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The effect of a polyurethane coating incorporating both a thrombin inhibitor and nitric oxide on hemocompatibility in extracorporeal circulation

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ABSTRACT

Nitric oxide (NO) releasing (NORel) materials have been extensively investigated to create localized increases in NO concentration by the proton driven diazeniumdiolate-containing polymer coatings and demonstrated to improve extracorporeal circulation (ECC) hemocompatibility. In this work, the NORel polymeric coating composed of a diazeniumdiolated dibutylhexanediamine (DBHD-N_2O_2)-containing hydrophobic Elast-eon™ (E2As) polyurethane was combined with a direct thrombin inhibitor, argatroban (AG), and evaluated in a 4 h rabbit thrombogenicity model without systemic anticoagulation. In addition, the immobilizing of argatroban to E2As polymer was achieved by either a polyethylene glycol-containing (PEGDI) or hexane methylene (HMDI) diisocyanate linker. The combined polymer film was coated on the inner walls of ECC circuits to yield significantly reduced ECC thrombus formation compared to argatroban alone ECC control after 4 h blood exposure (0.6 ± 0.1 AG/HMDI/NORel vs 1.7 ± 0.2 cm^2 AG/HMDI control). Platelet count (2.8 ± 0.3 AG/HMDI/NORel vs 1.9 ± 0.1 × 10^8/ml AG/HMDI control) and plasma fibrinogen levels were preserved after 4 h blood exposure with both the NORel/argatroban combination and the AG/HMDI control group compared to baseline. Platelet function as measured by aggregometry remained near normal in both the AG/HMDI/NORel (63 ± 5%) and AG/HMDI control (58 ± 7%) groups after 3 h compared to baseline (77 ± 1%). Platelet P-selectin mean fluorescence intensity (MFI) as measured by flow cytometry also remained near baseline levels after 4 h on ECC to *ex vivo* collagen stimulation (16 ± 3 AG/HMDI/NORel vs 11 ± 2 MFI baseline). These results suggest that the combined AG/HMDI/NORel polymer coating preserves platelets in blood exposure to ECCs to a better degree than AG/PEGDI/NORel, NORel alone or AG alone. These combined antithrombin, NO-mediated antiplatelet effects were shown to improve thromboresistance of the AG/HMDI/NORel polymer-coated ECCs and move potential nonthrombogenic polymers closer to mimicking vascular endothelium.

1. Introduction

Extracorporeal devices (i.e., cardio-pulmonary bypass, hemodialysis, hemofiltration, extracorporeal membrane oxygenation) require systemic anticoagulation. The major complications are clotting in the device and bleeding from the patient. Systemic anticoagulation blocks fibrin formation but does not prevent platelet adhesion and activation [1,2]. In an attempt to reduce the activation of platelets at the blood/material surface interface, we and other investigators have developed surface coatings that mimic the vascular endothelium and preserve platelet quiescence in the extracorporeal circuits (ECC). Nitric oxide, an endogenous platelet inhibitor and vasodilator, has been incorporated into polymeric tubings to mimic its endogenous effects on maintaining platelet quiescence. Indeed, a number of NO releasing (NORel) polymer coatings have been studied using polymer-embedded NO donor molecules, such as diazeniumdiolated dibutylhexanediamine (DBHD/N_2O_2) [3–9]. The NO released from the DBHD/N_2O_2

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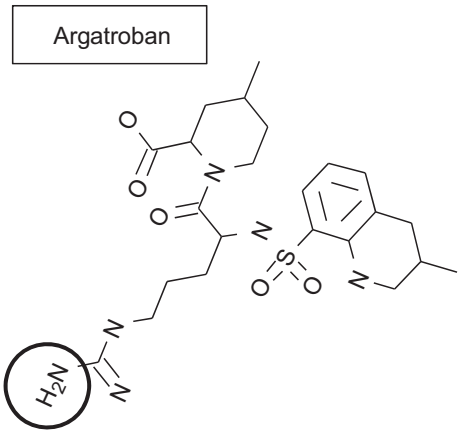

Fig. 1. Structure of argatroban ((2R,4R)-1-[(2S)-5-(diaminomethylideneamino)-2-[[(3R)-3-methyl-1,2,3,4-tetrahydroquinolin-8-yl]sulfonylamino]pentanoyl]-4-methylpiperidine-2-carboxylic acid). Chemically, argatroban (MW = 509) is the dipeptide between arginine and 4-methyl-2-piperidine carboxylic acid with the NH_2 closest to the carbonyl group of arginine bonded to a methyltetrahydroquinoline sulfonyl group. The immobilization of argatroban was accomplished by linking the free primary amine of the arginine 'tail' to an isocyanate group of either HMDI or PEG-DI (black circle).

polymer coating has proven to effectively maintain circulating platelets in a resting state and reduce thrombus formation in the ECCs even when no systemic anticoagulation is present. However, small fibrin thrombi are present in animal models. An immobilized anticoagulant in combination with the NORel polymer may provide the complete nonthrombogenic scenario needed for ECLS circuit longevity.

