Differential Effect of Heparin Coating and Complement Inhibition on Artificial Surface-Induced Eicosanoid Production

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Background. **Contact between blood and artificial surfaces induces an inflammatory response including activation of leukocytes and platelets, as well as complement and other plasma cascade systems. In the present study we investigated the roles of complement and surface modification in polyvinyl chloride-induced synthesis of eicosanoids (arachidonic acid metabolites).**

Methods. **Human whole blood was incubated in rotating loops of polyvinyl chloride or heparin-coated polyvinyl chloride tubing for 4 hours. Plasma concentrations of the eicosanoids leukotriene B4, prostaglandin E2 and thromboxane B2 were quantified.**

Results. **Polyvinyl chloride induced a substantial increase in leukotriene B4, prostaglandin E2, and thromboxane B2. Inhibition of complement activation by the complement factor 3 binding peptide compstatin or blockade of the complement factor 5a receptor with a specific antagonist significantly and specifically inhibited the synthesis of leukotriene B4, whereas thromboxane B2 and prostaglandin E2 synthesis were apparently complement independent. The increase in all three mediators was significantly reduced by the heparin coating. Indomethacin abolished the increase of the cyclooxygenase products prostaglandin E2 and thromboxane B2, but had no effect on the increase of the lipoxygenase product leukotriene B4, consistent with the specificity of indomethacin for the cyclooxygenase and confirming the specificity of complement inhibition.**

Conclusions. **Polyvinyl chloride-induced increase in all three eicosanoids was attenuated by heparin coating, whereas complement inhibition selectively reduced the synthesis of leukotriene B4.**

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Exposure of blood to artificial surfaces in medical devices, including extracorporeal circuit systems, leads to a reaction between the blood constituents and the surface. Polyvinyl chloride (PVC) is a frequently used artificial surface material in such devices. Clinical cardiopulmonary bypass (CPB) induces a systemic inflammatory response involving activation of both leukocytes, platelets, and plasma cascade systems [1, 2]. There are several contributors to these reactions; eg, the artificial surface itself, the membrane oxygenator, the surgical trauma, and the ischemia-reperfusion reaction. The relative contribution of these factors is only partly understood. It is well known, both from in vitro and in vivo experiments, that modification of the artificial surface by endpoint attachment of heparin attenuates several of the observed inflammatory responses [3–5]. Similar in vivo observations have been made with inhibitors of the complement system [6, 7].

Complement activation is responsible for a number of the inflammatory reactions taking place during CPB, and the complement inhibitory properties of the heparin coating may account for many of the beneficial effects of this surface. However, in an in vitro model, we have recently shown that various leukocyte responses, including expression of surface markers and synthesis of cytokines, differ in their dependence on complement and that surface modification with covalently attached heparin attenuates both complement-dependent and complement-independent reactions [8, 9]. The model is reductionistic in its nature as it aims to restrict the factors involved to the surface only and is thus not readily comparable to a clinical CPB setting. This should be kept in mind when comparing our results with those of previous studies.

Knowledge regarding the synthesis and release of eicosanoids in clinical CPB or in vitro models is scarce. To our knowledge, only two studies have investigated the effect of heparin coating on eicosanoids in simulated CPB

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Abbreviations and Acronyms

C3 = complement factor C3
C5a = complement factor 5a
C5aR = complement factor 5a receptor
C5aRA = complement factor 5a receptor antagonist
CBAS = Carmeda BioActive Surface heparin-coated PVC
CPB = cardiopulmonary bypass
ELISA = enzyme-linked immunosorbent assay
H-PVC = heparin-coated polyvinyl chloride
IL-8 = interleukin 8
LTB4 = leukotriene B4
PBS = phosphate-buffered saline
PGE2 = prostaglandin E2
PVC = polyvinyl chloride
T0 = baseline values
TXB2 = thromboxane B2
TCC = terminal complement complex

[10] or in vivo [11], whereas one group has compared uncoated and heparin-coated cardiac catheters [12]. All three studies were restricted to thromboxane B2 (TXB2). The effect of complement inhibition on PVC-induced eicosanoids has not been studied. Since eicosanoids play important roles in regulation and propagation of inflammatory processes [13] and are the common target of several antiinflammatory drugs [14], it is important to obtain information on artificial surface-induced eicosanoid synthesis and how to inhibit such a reaction. Thus, the aim of the present study was to investigate the role of complement and heparin coating in the PVC-induced synthesis of eicosanoids.

