

Effect of allergen-specific immunotherapy on plasma level of platelet factor 4 (PF-4) and beta-thromboglobulin (beta-TG), platelet activation markers in patients with house dust mite allergy

Alicja Kasperska-Zajac^{*}, Zenon Brzoza, Barbara Rogala

Clinical Department of Internal Diseases, Allergology and Clinical Immunology, Medical University of Silesia,
ul. 3-go Maja 13-15, 41-800 Zabrze, Poland

Received 10 October 2006; received in revised form 4 January 2007; accepted 11 January 2007
Available online 29 January 2007

Abstract

Patients suffering from house dust mite-sensitive persistent allergic rhinitis (PAR) seem to show changed platelet aggregability following mite allergen-specific immunotherapy (SIT). However, in patients with grass pollen-sensitive intermittent allergic rhinitis, platelet release reaction *in vivo*, measured by beta-TG, did not change significantly during the dose increase phase of subcutaneous SIT with grass pollen allergoids. The aim of this study was to evaluate the influence of SIT with house dust mite allergen extracts on the degree of circulating platelet activity in patients with PAR. The study comprised 14 patients sensitized to house dust mite allergens with PAR during the early phase of subcutaneous SIT with house dust mite allergen extracts—*Dermatophagoides farinae* 50 p.c. and *Dermatophagoides pteronyssinus* 50 p.c. (Novo-Helisen[®] Depot, Allergopharma, Germany). SIT comprised subcutaneous injection of allergen extracts during approximately 3 months. Blood was collected before SIT, as well as immediately before, 30 min and 24 h after maximum dose injection. Plasma levels of PF-4 and beta-TG, platelet activation markers were measured using commercial ELISA kit.

PF-4 and beta-TG basal levels were not significantly different between the patients and the healthy subjects. PAR patients treated with SIT showed no significant differences in plasma levels of the markers between the different points of the study. Thus, it seems that the degree of platelet activation measured by PF-4 and beta-TG does not change in PAR patients during hyposensitization with mite allergen extracts, after the maximum dose was reached.

© 2007 Elsevier Ltd. All rights reserved.

Keywords: Allergen-specific immunotherapy; House dust mite allergy; Platelet activation; Platelet factor 4; Beta-thromboglobulin

1. Introduction

IgE-mediated allergic processes play an important role in platelet activation induction, either in direct or indirect manner. Functional receptors for the Fc fragment of IgE, such as low affinity receptor (FcεRII) and high affinity receptor (FcεRI), have been identified on the platelet membrane [1–3]. Platelets could be activated directly upon contact with the corresponding allergen and are capable of releasing a wide range of biologically active mediators, which play an important role in IgE-mediated allergic reaction [3,4]. Among those, PF-4

and beta-TG show the chemotactic activity appearing then as good markers of platelet activation and degranulation [5].

Allergen-specific immunotherapy (SIT) is a form of systemic causative treatment of IgE-mediated allergic diseases [6]. It has been demonstrated that hyposensitization of allergic diseases may affect platelet function. Decrease of IgE-dependent platelet activation after specific rush desensitization has been observed in patients with *Hymenoptera* hypersensitivity [7]. Interestingly, platelet not only lost its reactivity to venom but also towards other allergens in two cases of polysensitized patients [7]. The previous study had shown that allergen-specific platelet activation as well as that mediated by IgE, may occur in *Dermatophagoides pteronyssinus* sensitive patients. Synthetic peptides derived

^{*} Corresponding author. Fax: +48 32 2718110.

E-mail address: kasperska@plusnet.pl (A. Kasperska-Zajac).

from house dust mite allergens activated platelets of such patients, failed however to activate platelets from healthy donors or platelets from non-*Dermatophagoides pteronyssinus* allergic patients [8]. Our earlier study failed to show any significant alteration in platelet release reaction *in vivo* measured by plasma level of beta-TG after the dose increase phase of SIT with grass pollen allergoids (Allergovit®) in patients suffering from allergic rhinitis symptoms [9]. It is known that chemically modified allergens, allergoids, have substantially reduced reactivity to allergen-specific IgE and preserved immunogenicity compared with native allergens. In order to gain further insight into the influence of allergic vaccines on platelet function, in the present we investigated changes in plasma levels of PF-4 and beta-TG in response to subcutaneous administration of the maximum dose of vaccine containing house dust mite allergen extracts after a brief course of SIT in patients who suffered from mite-sensitive allergic rhinitis without asthma symptoms.

