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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E xposure 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

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

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[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).
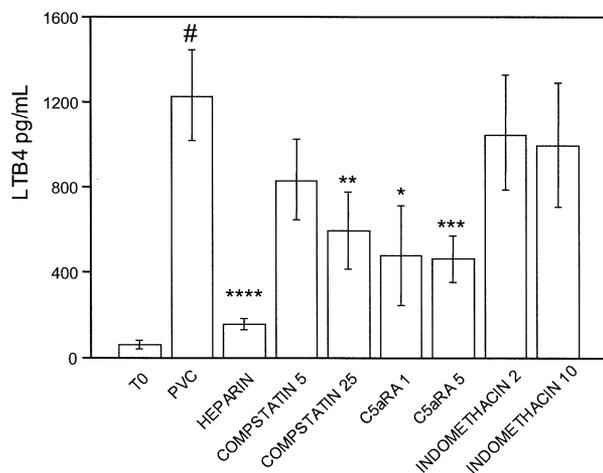

Fig 1. Polyvinyl chloride-induced (PVC) synthesis of leukotriene B4 (LTB4) (pg/mL) in human whole blood. Human whole blood, with or without complement inhibitors or indomethacin, was circulated for 4 hours in segments of tubing rotated as closed loops, whereupon the LTB4 concentration in plasma was measured 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
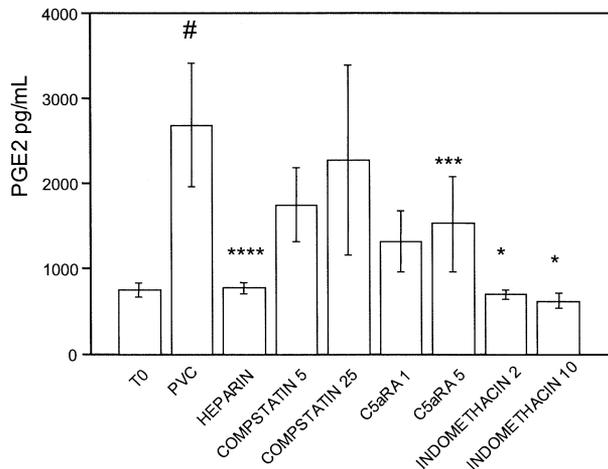
Fig 2. Polyvinyl chloride-induced (PVC) synthesis of prostaglandin E2 (PGE2) (pg/mL) in human whole blood. Human whole blood, with or without complement inhibitors or indomethacin, was circulated for 4 hours in segments of tubing rotated as closed loops, whereupon the PGE2 concentration in plasma was 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 mol/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 < 0.01; **** = p < 0.001; all versus PVC; data are presented as mean ± standard error of the mean).
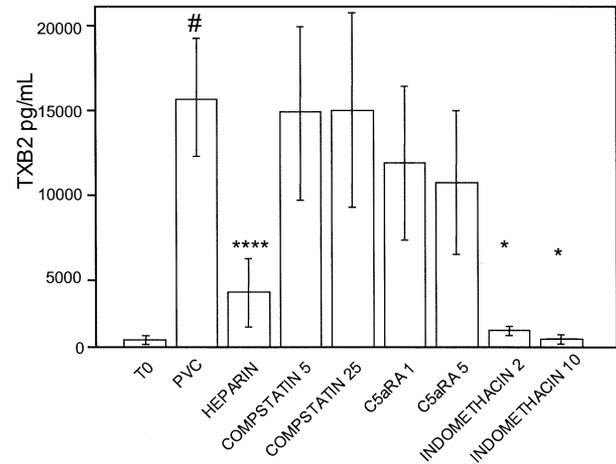

Fig 3. Polyvinyl chloride-induced (PVC) synthesis of thromboxane B2 (TXB2) (pg/mL) in human whole blood. Human whole blood, with or without complement inhibitors or indomethacin, was circulated for 4 hours in segments of tubing rotated as closed loops, whereupon the TXB2 concentration in plasma was measured 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; data are presented as mean ± standard error of the mean; # = p < 0.001 versus baseline; * = p < 0.05; **** = p < 0.001; both versus PVC.)

well as blood circulated in PVC tubing without inhibitor and blood circulated in H-PVC tubing were included in all experiments. All inhibitors were tested in blood from 6 to 8 different donors. Since baseline values as well as values from PVC tubing and H-PVC tubing were determined in every experiment, each donor served as his or her own control. After incubation, the blood was centrifuged at 3,220 × g for 15 minutes at 4°C. The plasma was frozen in aliquots at −70°C and stored until all samples were analyzed in one batch.

