Formation of C1,-C1-Inhibitor, Kallikrein-C1-Inhibitor, and Plasmin-α2-Plasmin-Inhibitor Complexes During Cardiopulmonary Bypass

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Stimulation of platelets and neutrophils occurs during clinical cardiopulmonary bypass. We investigated whether the classical complement, contact, or fibrinolytic pathways are activated under bypass conditions. Using enzyme-linked immunosorbent “sandwich” assays specific for C1,-C1-and kallikrein-C1-inhibitor complexes respectively, we found that there was a modest increase in plasma levels of each complex after clinical cardiopulmonary bypass was completed. The increased concentration of enzyme-inhibitor complexes reverted to baseline within 24 hours. Since these complexes are cleared in vivo, we measured their formation by assaying their plasma levels during in vitro simulated extracorporeal circulation. Over a period of two hours, C1,-C1-inhibitor complexes rose from a baseline of 2 ± 1 nmol/L to 21 ± 2 nmol/L, and kallikrein-C1-inhibitor complexes rose from 2 ± 1 nmol/L to 25 ± 5 nmol/L. However, there was no evidence of either in vivo or in vitro plasmin-α2-plasmin-inhibitor complex formation. These results indicate that the pathways of classical complement and contact activation, but probably not fibrinolysis, may be associated with neutrophil activation seen during clinical cardiopulmonary bypass.

DURING CARDIOPULMONARY BYPASS there is extensive contact between blood anticoagulated with heparin and synthetic surfaces of the extracorporeal circuit. Consequences of blood cell activation and plasma protein alterations prolong the bleeding time, increase postoperative blood loss, and may lead to pulmonary abnormalities. Extracorporeal circulation has been associated with major qualitative and quantitative alterations of platelets, including thrombocytopenia, reduced sensitivity to aggregating agents, platelet aggregate formation, decreased α2-adrenergic and fibrinogen receptors, secretion of thromboxane A2, and depletion of α-granule contents. Under bypass conditions, neutrophils release lysosomal hydrolases, the specific granule constituent, lactoferrin, the azurophilic neutrophils release lysosomal hydrolases, the specific granule constituent, lactoferrin, the azurophilic

PHYSICAL AND CHEMICAL AGENTS

Clinical cardiopulmonary bypass (CCB) circuits contained a centrifugal pump (Biomedicus, Eden Prairie, MN), bubble oxygenator (BOS-102; Bentley Labs, Irvine, CA), arterial line filter (model 1331) and cardiomyocyte reservoirs (Extracorporeal, Valley Forge, PA). Simulated extracorporeal circulation (SECC) perfusion circuits (surface area of 0.9 m2) included a spiral coil membrane oxygenator model 0800-2A (surface area of 0.8 m2) and a venous reservoir (model R-500-4), and were obtained from Sci-Med Life Systems, Minneapolis. Silicone tubing was obtained from Dow Chemical, Midland, MI. Blood was obtained from Upjohn, Kalamazoo, MI. Urokinase was obtained from Abbott Laboratories, Chicago, IL. This article must therefore be hereby marked in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

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connectors, and spiral coil membrane oxygenators as previously described. Blood and gas compartments were flushed with 100% carbon dioxide for 15 minutes before priming the circuit. Oxygenators were primed by applying a vacuum to the lower gas port. Five hundred milliliters of blood were drawn from random donors (who had abstained from any medications for 2 weeks) directly into circuit venous reservoirs containing heparin (5.0 U/mL blood) and glucose (3.3 mg/mL blood). Blood was permitted to enter the reservoir by gravity without foaming, and any air bubbles were removed from the system before starting the recirculation by a precisely shimmed, barely occlusive roller pump at a rate of 1.0 L/min for various lengths of time up to 120 minutes. The oxygenators were ventilated with a 95% oxygen-5% carbon dioxide mixture at a rate of 2.4 L/min. Blood temperature was maintained at 37°C by immersing the reservoir bag in a constant-temperature water bath. Plasma was harvested from blood samples drawn either directly from the donor (3.3 mg/mL blood) or from the reservoir at various times during recirculation as previously described.

Assays for measuring plasma enzyme-inhibitor complex formation. Enzyme-linked immunosorbent assays were performed as described, measuring Cl,-C1-inhibitor,26 kallikrein-C1-inhibitor,27 and plasmin-α2-plasmin-inhibitor complexes.

Statistical analysis. An analysis of variance (ANOVA) was used to test for significant differences between groups.28 In all cases ± values indicate the mean ± the SEM.

RESULTS

When either kallikrein or Cl1 is formed, the enzyme reacts with Cl-inhibitor to form an enzyme-inhibitor complex that can be detected by double antibody enzyme-linked immunosorbent assays. Similarly, plasmin forms a stable complex with α2-plasmin-inhibitor. We used three sensitive and specific assays to document whether activation of these enzymes occurs during extracorporeal circulation.

