Soluble Complement Receptor-1 Protects Heart, Lung, and Cardiac Myofilament Function From Cardiopulmonary Bypass Damage

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Background—Host defense system activation occurs with cardiopulmonary bypass (CPB) and is thought to contribute to the pathophysiological consequences of CPB. Complement inhibition effects on the post-CPB syndrome were tested with soluble complement receptor-1 (sCR1).

Methods and Results—Twenty neonatal pigs (weight 1.8 to 2.8 kg) were randomized to control and sCR1-treated groups. LV pressure and volume, left atrial pressure, pulmonary artery pressure and flow, and respiratory system compliance and resistance were measured. Preload recruitable stroke work, isovolumic diastolic relaxation time constant (τ), and pulmonary vascular resistance were determined. Pre-CPB measures were not statistically significantly different between the 2 groups. After CPB, preload recruitable stroke work was significantly higher in the sCR1 group (n=5, 46.8±3.2×10^3 vs n=6, 34.3±3.7×10^3 erg/cm^3, P=0.042); τ was significantly lower in the sCR1 group (26.4±1.5, 42.4±6.6 ms, P=0.003); pulmonary vascular resistance was significantly lower in the sCR1 group (5860±1360 vs 12 170±1200 dyn · s/cm^2, P=0.009); arterial Po2 in 100% FiO2 was significantly higher in the sCR1 group (406±63 vs 148±33 mm Hg, P=0.01); lung compliance and airway resistance did not differ significantly. The post-CPB Hill coefficient of atrial myocardium was higher in the sCR1 group (2.88±0.29 vs 1.88±0.16, P=0.023).

Conclusions—sCR1 meaningfully moderates the post-CPB syndrome, supporting the hypothesis that complement activation contributes to this syndrome. (Circulation. 2000;101:541-546.)

Key Words: cardiopulmonary bypass ■ diastole ■ myofilaments ■ calcium
pressure of 25 mm Hg and positive end-expiratory pressure of 3 mm Hg. A pneumotachometer (System 2600 Pediatric Pulmonary Cart, SensorMedics Corp) was placed in the ventilation circuit. Respiratory rate and inspired oxygen fraction were titrated to maintain a arterial Pco2 of 35 to 45 mm Hg and Po2 of 100 to 250 mm Hg. Sodium bicarbonate (8.5%, 10 to 15 mL/kg) was used to maintain a base excess between –3 and 3 mmol/L. Methyldprednisolone (25 mg/kg), given routinely to infants undergoing CPB in our institution, was given intravenously before CPB.

After a sternotomy was preformed, umbilical tape was placed around the superior vena cava and inferior vena cava and a 10-mm ultrasonic flow probe was placed around the main pulmonary artery (Transonic Systems Inc).

Ultrasonic crystals were sewn to the LV epicardium in the major and minor axes. Micromanometers (3F, Millar Instruments, Inc) were placed in the pulmonary artery, left atrium, and LV. The tip of the right atrial appendage and the LV apex were excised for in vitro experiments and protein characterization (see below).

Cardiopulmonary Bypass

Neonatal pigs in the sCR1 group were given sCR1 (10 mg/kg IV) before cannulation. Animals were given heparin (500 IU/kg), and arterial and venous cannulas (DLP, Inc) were placed. CPB was initiated 5 to 10 minutes after sCR1 infusion. The CPB circuit consisted of a Stockert Shiley roller pump (Shiley Inc model 10–10-00), Medtronic Minimax Plus oxygenator (Medtronic Inc), and Bio-Cal 370 heat exchanger (Bio-Medicus) to maintain piglet temperature at 37°C. The pump was primed with lactated Ringer's solution and fresh donor pig blood to maintain a circuit hematocrit of 18% to 22%. CPB flow rate was 100 mL·kg⁻¹·min⁻¹, and mean systemic arterial pressure was maintained at 50 to 60 mm Hg. The pigs were maintained on CPB for 90 minutes and then separated from CPB without the use of inotropic agents. Heparin was not reversed.

