Splenic Dynamics of Indium-111 Labeled Platelets in Idiopathic Thrombocytopenic Purpura

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Splenic dynamics of ^{111}In-labeled platelets and platelet-associated IgG in 33 patients with idiopathic thrombocytopenic purpura (ITP) were studied. Two half-lives were calculated for the biexponential splenic time-activity curve after i.v. injection of ^{111}In-labeled platelets. There was no difference in the mean half-life of the rapid component of the splenic curve (ST1) when patients with negative or slightly positive platelet suspension immunofluorescence test (PSIFT) were compared to those with strongly positive PSIFT (3.0 ± 0.7 min vs. 3.6 ± 0.4, $p > 0.05$). Mean half-life of the slow component of the splenic curve (ST2) was found to be longer in patients with a strongly positive than a negative or weakly positive PSIFT (26 ± 5 min vs. 13.2 ± 1.0 min, $p < 0.01$). It seems that determination of the two components of the splenic time-activity curve provides a useful method for studying platelet kinetics in ITP.

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The spleen is the major organ involved in platelet dynamics in normal and pathologic states (*1–3*). It is both the main site of platelet destruction and an important exchangeable platelet pool. The use of radioindium (^{111}In) as platelet label has made it possible to study platelet dynamics scintigraphically by recording time-activity curves over the liver, spleen, and heart following the injection of labeled platelets. Peters et al. (*2,4*) introduced methods for calculating splenic blood flow and intrasplenic platelet transit time by assuming that there is rapid exchange of platelets between the circulation and the spleen as in a well-mixed closed two compartmental system. This model has been validated in healthy persons with normal platelet dynamics (*3,5*). However, according to Peters et al. (*6*) this technique is not applicable to platelets with a very short life span and, instead, deconvolution analysis has been used for the study of splenic platelet dynamics in pathologic conditions. However, in deconvolution analysis it is assumed that the blood-pool signal is a true reflection of the arterial input into the spleen; therefore this analysis does not account for the possibility of platelet sequestration in the liver, lungs, and bone marrow.

We report a new biexponential model describing splenic platelet dynamics in patients with idiopathic thrombocytopenic purpura (ITP). This model was validated by relating data on platelet kinetics to results of platelet associated IgG.

MATERIALS AND METHODS

Patients

Of the 33 patients with ITP, including three patients with thrombocytopenia associated with systemic lupus erythematosus, 20 were females and 13 men with a mean age of 37 yr (range 6–75 yr). All patients had a shortened platelet survival time, peripheral thrombocytopenia and a normal or increased number of megakaryocytes in the bone marrow.

Platelet Labeling

Platelets were labeled by modifying a previously described method (*7*). Briefly, 18–43 ml whole blood was collected in a plastic syringe containing 3.3–7.5 ml acid-citric-dextrose (ACD). Platelet rich plasma (PRP) was obtained by centrifugation at 260 *g* for 10 min of the blood sample collected in a 30 ml rubber-capped polycarbonate (PC) tube (Nalgene, Oak

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Ridge, USA). PRP was then transferred into another PC tube to which was added ACD 1:10 (v/v) for adjusting pH to 6.5. The platelets were then centrifuged at 800 *g* for 10 min. The platelet poor plasma (PPP) was aspirated, the platelet pellet washed once with 5 ml and resuspended in 1 ml modified Tyrode's solution (MTS), whereafter 100–200 μCi [^{111}In]oxine (Amersham, UK) was added. After incubation for 5 min 3–4 ml PPP was added, the tube centrifuged at 800 *g* for 5 min and the supernatant containing the unbound isotope aspirated. The platelets were resuspended in 5 ml PPP and reinjected intravenously. In nine patients the platelet count was below 20×10^9/l and blood donor ABO- and Rh-compatible Hb_sAg and HIV negative platelets were used. The amount of unbound radioisotope in the suspension injected was < 5% and the erythrocyte-bound radioactivity measured from blood samples after injection was below 10%.

