

The Effect of Methylprednisolone on Complement Activation during Cardiopulmonary Bypass

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Abstract

A prospective randomized trial in 80 patients having heart surgery was undertaken to test the hypothesis that steroids (ST) (methylprednisolone, 30 mg/kg) would prevent or reduce complement activation during cardiopulmonary bypass (CPB) with membrane (MEM) or bubble (BUB) oxygenators. Group I patients had MEM and ST; Group II, MEM without ST; Group III, BUB and ST; Group IV, BUB without ST. Complement activation was assessed by C3a radioimmunoassay determinations obtained before, during and after CPB.

When Group I was compared to Group II, there was a significant increase in C3a generation in the presence of ST. Also, when Groups I and III combined — both with ST — were compared to Groups II and IV — both without ST — a significant increase in C3a with ST was observed. In contrast, comparing MEM to BUB (Group I vs. III or II vs. IV) no significant difference in C3a was seen.

Thus the hypothesis — that ST would prevent or reduce complement activation — was not confirmed and ST appears to actually increase complement activation. Also, MEM oxygenators were not associated with less activation of C3a than BUB oxygenators.

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Introduction

Complement activation occurs during cardiopulmonary bypass (CPB) because complement, a plasma protein, comes in contact with foreign surfaces of the pump oxygenator and the blood-gas interface. The degradation products, or split products, of complement activation include the anaphylatoxins, C3a and C5a. These anaphylatoxins are thought responsible for the occasionally observed damaging physiologic effects of CPB, including lung and kidney dysfunction as well as post-operative bleeding problems.

One current theory is that these degradation products cause a whole body inflammatory reaction of varying severity. The severity of damage varies with duration of CPB, age of the patient, and peak level of anaphylatoxin reached.^{1,2} If this theory is true, it would be of clinical importance to eliminate or minimize C3a and C5a production.²

A prospective randomized double blind trial in 80 adult patients having heart surgery with CPB was done to test the hypothesis that steroids, with their anti-inflammatory effect,^{3,4} would prevent or reduce complement activation. Additionally, the trial compared membrane (MEM) to bubble (BUB) oxygenators to test the hypothesis that the membrane oxygenator would be associated with less complement activation. A new, highly sensitive and reproducible radioimmunoassay for C3a was used for these studies.

Materials and Methods

Informed Consent — The Institutional Review Board gave approval of the study. All patients studied gave informed consent prior to their being

TABLE 1
Group Comparisons with Case Mix

	Group I	Group II	Group III	Group IV
Age	60.4 ± 11.0	60.4 ± 12.0	57.5 ± 9.5	57.6 ± 9.7
Sex	M = 70% (14)	M = 85% (17)	M = 80% (16)	M = 80% (16)
Weight	79.8 ± 114.0	81.2 ± 11.3	82.6 ± 9.3	82.4 ± 16.6
Bypass Time	93.1 ± 39.0	127.7 ± 47.2	130.6 ± 73.5	118.3 ± 45.7
X-Clamp Time	52.6 ± 23.4	71.7 ± 31.0	71.5 ± 34.1	66.0 ± 31.4
24 Hour				
Chest Drainage	603 ± 326	702 ± 491	660 ± 222	981 ± 702
Surgeon	A = 13, B = 5, C = 2	A = 10, B = 9, C = 1	A = 12, B = 8, C = 0	A = 9, B = 11, C = 0
CASE MIX				
CABGs	90% (18)	70% (14)	85% (17)	90% (15)
Re-op. CABGs	0	20% (4)	0	15% (3)
Single Valve	0	0	0	5% (1)
Double Valve	5% (1)	5% (1)	0	5% (1)
Valve & CABGs	0	5% (1)	10% (2)	0
ASD	5% (1)	0	5% (1)	0

A comparison of common variables demonstrated no significant difference between the four groups.

entered into the randomization process.

Study Design and Randomization Process — Eighty adult patients having heart surgery with CPB were studied. Half were to receive steroids (ST) (methylprednisolone, 30 mg/kg) and half no ST. Half were to have MEM and half BUB oxygenators.

