

Recurrent urticaria and reduced diamine oxidase activity

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Summary

An abnormal metabolism of histamine has been suspected in urticaria and the role of diamine oxidase (DAO; histaminase) is therefore of interest. We have studied DAO activity in plasma and jejunal biopsy material and have measured the post-heparin DAO release in 11 control subjects and nine with recurrent urticaria, three of whom had had concurrent episodes of abdominal pain. Two of the nine urticaria subjects had only a minimal rise in plasma DAO activity after heparin, three had a response which was at the lower end of the normal range, and four were normal. In four out of five cases in which jejunal biopsy activity was obtained, there was concordance between mucosal DAO activity and the post-heparin plasma DAO response. Those with abdominal symptoms had abnormally low mucosal DAO activity and the subject who was most severely affected had proven episodes of small bowel oedema.

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Introduction

Many patients with urticarial skin rashes do not have any of the classical features of allergy or any evidence that they are hypersensitive to an individual food or other substance [1]. Our previous work [2,3] has shown, however, that in some of these individuals, plasma levels of histamine rise abnormally rapidly in response to an intravenous histamine infusion and fall abnormally slowly when the infusion is stopped. This suggests a defect in the metabolism of histamine and raises the possibility that enzymes such as DAO (EC1.4.3.6) which normally degrades histamine, methyl histamine, and the diamine putrescine, may be deficient in these cases. The present studies have therefore been concerned with the function of DAO in urticarial subjects.

In normal subjects DAO activity is largely confined to the small bowel mucosa, especially in the ileum but also in the more easily studied jejunal mucosa. In the fasting state, DAO activity is barely detectable in plasma. However, the enzyme may be displaced from the intestinal mucosa into the peripheral circulation by intravenous heparin [4–6] and this has become the basis of a test in which plasma DAO levels are measured before and after

intravenous (i.v.) injections of heparin. We have studied the response in recurrent urticaria and, in consenting subjects, measured jejunal mucosal DAO activity.

Subjects and methods

Subjects

Nine subjects with urticaria were studied. Each had experienced five or more attacks of urticaria, at least one of which had occurred within 3 months before this study. In all cases, the urticarial skin lesions had been witnessed on at least one occasion by a physician. Table 1 shows the clinical details.

Three subjects (cases 4, 8 and 9) had also experienced severe attacks of abdominal pain in association with urticaria. One (case 4) had had four such attacks in which transient bowel wall thickening had been shown repeatedly by ultrasound and confirmed on one occasion by barium studies (Fig. 1). Case 4 had also had a laparotomy, which excluded Crohn's disease and showed some free fluid. Complement (C4) and C1 esterase inhibitor levels were normal, as were colonoscopy and stool culture. Case 8 had gastrointestinal colic during two severe attacks of urticaria. A third subject (case 9) had four to five loose stools daily and also reported an association between urticaria and gastrointestinal colic on six occasions.

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Table 1. Clinical features and investigations in nine urticaria subjects

Case number	Age (yr)	Sex	Associated abdominal colic	Post-heparin plasma DAO (5.6–31.7)*	Jejunal mucosal DAO (8.4–58.6)†
1	37	M	0	8.7	
2	70	F	0	6.7	
3	29	F	0	12.4	16.1
4	46	F	++	2.3	1.2
5	25	F	0	1.0	0.7
6	31	F	±‡	6.4	
7	38	F	0	22.6	
8	34	F	+	14.6	0.9
9	22	M	+	5.1	0.7

* Normal range in 11 subjects (16.0 ± 2.3 ; mean $\pm$ s.e.m.) expressed as area under the curve (AUC) in 2 hr ($\text{mU l}^{-1} 2 \text{ hr}^{-1}$).

† Normal values in 10 subjects expressed as mU/g mucosal protein (19.0 ± 2.37 ; mean $\pm$ s.e.m.).

‡ Pain and diarrhoea unassociated with attacks of urticaria.



Fig. 1. Small bowel meal (case 4) showing changes consistent with mucosal oedema.

