
Complement Activation During Long-Term Extracorporeal Membrane Oxygenation in Neonates

Edward M. Darling, William E. Harris, E. Shannon Cooper, James M. Hatchell, Allan A. Macphee

Ochsner Foundation Hospital
Division of Research
New Orleans, LA

Abstract

(*J. Extra-Corpor. Technol.* 20[1] pp. 19-23, 32 references, Spring 1988) Complement activation is known to occur in procedures where blood comes into contact with non-biological surfaces. We followed complement activation in 5 infants undergoing long term extracorporeal membrane oxygenation (ECMO). Duration of ECMO varied from 121 hours to 309 hours. Changes in plasma C3a, C4a, C5a, and serum CH₅₀ levels were measured before, during, and after ECMO. Upon initiation of ECMO, plasma C3a levels increased significantly during the first 2 hours. This was followed by a steady decline to pre-ECMO levels within 24 hours. Serum whole complement, measured by CH₅₀ units, dropped markedly by 10 minutes after initiation of ECMO, returning to pre-ECMO levels in 24 hours. Plasma levels of C5a (a factor with high affinity for neutrophils) did not change significantly; nor did plasma C4a (a factor produced via activation of the classical pathway) show significant changes. Chest X-ray images uniformly demonstrated the onset of dense pulmonary opacification during the first 24 hours of ECMO treatment. This condition slowly cleared and appeared normal at termination of ECMO. We conclude that current ECMO circuitry mediates complement activation in neonates, probably via the alternate pathway. Activation is primarily limited to the first hours of ECMO and is no longer evident after 24 hours.

Introduction

Extracorporeal handling of blood initiates the activation of several biological stress reaction systems, such as kinins, coagulation, fibrinolysis, and complement, due to the unavoidable contact between blood and foreign surfaces.¹⁻² Recent advances in the understand-

ing of the complement cascade and the properties of its activation products, C3a and C5a, have implicated complement activation in a variety of clinical sequelae, including respiratory distress and pulmonary edema.^{3,4} C3a and C5a are anaphalatoxins which stimulate the release of mast-cell histamine, contract smooth muscle, and increase vascular permeability.⁵ In addition, C5a binds with receptors on circulating neutrophils causing their aggregation, particularly in the pulmonary vasculature.⁶⁻⁸ This interaction initiates several cellular responses including superoxide generation, chemotaxis, and release of lysosomal enzymes.⁹ Evidence of complement activation has been reported in hemodialysis, apheresis, cardiopulmonary bypass (CPB), adult extracorporeal membrane CO₂ removal, and in storage of banked blood.¹⁰⁻¹⁸

Neonatal extracorporeal membrane oxygenation (ECMO) has emerged as an effective life-saving measure in acute respiratory failure for the 5%-10% of such infants who are unresponsive to conventional ventilatory and pharmacologic support. Our experiences with neonatal ECMO have consistently shown an increase of pulmonary edema (as revealed by chest x-rays) within 24 hours after the initiation of ECMO. If complement activation is occurring during neonatal ECMO, then the damaging effects of C3a and C5a could be a contributing factor to this observation.

We believe that complement activation may aggravate and prolong pre-existing pulmonary dysfunction in neonates who are undergoing ECMO. The objective of this report is to document the extent and duration of complement activation during neonatal ECMO.

Patients and Methods

Five newborn patients, 3 male and 2 female, who failed to respond to maximal ventilatory and pharmacological therapy were studied (Table 1). The decision to institute ECMO was made when the infants met strict selection criteria designed to predict a greater than 80% mortality, despite maximal conventional therapy. ECMO selection has been discussed in detail by the University of Michigan Hospitals, as well as others.¹⁹⁻²⁰

Direct communications to: Edward Darling, Department of Extracorporeal Technology, Ochsner Foundation Hospital, 1516 Jefferson Hwy., New Orleans, Louisiana 70121

TABLE I
CLINICAL DATA

Patient	Diagnosis	Hours on ECMO	Age at ECMO Initiation	Birth Weight (kg)	Outcome
A	MAS*	131	24 hr.	4.1	Survived
B	CDH*	309	19 hr.	4.2	Survived
C	MAS	121	40 hrs.	3.1	Survived
D	PPHN*	184	61 hrs.	4.3	Survived
E	CDH	129	34 hrs.	3.3	Survived

MAS = meconium aspiration syndrome, CHD = congenital diaphragmatic hernia, PPHN = persistent pulmonary hypertension

This study was approved by our Clinical Investigation Committee. Prior to initiation, parental informed consent was obtained for ECMO and the additional blood sampling for this study.