Group I patients (n = 20) were to get MEM and ST; Group II (n = 20), MEM without ST; Group III (n = 20), BUB and ST; Group IV (n = 20), BUB without ST.

The randomization was planned so as to create four groups of 20 patients by random number allocation using a standard table of random numbers.⁵ After the allocation to the four groups was determined the total number sequence from the first patient to the 80th patient was given to the pharmacy. The pharmacy then dispensed the drug or placebo and designated the appropriate oxygenator according to the patient assignment. Drugs were provided in coded syringes. Neither surgeon, perfusionist nor laboratory technician knew which patient was in which group. By necessity the perfusionist and the surgeon knew which oxygenator was being used but the assignment was done by the pharmacy department.

Patients — The exclusions to participating in the study were, 1) refusal to consent, 2) emergency operations, and 3) Friday operations (no C3a determinations were done that day). Also, only the first two patients on the operating schedule for each

day were asked to participate due to the time constraints of blood collection.

Types of operations in the 80 study patients were, coronary artery bypass graft (CABG) 64, redo CABG 7, single valve replacement 1, double valve replacement 3, valve replacement and CABG 3, and atrial septal defect 2. (Table 1) There were no deaths.

C3a Determinations — Blood samples for C3a were drawn, 1) pre-bypass, 2) two minutes, 3) 10 minutes, 4) 20 minutes, and 5) 30 minutes on bypass. Thereafter they were drawn at 30 minute intervals, plus at the end of bypass and one hour post-bypass. The samples were drawn from the patient's arterial line pre- and post-CPB and from the oxygenator arterial sampling port during CPB. blood was anticoagulated with 7 mM EDTA and the plasma separated within 10 minutes and frozen on dry ice. All plasma samples were stored at -85°C until assayed. Samples from each individual were analyzed in one run.

C3a was determined by radioimmunoassay. All reagents were prepared from a commercially available kit.^a I¹²⁵ C3a des Arg was mixed with patient's sample and a standardized quantity of rabbit Anti-C3a des Arg. The reaction mixture was incubated for 30 minutes at room temperature. A standardized amount of goat anti-rabbit antisera was added and incubation continued for an additional

^aUpjohn Diagnostics, Kalamazoo, MI 49001

30 minutes at room temperature. Two milliliters of isotonic saline were added and the precipitate separated by centrifugation at $2,000 \times g$ for 10 minutes. All samples were counted in a gamma counter such that the 0 ng (Bo) standard yielded 10,000 counts. Counts per minute in each standard were corrected for nonspecific binding and the per cent bound calculated as a ratio of the Bo standard. The results were plotted on log-logit paper as a function of C3a des Arg concentration. The C3a concentration in each sample was determined after correction for nonspecific binding.

Materials — The bubble oxygenator used was the Shiley S-100A^b and the membrane, the Travenol (TMO®)^c oxygenator. The methylprednisolone,^d or placebo, was added to the bypass prime and circulated prior to initiation of CPB.

Bypass Technique — Anticoagulation was managed with an activated clotting time (ACT) protocol. An initial loading dose of porcine mucosal heparin^e (2 mg/kg) was given and then adjusted to maintain an ACT of 460-520 seconds. Blood flows during CPB were maintained at 70 cc/kg, not to exceed 6.0 L/min. Moderate systemic hypothermia of 28°C was maintained for each patient. A crystalloid cardioplegia solution at 4°C was perfused into the aortic root to achieve and maintain cardiac standstill and myocardial hypothermia. In addition, topical cooling with Ringer's lactate slush was used.