Platelet Survival Studies

Samples of 3 ml ethylenediaminetetraacetic acid (EDTA) blood was collected 15 min, 30 min, 60 min, and 3 hr after the injection of the labeled platelets and thereafter 1–2 times daily over 2–5 successive days. The radioactivity in 1 ml whole blood samples was measured in a gamma counter (1282 Compugamma, LKB-Wallac, Finland). The whole blood activity was plotted against time and the platelet half-life was expressed as the time elapsed between the first sample and the moment when the radioactivity had decreased by 50%.

Scintigraphy

The labeled platelets were injected to the patient positioned supine over the gamma camera (General Electric Maxi Camera, equipped with a standard medium-energy parallel hole collimator) connected to a PDP 11/34 computer system with Gamma 11 software. Posterior images (PA) were accumulated every 15 sec for the first 10 min and every 2 min for the next 30 min, whereafter the liver and spleen were also imaged in the anterior direction (AP).

Serologic Studies

Platelet-associated IgG was measured by a platelet suspension immunofluorescence test (PSIFT) as described previously (*8*). The results were expressed as negative, weakly positive, or strongly positive.

Data Analysis

The following biexponential function was fitted to the inverted splenic time-activity curves:

$$y(t)=A_1e^{(-\ln 2/ST1)t}+A_2e^{-\ln 2/ST2)t} \quad (1)$$

Half-lives of the fast (ST1) and slow (ST2) components of the curves were determined by pealing technique (*9*). By use of least square regression analysis a line was fitted to the end portion of the semilog y(t) curve and extrapolated to t = 0 to give A_2 enabling calculation of ST2. The calculated values for $A_2e^{(-\ln 2/ST2)t}$ were subtracted from the y(t) curve and semilog plotted whereafter A_1 and ST1 were calculated as above. Corresponding half-lives (BT1 and BT2) were determined for the blood-pool (=cardiac) time-activity curve.

For calculation of the spleen/liver (S/L) ratio spleen and liver radioactivity in the frames at the end of the dynamic imaging was recorded using region of interest (ROI) and also radioactivity per pixel methods (geometric mean from AP and PA images).

Statistical Analysis

The results are presented as mean ± standard error of mean (s.e.m.). Statistical analysis of data was performed by the two-tailed Student's t-test for independent samples using a computer program (Stats+, StatSoft, Inc., Tulsa, USA).

RESULTS

The platelet blood half-life was 37 ± 5 hr for patients studied with autologous platelets and 13 ± 6 hr for those studied with homologous ones. The splenic time-activity curves fitted a biexponential function in all but four cases, whose curves run a monoexponential course. The results on ST1 and ST2 are presented in Figure 1. A typical splenic time-activity curve, a normalized inversion of this curve and its logarithmic transformation, are presented in Figure 2A–C (four patients excluded because PSIFT not performed; ST2 not determined in three additional patients because of patient movement during dynamic imaging).

Patients with a strongly positive PSIFT had a longer mean ST2 value than those with negative or weakly positive PSIFT (26 ± 5 min vs. 13.2 ± 1.0 min, $p < 0.01$). No relationship could be demonstrated between BT1 or BT2 and the other measured parameters. Hepatic time-activity curves showed irregular appearance and no single mathematic function was applicable and therefore quantitative analysis was not performed.

Patients with strongly positive PSIFT had significantly lower S/L-ratios than those with negative or weakly positive PSIFT (per pixel: 2.6 ± 0.4 vs. 7.8 ± 1.8, $p < 0.001$; ROI counts: 2.2 ± 0.7 vs. 6.6 ± 1.4, $p < 0.005$).

DISCUSSION

According to some previous reports the splenic time-activity curve runs a monoexponential course after i.v. injection of ^{111}In-labeled platelets (*2,4,5,10,11*), and therefore closed two-compartmental analysis should be applicable in the investigation of platelet dynamics. However, on the basis of observations in 29 of 33 ITP patients, in whom biexponential curves were found, we cannot share this opinion. If the closed two-compartmental model would be valid, an inverse relationship between the cardiac parameters and the splenic ones could be expected. We failed to demonstrate such a relationship and therefore a closed two-compartmental model cannot adequately explain splenic platelet dynamics. In four ITP patients the splenic time-activity curve ran a monoexponential course. According to Equation (1) this can be interpreted as if ST1 and ST2 could be equal.

