Plasma Concentrations of Platelet-Specific Proteins Correlated With Platelet Survival

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Relationships between $^{51}$Cr platelet survival and plasma concentrations of $\beta$-thromboglobulin ($\beta$TG) and platelet factor 4 (PF4) were analyzed in 91 studies of patients with coronary artery disease. $\beta$TG was significantly correlated with platelet life-span, turnover, and the number of hits in the multiple hit model. PF4 was significantly correlated with life-span and turnover. The most significant relationship involving platelet-specific protein concentrations and life-span estimates was between $\beta$TG and PF4 estimated using the multiple hit model ($r = -0.39$, $p < 0.001$). There was a high correlation between $\beta$TG and PF4 ($r = 0.62$, $p < 0.001$), and no improvement could be obtained by combining the measurements of the two proteins in any regression with life-span or turnover. The results indicate that the patients with the shortest platelet survival time in this group tended to have the highest plasma concentrations of $\beta$TG and PF4 and thus probably increased in vivo release of $\beta$TG and PF4. They strengthen the claim that these platelet-specific proteins may be indicators of platelet involvement in disease.

Evidence of platelet involvement in the development or progression of arterial disease and its associated thromboembolic complications has been supported by a number of reports of shortened platelet survival in such conditions, including some patients with coronary artery disease. Determination of platelet survival is time consuming, and the most appropriate method of data analysis is not clearly defined. Among other approaches suggested have been assays of the platelet-specific proteins, $\beta$-thromboglobulin ($\beta$TG), and platelet factor 4 (PF4), in carefully processed plasma samples. These closely related proteins are released simultaneously from the $\alpha$-granule of platelets, and increased plasma levels have been reported in a number of vascular and thrombotic disorders. It has been assumed that the increased levels in such situations are a reflection of in vivo platelet activation. This study reports significant correlations between platelet survival measurements and plasma concentrations of platelet-specific proteins in a group of patients with coronary artery disease (CAD) undergoing coronary artery bypass surgery (CABG).

Materials and Methods

Subjects

Ninety-one studies were carried out on 62 preoperative patients from the Melbourne University Open Heart Surgical Unit undergoing CABG and on 29 patients 6 mo after surgery. All had grade II–IV angina (NYHA) preoperatively and two patients had local arterial thrombosis related to cardiac catheterization.

Plasma Samples

Venous blood was taken for assay prior to reinfusion of the $^{51}$Cr-labeled platelet rich plasma (PRP). Plasma samples were prepared for PF4 and $\beta$TG assay from this blood that was anticoagulated with EDTA, theophylline, and prostaglandin E1 and prepared at 4°C as previously described. The plasma was stored at −70°C prior to assay.

$\beta$TG and PF4 Radioimmunoassay

Both assays were carried out using methods previously reported for PF4.

$^{51}$Cr Platelet Survival Studies

These were performed according to standard methods with the packed cells being returned to the patient after infusion of PRP. Blood samples were collected twice daily over an average of 5 days. Criteria for the inclusion of platelet survival data were established prior to initiation of the study, and of 99 survivals in all, only 91 with the following percentage labeling and recovery were accepted for analysis: platelet label >85%, red cell label <10%, plasma label <10%, and recovery 12 hr after infusion >40%. Radioactivity was measured in duplicate on heparinized and saponin-lysed whole blood samples.

Platelet Counts

Platelet counts were performed with a Technicon Autocounter Model 1A or a Coulter Thrombocounter Model C.