Data Acquisition

Before administration of sCR1 and after CPB, the blood gases were stabilized within the above limits (Surgical Procedures). LV pressure, left atrial pressure, pulmonary arterial pressure, right ventricular output, LV dimensions, arterial blood gases, heart rate, nasopharyngeal temperature, static pulmonary compliance, mean airway resistance, and systemic arterial blood pressure were recorded before CPB and at 5 minutes after CPB. Data were also recorded 30 and 60 minutes after CPB. Pressure and flow data were collected for 8 seconds (sampling rate, 500 Hz) in the presence of 3 mm Hg continuous positive airway pressure. Data for calculation of preload recruitable stroke work (PRSW) were collected for 16 seconds (sampling rate, 200 Hz) with gradual occlusion of the superior vena cava and inferior vena cava and a continuous positive airway pressure of 3 mm Hg.

At the completion of the in vivo experiment, right atrial and LV tissues were obtained to assess myofilament sensitivity to calcium and to examine the myofilament proteins (see below).

Data Analysis

Data were acquired and analyzed with software developed in-house. Pulmonary vascular resistance (PVR) was calculated. LV end-diastolic volume was obtained from the sonomicrometric data with the use of an ellipsoid model. PRSW was calculated as the slope of the LV pressure-volume loop. The time constant of LV pressure decay during isovolumetric relaxation (τ; References 13 and 14) was determined by fitting a single exponential function to the pressure waveform beginning at the minimum of the first derivative of LV pressure by nonlinear regression.

Isolated Muscle Studies

Preparations

The heart was washed in calcium-free physiological solution, then placed in skinnning solution (relaxing solution with 0.5% Triton X-100 added) for 30 minutes at 4°C. After CPB we used free-running atrial trabeculae (n = 12). The preparations had diameters of 70 to 300 μm (median 150 μm) and lengths of 0.5 to 2.8 mm (median 1.1 mm).

Solutions

Relaxing (pCa 9.0) and activating (pCa 4.5) solutions were prepared with the use of 10 mmol/L EGTA as a calcium buffer and 30 mmol/L/BES as a pH buffer. The compositions of the solutions were calculated with the use of a computer program to give an ionic strength of 190 mmol/L, pmg 3.14, pmgATP 2.50 at pH 7.10, 22°C. The stability constants were from Reference 18. The apparent stability constant used for Ca-EGTA, adjusted for ionic strength and temperature, was 3.702×10⁹ mol⁻¹.

The muscle bundle was placed in the cuvette of a Guth apparatus and illuminated with a He-Ne laser. Where first-order maxima of the diffraction pattern were discerned, the sarcomere length was set at 2.6 μm. Preparations whose first-order diffraction maxima were not discernible were stretched to what was judged to be a comparable amount.

The preparation was superfused with each of 12 test solutions (22°C, range pCa 8.0 to 4.75) until a steady-state force response was obtained (1 to 2 minutes). The force was recorded on disk using Digidata 1200 data acquisition system and Clampex software (Axon Instruments, Inc). A reading at pCa 8.0 was taken after every 3 or 4 measurements of active force to ascertain stability and provide a baseline for determining active force development. The preparation was then placed in sample buffer for SDS-PAGE and Western blot analysis (see below, Reference 20).

Data Analysis

The force versus free calcium data were fitted with the Hill equation: 

\[ F = F_\text{max} \frac{[Ca]^{n_H}}{[Ca]^{n_H} + K^{n_H}} \]

where \( F_\text{max} \) is the force at saturating calcium, \( K \) is the free calcium concentration at which \( F/F_\text{max} = 0.5 \), and \( n_H \) is the Hill coefficient; \( \text{pCa}_{\text{Ca}} = -\log_{10}(K) \).

The fit was carried out with the use of nonlinear least squares (Statgraphics-Plus version 7, Statistical Graphics Corp).

