

Assessment of platelet activity as expressed by plasma levels of platelet factor 4 and β -thromboglobulin in patients with chronic idiopathic urticaria

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Abstract: Blood platelet significance in inflammation is recognized but poorly characterized in urticaria. It is known that platelets are activated during inflammatory processes and are involved in modulating inflammatory and immune response via various mediator release. The aim of our study was to investigate the functional state of platelets, expressed by release reaction of C-X-C chemokines such as platelet factor 4 (PF-4) and β -thromboglobulin (β -TG) in chronic idiopathic urticaria (CIU). Plasma levels of PF-4 and β -TG, which are established markers of *in vivo* platelet activation and which play important role in inflammatory processes, were measured by enzyme-linked immunosorbent assay in 19 patients with CIU and in 25 healthy subjects. Mean plasma PF-4 level in CIU patients and control subjects was 5.01 ± 1.67 and 4.13 ± 2.05 IU/ml, respectively, whereas that for β -TG was 29.3 ± 14.0 and 25.2 ± 12.6 IU/ml, respectively. In our small study, there have been no significant differences found between the members of the control and CIU group regarding plasma levels of PF-4 and β -TG. Further studies should be performed to elucidate whether any systemic platelet activation occurs in CIU.

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Introduction

Pathogenic mechanisms of chronic idiopathic urticaria (CIU) have not been well understood; however, activation of skin mast cells plays a key role in inflammation associated with the disease. Mast cell activation and release of mediators, particularly histamine, can effect in cutaneous inflammatory processes and accumulation and activation of other cells (1). Histopathologically, CIU is characterized by an inflammatory infiltrate, consisting of lymphocytes, neutrophils, or eosinophils. Stimuli responsible for the skin infiltration with these cells are poorly recognized (2,3).

Also circulating cells, possibly basophils and platelets, may play a relevant role in urticaria (4,5). Besides their well-recognized role in hemostasis, platelets are involved in other biologic processes, including inflammation. Possible significance of

platelets in urticaria may be related to their proinflammatory properties. During inflammatory and immune response, platelets may be activated and might release inflammatory mediators. Among these are platelet factor 4 (PF-4) and β -thromboglobulin (β -TG), the better known C-X-C chemokine family members, which play a significant role in recruitment and activation of leukocytes from the circulation to sites of tissue inflammation (6). In addition to platelets, PF-4 is also found in tissue mast cell granules (7) and T lymphocytes (8). PF-4 and β -TG are important indices of platelet degranulation *in vivo*, and measurement of plasma levels of these chemokines can be applied to detect the activation of circulating platelets (9).

Platelets have been described as a source of inflammatory mediators that are implicated in histamine release from basophils (10,11) and

mast cells (12). Interestingly, data from experimental study prove that PF-4 fragment is able to induce rat mast cells to histamine release in a dose-dependent manner (12).

Moreover, it has been shown that histamine is released by human platelets in response to aggregatory and immunological stimuli. In turn, exogenous histamine potentiates platelet activity measured by the aggregation induced by different agonists, acting through histamine receptors (13).

A limited number of studies deal with the involvement of platelets in urticaria-associated processes. Wasserman and Ginsberg (14) demonstrated that induction of local angioedema after cold challenge of patients with cold urticaria is associated with the release of PF-4 into the circulation, suggesting that altered platelet function may occur in this disorder, and its active substances might participate in the genesis of different forms of urticaria (14).

Although inflammatory properties of platelets are well documented, their activity in CIU remains unclear. The objective of the present study was to deliver further evidence proving that the potent biologically active substances stored in platelet granules may be released into the blood stream upon urticaria. We investigated whether CIU may be manifested by enhanced platelet activity, expressed by PF-4 and β -TG plasma level.

Materials and methods

Subjects

The study group consisted of 19 non-smoking patients (15 females and 4 males; mean age 27.7 ± 5.52 years, range 18–39 years) with the duration of idiopathic chronic urticaria from 2 months to 15 years and in which all identified causes of urticaria had been excluded. Their disease was characterized by continuous or recurrent spontaneous wheals having appeared for more than 6 weeks. Four of these CIU patients had, in addition, angioedema. All the patients examined had clinical manifestation of urticaria within at least 4 days before and at the time of the sample collection. Disease intensity was measured by assessment of wheals and pruritus on a scale of 0–3 (sum of score: 0–6) proposed by Zuberbier et al. (15). Briefly, the number of the wheals and the severity of pruritus were scored as follows: number of wheals, 0 = no wheals, 1 = mild wheals (<20 wheals/24 h), 2 = moderate wheals (21–50 wheals/24 h), 3 = intense wheals (>50 wheals/24 h, or large confluent areas of wheals); pruritus, 0 = none, 1 = mild, 2 = moderate, 3 = intense. Only patients with a score of 4 or more were included in the study.