Enzyme Immunoassays

Assays for prostaglandin E2, thromboxane B2, and leukotriene B4 were purchased from Cayman Chemical Company (Ann Arbor, MI). Activation of the terminal complement pathway was determined in an enzyme immunoassay using the monoclonal antibody aE11 specific for a C9 neoepitope in the sC5b-9 complex using a modification of an assay described in detail previously [22].

Statistics

Wilcoxon's test for paired observations was used for comparison within groups. Statistical significance was defined as p less than 0.05 (two-tailed). Baseline values for the various eicosanoids, as well as control PVC and H-PVC loops were included in every experimental setup and thus represent a larger number of experiments than each of the inhibitors tested.

Results

PVC-Induced Synthesis of Eicosanoids

LEUKOTRIENE B4 (LTB4). Incubation of blood for 4 hours in PVC loops increased mean LTB4 concentration from 62 to 1,232 pg/mL (p < 0.001) (Fig 1). The increase was not affected by indomethacin (1,061 pg/mL and 1,000 pg/mL for 2 and 10 μmol/L indomethacin (p > 0.05 for both), respectively), consistent with the lack of effect of indomethacin on the lipoxygenase (Fig 1).

PROSTAGLANDIN E2 (PGE2). Incubation of blood for 4 hours in PVC loops increased mean PGE2 concentration from 569 to 2,557 pg/mL (p < 0.001) (Fig 2). By addition of 2 or 10 μmol/L indomethacin, this increase was significantly reduced to 517 and 380 pg/mL (p < 0.05 for both), respectively, indicating that the main fraction of PGE2 was synthesized during incubation (Fig 2).

THROMBOXANE B2 (TXB2). Incubation of blood for 4 hours in PVC loops increased mean TXB2 concentration from 78 to 15,747 pg/mL (p < 0.001) (Fig 3). By addition of 2 or 10 μmol/L indomethacin, this increase was reduced to 909 and 281 pg/mL (p < 0.05 for both), respectively, indicating that the main fraction of TXB2 was synthesized during incubation (Fig 3).

Effect of Heparin Coating on PVC-Induced Synthesis of Eicosanoids

Heparin-coated PVC reduced the mean PVC-induced increase in LTB4 from 1,232 to 157 pg/mL (p < 0.001) (Fig 1), the mean PVC-induced increase in PGE2 from 2,557 to
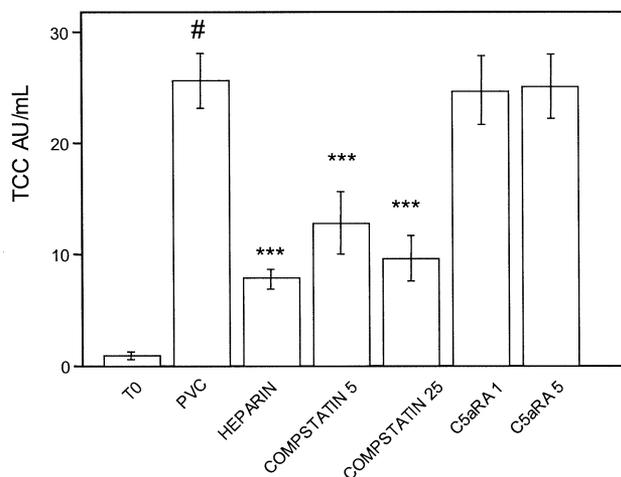
*Fig 4. Polyvinyl chloride-induced (PVC) complement activation in human whole blood. Human whole blood, with or without complement inhibitors, was circulated for 4 hours in segments of tubing rotated as closed loops, whereupon the concentration of the terminal complement complex (TCC) in plasma was measured 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; data are presented as mean ± standard error of the mean; AU = arbitrary units; # = p < 0.001 versus baseline; *** = p < 0.01 versus PVC.)*

587 pg/mL ($p < 0.001$) (Fig 2), and the mean PVC-induced increase in TXB2 from 15,747 to 3,284 pg/mL ($p < 0.001$) (Fig 3). Thus, the heparin-coated loops efficiently inhibited synthesis of all three eicosanoids.

