Complement and Neutrophil Activation in the Pathogenesis of Ischemic Myocardial Injury

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Complement depletion with cobra venom factor (CVF) before coronary artery ligation has been previously shown to reduce subsequent ischemic myocardial tissue injury in the baboon; however, whether complement depletion after the initiation of acute myocardial ischemia affords similar myocardial preservation is not known. Both complement depletion with CVF or the administration of certain nonsteroidal anti-inflammatory drugs, including ibuprofen, are thought to decrease myocardial infarct size by reducing polymorphonuclear leukocytic (PMN) infiltration; nevertheless, complement activation also could alter tissue injury by PMN-independent actions. Thus, the relative effects of CVF administered after coronary artery ligation on the subsequent development of myocardial tissue injury were assessed in a baboon myocardial infarction model. The animals were randomized into three treatment groups (n=6): either CVF (125 units/kg) or saline was given 30 minutes after coronary artery ligation, and ibuprofen (12.5 mg/kg) was administered 30 minutes and 4 hours after ligation. The extent of ischemic myocardial injury was assessed 24 hours later. Relative to saline-treated baboons, both CVF and ibuprofen reduced PMN infiltration (36±4% vs. 24±4% and 24±4% PMN/mm², respectively; mean±SEM) and histological evidence of transmural myocardial infarction (100% vs. 47% and 53%, respectively) in electrocardiographically designated, expected infarct sites. In both saline- and ibuprofen-treated animals, there was extensive localization of C4, C3, and C5 in all infarct sites; in contrast, there was only C4 localization in the CVF-treated baboons. When expected infarct sites were assessed for creatine kinase content as an indicator of tissue injury, there was significantly less epicardial and endocardial creatine kinase depletion in the CVF-treated animals (31.7±5.6% and 39.3±4.8%) than in the saline-treated animals (54.1±5.4% and 59.0±4.7%; p=0.012 and 0.011, respectively). The percent creatine kinase depletion in the ibuprofen-treated animals was intermediate between the two other groups. These results suggest that depletion of complement after coronary ligation has beneficial effects in reducing tissue injury that cannot be explained solely on the basis of reducing PMN infiltration into the ischemic myocardium. (Circulation 1988;78:1449–1458)

Considerable experimental evidence strongly implicates the acute inflammatory process in the pathogenesis of ischemic myocardial tissue injury.1–3 Therapeutic intervention with several nonsteroidal anti-inflammatory drugs (NSAID), including ibuprofen, nafazatrom, and BW755c, has been shown to significantly reduce myocardial infarct size.2,4–8 Paradoxically, other NSAID drugs, including indomethacin, aspirin, and meclofenamate, did not reduce and in some cases actually exacerbated ischemic myocardial tissue injury.8–10 One explanation for this disparity is that only the former group of NSAID drugs reduces neutrophil polymorphonuclear leukocyte (PMN) activation.8 These observations suggest that the PMN is involved in the pathogenesis of ischemic myocardial tissue damage. Indeed, depletion of circulating PMN has been shown to significantly reduce ischemic myocardial tissue injury and infarct size in dogs.11

Complement activation plays a pivotal role in the recruitment and activation of PMN within the ischemic myocardium. Depletion of complement by the administration of cobra venom factor (CVF) significantly reduced PMN infiltration into the ischemic myocardium.12–14 The relative importance of CVF is demonstrated by the fact that the extent of the infarct site in the CVF-treated animals (31.7±5.6%) was significantly less than that in the saline-treated animals (54.1±5.4%; p=0.011). The results of this study strongly suggest that complement activation is involved in the pathogenesis of ischemic myocardial tissue injury and that depletion of complement has beneficial effects in reducing tissue injury and infarct size. Additional studies are necessary to determine the exact role of complement in the pathogenesis of ischemic myocardial tissue injury.
rat myocardium. CVF activates the alternative pathway with the depletion of the third (C3) and terminal (C5–C9) components, thereby blocking the production of complement-derived leukotactic fragments (e.g., C5a). Complement depletion with CVF before coronary artery occlusion not only reduced PMN infiltration but also decreased the extent of tissue necrosis in canine, murine, and primate experimental myocardial infarction models.

