Total Complement Inhibition
An Effective Strategy to Limit Ischemic Injury During Coronary Revascularization on Cardiopulmonary Bypass

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Background—Activation of complement during revascularization of ischemic myocardium accentuates myocardial dysfunction. Soluble human complement receptor type 1 (sCR1) is a potent inhibitor of complement, as are heparin-bonded (HB) cardiopulmonary bypass (CPB) circuits. This study sought to determine whether total complement inhibition with the combination of sCR1 and HB-CPB limits damage during the revascularization of ischemic myocardium.

Methods and Results—In 40 pigs, the second and third diagonal coronary arteries were occluded for 90 minutes, followed by 45 minutes of cardiopulmonary arrest and 180 minutes of reperfusion. In 10 pigs, sCR1 (10 mg/kg) was infused 5 minutes after the onset of coronary occlusion (sCR1), 10 received HB-CPB only (HB-CPB), 10 received sCR1 and HB-CPB (sCR1+HB), and 10 received neither sCR1 or HB-CPB (unmodified). Addition of sCR1 to the HB group resulted in less myocardial tissue acidosis (ΔpH = −0.72±0.03 for unmodified; −0.46±0.05* for HB; −0.18±0.04** for sCR1; −0.13±0.01*** for sCR1+HB), better recovery of wall motion scores (4 = normal to −1 = dyskinesia; 1.67±0.17 for unmodified; 2.80±0.08* for HB; 3.35±0.10** for sCR1; 3.59±0.08*** for sCR1+HB), less lung water accumulation (5.46±0.28% for unmodified; 2.39±0.34%* for HB; 1.22±0.07%** for sCR1; 1.24±0.13%*** for sCR1+HB), and smaller infarct size (area necrosis/area risk=44.6±0.7% for unmodified; 33.2±1.9%* for HB; 19.0±2.4%*** for sCR1; 20.1±0.8%*** for sCR1+HB) (*P<0.05 versus unmodified; †P<0.05 versus unmodified and HB groups).

Conclusions—Total complement inhibition with sCR1 and sCR1+HB circuits optimizes recovery during the revascularization of ischemic myocardium. (Circulation. 1999;100:1438-1442.)

Key Words: heparin myocardial infarction

Activation of the complement system during cardiopulmonary bypass (CPB) can result in postoperative myocardial dysfunction and increased lung water accumulation, which increases morbidity and prolongs hospital stay.1,2 Interventions that lower levels of complement activation during CPB may contribute to better patient outcomes after cardiac surgery.

Recent advances in heparin bonding and coating techniques have resulted in development of heparin-bonded (HB) CPB circuits, which have been shown to reduce complement levels by mechanically rendering the foreign surface of the CPB circuit inaccessible to the adsorption of complement.3 Our experimental and clinical studies have shown that these circuits limit myocardial necrosis, minimize blood loss and the need for blood products, and contribute to better patient outcomes after both elective and emergent cardiac surgery.4−6 HB coating, however, cannot completely inhibit complement activity because it cannot prevent the activation that occurs on the gas-surface interface of the CPB circuit7 and the activation of complement that occurs during regional ischemia.8 Hence, use of a complement inhibitor in addition to HB circuits might further improve their biocompatibility and limit ischemic damage.

Soluble human complement receptor type 1 (sCR1), a recombinant form of human complement receptor, is a potent inhibitor of both the classic and alternative pathways of complement activation.9 It has been shown to reduce infarct size in rats and limit reperfusion edema after lung transplants in the pig.9,10 In a recent study using a porcine model of acute coronary occlusion and reperfusion with cardiopulmonary arrest on CPB, we demonstrated that pretreatment with sCR1 significantly decreased infarct size and preserved regional wall motion.11

This experimental study was therefore undertaken to determine whether achieving total complement inhibition with a combination of sCR1 and HB-CPB would limit myocardial damage during the revascularization of acutely ischemic myocardium.

