Abnormal adhesion of red blood cells to endothelium under flow conditions in diseases

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Abstract

Patients with Polythemia vera (PV) have a high incidence of thrombosis (25-75 \%). Vascular complications are responsible of retinopathy, renal failure and coronary disease in diabetes mellitus (DM). Retinal Vascular Vein Occlusion (RVO) is a cause of blindness. To evaluate is red blood cell (RBC) abnormalities may be one vascular risk in these disease we studied RBC adhesion to endothelial cells in flow conditions. We found that RBC of patients with DM had the higher number of adhering RBC at 0.03 and up to 0.2Pa (7-2x10\textsuperscript{-2} RBC per mm\textsuperscript{2}). RBC from patients with PV and RVO have a significantly increased adhesion up to 0.2Pa. Antibodies directed against Lu/BCAM inhibited adhesion of RBC from PV patients. The Receptor for Advanced Glycation End Products (RAGE) was shown to be a receptor for RBC glycated band 3. In this study we observed that soluble RAGE which binds to AGE RBC reduced adhesion in flow conditions. RBC from patients with RVO were shown to exhibit a higher level of phosphatidyl serine (PS). Using either anti PS receptor or Annexin V, which binds to PS, we significantly reduce RBC adhesion indicating that PS RBC and endothelial PS receptor are involved in RBC adhesion.

Keywords: Red Blood cells, polycythemia vera, retinal vein occlusion

1. Introduction

Blood cells essentially leukocytes and Red Blood Cells (RBC) adhere to endothelium while platelets are more reactive with the subendothelium. Several studies on adhesive properties of blood to the vascular wall components have been performed in static conditions but more recently in flow conditions either to characterized protein coated slides or to cultured endothelial cells (EC) \cite{1}.

We have studied RBC adhesion to EC, in flow conditions, in three different pathologies: Diabetes mellitus \cite{2}, Polycythemia Vera (PV) \cite{3} and Retinal Vascular Occlusion (RVO) \cite{4}. The adhesion to EC was secondary to abnormalities on RBC which lead to the binding to EC adhesion receptors.

2. Patients

All studies were approved by the Internal Review Boards from participating institutions and signed informed consent was obtained in accordance with the Declaration of Helsinki.

2.1. Polycythemia Vera (PV)

A total of 38 patients with PV in this study after red blood cell mass and plasma volume determination. The diagnosis of PV was made according to the Polycythemia Vera Study Group (PVSG).

2.2. Diabetes Mellitus

A group of 60 type 2 diabetic patients was studied.
2.3. Retinal Vein Occlusion (RVO)

A group of 20 patients with Retinal Vein Occlusion was studied. RVO was diagnosed by the presence of retinal hemorrhages and venous dilation/tortuosity with or without retinal/disc swelling in a defined venous retinal territory. All patients were compared to a sex and age matched group of 75 normal subjects.

3. Methods

3.1. Adhesion assay in flow conditions

Adhesion of RBC to Human Umbilical Vein Endothelial Cells (HUVEC) was determined according to a previously described technique [5]. Briefly HUVEC were cultured in gelatine (2%) coated glass capillaries (microslides; Camlab Ltd, Cambridge UK) for 24 h. Microslides containing confluent HUVEC were mounted on the stage of a videomicroscope, and one end of the microslide was attached to a Harvard syringe pump (Harvard Apparatus, South Natic, MA USA) allowing the control of the flow rate. The other end was connected to an electronic valve permitting switching between RBC suspension and cell-free buffer. RBC suspension was perfused through the microslide at a flow rate equivalent to a wall shear stress of 0.03 Pa for 10 minutes followed by washout of non adherent cells for 10 minutes, wall shear stress was then increased stepwise (from 0.07-1Pa) every 10 minutes. Adherent RBC were counted using a computerized image analysis system (Optimas Media Cybernetics MD USA, R & D Vision, Paris, France) averaged per field and expressed as number of RBC per mm².

3.2. Adhesion assay with antibodies or recombinant proteins

To investigate the receptors supporting adhesion, PV patient RBC were incubated with the following antibodies for 30 minutes at 37°C before assay: antibodies against Lu/BCAM (polyclonal anti-Lu², 50-100 µL, Biorad, Marnes La Coquette France, and mAb anti-Lu, 5-20 µg/ml, clone F241), mAb against CD59 (negative control).

