High fibrinogen levels promote erythrocyte-erythrocyte adhesion, becoming an important cardiovascular risk factor

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Introduction: Increased plasma fibrinogen levels result in changes in blood rheological properties, which are not completely clarified. Erythrocyte aggregation has become an issue of increasing interest, especially as an indicator of the associated cardiovascular risk, since it is influenced mostly by fibrinogen levels. A better understanding of the role of fibrinogen on erythrocyte aggregation in heart failure patients might be relevant for potential future drug interventions to reduce aggregation and enhance microcirculatory flow conditions. Our previous studies have shown that an αIIbβ3-like integrin is a specific receptor for fibrinogen on the erythrocyte membrane. The aim of this study was to understand how fibrinogen influences erythrocyte aggregation by cell-cell adhesion force spectroscopy measurements using an atomic force microscope (AFM). Additionally, we wanted to evaluate how this protein-cell interaction constitutes a cardiovascular risk factor in chronic heart failure (CHF) and essential arterial hypertension (EAH) patients. Material and Methods: 30 CHF patients (which were grouped according to two etiologies (ischemic or nonischemic) and 31 EAH were engaged in the study. We also included 15 healthy blood donors as a control group. Fibrinogen-erythrocyte binding measurements were conducted by AFM-based force spectroscopy, in buffer, with the protein covalently attached to the AFM tip. Erythrocyte-erythrocyte measurements were conducted only for healthy subjects so far, with one of the cells attached to AFM cantilevers without tip and the other on the solid substrate. Results: Cell-cell adhesion data showed that increasing fibrinogen concentrations there is an increase in the work necessary for cell detachment, from 0.45 ± 0.04 fJ without fibrinogen to 12.0 ± 0.13 fJ at 1 mg/ml fibrinogen (p<0.001). Concomitantly, average cell-cell detachment forces increase from 72.0 ± 2.9 pN without fibrinogen to 250.4 ± 3.2 pN at 1 mg/ml fibrinogen (p<0.001). Regarding the results from the protein-cell interactions, ischemic patients presented higher binding forces than healthy donors (74.9 ± 10.7 pN vs. 40.4 ± 3.0 pN; p=0.004), despite lower binding frequency (11.7 ± 2.1 % vs. 27.3 ± 4.2 %; p<0.002). Furthermore, ischemic patients presented higher forces than the non-ischemic (74.9 ± 10.7 pN vs. 45.4 ± 5.6 pN; p=0.021). Non-ischemic patients also had a lower binding frequency than the control group (14.3 ± 4.3 % vs. 27.3 ± 4.2 %, p=0.040). Concerning the EAH patients results, we observed that they had higher binding forces between erythrocyte and fibrinogen than the control group (101.0 ± 7.1 pN vs. 40.4 ± 3.0 pN, p<0.0001), despite a lower binding frequency observed for EAH patients (9.0 ± 0.3 % vs. 27.3 ± 4.2%, p<0.0001). Conclusions: Fibrinogen promotes erythrocyte-erythrocyte adhesion, and consequently erythrocyte aggregation, probably by transient simultaneous binding of the protein to two cells, bridging them. Fibrinogen-erythrocyte interactions were higher in ischemic CHF patients and in EAH patients than the control group. This could difficult the whole blood flow, representing a cardiovascular risk factor. These results are relevant to conclude on the degree of pathophysiological relevance of fibrinogen and erythrocyte aggregation, since an increment on both might induce a state of microcirculatory slower flow, increasing the probability of cardiovascular complications.

Keywords: fibrinogen, erythrocyte aggregation, biomarkers