Muscle Proteins

The myocardial proteins were resolved with the use of SDS-PAGE. Western blots were probed with cardiac troponin I (TnI)- and troponin T (TnT)-specific antibodies. Statistical analysis was carried out using R (version 0.63) computer software.

Statistical Analysis

Values are given as mean ± SEM unless indicated otherwise. Results were considered statistically significant at a value of \( P \leq 0.05 \). Unless stated otherwise, we used ANCOVA, with pre-CBP values used as the covariate, to compare results obtained 5 minutes after CPB from the control and sCR1 groups. Where necessary to meet the assumptions underlying the statistical tests, appropriate transformation of the data (the reciprocal of compliance and the squared reciprocal of \( r \)) or nonparametric significance tests (Wilcoxon’s rank sum test for the Hill coefficient) were used. Means and standard errors are shown for the original (untransformed) data. Statistical analysis was carried out using R (version 0.63) computer software.

Results

Animals Studied

Eleven animals fulfilled the criteria for acceptance in the study. The following animals were excluded: 1 control animal died before CPB, 4 (2 control and 2 sCR1-treated) could not be separated from CPB, 1 sCR1-treated animal had persistent respiratory acidosis after CPB, and 1 control animal was hypothermic after CPB. Two other animals were excluded because of equipment failure: The blood gas machine malfunctioned for 1 sCR1-treated animal and the physiological data for 1 control animal were lost.
**In Vivo Data**

The pre-CPB values of the measured and computed variables were not significantly different (see Table 1). We used the data collected 5 minutes after CPB for determining the effect of sCR1 on the in vivo measures (Tables 1 and 2). Pre-CPB and post-CPB results as a function of time are shown in Figure 1.

**Preload Recruitable Stroke Work**

Pre-CPB baseline PRSW (combined mean for both groups) was $63.0 \pm 6.6 \times 10^3$ erg/cm$^3$. Post-CPB PRSW was significantly higher in the sCR1 group than in the control group: $46.8 \pm 3.2 \times 10^3$ erg/cm$^3$, n=5, versus $34.3 \pm 3.7 \times 10^3$ erg/cm$^3$, n=6 ($P=0.042$, see Table 2). Heart rates were similar in the 2 groups: 157±9 for sCR1, 165±9 for controls ($P=0.54$). During the 60 minutes after CPB, mean PRSW in the control group increased gradually, but the sCR1 group did not change (Figure 1).

**Time Constant of Isovolumic Relaxation**

Baseline pre-CPB combined mean for $t$ was 25.5±1.5 ms. Post-CPB means were 26.4±1.5 ms for the sCR1 group and 42.4±6.6 ms for the control group ($P=0.003$, Table 2), which indicated that sCR1 prevented CPB-induced slowing of ventricular relaxation. Heart rates did not differ significantly between the 2 groups (155±8 sCR1, 163±9 controls, $P=0.51$). Over the 60 minutes after CPB, $\tau$ in the sCR1 group increased slightly (see Figure 1).

**PVR and Oxygenation**

The pre-CPB baseline PVR (combined mean for PVR) was 2720±400 dyn·s/cm$^5$. Post-CPB PVR was significantly lower in the sCR1 group: 5860±1360 vs 12 170±1200 dyn·s/cm$^5$ ($P=0.009$, Table 2). After CPB, the arterial Po$_2$ in 100% FIO$_2$ was significantly higher in the sCR1 group (406±63 vs 148±33 mm Hg, $P=0.01$). Figure 1 illustrates PVR as a function of time after CPB.
Pulmonary Mechanics
Pre-CPB pulmonary compliance (combined mean) was 3.09 ± 0.23 mL/cm H$_2$O. Post-CPB compliance adjusted means obtained by ANCOVA were 1.94 mL/cm H$_2$O for the sCR1 group and 1.60 mL/cm H$_2$O for the control group ($P$ = 0.16). Pre-CPB and post-CPB respiratory resistance did not differ significantly ($P$ = 0.19, Table 2).