The CIU group was limited to individuals without personal or family history of atopy. The patients had negative skin tests to common inhalant allergens, and all showed negative autologous serum skin test. Moreover, CIU patients had not taken oral steroids for more than 4 weeks preceding the study. Any other therapy was discontinued at least 2 weeks before the study.

The control group consisted of 25 non-atopic, non-smoking healthy subjects (20 women and 5 men; mean age 26.6 ± 6.52 years, range 18–40 years). The control subjects did not take any medication for at least 14 days before the study.

Serum creatinine concentration was normal in all subjects tested. Moreover, individuals were not enrolled into the study if they had personal history of disorders, which would affect platelet activity, like obesity, hypertension, dyslipidemia, or diabetes.

All participants gave written, informed consent, and the study was approved by the University Ethics Committee.

Blood sampling and assay of PF-4 and β -TG

The blood was obtained in the morning (7.00–8.00 hours; in the fasting state) after 25-min rest with slight or no stasis from the antecubital vein into Vacutainer tubes (containing anticoagulant CTAD), which were immediately placed in an ice/water bath for 20 min. The tubes were then centrifuged at $2500 \times g$ for 30 min at 4°C , and the top one-third of the volume of the resultant plasma supernatants was collected and frozen at -20°C for assay (not longer than 1 month). Plasma levels of PF-4 and β -TG as platelet-activation markers were measured by enzyme-linked immunosorbent assay (ELISA), using commercial kit (Asserachrom[®] PF-4 and β -TG, Diagnostica Stago, Paris, France). These ELISA methods were performed according to the manufacturer's recommendation. For all assays, intra- and interassay coefficients of variation were $<8\%$. The lower limit of detection for PF-4 and β -TG was 0.25 and 0.5 IU/ml, respectively.

Skin prick tests

Allergic status was evaluated by skin prick testing with a panel of 10 allergens (grass pollens, rye pollen, tree pollens, feather, house dust mite species, cat dander, dog dander, and molds; Allergopharma, Reinbeck, Germany). The test was regarded positive if the mean diameter of the wheal at 15 min was 3 mm or larger, and atopy was defined as at least one positive reaction in the presence of positive histamine and negative diluent controls.

Autologous serum skin test (ASST)

Intradermal tests with autologous serum were performed according to the method described by Sabroe et al. (16). We tested only patients with urticaria, as the ethical committee granted no consent to perform such test in controls subjects. A serum-induced wheal with a diameter of at least 1.5 mm greater than that of the control wheal induced with physiological saline was assessed as positive. Saline was used as a negative control and histamine served as a positive control.

Other laboratory investigations

Peripheral blood platelets were calculated by a direct method.

Statistical analyses

The results are presented as means $\pm$ standard deviations (SD). Because all data showed normal distribution, significance of differences between the means was tested by Student's *t*-test for unpaired groups with equal variance. Variance analysis was evaluated by means of the Levene test. *P*-values <0.05 were regarded as indicating a significant difference.

Results

Subject characteristics

The subject groups were comparable for sex and age. All the controls and patients had negative <3 -mm reactions to all tested aeroallergens and

were considered non-atopic. None of the urticaria patients had positive ≥ 1.5 -mm response to ASST.

Platelet tests

There were no statistically significant differences between CIU patients and controls in plasma levels of platelet-activation markers, PF-4 and β -TG, and in platelet count in the peripheral blood. The obtained values in the two examined groups, expressed as means and SD, are summarized in Table 1.

Discussion

Little is known about factors that stimulate mast cells to release histamine and other inflammatory mediators and about mediators released from incoming cells in CIU. Interestingly, altered platelet activity is regarded as an important factor in the pathogenesis of some inflammatory diseases (17).