Material and Methods

Reagents

Heparin-coated CBAS (Carmeda BioActive Surface) polyvinyl chloride (H-PVC) and uncoated PVC tubing were provided by Carmeda AB (Stockholm, Sweden). Sterile phosphate-buffered saline (PBS) was from Life Technologies (Paisley, UK), and lepirudin (Refludan) from Hoechst, (Frankfurt am Main, Germany). Indomethacin was purchased from Sigma-Aldrich (St. Louis, MO).

Complement Inhibitors

The cyclic hexapeptide AcF[OPdChaWR], a complement factor 5a receptor antagonist (C5aRA) [15] was synthesized as previously described [16]. Details of the synthesis, purification, mass spectrometry, and measurement of inhibitory activities of the compstatin analogue Ac-I[CVWQDWGAHRC]T-NH_2 are discussed elsewhere [17]. This peptide, an inhibitor of the C3 convertase [18, 19], is 45 times more active than the parent peptide I[CVVQDWGHHRC]T-NH_2. Both complement inhibitors were synthesized in the laboratory of one of the authors (JDL).

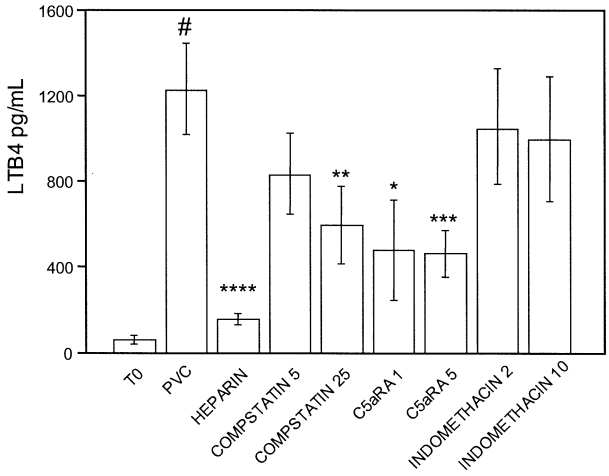

*Fig 1. Polyvinyl chloride-induced (*PVC*) synthesis of leukotriene B4 (*LTB4*) (pg/mL) in human whole blood. Human whole blood, with or without complement inhibitors or indomethacin, was circulated for 4 hours in segments of tubing rotated as closed loops, whereupon the LTB4 concentration in plasma was measured by enzyme-linked immunosorbent assay. (*T0 *= baseline values;* PVC *= PVC loops.* Heparin *= heparin-coated loops; Compstatin 5 or 25 μmol/L;* C5aRA *= C5a receptor antagonist 1 or 5 μmol/L; indomethacin 2 or 10 μmol/L. Inhibitors were added to blood incubated in uncoated PVC only; # = $p < 0.001$ versus baseline; * = $p < 0.05$; ** = $p < 0.02$; *** = $p < 0.01$; **** = $p < 0.001$; all versus PVC; data are presented as mean ± standard error of the mean).*

Experimental Model

The in vitro model for circulating whole blood through PVC loops has previously been described in detail [20]. The method was modified on the critical point of anticoagulation. Blood was drawn from healthy volunteers using lepirudin, a recombinant form of hirudin, instead of heparin as anticoagulant. Hirudin is a highly specific thrombin inhibitor and has no effect on the complement system. This is in contrast to heparin, which can either potentiate or attenuate complement activation, depending on the concentration used [21]. With regard to anticoagulation, lepirudin is equally effective as heparin. Fresh samples of blood were supplied with specific complement inhibitors (C5aRA 1 or 5 μmol/L, compstatin 5 or 25 μmol/L), indomethacin (2 or 10 μmol/L), or equal volumes of PBS. A volume of 750 μL blood was then transferred to segments of PVC or H-PVC tubing (length 30 cm, internal diameter 3 mm). The total volume of the tubing was 2,100 μL, and approximately 1/3 of the tubing was filled with blood, the remainder being air as to ascertain that the blood was actually circulating. Each segment was closed end-to-end and incubated by rotating slowly in an incubator at 37°C for 4 hours, based on pilot experiments designed for optimal conditions for incubation time. In previous experiments we have found that pH in the blood is stable during this period [21]. The blood was processed immediately after collection and in order to reduce delay in handling, the experimental setup did not allow for testing of all the different inhibitors in the same experiment. However, baseline values as