In addition to recruiting and activating PMN within the ischemic myocardium, complement-derived products also could exacerbate ischemic tissue injury and cardiac dysfunction by neutrophil-independent mechanisms (e.g., C3a, C4a, and C5a), increase vascular permeability, and induce smooth muscle contraction. Also, C3a initiates decreases in left ventricular force and coronary flow, induces tachycardia, and impairs atrioventricular conduction. Finally, if the terminal, cytotytic C5b-9 membrane attack complex (MAC) were assembled on the plasmalemma of myocytes, hydrophilic channels could be generated with the subsequent deleterious influx of extracellular calcium.

In view of the preceding, the present study was instituted to determine whether CVF given after coronary occlusion would reduce subsequent ischemic myocardial tissue injury in the baboon and to determine whether the therapeutic efficacy of CVF would be comparable with that of ibuprofen. Ibuprofen was selected as the NSAID drug of choice because it had been demonstrated to significantly reduce PMN infiltration after coronary artery occlusion or occlusion–reperfusion injury. Furthermore, ibuprofen does not block arachidonic acid lipooxygenase, and no evidence has been reported that ibuprofen interferes with the activation of complement. The results of our investigation indicate that both agents significantly reduced PMN infiltration and ischemic myocardial tissue injury and further suggest that complement-mediated injury to the ischemic myocardium may involve both PMN-dependent and -independent actions.

Materials and Methods

Experimental Protocol

Twenty-four feral baboons (Papio cynocephalus) were obtained from the Southwest Foundation for Biomedical Research (San Antonio, Texas). The animals were randomly assigned to one of three groups, each consisting of eight animals. Each group received two 30-minute intravenous infusions after left anterior descending coronary artery ligation; the first infusion was initiated 30 minutes after ligation and the second 4 hours after ligation. The first group of control baboons received saline for each infusion. The second group of animals received ibuprofen (total dose, 12.5 mg/kg) for each infusion. The third group received an infusion of purified CVF (total dose, 125 units/kg) 30 minutes after ligation and an infusion of saline 4 hours after ligation. Ibuprofen, obtained from the Upjohn Company (Kalamazoo, Michigan) as a sterile solution (50 mg/ml saline), was diluted in pyrogen-free saline immediately before use. CVF was purified as described and, after sterilization by filtration (pore size, 0.2 μm), was stored at 4°C until used.

The procedures used in our baboon myocardial infarction model have been described previously. Briefly, the baboons were sedated by an intramuscular injection of ketamine HCl (10 mg/kg), and, after endotracheal intubation, the animals were placed on a closed ventilator system containing 99% oxygen and 0.6–0.8% halothane. A left thoracotomy was performed, and the heart was suspended in a pericardial cradle. Lidocaine (1 mg/kg) was administered intravenously 2 minutes before ligation to prevent ventricular arrhythmias. A total of 12 anatomically identified sites on the anterior surface of the left ventricular free wall were selected for unipolar, epicardial electrocardiographic (ECG) monitoring. A detailed drawing relating the ECG sites to the anatomy of the coronary arteries, veins, and other cardiac structures was made and used in placing the electrode on the various sites before and after coronary artery ligation. The sites were selected to be distributed in the area likely to be supplied by the artery to be occluded as well as in areas far removed from it. After the control epicardial ECG map, the left anterior descending coronary artery was occluded with a mesoline tie just distal to the first major diagonal branch. Fifteen minutes later, epicardial ECG mapping was repeated to measure the extent of ST segment deviation at each site. ST segment amplitude was measured less than 0.08 seconds after the onset of the QRS complex in all recordings where the total QRS duration was less than 0.06 seconds (absence of bundle branch block). Similar recordings were repeated at 0.5, 1, 1.5, 2, 3, 4, 4.5, 5, 6, and 24 hours.

