Reduction of Platelet Thrombi and Emboli by L-Arginine during Cardiopulmonary Bypass in a Pig Model

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Abstract. We wanted to test the hypothesis that NO generation by L-arginine (LA) infusion will be beneficial in increasing blood flow to all organs to counteract the process of global ischemia during cardiopulmonary bypass (CPB) and to reduce platelet emboli by platelet inhibition. The effect of LA infusion on NO formation, vasodilation, and reduction of thromboembolic burden in organs and tissues after CPB was quantified with In-111-labeled autologous platelets in two major groups: 180 minutes CPB (CPB) and 90 minutes CPB plus 90 minutes reperfusion (RP). Platelets labeled with In-111 tropolone (650-780 ~Ci) were administered 24 hours before CPB and LA infusion (bolus, 10 mg/kg and infusion at 2 mg/kg/min, 21 pigs for 180 minutes CPB) in 8 groups of 30 Yorkshire pigs (30-35 kg, 6 pigs; LA 2 mg/kg/min, 3 pigs; sham-thoracotomy control, 6 pigs; unoperated control, 6 pigs). Two groups of 9 pigs (control CPB, 6 pigs; LA 2 mg/kg/min, 3 pigs) underwent 90 minutes of CPB and 90 minutes of reperfusion. All pigs were heparinized (ACT >400 seconds); CPB was instituted with a roller pump, an oxygenator (OX: Bentley Univox, 1.8 m2), and an arterial filter (AF: 0.25 m2, Bentley) at a blood flow of 2.5-3.5 l/min. Radioactive thrombi in OX and AF and emboli in viscera, brain, and connective tissues were imaged with a gamma camera and were finally measured with an ion chamber and a gamma counter. The percent of injected platelets (mean _+ SD) in the organs and tissues of all pigs was calculated.

Cerebral emboli were mapped in 25 regions of both hemispheres of pig brain. Flow cytometry with antibodies to CD61 (GPIIIa) and CD62P (GMP-140:control) of porcine platelets was carried out with blood samples taken before, during, and after CPB. Coronary bypass with LA infusion decreased the amount of adherent thrombi in OX and AF (p < 0.07). The embolic burden in brain and lung also decreased. Regional cerebral mapping of In-111 platelets showed reduced emboli in almost all regions, including the medulla, hippocampus, and posterior cerebral cortex in both LA-treated groups. Flow cytometry of blood samples demonstrated the shift of equilibria from single platelet to platelet-aggregate-microparticle during CPB and steady-state level after the first 5-10 minutes of initiation of CPB. The L-arginine infusion reduced thrombi and emboli during CPB in the pig model.

Key Words. cardiopulmonary bypass, Yorkshire pigs, indium-111 label, platelets, thrombi, emboli, flow-cytometry, trapped platelet-emboli, NOS gene, L-arginine

Thromboembolic complications in patients undergoing a cardiopulmonary bypass (CPB) procedure were reviewed extensively [1-15]. The foci of thrombus formation and embolization in the oxygenator and arterial filter, and in organs and tissues, were identified by our imaging and mapping studies with radiolabeled platelets. We developed a quantitative database of the amount of thrombi shed from the oxygenator and filter, and trapping of emboli in the arterial filter and organs [7-15]. Approximately 600,000 CPB procedures are performed annually worldwide. Significant modifications in the development of membrane oxygenators through several generations of change in biomaterials and change of design of the bloodflow path have increased oxygen delivery and reduced surface area and decreased the stagnation zones in the oxygenator and arterial filter, resulting in a significant...
reduction of risk of microemboli and macroemboli in patients undergoing CPB [3,5,7]. Development of hollow-fiber oxygenation technology with extraluminal blood flow, integrating a heat exchanger and blood reservoir, produced a significant improvement in oxygen delivery with a concomitant decrease in priming blood volume and platelet thrombus formation [9,13,15]. The current generation of oxygenators (Univox) satisfies the basic criteria of almost physiologic gas exchange of O₂ and CO₂, heat exchange with a low priming volume, a low pressure drop, hemocompatibility, an extended period of use without a high thrombus buildup, ease of operation, and low cost.