Effect of Complement Inhibition on PVC-Induced Synthesis of Eicosanoids

LEUKOTRIENE B4. Complement inhibition markedly suppressed the PVC-induced increase in LTB4 (Fig 1). Blocking the C3 convertase with compstatin 5 or 25 μmol/L reduced mean LTB4 from 1,232 pg/mL to 838 pg/mL (not significant) and 598 pg/mL ($p < 0.02$), respectively. Blocking the C5a receptor with 1 or 5 μmol/L C5a receptor antagonist reduced mean LTB4 to 482 pg/mL ($p < 0.05$) and 465 pg/mL ($p < 0.01$), respectively.

PROSTAGLANDIN E2. Complement inhibition did not affect PGE2 levels significantly, except for 5 μmol/L of the C5a receptor antagonist, which gave a reduction from 2,557 pg/mL to 1,385 pg/mL ($p < 0.01$) (Fig 2).

THROMBOXANE B2. Complement inhibition did not affect artificial surface-induced synthesis of thromboxane B2 (Fig 3).

PVC-Induced Complement Activation

The terminal complement complex (TCC) increased significantly ($p < 0.001$) in the PVC tubing, confirming that uncoated PVC activates the complement system. This activation was markedly lower ($p < 0.01$) in H-PVC, consistent with previous data that this heparin coating efficiently attenuates complement activation. Compstatin

reduced TCC generation in a dose-dependent manner, at the highest concentration tested the effect was comparable to that observed for H-PVC ($p < 0.01$ for both concentrations). As expected, the C5aRA had no effect on TCC formation since the antagonist blocks the receptor but does not interfere with the cleavage of C5 into C5a and C5b, or the subsequent assembly of TCC (Fig 4).

Comment

The systemic inflammatory response during and after CPB has been the subject of considerable research. This is due to both the frequency with which CPB is performed and to the potentially serious complications involved, as well as to the fact that CPB represents a fairly standardized model for studying in vivo human inflammatory reactions. However, as several factors influence the type and degree of the inflammatory response seen in such settings, results from different studies may not be directly comparable. For example, the type of tubing, pump, and oxygenator used, the duration and type of surgery, and the preoperative status of the patient could influence the results. In our model, we are able to selectively study the effect of the artificial surface, without interference from other, nonsurface-related factors mentioned above. By including a space with air, blood could be circulated simply by rotating the tubing. Thus, traumatizing pumps were avoided. The air in the tubing did not contribute to any of the inflammatory markers studied in this model [8, 9] nor those included in the present study since there were virtually no increases in the readouts in the heparin-coated tubing, which contained the same amount of air as the untreated PVC tubing. The most likely reason for the lack of blood-gas activation in this model is the limited contact surface area between the blood and the one single air bubble, in contrast to the large surface of blood-gas contact seen, eg, in bubble oxygenators. Thus, the model represents a selective mode of studying the effect of contact between blood and an artificial surface. This is both a strength and a limitation for this study and should be kept in mind when considering the results. Although our model is very different from a complete in vivo CPB setting, and our results thus cannot directly be extrapolated to a clinical situation, complement activation as measured by formation of TCC is remarkably similar to that seen in clinical studies [23].

