Effects of Flow Pulsatility on Platelet Adhesion to Subendothelium

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Platelet adhesion in the annular perfusion system developed by Baumgartner was studied under pulsatile, oscillatory, or steady flow conditions. To investigate in what way pulsatile flow affects platelet adhesion, we developed a flow system that produces a sinusoidal laminar flow superimposed on a constant component in the annular perfusion chamber. Frequencies and amplitudes of this sinusoidal flow were in the physiological range. Pulse frequencies varied between 30 and 120 beats/minute, and different amplitudes of the wall shear rate in the range 75 to 1000 s\(^{-1}\) were studied. Shear rates resulting from the constant flow component were between 500 s\(^{-1}\) and 1800 s\(^{-1}\). Under these conditions, no significant differences in platelet adhesion were observed between steady flow and pulsatile flow. In the case of an oscillatory flow (absence of constant component), a clear dependence of platelet adhesion on the amplitude of the pulse was seen. These data indicate that platelet adhesion in larger blood vessels, such as the aorta and larger arteries where backflow is limited, is not essentially influenced by the pulsatility in these vessels.

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A dhesion of blood platelets to human subendothelium and the effects of several physical and chemical parameters were extensively investigated in an in vitro perfusion system in which either rabbit aorta or umbilical cord arteries were perfused with citrated blood.1–4 The blood flow to which the vessel wall is exposed in this system is laminar and often constant.5–8

The main physical mechanisms involved in platelet adhesion induced by the flow are platelet transport toward the wall and platelet detachment from the wall, both influenced by the magnitude and probably the shape of the flow. For this reason, the effects of pulsatile flow in this system have been previously studied by Sakariassen et al.,9 but the frequencies used in this study were outside the physiological range.

We developed a pump that produces pulsatile flows in the perfusion system with pulse frequencies and flow amplitudes that can be varied independently within a physiologically relevant range. The effects of these parameters of blood flow on platelet adhesion to subendothelium were studied and were compared to the steady flow situation.

Methods

Flow System

Perfusions were performed in the annular perfusion chamber developed by Baumgartner by circulating 20 ml of reconstituted blood in a pulsatile flow system over a human umbilical artery segment (Figure 1).

Blood was pumped from a container by means of a roller pump, which was peristaltic and nonocclusive. To achieve a steady flow component, a funnel was used, causing a constant flow rate by means of gravity. Upon this steady component, a pulsatile wave was superimposed by pinching an elastic tube that had an internal diameter of 10 mm. This pinching was sinusoidal and was caused by an eccentric disc forcing a driver toward the tube. The frequency was changed by varying the rotation rate of the driving disc. The amplitude was changed by varying the length of the driving arm (Figure 2).

Frequency (f) was defined as the number of pulsations per minute, while the amplitude (γ) was defined as the height of the pulse wave superimposed on a constant component (γ0) (Figure 3). Both were expressed as wall shear rates. These wall shear rates were calculated by assuming laminar flow profiles. The shape of the pulse wave was checked by means of an electromagnetic flow probe and appeared to be close to sinusoidal. The range of frequencies achieved in this set-up varied from 30 beats/minute up to 120 beats/minute. The amplitude of the sinusoidal component could be varied between 0 and 2000 s\(^{-1}\), depending on the constant component that was varied between 0 and 1800 s\(^{-1}\).

Preparation of Perfusates and Detection of Platelet Adhesion

Perfusates were reconstituted from human volunteer donor blood collected into 0.1 volume of 0.11 M citrate and consisted of plasma, washed red blood cells, and washed platelets labeled with \(^{111}\)In\(^{10}\) which were treated with aspirin.11 The hematocrit was standardized to 0.4, and the platelet concentration in the plasma was \(1.9\times10^{11}\) l\(^{-1}\). The total volume of one perfusate was 20 ml.