Patient Management

Venoarterial bypass was used in each case. Cannulation was carried out in the neonatal intensive care unit. Vascular access was made through the right internal jugular vein and the right common carotid artery by inserting modified chest tubes^a as cannulae to the right atrium and the aortic arch respectively. The ECMO circuit was assembled and primed according to the methods previously described by Bartlett and Gazzaniga.²¹ Briefly, a modified servo-regulated roller-pump,^b a 0.8m² silicon membrane oxygenator, Omnitherm^R heat exchanger,^c and 1/4 inch I.D. PVC tubing completed the extracorporeal circuit (Figure 1). The circuit's prime consisted of 2 units of saline-washed packed red blood cells, 100 ml THAM, 80 ml albumin (25%), 600 mg calcium gluconate, and 200 units heparin. The final extracorporeal circuit volume was approximately 500 ml with a hematocrit of 45-50% and a pH of 7.5. Initial flows were calculated at approximately 120 ml per kilogram per minute. Once ECMO was established, ventilator settings were reduced to non-toxic levels: FiO₂ less than 0.40, rate = 10 to 20 cycles per minute, peak inspiratory pressure (PIP) = 16 to 20 cm H₂O, and continues distending pressure = 3 to 4 cm H₂O. Patients were maintained at normothermic temperatures.

Activated clotting times (ACTS) were measured hourly and maintained at 200-250 seconds. Hematocrit and platelet counts were maintained with transfusions of saline-washed packed red blood cells, fresh frozen plasma, and/or platelets concentrates as necessary.

Chest x-rays were taken daily; lung improvement was

a Argyle,^R Sherwood Medical, St. Louis, MO 63103

b Cobe Laboratories, Lakewood, CO, 80215

c Sci-Med Life Systems, Minneapolis, MN

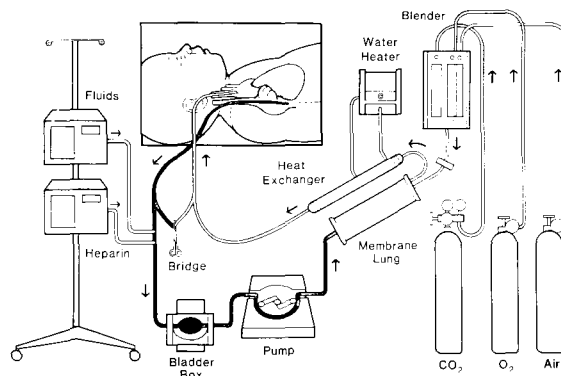


Figure 1. Schematic of ECMO Circuit.

reflected in increased umbilical PaO₂. The ECMO pump was gradually weaned to an idle flow of 50 ml per minute. When stable vital signs with acceptable arterial blood gases and urine output were achieved at this minimal flow for several hours the cannulae were clamped and the baby excluded from ECMO. When a stable course continued for several more hours the cannulae were removed. Details of this institution's ECMO program and protocol have been discussed previously.²²⁻²³

Sample Collection

Pre-ECMO and post-ECMO blood samples were collected from umbilical artery catheters. During ECMO, 5 ml blood samples were collected from a venous port on the circuit at 5, 10, 15, 30, 45 and 60 minutes, then at 2, 5, 10 and 24 hours, and daily thereafter. Samples were collected in disodium EDTA tubes and plasma separated by centrifugation. Plasma was immediately frozen and stored at -70 C.

Radioimmunoassay Determination of C3a, C4a, and C5a

Complement components, C3a, C4a, and C5a, were measured using kits from UpJohn Diagnostics.^d C3a and C5a assays followed the methods of Hulgi and Chenoweth.²⁴ C4a assay followed the methods described by Gorski.²⁵ Briefly, radioiodinated C3a, C4a, or C5a [des arg] was mixed with the patient's plasma sample and a known quantity of rabbit anti-C3a, -C4a, or -C5a des arg. After the prescribed room temperature incubation, a known amount of goat anti-rabbit antisera was added and followed by further incubation for 2 hours at room temperature. Bound antigen

d UpJohn Company, Kalamazoo, MI 49001

e Packard, Downer's Grove, IL 60603

f Cordis Laboratories, Miami, FL 33127

was separated from unbound by centrifugation at 2000xg for 10 minutes. Unbound ^{125}I was removed by aspiration and samples were counted in a Packard^e gamma scintillation counter. Quantitation of C3a, C4a, and C5a were obtained from duplicate determinations by means of standard curves (the relative amount of radioiodinated ligand versus the logarithm of the correlation of the ligand) whose correlation coefficients were >0.98 .