2. Material and methods

2.1. Subjects

Fourteen monoallergic house dust mite-sensitive patients (seven women and seven men; mean age: 22 years; range: 18–28 years) with persistent allergic rhinitis without asthma symptoms were included into the study. None of the subjects suffered from concomitant allergy and none had undergone treatment with any form of SIT before the study. They showed positive skin-prick tests to *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* as well as negative response to all other common aeroallergens. SIT was performed using Novo-Helisen® Depot (Allergopharma, Reinbek, Germany). SIT was administrated to all patients according to the standard administration schedule. No episodes of systemic side events were observed during the SIT course.

The control group consisted of 20 healthy volunteers, with no signs of atopy, 11 females and 9 males (mean age 22.5 years; range: 18–26 years).

The Ethics Committee of the Medical University of Silesia approved of the study and written, informed consent was obtained from all the participating subjects.

2.2. Blood collection for platelet markers analysis

Peripheral venous blood samples were collected during the early phase of mite SIT at fixed times: (a) immediately before SIT (T_0), (b) immediately before maximum dose injection (T_1) and (c) 30 min and 24 h after the maintenance dose was reached (T_2 and T_3 , respectively).

Blood samples were obtained and prepared according to the protocol described earlier [9].

2.3. PF-4 and beta-TG assay

Plasma levels of PF-4 and beta-TG were measured by enzyme-linked immunosorbent assay (ELISA), using commercial Asserachrom® kit by Diagnostica Stago, Paris, France, according to the manufacturers' instruction.

2.4. Skin-prick tests

Skin-prick testing was performed using a panel of common inhalant allergens (grass, weed and tree pollens, cat and dog dander, moulds, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*) (Allergopharma, Reinbek, Germany). Saline and histamine solutions were used as the negative and positive controls, respectively. A wheal of diameter by 3 mm greater than the negative control was accepted as a positive response. All the patients had a mean wheal diameter of >5 mm.

2.5. Immunotherapy protocol

SIT dose increase phase was carried out in accordance with the dosage scheme recommended by the manufacturer (Allergopharma, Reinbek, Germany). Immunotherapy consisted of subcutaneous injection of allergen extracts of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* during approximately 3 months, starting with 0.1 ml (Vial A, 50 TU/ml) and increasing until the patients reached the highest maintenance dose of 1 ml of Vial C, 5000 TU/ml.

2.6. Statistical analysis

Data are presented as median and range. The Mann–Whitney *U*-test was used to compare results between patients before the treatment and the controls. Data from all samples

Table 1

The values of plasma levels of PF-4 and beta-TG in the control subjects and the persistent allergic rhinitis patients, receiving SIT obtained during consecutive measurements

	T_0 (median range)	T_1 (median range)	T_2 (median range)	T_3 (median range)
Patients				
PF-4 level (IU/ml)	4.9 (1.5–6.2)	2.75 (0.5–5.5)	4.55 (0.8–7.5)	5.65 (2.2–6.8)
Beta-TG (IU/ml)	20.25 (8.6–48)	27 (6.6–35)	16.75 (9.5–30)	26 (10–45)
Controls				
PF-4 level (IU/ml)	4.1 (0.5–7.0)			
Beta-TG (IU/ml)	18.5 (8.5–42.5)			

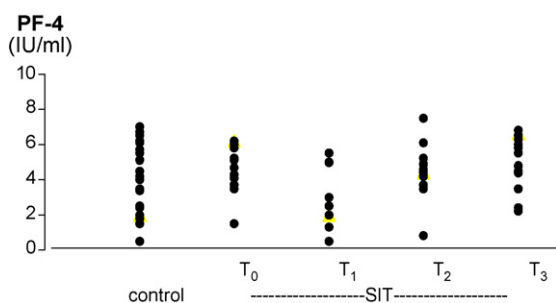


Fig. 1. Individual plasma levels of PF-4 in PAR patients in comparison to healthy subjects and during the early phase of SIT with mite allergen extracts (SIT, specific immunotherapy; PAR, persistent allergic rhinitis; T_0 , immediately before first dose injection; T_1 , immediately before maximum dose injection; T_2 , 30 min after the maximum dose injection; T_3 , 24 h after the maximum dose injection).