Autologous platelets labeled with the metallic radionuclide, indium-111, were found to be essential in identifying the site and amount of adherent thrombi on several types of oxygenators and filters, and the amount of trapped microemboli and macroemboli in the lung, heart, brain, and kidneys by biodistribution studies in control and CPB pigs [6-15]. In addition, the noninvasive imaging technique permitted us to follow the dynamic nature of thrombus buildup and embolization from the devices used during extracorporeal circulation with high blood flow oxygenation and low blood flow hemodialysis [15]. Our studies in the pig model also indicated a significant reduction of adherent thrombi from Univox of 0.2% to 2-3% in intraluminal flow oxygenators. Our studies definitely demonstrated that there was no decrease of platelet thrombi and emboli by heparin coating of components of the extracorporeal circuit, oxygenator, and arterial filter [8].

Previous studies with optical aggregometry indicated that platelet functions were impaired and the level of functional platelets necessary for hemostasis post-CPB was significantly reduced [1-5,11,16-21]. In addition, we and other investigators [14,16,18] observed loosely adherent smaller aggregates (7-10 μm) circulating in blood during CPB. These aggregates constitute only a small percentage of the total circulating platelets (5-12%) and are in equilibrium with single platelets bound by a variable amount of plasma proteins (fibrinogen, fibrin, von Willebrand factor, fibronectin). Only larger organized macroemboli may be responsible for inducing ischemic organ damage by blocking arteries. The microemboli distribute diffusely in all organs and tissues, reducing blood flow and access to substrates in arteriolar and capillary networks and inducing vasogenic edema, edema in addition to cytopathic edema, produced by activated white cells and cytokines (Figure 1).

Three forms of circulating platelet aggregates formed during cardipulmonary bypass in pigs—the single platelet, aggregate, and microparticle—were studied by flow cytometry with monoclonal antibodies to porcine platelet antigen (GPIIIa). Using empirical screening, we observed that murine monoclonal antibody to human GPIIIa also reacted with porcine platelets, giving us a powerful tool for flow cytometry assay of platelet aggregates formed during CPB in the pig model.

Metabolism of L-arginine, leading to NO formation (Figure 2A and 2B) has been identified as a very important bioregulatory step [23-55]. Macrophages [26,30], brain [24], and endothelial cells [23] from different species synthesize reactive nitrogen intermediates (NO, NO₂⁻, NO₃⁻) from the terminal guanidino nitrogen after exposure to a variety of microbial products and cytokines [51-55]. Production of NO from L-arginine is controlled by either a constitutive nitric oxide synthase (cNOS) enzyme present in endothelium and nerve tissue, or an inducible form in macrophage of the enzyme, called inducible nitric oxide synthase (iNOS). This activity could be blocked by the L-arginine analog, N⁰-monomethyl-L-arginine (NMMA) [41] or by other analogs.

At present, three different isoforms of NOS have been identified and cloned from brain, endothelial cells, and macrophages. High-level homology (50-60%) across different species of animals has been demonstrated. NOS from endothelial cells and the central nervous system is dependent on exogenous Ca²⁺ and calmodulin [23]. Macrophage NOS is not dependent on Ca²⁺ and calmodulin, and is inducible by cytokines [26,30]. In arteries, endothelial NO diffuses into the smooth muscle cells of media, where it reacts with the prosthetic heme group of guanylate cyclase, producing cyclic GMP (cGMP) and resulting in relaxation of smooth muscle cells (Figure 2B) and an increase of blood flow in all organs, including the hippocampus, in the brain [28]. NO formed by neutrophils is lower than that of macrophages [25,46-48]. Hepatocytes are also induced by Kupffer cells in inflammatory states to make more NO at the expense of protein synthesis [27]. NO also binds and inhibits iron-sulfur containing enzymes, including those in the electron transport chain of the citric acid cycle, and impairs oxidative metabolism in target cells [34-37], when they are generated in excess of consumption. Although several