Quantitative Assay of Human Whole Complement

Plasma samples were thawed, recalcified and allowed to clot according to the methods described by Issitt.²⁶ Serum was decanted and assayed for whole human complement. Whole human complement was measured by the standard methods of Mayer²⁷, using a commercially available kit supplied by Cordis Laboratories.^f Whole complement was measured as a function of sample sera's ability to lyse antibody sensitized sheep erythrocytes. The degree of hemolytic activity is expressed in CH_{50} units.

Statistical Analysis

One-way analysis of variance with repeated measures was used to test differences in C3a, C4a, C5a, and CH_{50} with time. Subsequent to finding a significant analysis of variance, Dunnett's test was used to compare pre-ECMO measurements to measurements taken during and post-ECMO.

Results

Plasma levels of C3a were measured in 5 neonates before, during, and after ECMO (Figure 2). During ECMO, C3a rose progressively from initial pre-ECMO levels of 394 ± 255 ng/ml plasma to a peak of 1360 ± 699 ng/ml ($p < .01$) after 2 hours of ECMO. C3a levels then dropped steadily, reaching pre-ECMO levels (410 ± 161 ng/ml) in 24 hours. C3a remained below pre-ECMO levels during the following 3 days. Post-ECMO C3a levels were 258 ± 76 ng/ml. The ECMO prime, consisting of saline-washed red blood cells, balanced electrolyte crystalloid, and albumin, had a C3a concentration of 256 ± 118 ng/ml.

Levels of plasma C4a showed no significant changes during ECMO, indicating the absence of classical pathway involvement. Also, plasma C5a did not show any measurable changes.

Whole complement hemolytic activity before initiation of ECMO was 246 ± 33 CH_{50} Units. After 10 minutes on ECMO, CH_{50} levels dropped significantly to 83 ± 41.5 ($p < 0.001$). After 1 hour, CH_{50} levels

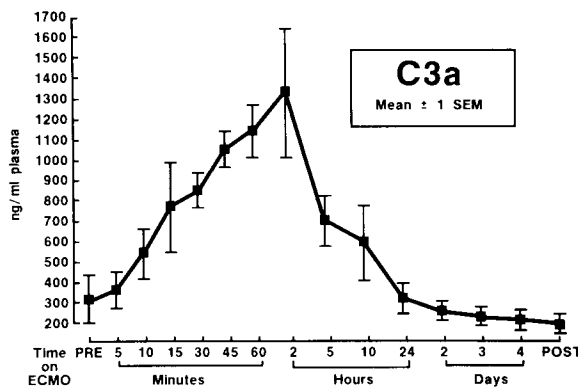


Figure 2. Plasma C3a mean levels in 5 neonates before, during and after ECMO

were 128 ± 50 Units. After 24 hours, CH_{50} levels approached pre-ECMO values (219 ± 24.6 CH_{50} units). After 4 days, CH_{50} levels were above pre-ECMO levels ($266.2 \pm \text{CH}_{50}$ Units), (Figure 3).

Observations of chest x-rays revealed increased radio-opaque images (interpreted as pulmonary edema) during the first 24 hours of ECMO. The x-rays demonstrated improvement at ECMO termination.

Discussion

There are three special considerations concerning complement activation during neonatal ECMO. First, the priming volume (500 ml) of the ECMO circuit can be over two times the blood volume of a 2-4 kilogram patient. We have previously avoided the use of whole blood in the prime because of the undesirable biochemical changes that occur in banked blood. Recent studies at our blood bank have demonstrated that complement activity is preserved in standard blood bank conditions (unpublished data). Therefore, another disadvantage to using whole blood as a prime additive would be the introduction of additional complement into the circulating volume. In our prime, there is theoretically a minimal amount of complement present since we use saline-washed red blood cells and balanced electrolyte crystalloid. C3a levels in our prime were comparatively low (258 ng/ml), suggesting low concentrations of complement in the prime. Even with the dilutional effect of this "complement poor" prime, we observed a tremendous generation of C3a during the initial hours of ECMO. Most of this is apparently from the activation of the patients' complement system rather than from the prime. We expected and saw a drop in the whole complement levels (CH_{50}) 10 minutes after ECMO initiation, due to both dilution and consumption as shown by activation products of the complement system.