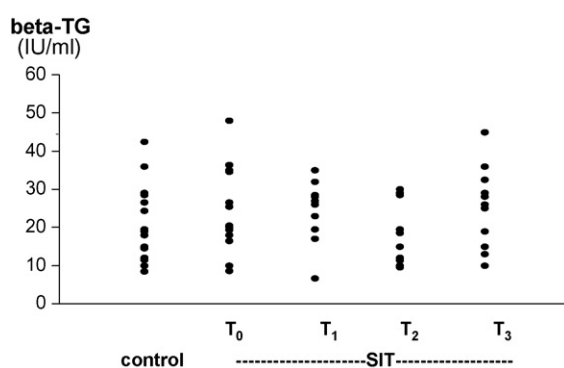


Fig. 2. Individual plasma levels of beta-TG in PAR patients in comparison to healthy subjects and during the early phase of SIT with mite allergen extracts (SIT, specific immunotherapy; PAR, persistent allergic rhinitis; T_0 , immediately before first dose injection; T_1 , immediately before maximum dose injection; T_2 , 30 min after the maximum dose injection; T_3 , 24 h after the maximum dose injection).

obtained during the course of SIT were compared using Friedman repeated measure analysis of variance. P -values lower than 0.05 were considered significant.

3. Results

Data in median and range are presented in Table 1. The baseline plasma PF-4 and beta-TG levels did not differ significantly between the patients and the healthy subjects. In patients treated with SIT for mite-sensitive allergic rhinitis, plasma level of the markers did not differ significantly between different times of the study ($T_0 - T_3$). Individual plasma levels of PF-4 and beta-TG in the patients in comparison to healthy subjects and during the course of SIT are presented on Figs. 1 and 2, respectively.

4. Discussion

Unfortunately, available data on functional platelet status in allergic diseases are limited and appear contradictory. Pre-

viously, we had observed significantly diminished platelet aggregation induced by adenosine diphosphate in house dust mite-sensitive patients with persistent allergic rhinitis, who partially improved following 2 year SIT with *D. pteronyssinus* extract, suggesting that such therapy may modulate platelet reactivity [10].

In the present study, we examined the influence of mite immunotherapy on circulating platelet activity as measured by plasma levels of PF-4 and beta-TG in patients suffering from persistent allergic rhinitis, sensitized to house dust mites.

The chemokines, such as PF-4 and beta-TG, are structurally similar and immunologically specific for platelets. Present in similar amounts within platelet alpha-granules they are released *in vitro* in similar quantities from the stimulated platelets. The half-life of PF-4 and beta-TG is about 5 and 100 min, respectively [5].

It seems that inhalation of a natural allergen, such as house dust mite, is not accompanied by increased platelet activity as expressed by baseline circulating levels of PF-4 and beta-TG in patients with house dust mite-sensitive persistent allergic rhinitis [11] and asthma symptoms [12]. However, in asthmatic patients bronchial allergen challenge with *D. pteronyssinus* resulted in activation of peripheral blood platelet measured by PF-4, beta-TG and sP-selectin [12]. In the present study, PF-4 and beta-TG levels were first evaluated in persistent allergic rhinitis patients immediately before SIT. No statistically significant differences were found in the chemokine plasma level between those patients and the healthy controls, which corresponds to our previous results [11]. Moreover, we did not observe any significant changes in plasma levels of PF-4 and beta-TG in sensitized patients following the dose increase phase of SIT for mite-sensitive persistent allergic rhinitis; both after 30 min and overnight the maximum dose administration of the allergen vaccine. Using previously a similar study protocol, we did not show any significant differences in plasma level of beta-TG in patients with pollen-allergens induced rhinitis after the induction phase of SIT with grass pollen allergoids [9]. Chemically modified allergens (allergoids) have substantially reduced reactivity to allergen-specific IgE compared with native allergens, but their immunogenicity is maintained. Therefore, our present study not only confirms the previous observation regarding the effect of subcutaneous SIT on platelet function (assessed by plasma level of platelet-derived chemokines) but also extends this observation on patients with mite allergy during subcutaneous desensitization with allergen extracts. In addition, in the present study was performed analysis of both PF-4 and beta-TG levels which may supply far more information and ensure deeper insight into the platelet function upon SIT. As mentioned above, the chemokines differ as regards to *in vivo* clearance, which is much faster for PF-4 than that for beta-TG [5]. Our study was however limited by the lack of parallel control group. The best control would be a placebo treated rhinitis group or at least “no treatment” rhinitis group evaluated at the selected timepoints. These pre-

liminary results should be further confirmed on larger group of patients and using other study protocols.

