Preservation of Platelet Count and Function in Extracorporeal Circulation via *In Situ* Nitric Oxide Generation at Polymer/Blood Interface

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Statement of Purpose: The preservation of platelet count and/or function in cardiopulmonary bypass and other ECC procedures has remained a great challenge. Current approaches use either a GPIIb/IIIa inhibitor to block platelet aggregation (Kondo N. Thromb Res.2004;113:303-310), or systemic heparin to inhibit thrombin activation (Muriithi EW. J Thorac Cardiovasc Surg. 2000;120:538-543). However, both methods fail to preserve platelet count and function at the same time and can lead to hemorrhage. Here we report a novel approach which utilizes the Cu(I/II)-catalyzed *S*-nitrosothiol (RSNO) decomposition (Williams DLH. Acc Chem Res. 1999;32:869-876) to generate nitric oxide (NO), a platelet inhibitor (Ignarro LJ. Proc Natl Acad Sci USA. 1987;84:9265–9269), *in situ* at the circuit/blood interface to simultaneously preserve both platelet count and platelet function.

Methods: Extracorporeal circulation was performed by an A/V bypass loop with varying diameters in rabbits for 4 h. The inner wall of the loop was coated with plasticized poly(vinyl chloride) (PVC:DOS = 2:1) containing 5 wt% of a lipophilic Cu(II)-DTTCT complex (Oh BK J Am Chem Soc. 2002;125:9552-9553) in the study set and plain PVC in the control set. Platelet count, platelet aggregation and activated clotting time were measured at 0, 1, 2, 3 and 4 h. At the same time, the endogenous RSNO concentration was determined by a pair of amperometric RSNO/NO sensors (Cha W. Langmuir. 2006;ASAP) to track changes of RSNO levels in blood as the result of Cu-catalyzed decomposition. An exogenous RSNO, *S*-nitrso-*N*-acetyl-penicillamine (SNAP), was supplemented to the animal at 80 nmol/dose at 1.5, 2.5 and 3.5 h during the circulation. Rabbits without circuits attached were used as resting-state controls. The platelet physiology data for rabbits with Cu-circuits attached but no SNAP supplement were also obtained.

Results/Discussion: *RSNO Concentrations:* After 1 h of circulation, ~ 80% of the initial RSNO is consumed in the Cu-circuits, while the RSNO levels in animals with control circuit or no circuit attached remain steady (see **Fig. 1**). Therefore, use of exogenous SNAP is necessary for the continuous NO generation from RSNOs.

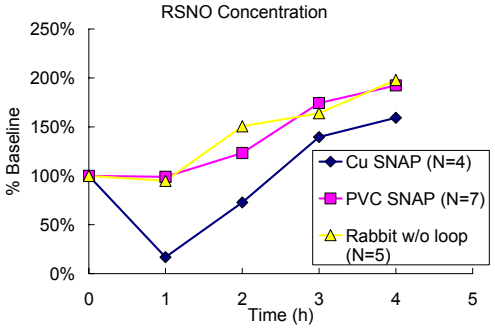

Fig. 1 The change in [RSNO] measured by RSNO and NO sensors

Platelet Count/Function: The platelet count (**Fig. 2**) and platelet function (**Fig. 3**) data illustrate the synergistic effect of combining Cu(II)-DTTCT coating and SNAP supplementation. The platelet counts in animals with Cu-circuits and SNAP infusion remained steady except for 1 h when endogenous RSNOs have been nearly completely consumed. The platelet function in this group is also best preserved among all animals under ECC. In addition, the ACT values remain steady (within ±5% of baseline) suggesting no risk of systemic bleeding. On the other hand, neither Cu-circuit nor SNAP infusion alone offers similar platelet preservation as observed with the combination of both.

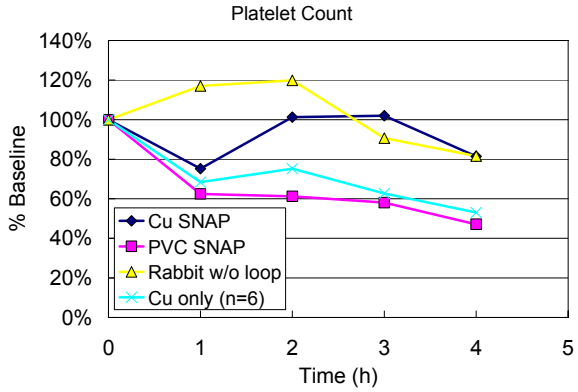

Fig. 2 Platelet Counts in rabbits with various circuits.

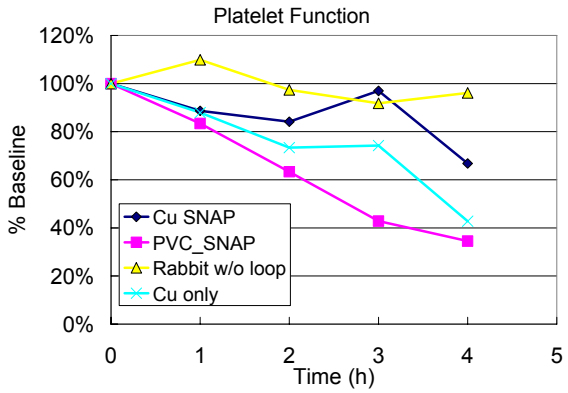

Fig. 3 Platelet function as determined by aggregometry

Conclusions: It is obvious that neither Cu(II)-DTTCT coating nor exogenous SNAP supplement alone preserves platelet count or function. Indeed, it is the combination of both that yields the most thromboresistant ECC circuit. These results suggest the critical role of locally generated NO from circulating RSNOs in the preservation of both platelet count and function without the risk of hemorrhage. This discovery has great potential for application in a variety of ECC procedures.