The extracorporeal circuit was composed of Tygon PVC and Travenol pumphead PVC tubing with an unfiltered Travenol cardiotomy reservoir and an Extracorporeal Intersept 20 u filter/bubble trap.^f Bypass systems were primed with 2,500 cc. Normosol-R,^g 50 gm. Mannitol,^h 40 mg heparin, and 44.6 mEq sodium bicarbonate.ⁱ Whole blood or packed cells were added to maintain a patient/pump hematocrit of approximately 24%. At the end of CPB heparin was neutralized with protamine sulfate.^j

^bShiley Inc., Irvine, CA 92714

^cTravenol Laboratories, Inc., Deerfield, IL 60015

^dUpjohn Company, Kalamazoo, MI 49001

^eElkins-Sinn, Inc., Cherry Hill, NJ 08034

^fExtracorporeal, Inc., King of Prussia, PA 19406

^gAbbott Laboratories, North Chicago, IL 60064

^hInvenex, Chagrin Falls, OH 44022

ⁱBristol Laboratories, Syracuse, NY 13201

^jEli Lilly and Company, Indianapolis, IN 46285

Statistical Analyses

Statistical comparisons were made with a package developed for microcomputers.^k Comparisons of more than two populations were performed by standard analysis of variance, while two population comparisons were made by the student-t test. Significance was defined as $p < 0.05$.

Results

Table 1 compares the four groups of patients studied in regard to age, sex, weight, bypass time, cross-clamp time, volume of 24 hour chest drainage, surgeon and case mix. None of these variables differed significantly in the four groups. Table 1 is an overall measure of how well the randomization process worked.

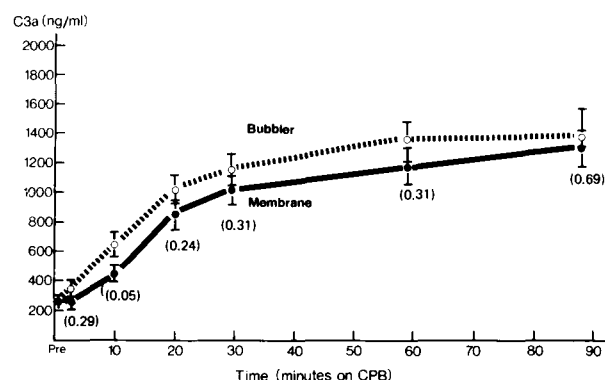


FIGURE 1. Oxygenator Effect on C3a Release Without Steroids. Comparison of Group II to Group IV.

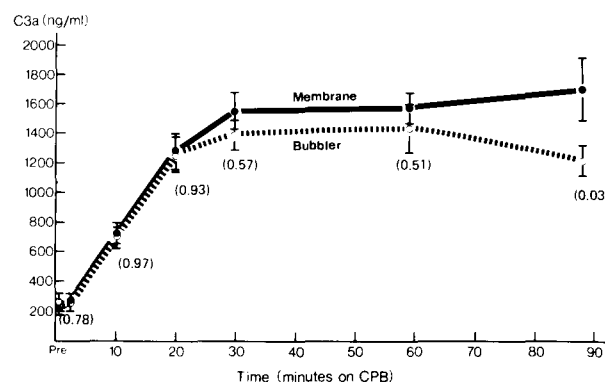


FIGURE 2. Oxygenator Effect of C3a Release With Steroids. Comparison of Group I to Group III.

The types of oxygenators are compared in Figures 1 and 2. Figure 1 compares the release of C3a in Group II patients (MEM, without ST) and Group

^k“The Number Cruncher” (J. Hintze), The Interactive Statistical Analysis System, Kaysville, UT 84037

IV patients (BUB, without ST). Figure 2 compares the release of C3a in Group I patients (MEM, with ST) and Group III patients (BUB, with ST). There is no statistically significant difference between the oxygenator types. A single unexplainable significant difference was seen at 90 minutes in the group receiving steroids. This is an isolated observation with no difference seen at other time periods.

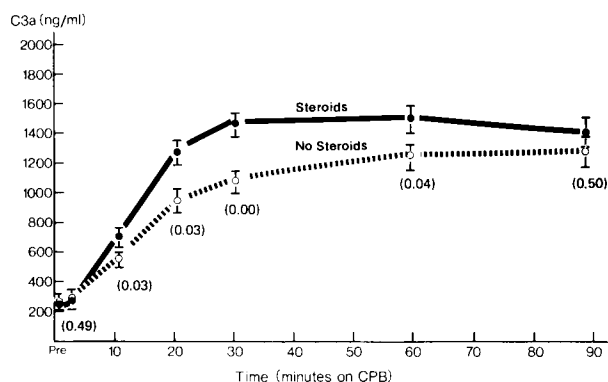


FIGURE 3. Steroid Effect on C3a Release. Comparison of Groups I and III combined to Groups II and IV combined.