Eleven control subjects were studied, 10 females and one male. Their ages ranged between 23 and 64 yr. None had had urticaria and none complained of abdominal pain.

Methods

The study was approved by the Guy's Hospital Ethical Committee and involved only subjects who had given their informed consent.

The post-heparin plasma DAO test. This test was performed as described by Rokkas *et al.*, [5]. Subjects who were taking antihistamines were asked to discontinue this treatment for 48 hr and all subjects fasted overnight before testing. Following 5000 IU of heparin (i.v. bolus dose) into an antecubital arm vein, 5 ml blood samples were withdrawn at 0, 5 and 15 min, and at 15 min intervals thereafter for a total of 2 hr. DAO activity was measured using Tryding's [7] modification of the method of Okuyama and Kobayashi [8]. In brief, under the influence of DAO, [^{14}C]putrescine is converted to γ -aminobutyraldehyde which then forms [^{14}C] Δ pyrroline when its conversion to γ -aminobutyric acid is blocked by the addition of acetaldehyde [9]. One unit of DAO activity = $1 \mu\text{mol } \Delta$ -pyrroline min^{-1} at 37°C . The results of plasma DAO concentration against time were also measured over 2 hr ($\text{mU l}^{-1} 2 \text{ hr}^{-1}$) after 5000 IU of i.v. heparin.

Jejunal mucosal DAO activity. Portions of peroral jejunal mucosal biopsies (Crosby capsule) were homogenized in 30 mM sodium phosphate buffer (pH 7.4; 1 ml buffer/5 mg tissue) using a tube homogenizer (Jencons Ltd, Leighton Buzzard, U.K.). The DAO activity in 100 μl aliquots of the resultant homogenate was then measured, as described above. The protein content of the homogenate was determined by the Lowry method [10] and mucosal DAO activity expressed as mU/g protein.

Results

In cases 1 and 4, fasting (unstimulated) plasma DAO activity was 7.5 and 3.8 mU/l but in all others was less than 2 mU/l.

Post-heparin plasma profiles

There was a wide variation in the plasma DAO response to the i.v. heparin, some subjects showing little or no increase (Fig. 2). Of nine subjects, two had only a minimal response as judged by the area under the curve (AUC) derived from data in Fig. 2. Three others had AUCs which were at the lower end of the range observed in our normal controls. The differences between the AUCs in the urticarial and control groups as a whole did not, however, reach statistical significance ($P=0.056$, Mann-Whitney *U*-test, two-tailed).

Jejunal mucosal DAO activity

There was a significant difference between the five subjects who were willing to have jejunal biopsy examinations and the range found in 10 volunteers ($P<0.02$, Mann-Whitney two-tailed test). In four of the five subjects studied, jejunal DAO activity was less than the

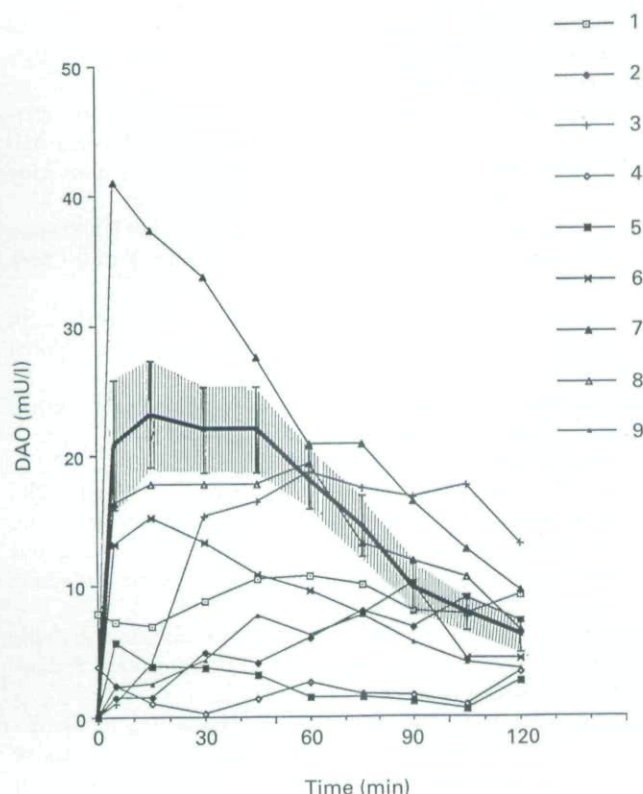


Fig. 2. Plasma DAO response to heparin. Numbers refer to subjects detailed in Table 1. The bold line represents the mean of values found in 11 normal subjects, shaded area is mean $\pm$ s.e.m.