Circulation of blood in PVC tubing induces complement activation primarily through the alternative pathway [8]. Increased levels of complexes between C1-inhibitor and its substrates, eg, C1s and kallikrein, has been demonstrated during in vivo as well as simulated extracorporeal circulation [24]. We have previously demonstrated increased C1rs-C1-inhibitor complexes by the heparin-coated surface in the present model [8]. This increase was not followed by C4 activation and therefore not indicative of classic pathway activation, but rather reflected the potentiation of heparin on C1-inhibitor binding to its substrate [25]. The effect of heparin on C1-inhibitor is thus operative both for solid-phase and

Ann Thorac Surg
2005;79:917–23

LAPPEGÅRD ET AL
BIOCOMPATIBILITY AND EICOSANOIDS

921

CARDIOVASCULAR

fluid-phase heparin. A similar effect of heparin has been described for the plasma contact activation system, where antithrombin bound to a heparinized surface was functionally accelerated by the heparin molecules and efficiently neutralized activated factor XII [26]. In general, therefore, increases in complexes between C1-inhibitor and its substrates should be interpreted with caution. To indicate that classic complement activation occurs, a combined increase in C1rs-C1-inhibitor complexes and C4 activation products should be documented.

Anticoagulation may directly affect complement, either by activation (eg, by low doses of heparin) or by inhibition, as seen with calcium binders and high doses of heparin. Thus, conflicting results from different experimental models may in part be due to the use of different anticoagulants. We have recently shown that lepirudin, in doses sufficient to achieve anticoagulation, has no effect on complement activation [21], and we suggest that lepirudin at present is the best anticoagulant available for such studies. Lepirudin has also been employed by others, both in whole blood models of inflammation [27] and in animal models of CPB [28].

It has previously been shown that heparin coating of the artificial surface markedly reduces the inflammatory response in blood [29], including complement activation [5]. However, it is largely unknown to what extent the complement inhibitory properties of the heparin coating are responsible for the antiinflammatory effects of this surface.

The dependency on complement of the different inflammatory reactions can be determined by the use of specific complement inhibitors. We have recently reported that certain inflammatory responses induced by PVC are strictly complement-dependent, some are partly dependent on complement, whereas others are totally complement-independent. For instance, formation of granulocyte-platelet conjugates, but not formation of monocyte-platelet conjugates could be attenuated by complement inhibition [8]. Furthermore, formation of IL-8 is totally complement-dependent in this model, whereas formation of monocyte chemoattractant protein 1 is only partially reduced by complement inhibition [9].

The eicosanoids are important mediators of inflammation [13]. The leukotrienes (eg, LTB4) are synthesized by the 5-lipoxygenase, whereas the cyclooxygenase is responsible for the synthesis of the prostaglandins (eg, PGE2) and thromboxanes (eg, TXB2). A connection between complement and eicosanoid production has been shown in several animal models of eicosanoid-mediated injury. In a model of rat membranous nephropathy it was shown that C5b-9 upregulated the cyclooxygenase-2 [30], and complement activation products induced synthesis of LTB4 [31]. Furthermore, in a rabbit model of dermal inflammation it was shown that C5a recruits leukocytes to the site of inflammation through an LTB4-dependent mechanism [32]. A complex interaction between complement and LTB4 is emphasized by the fact that a specific antagonist against LTB4 blocked LTB4-mediated, but not C5a-mediated neutrophil activation, as measured by release of calcium. Thus, complement-mediated leukocyte

activation may be both LTB4-dependent and independent [33].

The role of complement in PVC-induced eicosanoid production has not previously been investigated. In the present study we demonstrate that PVC-induced LTB4 synthesis to a large extent is complement-dependent since it was dose dependently reduced by the complement inhibitors. However, LTB4 was not completely complement-dependent since it was more efficiently reduced by the heparin coating than by complement inhibition. In contrast to LTB4, the production of PGE2 and TXB2 was virtually complement-independent, although the highest concentration of the C5a receptor antagonist reduced the levels of PGE2 to a certain degree but markedly less than the effect of complement inhibition on LTB4 production. However, the results obtained with indomethacin confirm that inhibition of PGE2 and TXB2 production in our model could be achieved, further emphasizing that the effect of complement inhibition on LTB4 production indicates substantial differences in modes of stimulation for the various eicosanoids. The LTB4 generated in our model is most likely a product of leukocytes [34, 35], whereas TXB2 and PGE2 originate from platelet [36] and monocyte [37] arachidonic acid. Consistently, we have previously shown that release of thrombospondin, a major constituent of platelet alpha granules, is unaffected by complement inhibition in this model [8]. Furthermore, in a previous study [9] we showed that synthesis of IL-8 in this model is totally C5a dependent. The present results show that blocking the C5aR markedly reduces PVC-induced LTB4 synthesis. It has previously been shown that IL-8 can stimulate human neutrophil 5-lipoxygenase and increase formation of LTB4 [38]. Collectively, these data suggest that there is complex interaction between complement, chemokines, and eicosanoids in the inflammatory reaction induced by PVC.