Methods

Randomization
Forty pigs were entered into the study and randomized to receive HB circuits, sCR1, sCR1+HB circuits, or no sCR1 or HB circuits (unmodified). Four pigs were excluded from the study: 2 had bilateral lobar pneumonia, 1 had pericarditis, and 1 developed intractable hypotension after anesthesia before coronary occlusion.

Received March 5, 1999; revision received May 18, 1999; accepted May 26, 1999.

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Preparation
Adult pigs (34 to 38 kg) were premedicated with ketamine (15 mg/kg IM) and xylazine (0.5 mg/kg IM), anesthetized with α-chloralose (75 mg/kg), and placed on positive-pressure endotracheal ventilation. After a median sternotomy and systemic heparinization (3 mg/kg), the second and third diagonal branches just distal to the takeoff of the left anterior descending artery were occluded with snares for 90 minutes. Pigs were then placed on CPB, followed by 45 minutes of multidose, antegrade/retrograde, cold-blood cardioplegic arrest (potassium 25 mEq/L, hematocrit 20%, pH 7.6, temperature 4°C) supplemented with topical hypothermia. After the aortic cross clamp was removed, the coronary snares were released, and all hearts were repurified for 180 minutes on CPB at 37°C.

Treatment Groups

Unmodified Group
Ten pigs received neither sCR1 or HB circuits. CPB was instituted by use of noncoated oxygenators and tubing (Membrane Oxygenator, Sarns Inc).

HB Group
No sCR1 was given to 10 pigs, but they received a completely heparinized CPB circuit (Durallo II heparin-coated circuit, Baxter-Bentley Laboratories Inc). This included heparin coating of all cannulas, arterial filters, and the cardiotomy reservoir.

cr1 Group
sCR1 (10 mg/kg IV, Avant Immunotherapeutics, Inc) was infused in 10 pigs over 30 minutes, beginning 5 minutes after the coronary arteries were snared. This plasma concentration of sCR1 has previously been shown to prevent complement activation in a porcine model.11 These pigs received nonbonded CPB circuits.

sCR1+HB Group
Ten pigs received both sCR1 and HB-CPB circuits.

Measurements and Statistical Analyses
Total hemolytic complement activity was determined by a modification of the method of Mayer.12 All complement titers were expressed as a percentage of preischemic values. Previous in vitro studies have demonstrated that human sCR1 is able to completely inactivate both the classic and alternative pathways of porcine complement.13 The concentrations of sC5b-9 in pig plasma samples, referred to as C5b-9 in the text, were determined by use of a dual monoclonal antibody enzyme immunoassay similar to a previously published method.14 The monoclonal antibodies are specific for components of activated complement.15 The monoclonal antibodies were purchased from Cappel (Lancaster, PA). The monoclonal antibodies were specific for human C9 neoanetant (clone aE11, Dako) and human C7 (Quidel) and were demonstrated to cross-react with components of activated porcine complement. Increased concentrations of C5b-9 are reported in arbitrary units per millimeter and directly reflect complement activation in vivo. Two data points from a single unmodified pig were excluded as statistical outliers (>5 SD).

Myocardial tissue pH was measured with a pH probe (Khuri Tissue Ischemia Monitor, Vascular Technology Inc) and standardized according to myocardial temperature as previously described.15 pH values were expressed as the change in pH from preischemic values, recorded for each experiment, and then averaged for all experimental groups.

Echocardiographic short- and long-axis sections were used to determine wall motion changes in the area of risk with previously described techniques.15 A numerical score was used (4 = normal, 3 = mild hypokinesis, 2 = moderate hypokinesis, 1 = severe hypokinesis, 0 = akinesia, −1 = dyskinesia) to indicate the degree of wall motion abnormalities. The sections were interpreted by an experienced echocardiographer in a blinded fashion, and the scores were averaged for coronary occlusion and reperfusion periods for all experimental groups.

