the normal range.

Though cold exposure for 15 min had no effect on the absolute kallikrein/kallikrein-inhibitor levels detectable in the patient's plasma (Fig. 1), other evidence indicated consumption of proteins of the blood effector systems *in vivo*. Table I shows that plasma fibrinogen levels decreased upon cold exposure in both mother and child. Moreover, significantly prolonged thrombin and Reptilase times were noted in the child's plasma after cold exposure (Table I); since fibrin split products, possibly interfering with thrombin action, were not detected, the fall in fibrinogen was presumably due to the action of activated plasmin.

The involvement of activated plasma enzymes is supported by the dynamics of some of the known protease inhibitors during exposure to cold. Thus α 1AT, measured both immunochemically and functionally (4), dropped to lower levels in both mother and child during cold exposure (Table II), indicating consumption by activated proteases; starting levels of α 1AT in both subjects already displayed rather low values (Table II). The low α 1AT level in the mother's serum 6 weeks prior to the test is quite remarkable in this respect: at that time she experi-

Table III. Influence of cold air provocation on complement factors C4 and C3-Activator (C3A), determined by single radial immunodiffusion

	Patient			Child			Controls	
	6 weeks previous	Exposure		6 weeks previous	Exposure		Adult	Children (7-9 yrs)
		Pre	Post		Pre	Post		
C4, mg/100 ml	42.0	59.6	46.8	34.8	35.2	32.8	33.2 $\pm$ 8.9	32.0 $\pm$ 12.4
C3A, mg/100 ml	23.6	26.8	23.6	20.6	18.0	18.0	20.7 $\pm$ 4.6	25.0 $\pm$ 8.6

enced an episode of acute urticaria, which is reflected in some other parameters as well (Tables II and III). Serum levels of $\alpha 2M$ —another known protease inhibitor—were elevated in the patient, but, for the child were within the normal range for her age group (Table II). A striking point, however, was that the ratios of $\alpha 2M$ and $\alpha 1AT$ were exceptionally high in both subjects. In an adult mixed control group of 102 normals and various patients, a mean ratio $\alpha 2M/\alpha 1AT$ of 0.86 ± 0.33 was found (Table II) (18). As shown in Table II, these values were quite considerably higher in both the mother and her child. In four other patients with acquired cold urticaria, we subsequently observed values ranging from 1.47 to 1.74. Work is under way to verify whether the unusual combination of elevated $\alpha 2M$ and lowered $\alpha 1AT$ may be a common feature in cold urticaria, or whether such findings indicate an inherited pattern in isolated cases.

Though CH50 values for haemolytic complement were low in both subjects, no alteration was observed upon exposure to cold. Participation of the complement system, however, was indicated by a drop in immunochemically detected C4 in both mother and child, and of C3A in the mother (Table III). Furthermore, the level of the inhibitor CIINA tended to decrease during cold exposure, as shown by electro-immunodiffusion (Table II). During later effective treatment of the patient with Cinnipirine®, moreover, CIINA showed a rise, which indicates depletion of this factor in symptomatic periods.

The generation of kallikrein and of complement split products is known to produce chemotactic factors. Also, leucocytosis has been observed after challenge in patients with hereditary essential cold urticaria (17). White blood cell counts in our subjects, performed shortly before and 5 hours after the 15 min cold exposure period, led to the following unusual results. Peripheral blood leucocytes in the mother rose from 7 700 to 8 400/ μl , whereas counts in the child's blood increased from 6 800 to 10 200/ μl ; the latter observation, especially, contradicts the proposed uniqueness of leucocytosis in the hereditary form of cold urticaria (17).

The results of this investigation raise more questions than can be answered. It seems certain that alterations of key factors in the fibrinolytic, kininogenase and complement pathways mirror the physiological changes even after brief cold exposure of a limited body surface area. The fact that measurable effects can be observed in each of the above inter-

related effector systems at an early stage, focuses attention on the possible intrinsic cold-lability of the precursor factors prekallikrein, plasminogen, or Factor XII. The unusual kaolin sensitivity of the patient's plasma (Fig. 1) and the depressed inhibitor levels may be consistent with this finding. Extended studies of the dynamics of these systems as described may be of value for unravelling the basic abnormalities and the patterns of inheritance of urticaria.

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