In circulation, platelets adhere to leukocytes forming relatively stable complexes that have been reported to be elevated in cases of unstable angina, myocardial infarction, coronary artery disease, and postangioplasty restenosis.\(^1-10\) For this reason, measurement of circulating platelet-leukocyte complexes has been proposed as an early and accurate marker of in vivo platelet activation and myocardial injury after infarction.\(^9,10\) Increased levels of such complexes have also been noted in a range of chronic inflammatory diseases, including rheumatoid arthritis, end-stage renal failure, type I diabetes, and systemic lupus erythematosus.\(^11-13\)

In the majority of published studies that have examined platelet-monocyte or platelet-polymorphonuclear (PMN) leukocyte complexes in human peripheral venous blood, sodium citrate (0.32 to 0.38%), a calcium-depleting agent, has been used as the blood anticoagulant. Because platelet adhesion to leukocytes is predominantly mediated by calcium-dependent interactions between platelet P-selectin and its leukocyte counter-receptor, P-selectin glycoprotein ligand-1 (PSGL-1),\(^1,14\) we aimed to determine whether calcium depletion by sodium citrate could affect platelet-leukocyte complex formation.

For this reason, platelet-monocyte and -PMN leukocyte complexes in citrated blood (0.38% wt/vol final concentration) were measured using 2-color flow cytometry (described in\(^1\)) and compared with those observed in blood anticoagulated by hirudin (200 U mL\(^{-1}\)), a calcium-depleting agent, has been used as the blood anticoagulant. Because platelet adhesion to leukocytes is predominantly mediated by calcium-dependent interactions between platelet P-selectin and its leukocyte counter-receptor, P-selectin glycoprotein ligand-1 (PSGL-1),\(^1,14\) we aimed to determine whether calcium depletion by sodium citrate could affect platelet-leukocyte complex formation.

We observed significantly lower percentages of platelet binding to monocytes (defined as CD42a\(^+/\)CD14\(^+\) events) in blood anticoagulated with sodium citrate compared with hirudin-, heparin-, or PPACK-anticoagulated blood (Figure). No significant differences in PMN-platelet binding were evident using the different anticoagulants (13.7% \(\pm\) 2.3 for citrate, 18.8% \(\pm\) 5 for heparin, 16.8% \(\pm\) 4.4 for hirudin, 19.3% \(\pm\) 4.3 for PPACK; \(n=6\)), an observation reflecting lower levels of cation-dependent platelet adhesion that might be attributed to lower levels of PSGL-1 expression on PMN leukocytes compared with monocytes (data not shown).

Incubation of blood with EDTA (10 mmol/L) resulted in a substantial decrease (to approximately 15%) in monocyte-platelet binding, an effect that was irrespective of the anticoagulant used (Figure). Because EDTA and sodium citrate are both calcium chelators but EDTA acts on other divalent cations as well, one interpretation could be the involvement of other divalent cation-dependent interactions (eg, Mg\(^+\)+- dependent integrin-mediated interactions). However, use of EGTA, a selective chelator of Ca\(^+\)+, resulted in comparable monocyte-platelet binding to that observed in EDTA (14.3% \(\pm\) 1.6 for EDTA; 14.1% \(\pm\) 1.4 for EGTA; \(n=4\)). We therefore suggest that the higher percentage of monocytes binding to platelets observed in blood anticoagulated with citrate compared with EDTA reflects the relative inefficiency of sodium citrate as calcium chelator.

We conclude that in the presence of sodium citrate, a substantial component of the Ca\(^+\)+-dependent interactions between platelets and monocytes is excluded, hence leading to underestimation of the actual percentage of monocyte-platelet binding in circulation. When compared with EDTA, sodium citrate has a limited capacity for calcium depletion and as a consequence, residual Ca\(^+\)+ ions could influence platelet adhesion to monocytes. The existence of divalent cation-dependent and -independent mechanisms for platelet adhesion to monocytes is well established.\(^3\) We suggest that previous studies describing an association of platelet-monocyte binding with disease pathogenesis should be carefully reexamined because the contribution of these component interactions cannot be precisely determined when citrate was used as blood anticoagulant.

In summary, without necessarily proposing the use of one anticoagulant in preference to another, our findings clearly indicate that the choice of blood anticoagulation is critically important in studies of platelet-leukocyte interactions. Particular caution is required when interpreting and comparing results from studies that have made use of different anticoagulants.

Disclosures

None.

References


**Figure.** Impact of different anticoagulation strategies on the percentage of platelet-monocyte complexes. Peripheral venous blood obtained from 6 healthy donors was anticoagulated with: sodium citrate (0.38% wt/vol), heparin (10 U ml⁻¹), hirudin (200 U ml⁻¹), or PPACK (75 μmol/L) and incubated with or without 10 mmol/L EDTA. Using 2-color flow cytometry, monocytes (CD14⁺) were identified and the percentage of platelet binding to them (defined as CD14⁺/CD42a⁺ events) was measured. A, In sodium citrate anticoagulated blood, significantly lower percentages of platelet-monocyte aggregates were observed compared with blood anticoagulated with heparin, hirudin, or PPACK. Incubation of blood with EDTA (10 mmol/L) resulted in all cases in a substantial decrease in platelet-monocyte complexes. Results are presented as mean±SD from 6 independent experiments in which platelet-monocyte complexes were measured in different donors. *P<0.01; **P<0.05, compared with sodium citrate. B, Representative flow cytometry histograms of monocytes (CD42a⁺) and platelet-monocyte complexes (CD42a⁺; M1 region) in blood anticoagulated with sodium citrate, heparin, hirudin, or PPACK. Histograms are gated only on CD14⁺ cells.
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