**Original Article**

**Effects on C3 and CH50 Levels During and Following Extracorporeal Whole Body Hyperthermia**

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**ABSTRACT**

Cardiopulmonary bypass can affect inflammatory reactions and evoke the “postperfusion syndrome,” manifested as multiple organ dysfunction in the recovery period. This syndrome is generated by the activation of complement, macrophages, neutrophils, and inflammatory cytokines. Following the use of hypothermia during cardiac procedures, active hyperthermic rewarming is used to reestablish body temperature. Complement levels and their interactions have been investigated during and following hypothermia. Hyperthermia is being used clinically; however, the effect of markedly elevated temperatures on complement is unknown and, therefore, needs to be investigated.

A pilot canine study was designed to begin to explore what role hyperthermia may play on complement levels during and following extracorporeal whole body hyperthermia. Five dogs were heated to a core temperature of approximately 42°C, held at this elevated temperature for 90 minutes, then cooled to normothermia. A decline in C3 levels at the end of warming with further declines through day 4 post treatment was observed. CH50 levels mimicked the C3 level decline; however, there was a trend for rebounding by day 4. The findings involving complement factors following hyperthermia signify that this increase in temperature causes a decrease in both C3 and CH50 levels.

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INTRODUCTION

The immune system acts continuously to combat infections, malignancies, and foreign invaders. In addition to the obvious production of lymphocytes, immunoglobulins, cytokines, and enzymes, the complement cascade exhibits biological activities centering on the mediation of acute and chronic reactions. The complement cascade system provides an effector function necessary for maintaining immunity.

Hyperthermia for medicinal treatments dates back centuries. Local, regional, and whole body are all methods in which hyperthermia may be administered. Extracorporeal is one type of whole body hyperthermia used in oncology, as well as infectious diseases (1, 2). Currently, whole body hyperthermia is being used in a variety of cancers, HIV, and hepatitis C. A core body temperature of 41.8 °C has generally been accepted in the use of whole body hyperthermia in humans (3). Given the higher normothermic temperature in dogs, a core temperature of 42 °C is routinely used in this species in order to produce a similar change between normothermia and a clinically relevant hyperthermic state (4). The effect of whole body hyperthermia on hematopoietic cells has been previously noted (5,6); however, the role that heat may play on the complement system has not been fully appreciated (7). This pilot study was performed to investigate further the relationship of C3 and CH50 levels following extracorporeal whole body hyperthermia.

MATERIALS AND METHODS

This study was conducted in compliance with the “Guide for the Care and Use of Laboratory Animals and Good Laboratory Practices.” Male canines comprised this study in which veno-venous, low flow extracorporeal whole body hyperthermia was induced. A low flow technique was chosen to reduce the toxicities seen in the past with high flow techniques (7). These animals weighed between 27.6–33.8 kg and were kept NPO (nothing per OS) the evening before the procedure. Dexamethasone was given the evening before the treatment and on the morning of the treatment. Intravenous fluids (D5W) were replaced at 75–100 ml/h before the beginning of heating, and perioperative antibiotics were administered.

General anesthesia was used during the procedure. Sodium pentothal (2.5%) and supplemental oxygen were used to maintain a pO2 >100 mmHg. Intra-procedure, isoflurane (1.5–2.0%) was used. Continuous electrocardiographic and urine assessment was performed. The access sites for insertion of cannulae involved surgical cutdowns to the cervical and femoral areas. An arterial catheter was placed in the right femoral artery for arterial pressure monitoring and for blood sampling. Complement C3 and CH50 levels were measured at baseline, the end of heating, and on postoperative day 3. C3 was assayed using radioimmunoassay, analyzing percentage change from normal canine sera. CH50 levels were measured utilizing a hemolytic assay with sheep red cells sensitized against antibodies. A 5Fr Swan–Ganz catheter was positioned properly in the main pulmonary artery via its initial insertion into the left jugular vein. Esophageal, rectal, and external jugular vein temperatures were monitored continuously. Main pulmonary artery blood temperature was recorded (Swan–Ganz catheter). Cardiac outputs were obtained throughout the procedure.

Heparin (150 μg/kg, bovine lung) was used to anticoagulate the animal. Each animal was placed on veno-venous extracorporeal circulation using the PS-1 centrifugal perfusion system. Activated clotting times were measured to assess anticoagulation status. Two straight cannulae were inserted, one residing in the inferior vena cava via the left femoral vein (18–21Fr). Efferent flow was from the left femoral vein cannula and in-flow from the perfusion circuit to the right internal jugular vein cannula. The perfusion circuit was primed with Plasmalyte A (280 mOsm), with no blood utilized as part of the priming solution. Blood replacement was neither added to the pump, nor given to the animal during the treatment. Flows on bypass ranged between 10–15% of the baseline cardiac output.