Lung water accumulation was assessed by use of wet-to-dry weight ratios. Lung samples were excised with a stapler before CPB and after 3 hours of reperfusion. Lung tissue was weighed before and after 48 hours of incubation at 100°C. Results were expressed as the percentile weight gain from pre-CPB values for each experiment and then averaged for each group.

The areas of risk and necrosis were determined by histochemical staining techniques with triphenyltetrazolium chloride (Sigma Chemical Co) as described in our previous study.16 Stained myocardial slices were planimetered to obtain the area of risk compared with the total left ventricular mass and the percent area of infarct in that area of risk.

All values represent mean ± SE. Differences in measurements between the various groups and across time were assessed by repeated measurements of ANOVA. StatView 4.5 (Abacus Concepts Inc) was used to compute these analyses. Data were considered significant at P values less than 0.05.

All pigs received humane care in compliance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH publication 86-23, revised 1985).

Results

Serum Complement Activity
Total hemolytic complement activity (CH50) is shown in Figure 1. After 90 minutes of coronary occlusion, CH50 values were 3.30 ± 0.3% of preischemic values in sCR1 animals and 6.4 ± 1.2% in the sCR1+HB group (both P < 0.01 versus the unmodified and HB groups). This indicated that virtually no hemolytic complement activity was present in sCR1-treated pigs during coronary occlusion. After 180 minutes of reperfusion on CPB, CH50 levels were only 3.2 ± 0.6% in sCR1 pigs and 3.5 ± 1.0% in sCR1+HB pigs compared with 18.0 ± 2.5% in the unmodified and 23.0 ± 2.9% in the HB group (P < 0.01 versus sCR1 and sCR1+HB).

Figure 2 summarizes the C5b-9 levels during coronary occlusion and reperfusion on CPB. Before coronary occlusion, there was no difference in C5b-9 levels among the 4 groups. After 90 minutes of coronary occlusion, C5b-9 levels decreased significantly in the sCR1 (11.6 ± 3.3 versus 2.8 ± 1.9 U/mL, P < 0.001) and sCR1+HB (13.9 ± 5.1 versus 0.0 ± 0.0 U/mL, P < 0.0001) groups. In contrast, C5b-9 levels increased significantly in the unmodified (12.6 ± 4.1 versus 20.4 ± 8.9 U/mL, P < 0.01) and HB (8.4 ± 1.6 versus 25.5 ± 5.7 U/mL).
U/mL, $P<0.01$) groups. C5b-9 levels continued to rise significantly in the unmodified group and reached its highest levels 180 minutes after reperfusion on CPB (73.5±13.2 U/mL). C5b-9 levels increased only slightly during CPB in the HB group and were significantly lower than the unmodified group (33.3±2.9 U/mL, $P<0.01$ versus the unmodified group). Hearts treated with sCR1 and sCR1+HB-CPB had virtually no circulating C5b-9 after 180 minutes of reperfusion.

Myocardial Tissue pH

Before coronary occlusion, myocardial tissue pH was similar in all groups (7.32±0.04 for unmodified, 7.31±0.05 for HB, 7.29±0.04 sCR1, and 7.33±0.05 for sCR1+HB; $P=NS$). The changes in pH after coronary occlusion are shown in Figure 3. Hearts treated with sCR1 had significantly less tissue acidosis than those in the unmodified and HB groups after 90 minutes of coronary occlusion ($\Delta$pH = −0.85±0.03 for unmodified, −0.70±0.05 for HB [$P<0.05$ versus unmodified], −0.30±0.05 for sCR1 [$P<0.01$ versus unmodified and HB], and −0.27±0.04 for sCR1+HB [$P<0.01$ versus unmodified and HB]). After 180 minutes of reperfusion, HB hearts had significantly less tissue acidosis compared with the unmodified group ($\Delta$pH = −0.72±0.03 for unmodified versus −0.46±0.05 HB; $P<0.03$). The least tissue acidosis was seen in the sCR1 ($−0.18±0.04, P<0.01$ versus unmodified and HB groups) and sCR1+HB ($−0.13±0.04, P<0.01$ versus unmodified and HB groups).