5. Conclusions

In the present study, the authors have mainly aimed at evaluating the differences in circulating platelet activity between the pretreatment period and after the early phase of mite SIT. We failed to detect any significant changes in the degree of platelet activation measured by PF-4 and beta-TG plasma levels in patients with persistent allergic rhinitis during the course of hyposensitization with mite extracts, after reaching the maximum dose. On the basis of the present study as well as our earlier investigations, it appears likely that platelet release reaction *in vivo*, expressed by such chemokines and measured following the protocol above, does not change throughout the induction phase of SIT with allergoid and allergen extract in patients with allergic rhinitis.

Acknowledgement

This study was supported by a research grant from the Medical University of Silesia (NN-1-246/03).

References

- [1] Capron M, Jouault T, Prin L, Joseph M, Ameisen JC, Butterworth AF, et al. Functional study of a monoclonal antibody to IgE Fc_ε receptor (Fc_εR2) of eosinophils, platelets and macrophages. *J Exp Med* 1986;164:72–89.
- [2] Joseph M, Gounni AS, Kusnierz JP, Vorng H, Sarfati M, Kinet JP, et al. Expression and function of the high-affinity IgE receptor on human platelets and megakaryocyte precursors. *Eur J Immunol* 1997;27:2212–8.
- [3] Hasegawa S, Pawankar R, Suzuki K, Nakahata T, Furukawa S, Okumura K, et al. Functional expression of the high affinity receptor for IgE (Fc_εRI) in human platelets and its' intracellular expression in human megakaryocytes. *Blood* 1999;93:2543–51.
- [4] Joseph M, Capron A, Ameisen JC, Capron M, Vorng H, Pancré V, et al. The receptor for IgE on blood platelets. *Eur J Immunol* 1986;16:306–12.
- [5] Kaplan KL, Owen J. Plasma levels of β -thromboglobulin and platelet factor 4 as indices of platelet activation *in vivo*. *Blood* 1981;57:199–202.
- [6] Bousquet J, Lockey R, Malling HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. *J Allergy Clin Immunol* 1998;102:558–62.
- [7] Tsicopoulos A, Tonnel AB, Wallaert B, Joseph M, Ameisen JC, Ramon P, et al. Decrease of IgE-dependent platelet activation in hymenoptera hypersensitivity after specific rush desensitization. *Clin Exp Immunol* 1988;71:433–8.
- [8] Cardot E, Pestel J, Callebaut I, Lassalle P, Tsicopoulos A, Gras-Masse H, et al. Specific activation of platelets from patients allergic to *Dermatophagoides pteronyssinus* by synthetic peptides derived from the allergen Der p 1. *Int Arch Allergy Immunol* 1992;98:127–34.
- [9] Kasperska-Zajac A, Brzoza Z, Rogala B. Effect of allergen-specific immunotherapy on platelet secretory activity in patients with grass-pollen allergy. *Vaccine* 2006;24:6990–3.
- [10] Rogala B, Gumprecht J, Gawlik R, Strojek K. Platelet aggregation in IgE-mediated allergy with elevated soluble Fc ϵ R2/CD23 level. *J Invest Allergol Clin Immunol* 1995;5:161–5.
- [11] Kasperska-Zajac A, Rogala B. Markers of platelet activation in plasma of patients suffering from persistent allergic rhinitis with or without asthma symptoms. *Clin Exp Allergy* 2005;35:1462–5.
- [12] Kowal K, Pampuch A, Kowal-Bielecka O, DuBuske LM, Bodzenta-Łukaszyk A. Platelet activation in allergic asthma patients during allergen challenge with *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 2005;36:426–32.