Deconvolution analysis is based on the assumption that the blood signal recorded by gamma camera is a

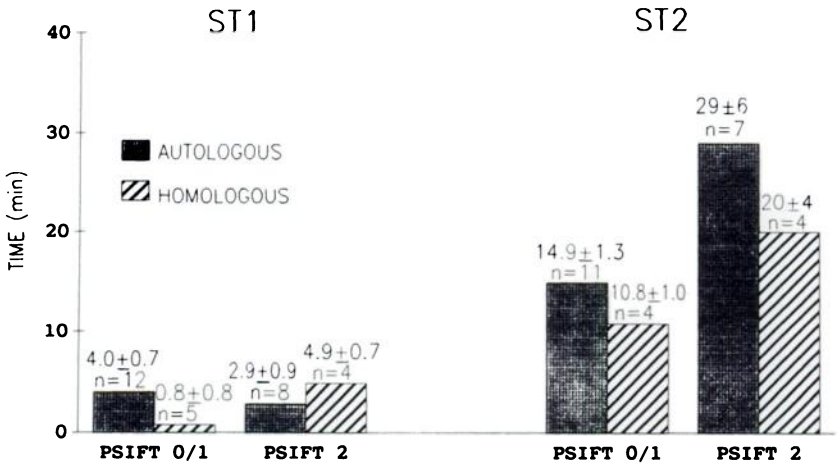

FIGURE 1
Splenic half-lives (mean ± s.e.m.) for rapid (ST1) and slow component (ST2) and their relationship to PSIFT (studies with autologous and homologous platelets presented separately). PSIFT = platelet suspension immunofluorescence test: 0/1 = negative or slightly positive PSIFT, 2 = strongly positive PSIFT.

true reflection of the arterial input into the spleen, and that only the spleen is involved in platelet dynamics (*6*). In practice one must, however, account for the possibility of platelet sequestration occurring also in other organ systems. In this study the liver showed an active role in platelet dynamics, as demonstrated by the relationship between the presence of platelet antibodies and the S/L-ratio. This demonstrates that the role of the liver cannot be dismissed as a closed two-compartmental model as deconvolution analysis would require. However, data from dynamic liver imaging was difficult to analyze because of the irregular behavior of the liver time-activity curves. Three patterns of curves were detected: decreasing, constant and increasing making quantitative analysis of the curves difficult. Further investigation is required to find the most reliable mathematical models enabling quantitative analysis of liver platelet dynamics.

Fitting the splenic time-activity curve to a biexponential function facilitates the determination of half-lives of the two components of the curve. The half-life of the slower component (ST2) seems to be pathophys-