Wall Motion Scores

Wall motion scores for the area at risk are summarized in Figure 4. Ninety minutes of coronary occlusion resulted in significant depression in wall motion scores in both the unmodified (1.20±0.16, $P<0.001$ versus preischemia) and HB (2.03±1.17, $P<0.001$ versus preischemia) groups. In contrast, wall motion scores were significantly higher in both the sCR1 and sCR1+HB groups (3.30±0.14 and 3.15±0.07, $P<0.05$ versus unmodified and HB groups). Wall motion remained depressed in the unmodified hearts after 180 minutes of reperfusion but improved significantly in the HB group (1.67±0.17 versus 2.80±0.08, $P<0.05$). However, the best preservation of wall motion scores were seen in both the sCR1 and sCR1+HB groups (3.35±0.10 versus 3.59±0.08, $P<0.05$ versus both the unmodified and HB groups).

Lung Water Content and Infarct Size

The greatest accumulation of lung water occurred in the unmodified group (5.46±0.28%). Although pigs treated with HB circuits had significantly less water accumulation (2.39±0.34%, $P<0.01$ versus unmodified), the least water accumulation was seen in the sCR1 and sCR1+HB groups (1.22±0.07% and 1.24±0.13%, both $P<0.01$ versus the unmodified and HB groups).

The area of myocardium at risk was similar in all groups (17.2±1.3% for unmodified, 16.5±0.9% for HB, 17.1±1.4% for sCR1, and 16.8±1.9% for sCR1+HB; $P=NS$). The amount of myocardial necrosis in the area at risk was greatest in the unmodified group (44.6±0.7%). Hearts treated with HB circuits alone had a significantly lower area of necrosis (33.2±1.89%, $P<0.01$ versus unmodified). The lowest area of necrosis was seen in the sCR1 and sCR1+HB groups (19.0±2.4 and 20.0±1.0, $P<0.01$ versus both the unmodified and HB groups).

Discussion

Growing evidence suggests that the inflammatory response that results from complement activation during CPB contributes to postoperative myocardial dysfunction and poor clini-
C5b-9 is an important mediator of ischemic tissue injury and can directly result in myocardial dysfunction by altering intracellular Ca²⁺ and creating water and electrolyte imbalances in the myocyte. Increased levels of C5b-9 have been observed in the plasma of patients after an acute myocardial infarction and can be seen deposited in myocardial and endothelial cells in infarcted tissue and after reperfusion of ischemic myocardium. Formation of C5b-9 directly alters endothelial function by creating transmembrane channels that may ultimately result in cell lysis. Even nonlytic amounts of C5b-9 can result in the release of oxygen free radicals, prostaglandins, and leukotrienes and secretion of cytokines. Complement activation and C5b-9 formation have also been shown to upregulate the leukocyte adherence protein P-selectin on endothelial cells, which is necessary for leukocyte endothelial cell adherence and subsequent transendothelial migration. Hence, complement activation may also play an important role in transcellular migration of leukocytes during ischemia and reperfusion.

HB-CPB circuits have been shown to lower complement levels, decrease neutrophil activation, and reduce serum levels of interleukin-6 and interleukin-8. In our porcine model of acute coronary occlusion and reperfusion with cardioplegic arrest on CPB, we demonstrated that HB circuits preserved regional wall motion, minimized tissue acidosis and lung edema, and reduced infarct size. Our experimental findings were corroborated by several clinical studies. Jansen and coworkers measured C5a and C5b-9 levels in patients with and without HB circuits undergoing coronary bypass surgery. Although C5a and C5b-9 levels were significantly elevated from pre-CPB levels in both groups, they were lower in the HB patients. HB patients had significantly better recovery scores that reflected fluid balance, weight gain, and postoperative intubation time. In a prospective clinical study involving 234 patients undergoing CABG, patients treated with HB circuits had a lower incidence of myocardial infarction, less need for inotropic support, a lower incidence of prolonged ventilation, and fewer postoperative complications. In a recent study involving 206 patients undergoing emergent CABG, patients treated with HB circuits required fewer homologous donor units and less inotropic support and had a lower incidence of perioperative myocardial infarctions and a shorter duration of ventilatory support, ICU, and hospital stays.