Clinical cardiopulmonary bypass. Levels of Cl,-Cl-inhibitor complex formed were found to be 12 ± 2 nmol/L prebypass, 10 ± 1 nmol/L after 45 minutes of bypass, 24 ± 4 nmol/L by 60 minutes postbypass, and 8 ± 1 nmol/L by 24 hours postbypass (Fig 1). The concentration of kallikrein-

DISCUSSION

Cardiopulmonary bypass is performed in approximately 200,000 Americans annually. Stimulation of platelets and neutrophils occurs as a direct result of the blood-surface interaction. The consequences of this cellular activation prevent the application of prolonged extracorporeal recircu-
lation to the treatment of severe pulmonary injury. In addition, anticoagulation with heparin, degranulation, and membrane alterations of platelets and neutrophils result in much of the morbidity associated with extracorporeal bypass. In this study we chose to examine the possible mechanisms of neutrophil activation. We postulated that activation of the classical complement, contact, and/or fibrinolytic pathways during cardiopulmonary bypass would produce agonists capable of causing neutrophil degranulation. Due to the probable in vivo clearance of the enzyme-inhibitor complexes, we also measured the formation of the above complexes in a well-defined simulated extracorporeal circulation system, which allows the blood-surface interaction to occur in the absence of hepatic or reticuloendothelial cell clearance.

Neutrophil activation occurs in response to the plasma chemotaxin, C5a. Since the active component of C5 is common to both the alternative and classical pathways of complement, elevation of C3a levels during CCB has led investigators to implicate activation of the alternative pathway. However, the classical complement pathway must now be considered, since levels of C1,-C1-inhibitor complex, unique to the classical system, are elevated as a result of the extracorporeal circulation in both CCB and SECC (Figs 1 and 2). It is apparent that in vivo clearance acts to mask the full extent of the activation that has occurred.

Two components of the intrinsic pathway of contact coagulation, human plasma kallikrein and factor XIa, stimulate neutrophils to aggregate and degranulate. Additionally, kallikrein induces neutrophil chemotaxis and aortic glycolysis. Levels of kallikrein-C1-inhibitor complex are elevated as a result of extracorporeal circulation (Figs 1 and 2) indicating that plasma prekallikrein has been converted to the active enzyme. Kallikrein activates factor XII to factor XIIa and subsequently to factor XII fragments; factor XII fragments activate the classical complement pathway. Surface contact also activates factor XII directly. Thus, the contact coagulation system, both directly through factor XIIa and kallikrein, and indirectly through the classical complement pathway (C5a), has the potential to activate neutrophils.

Kallikrein-C1-inhibitor complexes form much faster than the C1,-C1-inhibitor complexes (Fig 2). This effect is probably due to a combination of two factors. First, the second order rate constant for the formation of kallikrein-C1-inhibitor complexes is $3.0 \times 10^5$ mol/L$^{-1}$s$^{-1}$ while that for C1,-C1-inhibitor complexes is $4.4 \times 10^4$ mol/L$^{-1}$s$^{-1}$. Second, the rate of activation for prekallikrein by factor XIIa is accelerated by high molecular weight kininogen while that of C1 by activated factor XII is not. Therefore, kallikrein formation is probably more rapid than that of C1. In addition, C1 must then activate C2 and C4 which in turn activate C3 and finally C5 to form C5a.

Levels of plasmin-$\alpha$-plasmin-inhibitor complex did not change in either CCB or SECC. This rules out fibrinolysis during SECC. In this system the lack of endothelial cell products, such as tissue plasminogen activator and/or urokinase, may explain the absence of fibrinolytic system activation. However, in CCB the rate of clearance may completely mask the production of plasmin-$\alpha$-plasmin-inhibitor complex and prevent detection of elevated levels. Thus, while we found no evidence of activation of the fibrinolytic system in SECC, we cannot exclude that possibility in CCB.

Levels of C1,-C1-inhibitor rose significantly within 60 minutes postbypass to 20 ± 4 nmol/L but returned to a normal level of 8 ± 1 nmol/L by 24 hours postbypass (Fig 1). However, levels of both kallikrein-C1-inhibitor and plasmin-$\alpha$-plasmin-inhibitor complexes showed no significant rise at any time postbypass (Fig 1). The mechanism of activation of the complement cascade is unknown, but formation of heparin-protease complexes in vitro is known to activate the classical complement pathway. Moreover, two of 48 patients, undergoing cardiopulmonary bypass in a prospective study, presented with a sudden rise of airway pressure, acute pulmonary hypertension, and systemic hypotension shortly after protamine injection, consistent with activation of the complement and/or kinin forming systems. Previous work has shown that cardiopulmonary bypass with either bubble or membrane oxygenators sharply increases plasma levels of C3a and causes sequestration of leukocytes within the lungs. Although Collett et al reported direct evidence of alternative pathway activation, most investigators do not observe a consistent reduction of C4 and thus conclude that complement is activated by the alternative pathway.

This study provides the first evidence that cardiopulmonary bypass activates both the contact and classical complement pathways. Presumably both pathways are initiated by activation of factor XII on the surface of the bypass circuit. Activation of both pathways during cardiopulmonary bypass suggests, but does definitively prove, that components of the contact enzyme system are wholly or partially responsible for the previously observed neutrophil degranulation. The use of specific inhibitors of factor XIIa, kallikrein, or C1, may help to delineate the role of these pathways in cardiopulmonary bypass.

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