Letters to the Editor

Formation of mixed platelet-PMN leukocyte aggregates in the platelet function analyzer (PFA-100) device

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Dear Sir,

Platelet-polymorphonuclear leukocyte (PMN) mixed aggregates were described by Giulio Bizzozero in blood flowing from a bleeding injury, during the process of hemostasis (1).

In the last two decades, interest for mixed platelet-PMN aggregates has been renewed by the possible relevance of this phenomenon to thrombosis and vascular ischaemic injury (2–10).

We report here that mixed platelet-PMN aggregates are detectable in the blood emerging from the Platelet Function Analyzer (PFA-100), a device currently used to evaluate platelet disorders and platelet function (11). The system mimics the high shear of small arterioles and some biochemical interactions occurring when damaged subendothelium is exposed. In the PFA-100, citrated whole blood is challenged by standardized shear forces (negative pressure through a capillary) and by a “biochemically” active membrane (cardio). We present here the results obtained with collagen-ADP cartridges. Preliminary data obtained with collagen-epinephrine cartridges were too variable, possibly due to the high inter-individual variability of platelet response to epinephrine (12). Further studies with the latter cartridge are currently under way.

Venous blood was collected on 3.8% trisodium citrate (9:1, v:v) from nine healthy volunteers (all blood donors from the Transfusion Centre of the “Cardarelli” Hospital, Campobasso) (5 males, 4 females, aged 44 ± 3 years). All subjects, previously informed that part of their blood would be collected for research purposes and that their privacy would be protected, signed a written consent. They were all non-smokers, denied having received any medication (including oral contraceptives) during the last two weeks and had hematoo-chemical values within the normal ranges. The blood group was 0 in five subjects (four Rh negative, one positive), A in two (one negative, one positive), and B in two (both positive).

Aliquots of the blood emerging from the PFA-100 device, aspirated through the capillary and the aperture of the PFA-100, were fixed and labelled with specific antibodies to determine cell aggregates and platelet P-selectin expression (7, 10, 13). Briefly, platelet-PMN aggregates were determined by three-color flow-cytometry as the percent of CD61-positive platelets in PMN cluster (CD45 positive and according to their forward and scatter characteristics). Platelet P-selectin expression was determined by double-color flow cytometry as the percent of P-selectin-positive platelet cluster (200,000 CD61-positive events). Blood samples were simultaneously labeled with specific MoAb and fixed with Thrombofix® (Instrumentation Laboratory, Milan, Italy), and analyses were performed four hours after sample preparation.

We found that platelet-PMN aggregates increased from 10.3 ± 2.4% (in the residual blood that was not aspirated from the “reservoir” through the PFA-100 device) to 30.3 ± 6.8% (in the blood emerging from the device) (mean ± sem, n=9; p<0.05) (Fig. 1). The formation of mixed platelet-PMN aggregates might be even greater than suggested by these data. Indeed, the background levels of platelet-PMN complexes in the samples examined for this study were higher than expected (4.4 ± 1.1%, n=9) as in our previous experience (13). Moreover, these data do not take into account the possible capture of activated platelets and/or PMN into forming a hemostatic plug within the aperture of the cartridge. Further studies will indicate whether a proportion of mixed (possibly larger) aggregates are formed and retained within the cartridge, thus escaping detection in the emerging blood. The percent of mixed aggregates apparently did not correlate with the PFA-100 closure time (91 ± 6 sec; mean ± sem, n=9), suggesting that in the device, platelet-PMN interaction does not substantially contribute to the closure time. This conclusion is, however, indirect and needs further investigation, including leukocyte-depleted or -enriched blood samples. This phenomenon is mainly, if not exclusively, mediated by platelet aggregation triggered by the ADP- and collagen-rich cartridge (11). Platelet P-selectin expression was indeed increased from 2.6 ± 0.5% (in the residual blood that was not aspirated through the PFA-100 device) to 43.1 ± 2.8% (in the blood emerging from the device) (mean ± sem, n=5; p<0.01) (Fig. 1). The platelet P-selectin valu-

156–157

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Platelet-PMN aggregates and platelet P-selectin expression were inversely correlated with the PFA-100 closure time \((r=0.86; p<0.001)\).

While our data confirm that platelet activation plays a major role in the mechanism leading to PFA-100 closure (11), the pathophysiological and clinical significance of mixed platelet-PMN aggregates formed within and emerging from the device remains to be defined.

Since PFA-100 is a rather simple, well standardized, easily available device (11), it could offer a possibility to implement collaborative studies on the physiopathology and pharmacology of platelet-PMN interaction formed in human whole blood, in relation to either hemostasis and thrombosis. As an example, preliminary experiments with low-molecular-weight heparins suggest that interference with P-selectin by these compounds (10, 14) induces a marked reduction in mixed-cell aggregate formation, but only a modest prolongation of the closure time (data not shown).

References