Figure 3 compares the effect of steroids on the release of C3a by contrasting Groups I and III combined (MEM and BUB, with ST) to II and IV (MEM and BUB, without ST). This comparison ignores differences in effects of oxygenator type. There was a significant difference between the two at all points except at 90 minutes when the number of observations was small and the validity of the difference suspect.

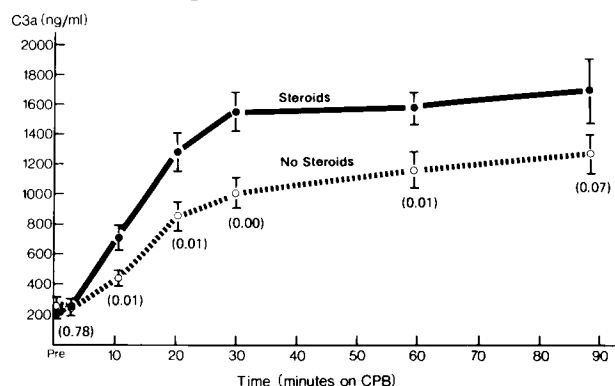


FIGURE 4. Steroid Effect on C3a Release With Membrane Oxygenator. Comparison of Group I to Group II.

In Figure 4, the release of C3a is compared between Group I patients (MEM, with ST) and Group II patients (MEM, without ST). There was a statistically significant difference seen between these two groups.

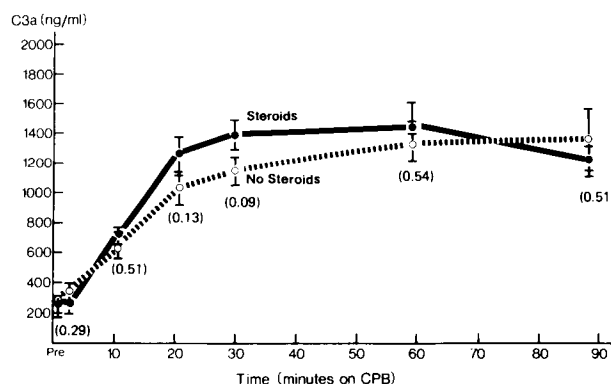


FIGURE 5. Steroid Effect on C3a Release With Bubble Oxygenator. Comparison of Group III to Group IV.

Figure 5 compares the release of C3a in Group III patients (BUB, with ST) and Group IV patients (BUB, without ST). Patients receiving steroids had a definite trend toward higher C3a levels at multiple time periods. This trend was not statistically significant, however.

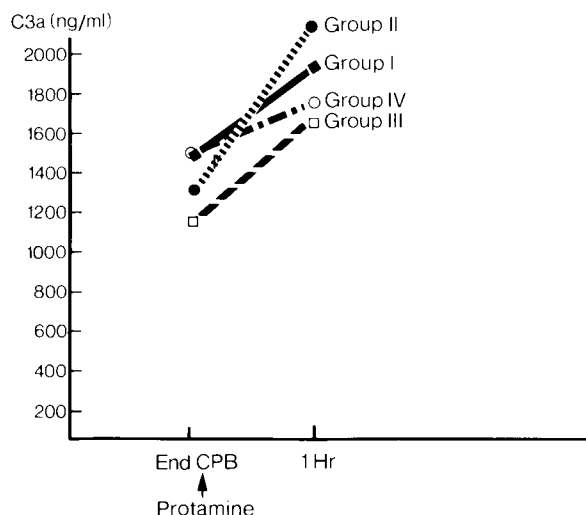


FIGURE 6. C3a Release After Bypass; Protamine Effect? Comparison of end-bypass to 1 hr. post bypass C3a levels in all four groups.

In Figure 6, the level of C3a at one hour post bypass in the four groups is compared to C3a levels at end bypass. There was a further rise in C3a levels in the four groups, whereas from one hour on bypass to the end of bypass the C3a values had been high but relatively stable. It was during this time period that protamine sulfate was administered.