In Vitro Data
Sensitivity of Myofilaments to Calcium
Post-CPB pCa$_{50}$ did not differ between the 2 groups (6.08 ± 0.05, n = 5 sCR1, vs 6.09 ± 0.05, n = 7 control, $P$ = 0.83, Table 3), but the Hill coefficient ($n_h$) was significantly higher in the sCR1 group: 2.88 ± 0.29 versus 1.88 ± 0.16 ($P$ = 0.023, Table 3 and Figure 2). The higher $n_h$ suggests that sCR1 is effective in maintaining myofilament function in myocardium exposed to CPB.

Myofilament Proteins
The profiles of the myofilament proteins in atrial and ventricular myocardium from control and sCR1-treated groups demonstrated in pre-CPB and post-CPB specimens a single band of TnI with the same electrophoretic mobility (Figure 3), indicating no difference in cAMP-dependent phosphorylation$^{21}$ and no TnI proteolysis (Figure 3). Small amounts of a TnT proteolytic product were evident in post-CPB ventricular myocardium from 4 control and 3 sCR1-treated animals (see Figure 3).

Discussion
In the human neonate, the post-CPB syndrome is both frequent and severe. Clinically important generalized edema, abnormal lung compliance, and impaired LV filling affect up to 50% of infants undergoing CPB.$^{2,3,24}$ The mechanisms underlying the pathophysiological consequences of CPB remain to be established. CPB activates the host defense cascades.$^{1-3}$ The complement products increase vascular permeability, smooth muscle contraction, neutrophil activation, and cytosolic calcium concentration, leading to pathophysiological effects similar to those seen after CPB.$^{1,2,25,26}$

The classical and alternative complement pathways are inhibited by the membrane-bound CR1 and sCR1.$^4$ Recombinant sCR1 blocks activation of both complement pathways in vivo and in vitro,$^5$ decreases postischemia myocardial damage,$^3$ and moderates CPB-associated increase in PVR.$^6$ We used recombinant sCR1 in the neonatal pig to test the effect of blocking CPB-induced complement activation on heart and lung function.

Myocardial contraction and relaxation depend, among other things, on the instantaneous concentration of cytosolic calcium and on myofilament sensitivity to calcium. Myocardial ischemia alters these regulators of contraction.$^{27-29}$ To minimize the likelihood of ischemia and reperfusion injury, the CPB strategy used in this study included neither circulatory arrest nor aortic cross-clamp. Although our flow rates were similar to those used clinically, our use of normothermia
may have resulted in decreased energy stores. The interaction of all these effects could alter both systolic and diastolic function.

**Diastolic Function**

The time constant of isovolumic diastolic relaxation, \( \tau \), has been used in the piglet and other animals as a measure of diastolic function.\(^1\)\(^,\)\(^2\)\(^,\)\(^3\)\(^,\)\(^4\)\(^,\)\(^5\)\(^,\)\(^6\) Pigs exposed to CPB and deep hypothermic circulatory arrest showed evidence of slower ventricular relaxation.\(^6\) Our CPB study, which did not include hypothermic circulatory arrest, also showed a post-CPB increase in \( \tau \) in the controls, which suggests that CPB itself is a contributor to this increase. In a study of human neonates, we found that many had acquired diastolic dysfunction after CPB (measured by reversal of mitral diastolic inflow).\(^2\) In vitro exposure of isolated myocytes to hyperkalemia and hypothermia to mimic aspects of CPB strategies resulted in decreased cell shortening and relaxation rate. The relation of these changes in the myocyte to the in vivo effects of CPB remains to be established.