The platelet adhesion to the subendothelium was determined by \(^{111}\)In counting of the artery segments in a gamma counter.12
Results

Perfusion experiments were performed by varying the amplitudes of the wall shear rate between 200 s\(^{-1}\) and 1000 s\(^{-1}\). The steady component was held constant at 1800 s\(^{-1}\) with a pulse frequency of 70 beats/minute. The adhesion values under these conditions were compared to those in a situation in which the pulse pump was completely open and a steady flow situation was present. The perfusion time was 5 minutes. The data are summarized in Figure 4. Within the precision of detection of platelet adhesion, we could not distinguish any difference between the steady and the pulsatile flow situation, and no effect of an increasing amplitude could be seen.

Similar results were obtained in an experiment in which a steady component of 500 s\(^{-1}\) and amplitudes of the pulse up to 500 s\(^{-1}\) were used. Again, the perfusion time was 5 minutes. These data are summarized in Figure 5. These results point in the same direction as the previous experiment: that no difference between steady and pulsatile flow can be detected. Differences in absolute platelet adhesion values between the results in Figure 4 and Figure 5 were due to the individual characteristics of umbilical cords and blood donors which were different in these experiments.

Figure 6 presents the results of a study to investigate the effects of a pulsatile flow without a steady component, an oscillating flow. Under these conditions, an amplitude-dependent, platelet adhesion was observed. When backflow in this situation was considered in an absolute way, i.e., without the effect of flow direction, the mean absolute flow was linear with the amplitude of the
pulse, while in the previous situations, the mean flow was constant and equal to the steady component. It has previously been shown\textsuperscript{13-16} that platelet adhesion increases with increasing shear rate under steady flow. The data obtained with oscillating flow show this same effect of increasing shear rate causing increasing platelet adhesion.

To look at the effect of frequency of the pulse, we used frequencies between 30 min\textsuperscript{−1} and 120 min\textsuperscript{−1}. The steady component and the amplitude were both held constant at a value of 800 s\textsuperscript{−1} during 5 minutes of perfusion (Figure 7). No effect of frequency on platelet adhesion was found.

Discussion

The effect of pulsatility on platelet adhesion to subendothelium was not significantly different from that of a steady flow in our system. Neither a change in frequency nor a change in amplitude gave any change in platelet adhesion.

In vivo pulsatility is mainly present in the arteries and arterioles. The shape of the flow there is different from the one presented here. The main difference is that in vivo the amplitude is not symmetrical around a constant component. The reason we chose a symmetrical and sinusoidal flow shape is that the flow parameters are better defined and easier to modify in a controlled way. If there were a difference between steady and pulsatile flow, it would be mainly due to the accelerating effect of pulsatility, and any effect of this would then also appear in our simplified system.

Since platelet adhesion is less dependent on shear rate at higher shear rates than at lower shear rates,\textsuperscript{17} hardly any effect in platelet adhesion was expected at higher steady components of the pulsatile flow (1800 s\textsuperscript{−1}). But even at low, steady components (500 s\textsuperscript{−1}), no effect of pulsatility was observed.

Calculation of the theoretical values of diffusion of particles toward the wall in a tube\textsuperscript{18-21} by integrating the diffusion in small time intervals over a single period only gave a slightly decreased value compared to steady flow. Because of the acceleration occurring with pulsatile flow, one would expect the detachment of platelets from the vessel wall to be larger, resulting in an overall decrease in adhered platelets. This expectation was not borne out by the experimental data, which may mean that acceleration is of little importance or that there are various factors compensating each other.

In pulsatile flow, wall shear rates are not in phase with the mean flow rate and its direction, and velocity profiles are changed during a pulse wave cycle,\textsuperscript{22} which makes it difficult to describe the diffusion process in a pulsatile flow. To obtain further insight about the precise mechanisms of diffusion and detachment under pulsatile flow conditions, we will have to estimate the local platelet concentrations at the vessel wall and we will have to study platelet detachment in separate experiments.

From a previous study,\textsuperscript{9} we know that there are situa-
tions in which the use of a funnel in the perfusion system with a roller pump gives an increase in platelet adhesion compared to the situation without a funnel. These experiments were done in frequency ranges far from the physiologically relevant ones (450 beats/minute), because the question was asked whether the pulsatility generated by the roller pump at high flow rates would influence platelet adhesion. It now appears that the results of experiments at high frequencies cannot be extrapolated to those in the physiological range.

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