The heparin coating essentially eliminated both the PVC-induced formation of LTB4, as well as PVC-induced complement activation, as judged by the formation of TCC. However, the fact that the same effect was observed for release of PGE2 and TXB2 shows that the heparin coating prevents eicosanoid synthesis through a mechanism that is independent of complement. The beneficial properties of the heparin coating as compared to uncoated PVC have previously been documented for several factors, including complement activation [5], synthesis of chemokines [9], leukocyte CD11b-expression [8], and neutrophil and platelet degranulation [8]. In the present study we clearly show that this also applies to synthesis of LTB4, PGE2, and TXB2. Thus, the heparin coating makes the artificial surface highly biocompatible, and this may account for the beneficial results seen when used in clinical cardiopulmonary bypass [39]. The platelet-compatible properties of the heparin coating may decrease the risks of microemboli in clinical settings, which is in line with previous observations that the use of heparin-coated surfaces can inhibit deterioration of mental function after coronary bypass [40]. There are not many clinical trials with complement inhibition in car-

diopulmonary bypass, but in one study a monoclonal antibody against C5 reduced complications after coronary artery bypass graft [6].

In conclusion, the PVC surface induced a marked increase in the concentrations of LTB4, PGE2, and TXB2. The increase was almost completely inhibited by heparin coating of the surface, whereas complement inhibition specifically reduced LTB4 formation. Thus, the attenuating effects of the heparin coating on eicosanoid synthesis can only partly be explained by its complement inhibitory properties. As the model focuses on the interaction between blood and the artificial surface, and as a clinical situation involving CPB also contains numerous other inflammatory stimuli including the wound, direct extrapolations of our results to a clinical setting are not warranted.

