Reticulated platelets and platelet reactivity in renal transplant recipients receiving antiplatelet therapy

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Reticulated Platelets and Platelet Reactivity in Renal Transplant Recipients Receiving Antiplatelet Therapy

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ABSTRACT

Introduction. Renal transplant recipients are at increased risk of cardiovascular morbidity and mortality. We assessed platelet reactivity and reticulated platelets (RPs) in 90 recipients, 51 (56.6%) of whom were not receiving acetylsalicylic acid (ASA) therapy (group A) and 39 (43.3%) who were receiving ASA therapy, 100 mg (group B), and in 60 healthy controls (group C).

Methods. Reticulated platelets were measured using a hematology automated analyzer (XE-2100; Sysmex Corp, Kobe, Japan) and were expressed as the percentage of RPs in the total optical platelet count (immature platelet fraction [IPF]), as the percentage of highly fluorescent RPs, and as the absolute number of RPs (IPF#). Platelet function was assessed using optical aggregometry (platelet aggregation) induced using 1 mmol/L of arachidonic acid, 2 or 10 μmol/L of adenosine diphosphate, or 2 μg/mL of collagen.

Results. Group A demonstrated significantly higher values of RP compared with group B or group C. Group B demonstrated a substantially higher percentage of RPs compared with group C, which was significant only for the IPF parameter. Multiple regression analysis demonstrated that IPF and IPF# were significantly and positively related to collagen-induced platelet aggregation.

Conclusion. We documented the presence of higher concentrations of RPs in transplant recipients compared with a control population, and a significant association between RPs and platelet function.

OVER THE LAST YEARS, a number of studies have demonstrated the importance of some classic risk factors such as hyperlipidemia and hypertension in determining cardiovascular disease in renal transplant recipients; however, the presence of risk factors did not enable prediction of patient survival.[1] Various mechanisms can be responsible for cardiovascular complications in renal transplant recipients. Their platelets demonstrate enhanced thromboxane biosynthesis,[2] and some immunosuppression agents such as cyclosporine are associated with increased platelet procoagulant activity.[3,4] However, to our knowledge, no data on the presence of reticulated platelets (RPs), and in particular platelet activity, are available. The objective of the present study was to assess RPs and platelet aggregation (PA) in renal transplant recipients who were or were not receiving acetylsalicylic acid (ASA) therapy and in a population of healthy controls.

METHODS

The study population included 90 renal transplant recipients observed according to American Society of Nephrology guiidelines.[5] Fifty-nine patients were men, and 31 were women, with median (range) age of 55 (20–70) years. Group A included 51 recipients (56.6%) not receiving ASA therapy, and group B included 39 recipients (43.3%) receiving ASA therapy for at least 6 months posttransplantation. Group C (control group) included 60 healthy individuals. Forty-one were men, and 19 were women, with median (range) age of 52 (21–72) years, who were free of vascular and renal diseases. All 3 groups were comparable for age and sex.

Venous blood samples were obtained from each study participant in the morning, after an overnight fast, and were anticoagulated using 0.129 mol/L of sodium citrate, and examined using

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platelet aggregometry and ethylenediamine tetraacetic acid, 0.17 mol/L.

Turbidimetric PA was used to measure agonist-induced PA in response to various stimuli including 2 or 10 μmol/L of adenine diphosphate (ADP2-PA and ADP10-PA, respectively), 1 mmol of arachidonic acid (AA-PA), or 2 μg/mL of collagen (Coll-PA), as previously described.[6]

Reticulated platelets were measured as previously described[6] using a hematology automated analyzer (XE-2100; Sysmex Corp, Kobe, Japan). They were expressed as a percentage of the total optical platelet count (immature platelet fraction [IPF]), the percentage of highly fluorescent RPs (H-IPF), or absolute number of RPs (IPF#).

Statistical analysis was performed using commercially available software (SPSS version 13.0 for Windows; SPSS, Inc, Chicago, Illinois). Data are given as median (range). The Mann-Whitney test was used to compare unpaired data between the various groups. A multivariate linear regression model adjusted for cardiovascular risk factors; hematocrit; ASA, erythropoietin, and cyclosporine therapy; and glomerular filtration rate was used to test independent associations of RPs with platelet reactivity. $P < .05$ was considered significant.

RESULTS

Group A demonstrated significantly higher values for RPs compared with groups B or C. Group B demonstrated a significantly higher percentage of RPs compared with group C, which was significant only for the IPF parameter.

To evaluate the influence of RPs on PA, we performed a linear regression analysis. In the univariate model, IPF was significantly and positively related to PA by arachidonic acid (AA) (β [SE], 1nAA and 1nIPF, 0.42 [0.16]; $P = .01$). However, in the multivariate model, after adjusting for age and sex; hypertension, dyslipidemia, and diabetes mellitus; smoking status; hematocrit; ASA, cyclosporine, and erythropoietin therapy; and glomerular filtration rate, the association lost its significance. Insofar as the association of RPs and Coll-PA, the univariate model demonstrated that IPF, H-IPF, and IPF# were positively and significantly related to Coll-PA (β [SE]: 1nColl and 1nIPF, 0.30 [0.06], $P = .01$; 1nColl and 1nH-IPF, 0.18 [0.07]; 1nColl and 1nIPF#, 0.25 [0.07]). A multiple regression analysis adjusted for all confounding factors demonstrated that these associations remained significant (β [SE]: 1nColl and 1nIPF, 0.25 [0.06, $P < .05$; 1nColl and 1nIPF#, 0.18 [0.07], $P < .05$).

DISCUSSION

This article provides new information about the presence of increased platelet turnover in renal transplant recipients and the relationship between the presence of RPs and platelet reactivity induced by AA or collagen. Reticulated platelets are immature platelets with a greater mass and greater prothrombotic potential compared with smaller platelets. They aggregate more rapidly with collagen, and demonstrate a higher concentration of thromboxane A_2 and increased concentrations of procoagulant surface proteins such as P-selectin and glycoproteins IIb and IIIa.[7] Moreover, RPs are associated with diminished antiplatelet effects of ASA and increased ASA resistance, possibly because of increased reactivity and the presence of uninhibited cyclooxygenase-1 and cyclooxygenase-2 activity.[8]

We documented the presence of the higher concentrations of RPs in renal transplant recipients compared with a control population of healthy individuals, and a significant association between RPs and platelet function.

The number of newly produced circulating platelets depends on stimulation of the thrombopoietic capacity of bone marrow and on the degree of platelet removal from the bloodstream via activation-dependent mechanisms that enhance platelet turnover. Both mechanisms may be important in determining the number of RPs. Some cytokines augment megakaryopoiesis quantitatively, and in renal transplant recipients, higher concentrations of tumor necrosis factor-α, a cytokine that can act to promote commitment along the megakaryocytic lineage to increased thrombopoiesis, have been reported.[9]

In conclusion, the presence of an increased percentage of RPs may be another mechanism involved in the increased cardiovascular risk profile documented in renal transplant recipients.

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