Systemic arterial pressures were monitored continuously by a femoral artery catheter by a P23Db pressure gauge (Statham Instruments, Gould, Oxnard, California). Pulmonary artery pressures were measured by a flow-directed 7F balloon-tipped catheter (Electronics for Medicine, White Plains, New York) inserted through the femoral vein into the pulmonary artery. The hemodynamic variables were recorded before coronary artery ligation and at 0.5, 1, 1.5, 2, 3, 4, 4.5, 5, and 6 hours after ligation. Additionally, a lead II electrocardiogram was monitored throughout the 6-hour period to measure heart rate. Before and 0.5, 1, 1.5, 2, 3, 4, 4.5, 5, 6, and 24 hours after ligation, blood samples were obtained for the measurement of functional plasma levels of C3, C4, C5, and C6 in hemolytic assays as described. Six hours after coronary artery ligation, the chest was closed in layers and the animal allowed to recover. At this time, six baboons were excluded from further study because of previously established exclusion criteria applied 6 hours after coronary artery ligation: a mean arterial pressure of less than
60 mm Hg for 30 minutes despite maintenance of pulmonary artery wedge pressure at more than 15 mm Hg by saline infusion (n=2) or ventricular fibrillation (n=4). These six animals consisted of two from each treatment group.

Immediately before the baboons were killed (24 hours after coronary artery ligation), epicardial ECG mapping was performed on each of the 12 anatomically defined sites. Immediately after excision, the baboon hearts were rinsed in physiological saline, and the 12 ECG-mapped sites were demarcated by application of Evans blue dye with a cotton swab of identical diameter (4 mm) as the unipolar epicardial ECG recording electrode. Each of the Evans blue–delineated sites was then excised and subdivided into three transmural sections (1–2 mm thick) for measurement of creatine kinase content and for histopathological and immunohistochemical examination.

One portion of each transmural biopsy was subdivided into the endocardial half and epicardial halves; after homogenization, the creatine kinase and protein content of cell-free supernatants was assessed as described. The transmural sections for histopathology were placed in 10% neutral buffered formalin and subsequently were embedded in paraffin, sectioned (6 μm), and stained with hematoxylin and eosin. The transmural sections for immunohistochemistry were placed in ornithine carbamyltransferase (Lab-Tek Products, Naperville, Illinois), quickly frozen, and subsequently used for the light microscopic immunohistochemical localization of various complement components with horseradish peroxidase (HRP)-labeled Fab' antibody fragments as described. Complement C3, C4, and C5 localization was evaluated in all sites of myocardium obtained from every animal; normal goat Fab'-HRP was used as a negative control for each tissue block examined.

All ECG-mapped myocardial sites were carefully examined histologically for evidence of ischemic injury and the extent of PMN infiltration. All histological evaluations were performed in a blinded manner in that the tissues were not identified with respect to either animal, ECG site number, experimental conditions, or electrophysiological evaluation. Alterations associated with ischemic injury of myofibrils included nuclear pyknosis or karyorrhexis as well as cytoplasmic shrinkage and cosinophilia. All sites were classified as either transmural or nontransmural infarction; the distinction between these designations was based on the extent of tissue injury. A site was considered transmurally infarcted only when myocardial fiber destruction was observed to extend from the subendocardium to the subepicardium (excluding consideration of the few cell layers immediately below the endocardium and epicardium, which were regularly spared). Infarct sites that had sparing of myocardial fibers were classified as nontransmural. In only one case (a CVF-treated animal) did a site predicted to be infarcted by electrocardiography have a completely normal histological appearance; some evidence of tissue damage was present in all of the other ECG-designated infarct sites.

