Contact system activation and bradykinin generation in patients with idiopathic angioedema

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On the basis of the underlying pathogenetic mechanism, angioedema can be classified into three major categories: 1) histaminergic angioedema, which responds to antihistamine therapy; 2) bradykinin-mediated angioedema, which can be hereditary, angiotensin-converting enzyme (ACE) inhibitor-related and acquired C1-inhibitor (C1-INH) deficiency angioedema; 3) idiopathic angioedema, the causes of which are still unknown [1,2]. Some forms of idiopathic angioedema respond to antihistamines (idiopathic histaminergic angioedema) and others do not respond (idiopathic non-histaminergic angioedema) [1]. Since in about 10% of angioedema cases, the pathogenesis of increased vascular permeability is still unknown [3] and these patients do not respond to antihistamines, the study of an alternative mediator like bradykinin may open new diagnostic and therapeutic perspectives. A role of bradykinin has also been demonstrated in the pathophysiology of anaphylaxis and chronic urticaria [4,5], conditions in which mast-cells involvement is considered predominant. Mast-cell activation may be important also in angioedema non-responding to antihistamines, indeed several cases of idiopathic non-histaminergic angioedema respond to the anti-IgE treatment with omalizumab [6]. Bradykinin is a potent vasoactive peptide that is released from high-molecular-weight kininogen (HK) by plasma kallikrein during the
activation of the contact system. The cleavage of HK occurs at several points, enabling the release of bradykinin and resulting in breakdown products (cleaved HK) [7]. To date, the evaluation of contact system activation and bradykinin generation in vivo has presented methodological difficulties and consequently reliable data are not always available [8]. However, the main problems, such as in vitro generation and degradation of bradykinin, can be solved by using a meticulous procedure for the blood collection [9-11]. The aim of the present study was to evaluate contact system activation and bradykinin generation during maximum disease activity, i.e. at the exact moment the patient arrived in the emergency department.

We studied 9 patients during attacks of angioedema (4 men and 5 women; age range: 32-83 years) with no family history of angioedema, no known allergy, no C1-inhibitor deficiency, no therapy with angiotensin-converting enzyme inhibitors or non-steroidal anti-inflammatory drugs and with previous episodes of angioedema not responding to antihistamines. A trained researcher collected blood samples in the emergency room. Nine healthy subjects, sex- and age-matched with patients, served as controls. The study was approved by Ethics Committee Valpadana of ASST Ospedale Maggiore Crema (No. 104, 22 march 2019). We measured plasma levels of cleaved high-molecular-weight kininogen by SDS-PAGE/immunoblotting and bradykinin by an enzyme immunoassay. Angioedema related genes were also analysed [12]. Details on patients, methods and statistical analysis are given as supplementary material.

All patients had acute angioedema involving the face in 5 patients, lips in 3 patients and both abdominal wall and feet in one patient. Demographic, clinical and laboratory characteristics of patients are reported in supplementary Table 1. Cleaved HK levels (Figure 1) were significantly higher in angioedema patients (median 27.1 %, range 22-61.3 %) than in normal subjects (20.5 %, 9.5-23.4 %) (p = 0.0001). Bradykinin levels (Figure 1) were higher in angioedema patients (8.21
ng/ml, 7.05-21.79 ng/ml) than in normal subjects (2.83 ng/ml, 1.96-3.99 ng/ml) (p=0.0001). Bradykinin levels were directly correlated with levels of cleaved HK (r =0.893, p=0.0001). No mutation was found in the genes implicated in the pathogenesis of hereditary angioedema (SERPING1, ANGPT1, PLG, MYOF, KNG1 and F12). All patients were treated with systemic corticosteroids (methylprednisolone 80 mg i.v.) and antihistamines (chlorphenamine 10 mg i.v.) and, on the basis of oral or tongue involvement, also with epinephrine (1 mg i.m.) (supplementary Table 2). The symptom resolution time (i.e. the time corresponding to the complete disappearance of angioedema reported by the patient after discharge) had a median of 30 hours (range 15-48 hours) and was directly correlated with bradykinin levels (r=0.819; p=0.007).

Our study shows that patients with idiopathic non-histaminergic angioedema may have an increased generation of the vasoactive peptide bradykinin due to contact system activation. Indeed, we found high plasma levels of bradykinin directly correlated with the levels of a marker of contact system activation, i.e. cleaved HK. The pathophysiological involvement of bradykinin in our patients is also indicated by the direct correlation between plasma levels of bradykinin and the angioedema resolution time. The absence of angioedema-related genetic mutations we observed in our patients indicates an acquired nature of the disease. To the best of our knowledge, this is the first demonstration that bradykinin can be generated through contact system activation in idiopathic angioedema; indeed, we measured at the same time both the levels of bradykinin and those of the marker of its generation through contact system activation (cleaved HK). Therefore, in non-histaminergic idiopathic angioedema, the increase of bradykinin, which we observed here and preliminarily in a previous report [13], is due to the activation of the contact system, as in angioedema due to C1-INH deficiency [9-11]. However, in angioedema due to C1-INH deficiency, previous data indicate that the contact system is 2 to 5 times more activated during attacks [9-11].
Levels of bradykinin are high also in patients with acute angioedema due to ACE inhibitors [9,14] but their cleaved HK is normal [14]. Thus, in ACE inhibitor-associated angioedema, the increase of bradykinin is not due to increased generation of bradykinin but rather to reduction in its catabolism, which is normally sustained by ACE and, in this case, inhibited by therapy.

Limitations of our study were the small number of subjects (due to the low incidence of the condition) and the lack of plasma samples from patients during remission; however, the sample size allowed a good power (80% with an alpha error of 5%), and the blood sampling procedure performed in the emergency department represents a strength of our study, allowing us to obtain reliable measurements of contact system and bradykinin just at the peak of the acute attack of angioedema.

In conclusion, our data indicate that the contact/kinin system is involved in the pathophysiology of some cases of idiopathic angioedema and may influence the duration of symptoms. If confirmed by larger studies, they may open new perspectives in this condition for evaluation of drugs that inhibit the contact/kinin system as previously reported [15].

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Conflicts of interest

All authors have no conflict of interest to declare within the scope of the submitted work.
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Figure 1

Upper panel. Immunoblotting of cleaved high-molecular-weight kininogen (HK) in plasma collected from normal subjects (N, left) and from patients (P, right). K: normal plasma treated with kaolin. Lower panel. Cleaved high-molecular-weight kininogen (HK) (left) and bradykinin (right) plasma levels in angioedema patients and in normal subjects.