Discussion

We were pleased the randomization process used, 1) created four groups of patients that were comparable, 2) apportioned operations of like se-

verity to the four groups, and 3) distributed the four groups evenly between three surgeons.

We were surprised that neither of our two hypotheses were confirmed.

The major hypothesis of our study was that steroid use would lessen the release of C3a. However, steroid use was associated with a significantly greater release of C3a in membrane oxygenator patients in our study. This difference is particularly striking since Group I patients had shorter CPB and cross-clamp times than Group II patients. The hypothesis that steroid use would lessen C3a release was not found to be true and methylprednisolone appears to actually be associated with greater C3a release.

Another hypothesis tested in this study was that membrane oxygenators would cause less C3a release than bubble oxygenators. We felt that bubble oxygenators with their direct blood-gas interface would be more injurious to plasma proteins and cause greater C3a generation. However, in our study both types of oxygenators caused a statistically similar and striking release of C3a which rises sharply, and then tends to level off after one hour of CPB.

Much interest has been focused recently on protamine sulfate administration and C3a release.^{6,7} Our study, although not specifically designed to study this question, gave indirect support to the notion that protamine sulfate administration may cause C3a release. By one hour post-bypass, when all the protamine sulfate had been given, the C3a level in all four groups was markedly higher than it had been at any time during the bypass itself. In a recent study by Chiu et al, complement components, the forerunners of activation products C3a and C5a, were not changed after the slow intravenous infusion of protamine sulfate.⁶ The authors interpreted this to mean that protamine sulfate, at least when given slowly, did not cause anaphylatoxin release. Kirklin, however, feels that protamine sulfate administration does cause C3a release.¹

In this study we did not prospectively ask the question, "Were increases in C3a related to post-operative complications and increased post-operative morbidity?" Retrospectively we did try to see whether we could associate any subsystem dysfunction—cardiac, pulmonary, renal or coagulation—with elevated C3a levels. We were not able

to make such correlations nor could we find such a trend. There was no statistically significant difference among the four groups of patients in respect to their day of discharge from the hospital. In a prospective study of 128 cardiac surgical patients Kirklin et al did find that elevated levels of C3a were associated with increased morbidity and mortality.¹ This important finding needs to be corroborated in other centers, however.

The prophylactic use of steroids during CPB is a controversial practice.^{3,4} A recent survey showed only 3% of cardiac centers using steroids.⁸ Proponents of steroid use say that steroids protect against pump lung or shock lung and the shock-like state of CPB and thereby reduce the morbidity of cardiac surgery.^{3,4} Our study did not focus on the question of whether steroids were of benefit or not. But our results might not be incompatible with those that theorize steroids protect the lungs from damage by preventing granulocyte aggregation.⁹ Steroids generally act to stabilize cell membranes, and more specifically the lysosomal membrane. One might easily suppose that steroid inhibition of granulocyte aggregation, demonstrated by other investigators,^{10,11} could be occurring at the same time as steroid activation of the complement cascade—which is apparently what occurred during our study—was taking place. Steroids may well have several sites of action and some effects might be beneficial while others were neutral or even detrimental.

The recurring clinical question of which oxygenator is best—bubbler or membrane—was not answered by our study. The often cited theoretical superiority of the membrane oxygenators has recently been questioned by Maurer.¹² If one believes that C3a anaphylatoxins are indicators of a whole body inflammatory reaction, then both types of oxygenators can be incriminated by our study as not being gentle enough during CPB.

The most critical intellectual question—"Where exactly is complement activation taking place?"—was not assessed in our study. It could be all or part of the extracorporeal circuit or else it could be due to the pump prime or its additives. As suggested by Chenoweth,¹³ the search for the exact site of complement activation will be greatly aided by the commercial availability of kits for the radioimmunoassay of anaphylatoxins.

Further investigation is certainly needed, 1) to

identify the causative mechanisms of complement activation and C3a generation during CPB, 2) to make certain that C3a is indeed the acting mediator of post CPB subsystem dysfunction, 3) to develop equipment and/or drug therapies to lessen the detrimental physiologic effects which occur during CPB.

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