Second, while CPB and hemodialysis may last sev-

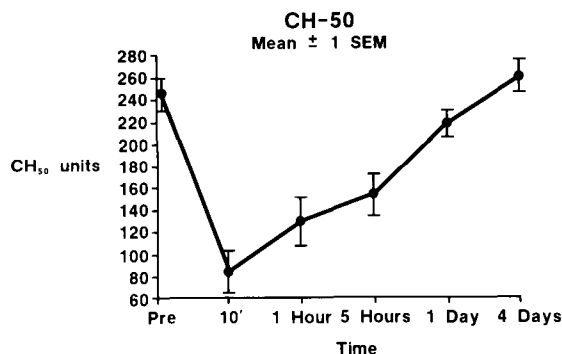


Figure 3. Measured mean CH₅₀ units in 5 neonates before and during ECMO.

eral hours, ECMO is a relatively long-term procedure that can last for days or even weeks. It was of interest to us to document the course of complement activation throughout the duration of ECMO. The levels of C3a peaked after 2 hours on ECMO, only to fall back to near baseline levels by 24 hours. After this initial surge of C3a generation, the levels then remained low during the next 4 days, until sampling was discontinued. Whole complement (CH₅₀) fell within the first 10 minutes of ECMO, only to rebound significantly during the ensuing 24 hours. After 24 hours, CH₅₀ levels were nearly as high as pre-ECMO levels and by 4 days, CH₅₀ levels actually exceeded pre-ECMO levels. This seems to indicate that the circuit induced complement activation is limited to the initial hours of ECMO. We have speculated that there is the adherence of a protein coat to the surfaces of the circuit and this protects against further activation of complement.

Finally, neonatal ECMO patients are being treated primarily for acute pulmonary dysfunction. The link between complement activation and the initiation or prolongation of pulmonary dysfunction is another consideration for the already high risk neonate on ECMO.

Ideally, preventing complement activation would eliminate the threat of additional pulmonary damage to the neonate. This could be accomplished with the development of more biocompatible materials in the circuit or by the introduction of a substance(s) which could block activation. The steroid, methylprednisolone, has been thought to inhibit the alternate pathway of complement.²⁸ Studies of methylprednisolone's effectiveness at reducing complement activation during CPB are presently controversial. Some investigators have not seen any reduction in activation with steroid treatment during CPB with either bubble or membrane oxygenators.²⁹⁻³⁰ In contrast, Cavarocchi and associates demonstrated a reduction in C3a generation by using a 20-30 minute steroid treatment prior to CPB and a high dose steroid prime.³¹ The effects of steroids on the neonate may prohibit its use in ECMO even if it can be

proven effective at reducing complement activation.

Once activation of the complement system occurs, the cleavage fragment C5a seems to be the critical effector of pulmonary leukosequestration by binding to polymorphonuclear leukocytes.³² This may explain why we were unable to detect C5a in the plasma. The activation and subsequent intrapulmonary capillary sequestration of neutrophils are thought to liberate harmful amounts of superoxide anion, hydrogen peroxide, and hydroxyl radical causing damage to endothelial cells in the lungs.³³⁻³⁴ Recently, Cavarocchi and associates have demonstrated a correlation between the generation of oxygen-free-radicals and complement activation during CPB.³⁵ Several studies provide evidence that oxygen-free-radical scavengers can circumvent the damage mediated by these molecules.³⁶⁻³⁷ Therefore, if complement activation cannot be fully avoided, then scavenging the toxic oxygen products of activated neutrophils may be the next defense against complement-mediated microvascular lung injury during ECMO.

We have demonstrated complement activation in neonates undergoing ECMO. Further research must be done to ascertain the clinical significance of complement activation in neonatal ECMO. We believe complement activation is a greater threat to the neonate on ECMO than to patients having other extracorporeal procedures because of their pre-existing pulmonary problems. Blocking complement activation or scavenging dangerous oxygen-free-radicals produced as a result of complement activation, are avenues to investigate with hopes of improving survival and reducing time on ECMO; thereby, allowing greater access to ECMO therapy that is not widely available.

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