References

1. Levy JH, Tanaka KA. Inflammatory response to cardiopulmonary bypass. Ann Thorac Surg 2003;75:S715–20.
2. Edmunds LH Jr. Inflammatory response to cardiopulmonary bypass. Ann Thorac Surg 1998;66:S12–6.
3. Olsson P, Sanchez J, Mollnes TE, Riesenfeld J. On the blood compatibility of end-point immobilized heparin. J Biomater Sci Polym Ed 2000;11:1261–73.
4. Hsu LC. Heparin-coated cardiopulmonary bypass circuits: current status. Perfusion 2001;16:417–28.
5. Videm V, Mollnes TE, Bergh K, et al. Heparin-coated cardiopulmonary bypass equipment. II. Mechanisms for reduced complement activation in vivo. J Thorac Cardiovasc Surg 1999;117:803–9.
6. Fitch JC, Rollins S, Matis L, et al. Pharmacology and biological efficacy of a recombinant, humanized, single-chain antibody C5 complement inhibitor in patients undergoing coronary artery bypass graft surgery with cardiopulmonary bypass. Circulation 1999;100:2499–506.
7. Undar A, Eichstaedt HC, Clubb FJ Jr, et al. Novel anti-factor D monoclonal antibody inhibits complement and leukocyte activation in a baboon model of cardiopulmonary bypass. Ann Thorac Surg 2002;74:355–62.
8. Lappegård KT, Fung M, Bergseth G, et al. Effect of complement inhibition and heparin coating on artificial surface-induced leukocyte and platelet activation. Ann Thorac Surg 2004;77:932–41.
9. Lappegård KT, Fung M, Bergseth G, Riesenfeld J, Mollnes TE. Artificial surface-induced cytokine synthesis: effect of heparin coating and complement inhibition. Ann Thorac Surg 2004;78: 38–44.
10. Korn RL, Fisher CA, Livingston ER, et al. The effects of Carmeda Bioactive Surface on human blood components during simulated extracorporeal circulation. J Thorac Cardiovasc Surg 1996;111:1073–84.
11. Hatori N, Yoshizu H, Haga Y, et al. Biocompatibility of heparin-coated membrane oxygenator during cardiopulmonary bypass. Artif Organs 1994;18:904–10.
12. Nichols AB, Owen J, Grossman BA, Marcella JJ, Fleisher LN, Lee MM. Effect of heparin bonding on catheter-induced fibrin formation and platelet activation. Circulation 1984;70: 843–50.
13. Yoshikai Y. Roles of prostaglandins and leukotrienes in acute inflammation caused by bacterial infection. Curr Opin Infect Dis 2001;14:257–63.
14. Kontogiorgis CA, Hadjipavlou-Litina DJ. Non steroidal anti-inflammatory and anti-allergy agents. Curr Med Chem 2002; 9:89–98.
15. Finch AM, Wong AK, Paczkowski NJ, et al. Low-molecular-weight peptidic and cyclic antagonists of the receptor for the complement factor C5a. J Med Chem 1999;42:1965–74.
16. Mastellos D, Papadimitriou JC, Franchini S, Tsonis PA, Lambris JD. A novel role of complement: mice deficient in the fifth component of complement (C5) exhibit impaired liver regeneration. J Immunol 2001;166:2479–86.
17. Mallik B, Spruce L, Katragadda M, Carafides C, Morikis D, Lambris JD. Design and characterization of active hybrid peptide-peptidomimetic analogs of compstatin. Manuscript in preparation 2004.
18. Sahu A, Kay BK, Lambris JD. Inhibition of human complement by a C3-binding peptide isolated from a phage-displayed random peptide library. J Immunol 1996;157:884–91.
19. Morikis D, Soulika AM, Mallik B, Klepeis JL, Floudas CA, Lambris JD. Improvement of the anti-C3 activity of compstatin using rational and combinatorial approaches. Biochem Soc Trans 2004;32:28–32.
20. Mollnes TE, Riesenfeld J, Garred P, et al. A new model for evaluation of biocompatibility: combined determination of neoepitopes in blood and on artificial surfaces demonstrates reduced complement activation by immobilization of heparin. Artif Organs 1995;19:909–17.
21. Mollnes TE, Brekke OL, Fung M, et al. Essential role of the C5a receptor in E coli-induced oxidative burst and phagocytosis revealed by a novel lepirudin-based human whole blood model of inflammation. Blood 2002;100:1869–77.
22. Mollnes TE, Lea T, Froland SS, Harboe M. Quantification of the terminal complement complex in human plasma by an enzyme-linked immunosorbent assay based on monoclonal antibodies against a neoantigen of the complex. Scand J Immunol 1985;22:197–202.
23. Videm V, Svennevig JL, Fosse E, Semb G, Osterud A, Mollnes TE. Reduced complement activation with heparin-coated oxygenator and tubings in coronary bypass operations. J Thorac Cardiovasc Surg 1992;103:806–13.
24. Wachtfogel YT, Harpel PC, Edmunds LH Jr, Colman RW. Formation of C1s-C1-inhibitor, kallikrein-C1-inhibitor, and plasmin-alpha 2-plasmin-inhibitor complexes during cardiopulmonary bypass. Blood 1989;73:468–71.
25. Caldwell EE, Andreasen AM, Blietz MA, et al. Heparin binding and augmentation of C1 inhibitor activity. Arch Biochem Biophys 1999;361:215–22.
26. Sanchez J, Elgue G, Riesenfeld J, Olsson P. Control of contact activation on end-point immobilized heparin: the role of antithrombin and the specific antithrombin-binding sequence. J Biomed Mater Res 1995;29:655–61.
27. Levy O, Jean-Jacques RM, Cywes C, et al. Critical role of the complement system in group B streptococcus-induced tumor necrosis factor alpha release. Infect Immun 2003;71: 6344–53.
28. Jormalainen M, Vento AE, Wartiovaara-Kautto U, Suojaranta-Ylinen R, Ramo OJ, Petaja J. Recombinant hirudin enhances cardiac output and decreases systemic vascular resistance during reperfusion after cardiopulmonary bypass in a porcine model. J Thorac Cardiovasc Surg 2004;128:189–96.
29. Solberg R, Scholz T, Videm V, Okkenhaug C, Aasen AO. Heparin coating reduces cell activation and mediator release in an in vitro venovenous bypass model for liver transplantation. Transpl Int 1998;11:252–8.
30. Takano T, Cybulsky AV, Yang X, Aoudjit L. Complement C5b-9 induces cyclooxygenase-2 gene transcription in glomerular epithelial cells. Am J Physiol Renal Physiol 2001;281: F841–50.
31. Crooks SW, Stockley RA. Leukotriene B4. Int J Biochem Cell Biol 1998;30:173–8.