The animal was gradually heated to a core body temperature of approximately 42.0 °C. This heating phase ranged from 45–60 minutes. The blood temperature ranged between 45.2–48.0 °C, during this warm-up phase. The plateau phase of 90 minutes began once the core body temperature reached 42.0 °C, which was identified when either the rectal or esophageal temperature reached this point. The rate of intravenous fluid replacement during the warming and plateau phases increased to 150–200 ml/h. Ice packs were placed at the back of the neck when the core temperature reached 40.0 °C.

The plateau phase lasted 90 minutes. Mannitol (10 gm) was given intravascularly during the plateau phase. The cooling phase was initiated at the conclusion of the plateau phase. Passive and active cooling phases were utilized. After passive cooling had occurred, a cooling coil connected to the afferent end of the heater/cooler was put into an ice water bath to effect active cooling.

Once the animal reached 39.0 °C, perfusion was discontinued. All cannulae were removed, and the animal’s heparinization was reversed with protamine sulfate (10 mg of protamine: 1000 units of heparin) based on the use of a dose-response curve. All vascular cannula/catheter sites were either ligated or repaired. All animals emerged from anesthesia within 0.5–1.0 h.

Animals were housed for 7–10 days, at which time, a formal
postmortem examination was performed. Serial blood values were obtained at days 1, 4, and 7. At the time of necropsy, tissue samples were retrieved for further histological analysis. Gross and microscopic examination was performed on: lungs, heart, brain (cerebral cortex, cerebellum), spinal cord, thyroid, parathyroids, thymus, adrenals, liver, spleen, pancreas, kidneys, vena cava, aorta, gastrointestinal tract, skeletal muscle and lymph nodes (regional and mesentery). Full results of hemodynamic and pathology data for this study have been previously reported (7). Changes in C3 and CH50 levels were analyzed by using the baseline value as the zero time point and calculating the percentage difference in values seen at plateau-0, plateau-90, postprotamine, days 1, 4, and 7. Average values of the percentage differences, as well as the standard deviation of the mean, for these averages are given.

RESULTS

All animals survived the therapy and remained healthy during the postprocedure interval before necropsy. Table 1 reveals a decline in the percentage difference in C3 levels in all animals at the end of the plateau phase, with a further decline at day 1. Elevation in these values from the largest difference occurred by day 7. CH50 level percentage change from baseline is shown in Table 2. All animals experienced a decline in values until day 4, when one animal had returned to baseline.

DISCUSSION

Some medical conditions and procedures can strikingly alter complement levels, which can ultimately affect the organism’s immunological state. Isolated hyperthermic liver perfusion has shown an activation of the complement cascade (8). Cardiopulmonary bypass has been theorized to activate complement. The observed “postperfusion syndrome” following cardiopulmonary bypass activates complement, inflammatory cytokines, and specific immune cellular parameters (5).

An efficient complement system includes the factors required to provide a line of action in our immune system. C3 may have the largest role in the complement system, by potentiating antibody-mediated phagocytosis. CH50 may act in an adjunctive manner. Both components may directly link to providing a line of action in immune response. Ferencikova and Kolesar demonstrated that a short exposure of mild body heating (38–38.5 °C) caused a drop in CH50 and C3 components in both healthy and ischemic heart disease patients (9). They argued that hyperthermia can influence these levels and the potential activity of complement. Chiu et al. found a rapid reduction in C3 levels in human patients undergoing cardiopulmonary bypass, which returned to normal within 24 hours (10). This rapid activation of the complement system by cardiopulmonary bypass revealed no long lasting effect. Ohata et al. reported a beneficial effect of cardiopulmonary bypass under tepid temperature, which they contended could...
possibly attenuate the postperfusion syndrome (5). Furthermore, others have argued that altered levels of complement may produce a beneficial effect. Gillnov et al. reported that dogs genetically deficient in C3 levels demonstrated no C3 deposition in their lungs post cardiopulmonary bypass, in comparison to animals with normal levels of C3 (11). We found extended low levels of C3 and CH50 in heated animals during and post heating. 

Previously reported findings concerning heating of normal and malignant cells in humans, as well as the data collected in our canine study, pose a question as to the relationship that heat plays on the complement system. This study has begun to address this issue; however, the sequencing, duration, and precise temperature requires further exploration. This was not meant to be an exhaustive study, rather it is a small study to begin to observe the role that hyperthermia might play in a variety of systems, including the immune system. Certainly, no conclusive statements about the immune system can be made without accumulation of a larger data set.

REFERENCES