Effect of Blood Shear Forces on Platelet Mediated Thrombosis Inside Arterial Stenosis

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Hemostasis provides a mechanism whereby the body prevents severe blood loss from damaged vessels. When a blood vessel is damaged due to a chronic or acute injury, platelets adhere to the newly exposed surface. Then, circulating platelets form platelet aggregates on top of the initial layer of adhering platelets constituting a hemostatic plug within several minutes. However, when platelets become hyperactive, they can exacerbate the development of atherosclerosis leading to life threatening complications such as stroke and acute coronary thrombosis that leads to coronary occlusion, the leading cause of death in the U.S.

It was our hypothesis that the fluid dynamic shear in acute stenosed arteries plays a pivotal role in initiating and maintaining events that lead to acute thrombotic occlusion. Although some in vitro and ex vivo models were useful in addressing some aspects of acute platelet thrombus formation, they fail to duplicate the complex rheologic and physiologic environments of stenosed arteries. To date there is no study which demonstrates the role of shear on platelet activation and aggregation under in vivo conditions. We have designed new in vivo experimental methods to study the effect of blood hemodynamic shear on acute platelet mediated thrombus formation in canine stenosed arteries.

To study platelet kinetics and blood hemodynamics, we used the Folts model of mechanically stenosed arteries to create conditions similar to patient arterial stenosis leading to thrombosis. The Folts cyclic flow model is a well accepted means of assessing in vivo platelet function and platelet-vessel wall interaction in mechanically injured and stenosed coronary, carotid, or other arteries (MSCA) with intimal damage. Acute platelet mediated thrombus formation in the MSCA followed by distal embolization produces cyclical flow reductions (CFRs) in blood flow. We instrumented the cylindrical constrictor of the artery with two ultrasound crystals. Using a pulsed Doppler range gated instrument, we measured the velocity profile inside the lumen of the stenosis and distal to the stenosis. We also measured the blood volume flow in the artery using an electromagnetic flow-meter (EMF) probe proximal to the stenosis. We used digital subtraction angiography to obtain X-ray arteriograms of the stenosed artery. Using digital video image processing and quantitative arteriography, developed at the University of Wisconsin, we were able to obtain the geometry of the stenosis. The geometric measurements and the blood flow and pressure measurements were used in a flow modeling program (FLUENT) to solve for the velocity profiles and wall shear stresses (SS) inside the stenosis. FLUENT uses finite difference numerical methods to solve the Navier-Stokes equations. The flow modeling allowed us to obtain quantitative and qualitative characteristics of flow in stenosed arteries.

In order to measure platelet accumulation (PA) inside the stenosis during thrombus formation, we labeled the autologous platelets with Indium111-Oxine, and injected them back in the blood stream. A NaI scintillation detector was shielded and collimated to the stenosis. The detector allowed us to detect radio-labeled platelet accumulation at the site of arterial damage for different levels of stenosis. This method allowed us to dynamically monitor radio-labeled platelet accumulation during acute platelet mediated thrombus formation.

Increased shear stress (SS) has been postulated to reverse the antithrombotic effect of some drugs such as aspirin. Experiments were conducted in five dogs to determine the minimal SS levels that produce acute platelet thrombus formation in mechanically stenosed arteries and the increase in shear required to reverse the antithrombotic effect of aspirin. After intimal and medial damage, a stenosis was produced in the circumflex coronary artery. At 70ñ5% coronary diameter reduction, cyclic flow reductions (CFRs) were observed caused by acute platelet thrombus formation in the stenosed lumen. At this level of stenosis, the SS was 144ñ15 pascals (Pa). Aspirin given IV at a dose of 5 mg/Kg inhibited in vivo acute platelet mediated thrombus formation and abolished CFRs in all dogs. However, increasing the stenosis level to 80ñ5% caused the CFRs to appear again. The SS increased with the increased level of stenosis to 226ñ22 Pa. Thus, a 10-20 % increase in diameter narrowing caused a 56ñ20% increase in SS ($p < 0.005$) and renewed platelet activation and thrombus formation in spite of the aspirin pretreatment. Individuals who take ASA daily to prevent coronary artery thrombus formation may not be well protected when a change in hemodynamics, such as an acute hypertensive episode, or an increase in stenosis severity due to a ruptured atherosclerotic plaque, causes an increase in shear stress.

We also investigated the effect of shear in vivo on platelet accumulation (PA) inside canine stenosed carotid arteries in 10 dogs. A carotid artery/jugular vein anastomotic shunt was produced. Intimal damage and controlled variations in the degree of stenosis were produced on the carotid artery. An inflatable cuff was placed distally around the jugular vein to control vascular resistance distal to the stenosis. The radioactive count rate increased in an inverse parallel fashion to the decline in flow rate during thrombus formation. The rate of flow decline correlated directly with the rate of PA ($r2 > 0.9$). This confirms that the acute occlusive thrombus is platelet mediated, and that the rate of flow decline is a measure of in vivo platelet activity and platelet-vessel wall interaction at the site of a damaged and stenosed artery.

We also observed that the SS varies dramatically from point to point along the irregular geometry of the stenosis. The sudden narrowing of the stenosis lumen leads to characteristic disturbances of the flow profile, high local SS at the arterial stenosis and post-stenotic vortices. The post-stenotc vortices were confirmed by the measurement of flow reversal as we range gated the ultrasound measurement of flow distal to the stenosis. The highest levels of shear occur at the apex of the stenosis, where the lumen is the smallest. The rate of PA in the MSCA did not significantly change until the pressure gradient across the stenosis exceeded 50ñ10 mmHg. PA increased with the amount of stenosis and leads to totally occlusive thrombus formation at levels of stenosis higher than 70ñ5 % (diameter narrowing). Totally occlusive thrombus formation was only observed for SS greater than 100 ñ10 Pa. Hence, at critical levels of stenosis, high trans-stenotic pressures produce critical levels of SS that exacerbate acute thrombus formation. From ex vivo measurements of platelet accumulation on the excised stenosed vessel, we also observed that the platelet accumulation is maximal inside the stenosed lumen where the shear force is the highest. Critical levels of shear might be produced clinically at sites of arterial lesion by a sudden change in blood hemodynamics or flow geometry. This may put a patient with arterial stenosis at greater risk of acute thrombus formation leading to stroke or myocardial infarction. Hence, the measurement of wall shears tress inside stenosed arteries of patients with stable and unstable angina might prove to be a valuable diagnostic parameter to assess the severity of the stenosis and the risk of thrombotic occlusion.