lowest value previously found in normal subjects [11]. In three of these there was a correspondingly low AUC (Table 1), but in the fourth the AUC, measured on the same day as the jejunal biopsy, was normal. In this fourth case a discrepancy between absent mucosal enzyme activity and a normal DAO curve was again present when the tests were repeated 4 months later (case 8).

Discussion

The importance of histamine-degrading enzymes has been noted in the pathogenesis of symptoms which are associated with histamine release or histamine infusion [3,12,13]. The role of DAO in catabolizing histamine has often been emphasized [14,15], but plasma DAO activity is variable and may be undetectable in normal subjects. The rise in plasma DAO which occurs after heparin challenge has therefore attracted considerable attention, especially because of the association between deficient post-heparin plasma DAO profile and small bowel mucosal damage [4–6]. The heparin response has not, however, been studied systematically in urticaria or in other conditions suspected of having an allergic origin.

Two out of nine subjects in the present study with

urticaria had only a minimal rise in plasma DAO activity after i.v. heparin, and jejunal mucosal DAO activity was below the levels found in normal subjects in four out of the five cases in which it was measured. There was a good correlation between the two measurements in all but one case, which accords with our previous demonstration [5] of a linear relationship between the post-heparin DAO profile and jejunal mucosal DAO activity. In ilectomized animals [6,16], in subjects who have undergone small bowel resection, and in those with active ileal Crohn's disease [11], the heparin plasma DAO response depends on both jejunal and ileal mucosal activity, which may explain why jejunal mucosal activity may not invariably correlate with the displacement of DAO into the plasma after heparin. Taking both the heparin response and the jejunal mucosal findings together, our evidence nevertheless points to defective enzyme activity in the intestine in some, but not in all, urticarial subjects. This was particularly striking in three subjects (cases 4, 8 and 9) who had had simultaneous urticaria and abdominal colic. In one of these three we found evidence of small bowel mucosal thickening similar to that noted by Robertson and Wright in a recent case report [17]. Ultrasound studies showed that this bowel wall thickening was reversible, as was demonstrated in Robertson's subject by means of a repeat barium study.

The cause of the urticaria in these cases remains in doubt and as yet there is no evidence that a reduced DAO activity is of primary importance and not a secondary phenomenon. D'Agostino [16] showed that in the rat, after very large doses of heparin, jejunal mucosal DAO levels fall sharply and take up to 4 days to recover. It is therefore possible that low mucosal DAO levels and a poor response to heparin challenge could be a sequel to a previous depletion of intestinal mucosal DAO activity, for example following mast cell activation and the endogenous release of heparin. Almost identical plasma results were obtained in one of our subjects after a 4 month interval, but further studies will be required to establish this point.

The relative importance of gastrointestinal and circulating DAO also requires consideration. The loss of the protective role of mucosal DAO, whether primary or secondary, might allow an increased absorption of biologically active amines, while a lack of DAO in the circulation may delay their elimination, as our previous studies have suggested [2,3]. Endogenous histamine release or the DAO-blocking effects of common drugs, alcohol or spoiled food [13] could also play a part, as could the inhibitory effect of other circulating factors. In addition, we need to study other enzymes such as type-B monoamine oxidase [18] and hepatic *N*-methyl transferase [19].

While the above findings do not show a definitive association between urticaria and reduced DAO activity, this appeared to be present in some cases, notably in subjects with concurrent abdominal symptoms who had a low mucosal DAO activity. The nature of that association still requires elucidation.

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