Commercial availability of heparin-coated components for ECLS circuits such as catheters and oxygenators has brought immobilized anticoagulants to the forefront for over 20 years. Unfortunately systemic anticoagulation is still required in all clinical ECLS procedures, albeit at a reduced level [2,10]. The use of heparin-coated ECLS components is dependent upon the patient plasma levels of antithrombin III (ATIII) to which heparin needs to bind before the heparin/ATIII complex directly inhibits any released thrombin. Varying ATIII plasma concentrations could be an important explanation for varying nonthrombogenic effects of the heparin-coated tubing and devices. Therefore, use of a direct thrombin inhibitor such as argatroban would be an effective means to inhibit thrombin activity regardless of the plasma ATIII concentrations.

Argatroban, a synthetic small molecule (Fig. 1), was developed to provide better nonthrombotic efficacy over heparin [11]. After 2 decades of research, direct thrombin inhibition particularly with argatroban has proven to provide adequate anticoagulation clinically [12]. Studying the effects of the combined argatroban and NORel polymer coatings in blocking both fibrin formation and platelet activation is a logical next step of investigation (Fig. 2).

The present study was designed to evaluate by the NORel and argatroban combination. This combination of the direct thrombin inhibitor, argatroban, and NORel would provide clinicians medical devices for patients who demonstrate the adverse immune-mediated response to administered heparin namely heparin-induced thrombocytopenia (HITS) [13].

2. Materials and methods

2.1. Materials

Tygon® poly(vinyl chloride) (PVC) tubing was purchased from Fisher Healthcare (Houston, TX). The hydrophobic polyurethane, Elast-Eon (E2As), was a product of AorTech Biomaterials, Inc. (Rogers, MN). Anhydrous tetrahydrofuran (THF), hexamethylene diisocyanate (HMDI) and poly(propylene glycol), tolylene 2, 4-

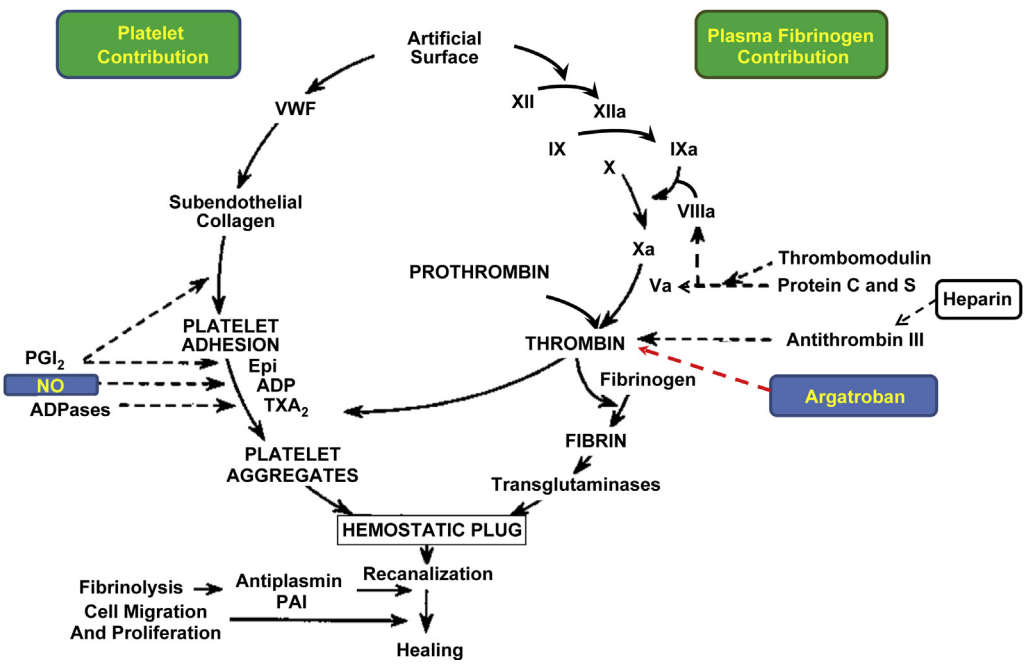

Fig. 2. Overview of hemostasis. Hemostasis for maintaining a thrombotic environment when blood comes into contact with foreign surfaces such as extracorporeal life support (ECLS) circuits consists of two contributions. One is the intrinsic coagulation pathway (right) that initiates with factor XII activation and ending with the formation of a fibrin clot. The second contribution is the activation of blood cell components particularly the circulating platelets (left). Both contribution pathways converge at a point of thrombin formation which forms the beginning of the common thrombotic pathway and ultimate hemostatic plug which consists of the fibrin clot and aggregated platelets. As the hemostatic plug matures other blood cell components such as red blood cells (RBC) and activated white blood cells (WBC) also bind in the plug. Fibrinolysis of the plug in the ECLS circuit may not be as important of a process as it is in vessel wounds and healing (i.e., extrinsic coagulation pathway; not shown). The 'a' following the various coagulation factors (right) means activated form of factor. On the left side of hemostasis, vWF is von Willibrand factor, epi = epinephrine, ADP = adenosine diphosphate, TXA_2 = thromboxane A_2, PGI_2 = prostacyclin and ADPases = adenosine diphosphatases. Two inhibitors of thrombosis are shown on schematic with heparin used to block fibrin formation and endothelial-derived nitric oxide (NO) which inhibits platelet activation.

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