Statistical Methods

Five indices of platelet survival were determined for each study. These comprised platelet life-span calculated using (1) linear (L), (2) exponential (E), (3) weighted mean (WM), (4) multiple hit (MH) models, and (5) the number of hits (n) in the multiple hit
model (i.e., the order of the survival curve) was also estimated. These indices were determined using standard methods, except that E was obtained by fitting the function $N_t = ce^{-kt}$ explicitly rather than by first taking logarithms. This is consistent with the determination of weights for the WM, and unlike the standard method, gives the same results as a one-hit MH. The maximum number of hits considered in the MH was set at 25 on grounds of practicability. Correlation coefficients, both parametric (correlation coefficient, $r$) and nonparametric (Spearman’s $\rho$), were calculated between the five measures of platelet life-span, platelet counts, corresponding turnover measures (platelet counts/life-span), and $\beta$TG and PF4 (singly and in combination). In addition all platelet survival indices were correlated with the date of entry into the study in order to determine whether any change occurred with time as previously reported. Twenty-six patients were studied on 2 occasions, namely preoperatively and 6 mo postoperatively. Since the within-subject variation exceeded the between-subject variation (e.g., for $\beta$TG, $\rho = 0.826$ and for MH, $F$ (between/within) = 0.794) it was considered statistically desirable to include all data in the correlation analyses.

Ethical Considerations

Informed consent was obtained. The protocol was approved by the Ethics Committees of both hospitals and is in accord with the recommendations of the National Health and Medical Research Council of Australia.

RESULTS

The values for the platelet survival indices, $\beta$TG, PF4, and platelet counts are shown in Table 1.

Platelet Survival

Based on skewness and kurtosis, the values for platelet life-span approximated a normal distribution. The values for $\beta$TG were not normally distributed, with 55% 3 hits or less and 31% 25 hits. The platelet label (mean $\pm$ SD) was 95% $\pm$ 3%, red cell label 2%$\pm$2%, plasma label 3%$\pm$2%, and recovery 58%$\pm$14%. There was no change in any of the platelet survival indices with time of entry into the study.

$\beta$TG and PF4

For $\beta$TG, the coefficient of variation between assays was 14% and within assay was 8%. For PF4, these values were 20% and 8%, respectively.

Correlations (Table 2)

Platelet survival. Apart from $n$, all indices correlated significantly with each other. The values of $r$ and Spearman’s $\rho$ both ranged from 0.68 to 0.96 ($p<0.001$); $n$ correlated only with MH.

$\beta$TG and PF4. These were significantly correlated ($r = 0.62, \rho = 0.60, p<0.001$ for both). The correlation was greater under a logarithmic transformation ($r = 0.65, \rho < 0.001$).

$\beta$TG and platelet survival. There were significant correlations between $\beta$TG and all indices of platelet survival. The highest was with WM ($r = -0.39, p<0.001; \rho = -0.34, p<0.01$) (Fig. 1). Significant correlations were also found with all estimates of platelet turnover except that based on L.

PF4 and platelet survival. There were significant correlations between PF4 and all indices of platelet survival except $n$. The highest was with WM ($r = -0.33, p<0.01; \rho = -0.24, p<0.05$). Significant correlations were also found with all estimates of platelet turnover except that based on L.

Platelet counts did not correlate with any other measurement.

DISCUSSION

In a group of patients with proven CAD, this study has demonstrated a relationship between plasma concentrations of $\beta$TG and PF4 with platelet survival
measurements. Patients with the shortest platelet survivals tended to have the highest plasma levels of \( \beta \)TG and PF4, suggesting platelet granule release in vivo. The possibility of enhanced in vitro release in situations associated with reduced platelet survival cannot be completely excluded. A similar correlation has been reported for \( \beta \)TG in normal subjects, but other investigators have not found this in one group of patients with coronary artery disease. Furthermore, in a small number of patients with immune thrombocytopenia, plasma PF4 concentrations were consistently low, suggesting an alternative mechanism for removal of platelets from the circulation in this condition.

Of interest is the correlation between plasma \( \beta \)TG and number of hits. This relationship suggests that the closer the curve approaches the exponential \((1 \text{ hit})\), the more likely is it that platelet protein release occurs. The close correlation between plasma concentration of \( \beta \)TG and PF4 and the fact that they are interchangeable in regression analysis with platelet life-span provides further evidence for the comparability of their release from platelets and clearance from plasma. Because of the relative simplicity of their measurement and their significant correlation with platelet survival, either \( \beta \)TG or PF4 radioimmunoassay could be useful in further studies of platelet involvement in disease and in the assessment of potential therapeutic interventions.

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**REFERENCES**

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