**Systolic Function**

PRSW has been used previously to characterize the decrease in LV systolic function (contractility) in the neonatal pig exposed to bypass because it is little affected by changes in loading conditions.\(^1\)\(^,\)\(^2\)\(^,\) The decrease in systolic function observed in our control piglets is similar in magnitude to that described previously.\(^6\) In contrast, in our study of post-CPB human neonates, we found abnormal systolic function.\(^2\) The apparent discrepancy is probably caused by inotropic agents being administered to all the infants but not to the pigs. Additionally, the echocardiographically derived ejection fraction used in the infants is more sensitive to alterations in \( t \) in the controls, which suggests that CPB itself is a contributor to this increase. In a study of human neonates, we found that many had acquired diastolic dysfunction after CPB (measured by reversal of mitral diastolic inflow). In vitro exposure of isolated myocytes to hyperkalemia and hypothermia to mimic aspects of CPB strategies resulted in decreased cell shortening and relaxation rate. The relation of these changes in the myocyte to the in vivo effects of CPB remains to be established.

**Myofilament Function and Structure**

**Function**

We used chemically skinned myocardial preparations to measure the sensitivity of the myofilaments to calcium: the relation between free calcium concentration and force (Reference 32, Figure 2) in terms of \( \text{PcA}_{\text{m}} \) (the \( \text{PcA} \) at half the force generated under a saturating calcium concentration) and \( n_{h} \) (a measure of the slope of the relation between force and \( \text{PcA} \)). Alterations in the myofilaments’ sensitivity to calcium can alter ventricular function. For example, \( \beta \)-tropomyosin overexpression increases the sensitivity of the myofilaments to calcium in vitro as measured by \( \text{PcA}_{\text{m}} \) and results in slower diastolic relaxation in vivo.\(^3\) \(^,\)\(^3\)

\( n_{h} \) was higher in the sCR1 group than in the controls (Figure 2). In the absence of a shift in the \( \text{PcA}_{\text{m}} \), a smaller \( n_{h} \) would be expected to result in a lower rate of diastolic relaxation. Our finding in the post-CPB sCR1 group of a higher \( n_{h} \) and faster isovolumic ventricular relaxation is consistent with the effect of sCR1 in maintaining normal interaction between the myofilaments and calcium and preserving ventricular diastolic function.

**Protein Structure**

We examined the myofilament proteins with the use of SDS-PAGE and Western blot analysis to search for possible mechanisms underlying the deleterious effects of CPB and the protective effects of sCR1. We were unable to demonstrate a difference in TnT proteolysis of post-CPB myocardium between the 2 groups, making it unlikely that proteolysis of myofilament proteins was a significant factor in the effects of sCR1 on post-CPB ventricular function in our piglets.

**Pulmonary Function**

CPB in the neonatal human and pig affects pulmonary function.\(^1\)\(^,\)\(^2\)\(^,\)\(^3\)\(^,\)\(^4\)\(^,\)\(^5\)\(^,\)\(^6\)\(^,\)\(^7\) In our previous study of the effects of CPB on the neonatal human, ventricular diastolic and pulmonary dysfunction in affected infants contributed to post-CPB morbidity, tripling the duration of mechanical ventilation after surgery. The significantly higher arterial \( \text{PO}_{2} \) after CPB in the sCR1 animals suggests that sCR1 protects lung function. Gillinov et al\(^6\) reported, in young pigs, that after CPB, PVR increased by 338% in control but only by 147% in sCR1-treated animals. Our results from neonatal pigs show very similar results: After CPB, PVR increased by 350% and 120% in the control and sCR1 groups, respectively.

**Summary**

This study demonstrates that sCR1 protects the myocardium and lungs from some of the deleterious effects of CPB. A greater effect is seen early after CPB. Given that sCR1 is a specific inhibitor of complement activation, our results support the hypothesis that complement activation is an important contributor to the post-CPB syndrome. The protection by sCR1 of ventricular diastolic function appears to be based, at least in part, on preservation of myofilament cooperativity at the sarcomere level. The decrease in the pathophysiological consequences of CPB in pigs that have received sCR1 suggests that sCR1 may be useful in decreasing the effects of CPB on the infant.

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