Another method of decreasing complement activation is the use of soluble inhibitors. sCR1 exerts its biologic actions by binding C3b and C4b to distinct sites, displacing the catalytic subunits from C3 and C5 convertases, and acting as a cofactor in promoting the degradation of C3b and C4b by factor I. By inhibiting complement activation by both the classic and alternative pathways, sCR1 directly inhibits C5b-9 production and indirectly inhibits the generation of interleukin-8, which has been associated with the “capillary-leak syndrome” seen in post-CPB patients. Although HB circuits result in significantly less complement activation than non-HB circuits, a large amount of complement is still generated. This is partly because heparin-coating techniques cannot inhibit the complement activation that occurs at the gas-surface interface. Generation of C3a is dependent on both the size of the gas and biomaterial surfaces. Larsson and coworkers showed the advantages of sCR1 in inhibiting both gas and biomaterial surfaces in artificial conditions resembling a CPB circuit. The addition of sCR1 to whole blood in tubing loops inhibited the production of both C3a and C5b-9. In contrast to C5b-9, which was almost completely inhibited by sCR1, ∼30% of C3a was unaffected. The results of this study strongly suggested that the addition of a complement inhibitor such as sCR1 could further improve the biocompatibility of HB-CPB circuits.

Our study confirms previous investigations showing that periods of regional ischemia can activate the complement cascade. Levels of C5b-9 were twice as great in the unmodified and HB groups after 90 minutes of coronary occlusion and 3.5 times higher than preischemic levels in the unmodified group after 180 minutes of CPB. The highest levels of C5b-9 were associated with the greatest lung water accumulation, poorest recovery of wall motion, most tissue acidosis, and largest infarct size. Although HB circuits decreased the activation of C5b-9 on CPB, the levels of complement were significantly higher than the sCR1-treated animals, and the recovery was not as good. The addition of sCR1 to the HB-treated pigs virtually abolished all complement activation and resulted in more complete recovery of ischemic damage. Because equal concentrations of heparin (3 mg/kg) were used in all study groups, heparin alone could not be responsible for the total complement inhibition seen in the sCR1 + HB groups.

Although both sCR1 and sCR1 + HB-CPB groups had similar recovery of regional wall motion and identical areas of infarct size, we continue to advocate the use of HB-CPB circuits despite the beneficial effects of sCR1 alone. HB circuits by themselves provide benefits unrelated to decreased complement activation that have resulted in less blood loss, have minimized the need for blood products, and have decreased the incidence of myocardial infarctions and strokes. In contrast, there is no evidence that sCR1 alone will decrease perioperative blood loss and the need for blood products.

Our data show that strategies that result in more complete inhibition of complement activation result in the least myocardial damage during the revascularization of acutely ischemic myocardium. The addition of sCR1 to HB-CPB and non–HB-CPB circuits will help decrease the deleterious effects of the increased inflammatory response associated with CPB. Inhibiting C5b-9 activation may better preserve endothelial function and prevent the accumulation of lung water and systemic weight gain that contribute to prolonged ventilatory support, myocardial dysfunction, and longer hospital stays. Clinical trials are being planned to determine whether the beneficial experimental results using the strategy of total complement inhibition with sCR1 and HB circuits...
will result in less morbidity and mortality in patients undergoing cardiac surgical procedures on CPB.

Acknowledgment
This work was supported in part by a grant from Avant Immunotherapeutics, Inc, Needham, Mass.

References
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Circulation. 1999;100:1438-1442
doi: 10.1161/01.CIR.100.13.1438

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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