RBC from patients with diabetes (DRBC) were incubated with recombinant rat-RAGE (60 and 100µg/mL) corresponding to the optimal dose-response of AGE/RAGE interaction blocking effect on HUVEC as previously demonstrated [6]. The recombinant rat-RAGE (rR-RAGE) was prepared as previously described [7]. The protein corresponds to the RAGE extra-cellular domain and acts by binding to AGEs, thereby blocking their interaction with endogenous RAGE.

Inhibition of adhesion of RBC from RVO patients was assessed using endothelial cells preincubated with specific antibodies directed against PS receptor (polyclonal anti-PS receptor antibody, Sigma St Louis MO, USA),Isotype-matched rabbit IgG were used as controls as appropriate, or after RBC incubation with annexin V.

4. Results

4.1. Polycythemia Vera (PV)

RBC from PV patients adhered in greater number at 0.07Pa and were much more resistant to washout than control RBC (PV patients 2.5±0.25, normal subjects 0.15±0.05 RBCx10²/mm² at 0.07 Pa) . The number of RBC remaining adherent, after washout at the highest shear stress (1Pa), was 0.6±0.06 RBCx10²/mm² for the PV patients and less than 0.001 RBCx10²/mm² for the control group (Fig 1). Treatment of RBC with mAb against Lu/BCAM (F241) abolished RBC adhesion to HUVEC at 0.07Pa and above (Fig 2).
RBC from patients with RVO (n = 20) adhere more efficiently to HUVEC than RBC from healthy volunteers under flow conditions and are resistant to washout by increasing shear stress (p < 0.001). The more adherent were RBC from patients with type 2 diabetes (n=60) (p < 0.001) , adhesion of RBC from patients with polycythemia vera (n=38) was higher at low shear stress but was similar to that of patients with RVO at 0.1 and 0.2 Pa and significantly adhere at 1 Pa (p < 0.001).

**Fig. 2.** Inhibition of PV RBC adhesion to HUVEC by mAb against Lu/BCAM measured under flow conditions.

Anti-Lu/BCAM inhibited the adhesion of PV RBC to HUVEC at different shear stresses and the inhibition at 0.07Pa is highly significant (p<0.001).

**4.2. Diabetes mellitus**

RBCs from diabetic patients bound to HUVEC and had a significantly higher adhesion than that observed in the controls, irrespective of shear stresses applied up to 0.2 Pa (Fig 1).
Incubation of diabetic RBCs with rR-RAGE resulted in significantly reduced adhesion at high shear stresses. In contrast, there was no change at lower shear stresses (0.03 Pa and 0.07 Pa). These findings indicate that stable adhesion is mediated by the interaction between AGEs located on the surface of RBC and endothelial RAGE (Fig 3).

When RBCs were preincubated with rR-RAGE the adhesion on endothelial cells was significantly inhibited at a shear stress of 0.1 to 0.2 Pa (p<0.001).

4.3. Retinal Vein Occlusion

RBC from patients with RVO adhered in greater numbers and were more resistant to washout than control RBC: at 0.07 Pa 1,90±0.08 x10^2 per mm^2 and 0.30±4 per mm^2 RBC respectively, (p<0.001). The number of RBC remaining adherent after washout at the highest shear stress (0.2 Pa) was 1.2±0.08 x10^2 per mm^2 for the patients with RVO and less than one per mm^2 for the control group (Fig 1). Anti-PS receptor significantly inhibited RBC adhesion (Fig. 4). Annexin V is known to bind to PS and when preincubated with RBC from RVO it reduced the adhesion measured in flow conditions between 60 and 72 % according to the shear stress (Fig.4).

Anti PSR exhibited a high inhibitory effect at 0.1Pa (62±8% and 70±4% respectively), Annexin V was the strongest adhesion blocker at 0.1 and 0.2Pa (75±6% and 74±8% respectively).
5. Conclusion

In previous studies we reported increased adhesion of RBC in Diabetes mellitus, PV, RVO, observed in static conditions. In flow conditions we were able to evaluate the strength of adhesion and found that the differences could be related to the molecules involved in the adhesion process and could explain the differences of the vascular consequences location of vascular occlusion, damage and severity of the vascular disease. This type of investigation can help the clinician to decide which type of treatment may be efficient and should be applied. On the other hand new drugs can be discovered and applied to disease such as RVO which cannot be now days efficiently treated. New compounds, mostly peptides, are currently under evaluation for efficiency in preventing vascular thrombosis or vascular disorders in diabetes mellitus, RVO.

References