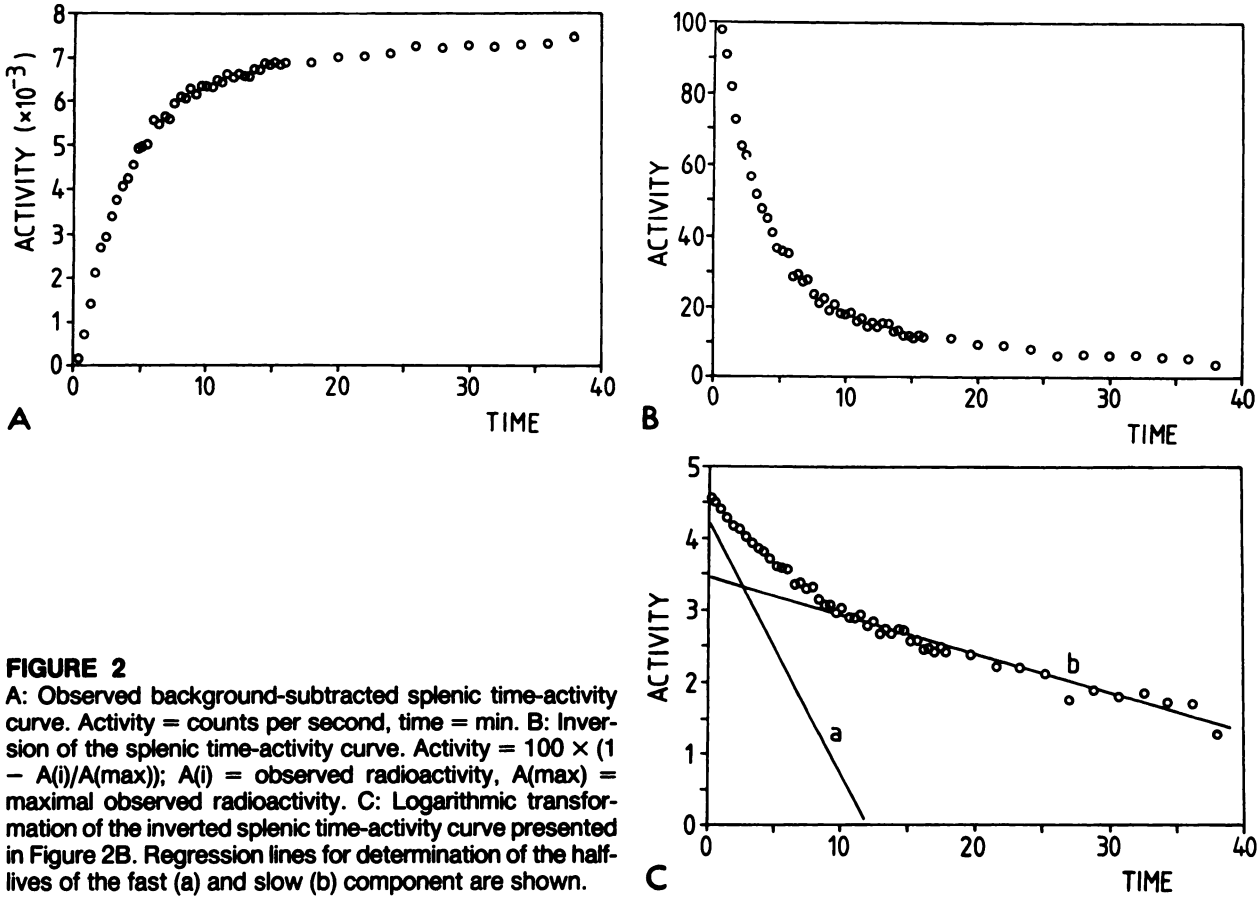

FIGURE 2
A: Observed background-subtracted splenic time-activity curve. Activity = counts per second, time = min. B: Inversion of the splenic time-activity curve. Activity = 100 × (1 − A(i)/A(max)); A(i) = observed radioactivity, A(max) = maximal observed radioactivity. C: Logarithmic transformation of the inverted splenic time-activity curve presented in Figure 2B. Regression lines for determination of the half-lives of the fast (a) and slow (b) component are shown.

iologically relevant as demonstrated by its association with the level of platelet-associated IgG. Platelets covered with high amounts of antibodies seem to have longer ST2 than those without. Thus, it seems as if the transit of antibody-coated platelets through the spleen is delayed, possibly as a preliminary preparation for their removal from the circulation and ultimate destruction. Patients with a strongly positive PSIFT also had a lower S/L-ratio than those with a negative PSIFT. This is in accordance with a previous observation that increasing amounts of antibody bound to platelets increase platelet sequestration in the liver (*12*), although opposite observations have been reported by others (*13*). In patients with negative or weakly positive PSIFT the values for ST1, ST2 and the S/L-ratio were in the same range as for three healthy volunteers studied (data not shown).

The clinical and pathophysiologic relevance of ST1 remains unknown. There was no significant difference in ST1 values between PSIFT positive and negative patients. Therefore ST1 might reflect the distribution of the labeled platelets during the first few minutes after the injection. The clearly different nature of the two components emphasize the importance of separating them in the interpretation of platelet dynamics. We conclude that closed two-compartmental analysis of splenic platelet dynamics is not universally applicable to short-lived platelets and fitting a biexponential function to the inverted splenic time-activity curve for calculating half-lives provides a more universal approach to the analysis of splenic platelet dynamics.

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