Raised plasma concentrations of platelet factor 4 (PF4) in Crohn's disease

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SUMMARY Plasma platelet factor 4 (PF4), secreted by the platelets, is an index of platelet aggregation and thromboembolic risk. The authors assessed PF4 in 20 patients with Crohn's disease (ileitis in 13 patients, ileocolitis in seven) and in 20 healthy volunteers. Disease activity was low (Crohn's Disease Activity Index <150) in 11 patients and high in nine. Radioimmunoassay of PF4 using Abbott's Kit was performed on one sample of plasma from each subject ($nv \leq 0.324$ nmol/ml), ($nv \leq 10$ ng/ml). A significantly higher concentration of PF4 was found in Crohn's disease patients: 4.625 ± 1.1 nmol/ml (142.5 ± 36 ng/ml) than in the control group: 0.189 ± 0.07 nmol/ml (5.6 ± 4.8 ng/ml) ($Z = 5.396$, $p < 0.0001$). No correlation was present between PF4 levels and activity, the site of disease, or medical treatment with or without prednisone.

In chronic inflammatory bowel disease a high incidence of thromboemboli has been observed in clinical (7.1%,¹ 6.4%,² 30%,³ 11.7%⁴) and necropsy studies (32%,⁵ 39%⁶).

These complications result from altered haemostatic mechanisms with consequent hypercoagulation.⁷⁻⁹ The main changes are thrombocytosis and hyperfibrinogenaemia.¹⁰⁻¹² It is not known whether the thrombocytosis seen in these patients does in fact correspond to an increase in platelet aggregation – that is, the event that triggers thrombosis.^{13,14}

Platelet aggregation activity can now be determined by assay of plasma endothrombocyte substances such as platelet factor 4 (PF4)¹⁵⁻¹⁸ and beta-thromboglobulin.^{15,17,18} The quantity of these substances released by the platelets at the moment of aggregation is directly proportional to the degree of aggregation occurring.

This study was undertaken to verify, by means of PF4 radioimmunoassay (RIA), whether platelet aggregation is really increased in Crohn's disease and if so, to consider the possible implications.

Methods

PATIENTS

Twenty patients with Crohn's disease were studied (10 men and 10 women, median age 38 years, range 25–70 years).

The diagnosis of Crohn's disease was based on clinical and radiological findings, confirmed whenever possible endoscopically and/or histologically.¹⁹

The characteristic lesions of Crohn's disease were sited in the ileum in 13 cases and in the ileocolon in seven cases. Lesions were recurrent in nine patients who had undergone intestinal resection two to 10 years previously; the other 11 patients had never been operated. Disease was active (Crohn's Disease Activity Index >150²⁰) in nine cases and inactive in 11.

At the time of the study, 16 patients were being treated with prednisone (4–20 mg/day) and five of these also with sulphasalazine (1.5–3 g/day). The remaining four patients were receiving no medical treatment. None of the patients had a previous history of thromboemboli or clinical signs of thromboembolic disease.

Blood samples for plasma PF4-RIA were taken by antecubital venipuncture and added to standard 10

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cc vacutainer tubes containing EDTA. Immediately after collection each tube was inverted gently three times and placed straight into an ice water bath. Within 15 minutes, the plasma samples were centrifuged at 2000 g for 30 minutes at 4°C and stored at -20°C until assay. This was done by the radio-immunologic method using I¹²⁵ PF4 (human) and PF4 antiserum (goat) (Abbott Kit) (nv≤0.324 nmol/ml (10 ng/ml)). Plasma PF4 concentrations were determined in the same way in a control group of 20 healthy volunteers matched for sex and age.

STATISTICAL ANALYSIS

The statistical significance of the results was calculated using the Mann-Whitney test for non-parametric data.

Results

In the control group, plasma PF4 concentrations were normal, 0.189±0.07 nmol/ml (5.6±4.8 ng/ml), but in all the patients with Crohn's disease they were

Figure Plasma concentrations of PF4 in 20 patients with Crohn's disease and in the control group. The mean values of PF4 were: 4.625±1.1 nmol/ml (142.5±36 ng/ml) (Crohn's disease); 0.189±0.07 nmol/ml (5.6±4.8 ng/ml) (control group) (PF4 nv≤0.324 nmol/ml (10 ng/ml)).

significantly increased, 4.625±1.1 nmol/ml (142.5±36 ng/ml) (Z=5.396, p<0.0001) (Figure).

No significant correlation was found between increased plasma PF4 concentrations and disease activity, the site of lesions or medical treatment with or without prednisone.

Discussion

Platelet factor 4 is a tetrameric protein stored in the alpha-granules of the platelets.²¹ It is released into the blood during platelet aggregation, the trigger phase of coagulation.¹⁴

The main role of PF4 seems to be inhibition of the anticoagulant effect of heparin, but other activities await explanation.¹⁸

A significant relationship has been shown between PF4 plasma level and thrombocytosis¹⁷ and in particular between PF4 plasma level and platelet aggregation activity.^{13,17,18} Plasma PF4-RIA thus provides a valid index of thromboembolic risk.²²⁻²⁴ Raised concentrations of plasma PF4 have been found in subjects at high risk of thromboemboli, such as those with myocardial infarction,²⁵ disseminated intravascular coagulation,²⁴ diabetes mellitus,²⁶ and prosthetic heart valve implants.²⁷

In this study we found significantly raised concentrations of PF4 (Z=5.396, p<0.0001) in patients with Crohn's disease (Figure). Increased platelet aggregation, together with a high platelet count as already shown,^{8,11,12} might therefore be the major step in the pathogenesis of the hypercoagulation phenomena which are responsible for the high incidence of postoperative thromboembolic complications seen in these patients. Although Lake *et al*¹¹ suggested this mechanism, they were not able to confirm it *in vitro*.

Unlike observations in thrombocytosis,^{10,28} in this study increased platelet aggregation did not correlate to disease activity, as raised plasma concentrations of PF4 were seen both in patients with active and inactive Crohn's disease. Whereas others have reported increased thrombocytosis particularly in patients with colonic disease,^{7,10} we found that the ileal or ileocolonic localisation of disease did not apparently influence PF4 plasma level. Similarly, medical treatment with or without prednisone had no effect on the degree of platelet aggregation.

This study does not clarify whether thrombocytosis and increased platelet aggregation have the same pathogenesis, or which, if either, is the trigger factor. The results of previous investigations into the role of prostanooids might, however, help to provide a link in the pathogenetic chain. As stated by Mehta and Mehta,¹⁴ thromboxane A₂ is the most potent stimulus known for platelet aggregation and its action is balanced by prostacyclin. Furthermore, a significant

rise in thromboxane synthesis and reduction in the prostacyclin/thromboxane ratio have recently been reported in patients with Crohn's disease.²⁹ This reduction might explain the pathological platelet aggregation seen in our study. Further investigations are needed to show this and identify the mechanism responsible for the increased platelet aggregation found in Crohn's disease.

In practice, PF4-RIA may prove to be a useful method for identifying the cases of Crohn's disease most at risk for thromboembolism, particularly those undergoing surgery. As surgical therapy *per se* enhances this risk, prophylaxis against thromboembolism is especially advisable when raised plasma concentrations of PF4 are found. It is important to remember that these concentrations would inhibit the anticoagulant properties of heparin.^{18,30} Peri-operative prophylactic treatment should therefore be carried out with antiaggregatory drugs, such as dipyridamole, but not with heparin.³¹

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