Ann Thorac Surg
2005;79:917–23

LAPPEGÅRD ET AL
BIOCOMPATIBILITY AND EICOSANOIDS

923

CARDIOVASCULAR

32. Marleau S, Fruteau de Laclos B, Sanchez AB, Poubelle PE, Borgeat P. Role of 5-lipoxygenase products in the local accumulation of neutrophils in dermal inflammation in the rabbit. J Immunol 1999;163:3449–58.

33. Marder P, Sawyer JS, Froelich LL, Mann LL, Spaethe SM. Blockade of human neutrophil activation by 2-2[2-propyl-3-[3-[2-ethyl-4-(4-fluorophenyl)-5-hydroxyphenoxy]propoxy]-phenoxy]benzoic acid (LY293111), a novel leukotriene B4 receptor antagonist. Biochem Pharmacol 1995;49:1683–90.

34. Pawlowski NA, Abraham E, Hamill A, Scott WA. The cyclo-oxygenase and lipoxygenase activities of platelet-depleted human monocytes. J Allergy Clin Immunol 1984;74:324–30.

35. Claesson HE, Lundberg U, Malmsten C. Serum-coated zymosan stimulates the synthesis of leukotriene B4 in human polymorphonuclear leukocytes. Inhibition by cyclic AMP. Biochem Biophys Res Commun 1981;99:1230–7.

36. Minkes M, Stanford N, Chi MM, et al. Cyclic adenosine 3′,5′-monophosphate inhibits the availability of arachidonate to prostaglandin synthetase in human platelet suspensions. J Clin Invest 1977;59:449–54.

37. Kennedy MS, Stobo JD, Goldyne ME. In vitro synthesis of prostaglandins and related lipids by populations of human peripheral blood mononuclear cells. Prostaglandins 1980;20:135–45.

38. Schroder JM. The monocyte-derived neutrophil activating peptide (NAP/interleukin 8) stimulates human neutrophil arachidonate-5-lipoxygenase, but not the release of cellular arachidonate. J Exp Med 1989;170:847–63.

39. Øvrum E, Tangen G, Tølløfsrud S, Ringdal MA. Heparin-coated circuits and reduced systemic anticoagulation applied to 2500 consecutive first-time coronary artery bypass grafting procedures. Ann Thorac Surg 2003;76:1144–8.

40. Svenmarker S, Haggmark S, Jansson E, et al. Use of heparin-bonded circuits in cardiopulmonary bypass improves clinical outcome. Scand Cardiovasc J 2002;36:241–6.

Southern Thoracic Surgical Association: Fifty-Second Annual Meeting

The Fifty-Second Annual Meeting of the Southern Thoracic Surgical Association (STSA) will be held November 10–12, 2005, in Orlando, Florida.

Members wishing to participate in the Scientific Program should submit an abstract by April 8, 2005, 12:00 Midnight, Central Daylight Time. Abstracts must be submitted electronically. Instructions for the abstract submission process can be found on the STSA Web site at www.stsa.org; on the CTSNet Web site at www.ctsnet.org; or in the back of the issue of *The Annals of Thoracic Surgery*.

Manuscripts accepted for the Resident Competition must be submitted to the STSA headquarters office no later than September 15, 2005. The Resident Award will be based on abstract, presentation, and manuscript.