We determined platelet functional state by analyzing the plasma level of PF-4 and β -TG, because these chemokines are good indicators of the degree of platelet activation and have potent inflammatory properties, which might play some roles in inflammatory response in urticaria.

In the present study, plasma levels of PF-4 and β -TG in CIU patients were not significantly increased compared to those in controls. The study delivers then no evidence, proving that circulating platelets of CIU patients have greater activity than blood platelets of normal subjects, when their activation state is measured by the plasma level of PF-4 and β -TG.

Release of the platelet-derived chemokines PF-4 and β -TG in CIU has to our knowledge not been documented yet. On the other hand, the role of blood platelets in cold urticaria is controversial. Early studies had suggested blood platelet activation in physical urticaria on the basis of the finding of enhanced plasma level of PF-4 after cold stimulus (14). In contrast to these results, Rosenkranz et al. (18) did not observe any difference concerning PF-4 release between patients and controls

after cold challenge test, suggesting that platelets are not directly involved in cold urticaria.

Our study does not allow for determination of whether platelets or platelet α -granule proteins play any role in the mechanisms involved in its pathogenesis. This is because platelet activation and release of their mediators might occur mainly locally, at the site of urticarial inflammation. Activated platelets might stimulate the production of histamine or other mediators through enhanced chemokine secretion and by direct contact with different inflammatory cells, which would be mediated by the expression of adhesion molecules on the platelet surface. Interestingly, the presence of platelet aggregates within skin biopsy specimens in cold urticaria with vasculitis has been reported (19).

To our knowledge, no prior demonstration of local platelet hyperactivity in CIU measured by PF-4 or β -TG has been described. The local platelet activation process may not be intense enough to cause significant increase in the plasma levels of these chemokines. Alternatively, it is possible that PF-4 or β -TG released locally may be rapidly neutralized or bound by different cells, so that their secretion is not reflected by an increase in the levels of these chemokines in the systemic circulation.

On the other hand, other types of mediators could be released from activated platelets into the circulation of CIU patients and could be involved in the CIU-inflammatory process. Indeed, a marked increase in serum level of soluble P-selectin, which is released by activated platelets and endothelial cells, has been described in both chronic and dermographic urticaria as compared with healthy controls and allergic rhinitis patients (20).

Urticaria is a mast-cell-dependent disease; these cells are also known to play the central role in IgE-mediated allergic diseases. Elevated circulating levels of PF-4 and β -TG have been noted in patients with bronchial asthma (21) and Atopic Eczema/Dermatitis Syndrome (AEDS) (22), but not in patients with seasonal allergic rhinitis (23). The importance of PF-4 in AEDS pathogenesis is emphasized by experimental results in mouse models of AEDS, which demonstrate enhanced expression of PF-4 (24). PF-4 and β -TG may play a role during allergic inflammatory reactions, particularly in the regulation of eosinophil activity (25,26).

Moreover, it has been demonstrated that *in vivo* platelet activation, as measured by β -TG, occurs in patients with psoriasis, another common skin disease. As platelets release both inflammatory and mitogenic substances, it has been suggested

Table 1. Levels of PF-4 and β -TG and the number of platelets in the blood of the control and CIU groups

Analyzed parameters (unit)	Control ($n=25$)	CIU ($n=19$)
PF-4 (IU/ml)	4.13 ± 2.05	5.01 ± 1.67
β -TG (IU/ml)	25.2 ± 12.6	29.3 ± 14.0
Blood platelets ($\times 10^9/l$)	233 ± 43.6	226 ± 46.9

CIU, chronic idiopathic urticaria; β -TG; β -thromboglobulin; PF-4, platelet factor 4; n , number of subjects. Values are means $\pm$ SD.

that they could well be involved in the histogenesis of psoriatic lesions (27).

The present study has also demonstrated no significant difference in platelet counts in peripheral blood between the CIU patients and the control group.

In conclusion, we investigated platelet activation state to achieve some better understanding of biologic processes that are associated with CIU. Hyperactivity of platelets, as detected by rising plasma PF-4 and β -TG, was not observed in patients with CIU. Such data seem to indicate that the inflammatory response in CIU is not manifested by enhanced systemic platelet activity, characterized by these chemokines. Whether systemic activation of platelets is associated with CIU remains to be elucidated using other methods, including flow cytometric detection of activated platelets.

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