PMN in each of the ECG-mapped, hematoxylin and eosin–stained transmural myocardial sites were counted with a light microscope with a square reticle in a ×10 eyepiece with a ×40 objective (0.625 mm²). In the center of the histological tissue section and beginning in the muscle layer at one edge of the myocardium (either endocardial or epicardial), successive enumerations across the myocardium were accumulated and used to calculate the average PMN per millimeter squared within the transmural area evaluated. For each animal, the data were averaged for all normal or ECG-designated infarct sites and subsequently used to calculate the mean for the various treatment groups.

Analysis of Data

For each animal, the mean creatine kinase content in the expected infarct sites based on the 15-minute postligation ECG recordings was computed for the epicardium and endocardium. There was an average of three infarct sites for each baboon. The creatine kinase data were then expressed as a percent reduction relative to the average creatine kinase content of normal myocardium obtained from the same heart. The normal sites were distal to the observed epicardial cyanotic areas after ligation and showed no ECG changes throughout the experimental procedure. There was an average of seven normal sites for each baboon. The mean percent creatine kinase reduction of ECG-designated infarct sites was computed for each animal. These means were analyzed with a weighted analysis of variance where the weights were the number of ECG-designated infarct sites obtained for each animal. The assumptions underlying the analysis of variance appeared not to be violated. The log of the proportion of the ECG-designated infarct sites for each animal that were histologically classified nontransmural were analyzed with a weighted analysis of variance. Because of an outlier in the PMN sequestration data, we used a robust analysis of variance that gave less weight to the outlier. The hemodynamic data were analyzed with a two-way analysis of variance for repeated measurements. All data are expressed as mean±SEM, and p<0.05 was considered significant.

Results

Creatine Kinase Depletion

In the six saline-infused (control) animals, the maximum change in ST segment amplitude from the preligation recordings on the serial epicardial ECG maps occurred 15 minutes after coronary artery ligation. This value (ΔST15) for each site was then compared with the creatine kinase content of these sites at 24 hours (Figures 1 and 2). Although linear regression analysis between creatine kinase content and ΔST15 has been used to characterize canine
experimental myocardial infarcts, this analysis was not appropriate in the present study. As can be seen in Figures 1 and 2, the linear regression lines obtained did not reflect an adequate description of the data. Inspection of the data indicated there was an all or none phenomenon with two distinct clusters of points: the first where ΔST₁₅ was 3 mV or greater with an associated creatine kinase less than 20 IU/mg protein, and the second where ΔST₁₅ was less than 3 mV with an associated creatine kinase equal to or more than 20 IU/mg protein. Thus, the data could only be adequately analyzed among the three groups of animals by comparing the mean creatine kinase contents of ECG-designated infarct sites (i.e., where ΔST₁₅=3 mV).

There were no significant differences in the mean creatine kinase content in the epicardial or endocardial halves of normal myocardial sites between the three groups of six animals (Table 1). Nevertheless, to adjust for the variation in absolute creatine kinase content among animals within and between groups, the mean percent creatine kinase depletion in ECG-designated infarct sites was determined relative to the mean creatine kinase content of normal sites for each individual animal. These data then were used for weighted analysis of variance of the mean myocardial percent creatine kinase depletion among the experimental groups.

There was an overall significant difference between the three groups with respect to the mean percent creatine kinase reductions in the epicardial (p=0.037) and endocardial (p=0.034) halves of the ECG-designated infarct sites (Table 2). Individual comparisons between groups indicated that the mean percent reduction in epicardial creatine kinase was significantly less in the CVF-treated group (31.7±5.6%) than in the saline-treated group (54.1±5.4%, p=0.012). Similarly, the mean percent reduction in creatine kinase in the endocardial halves of ECG-designated infarct sites also was significantly less in the CVF-treated group (39.3±4.8%) than in the saline-treated group (59.0±4.7%, p=0.011).

In the six ibuprofen-treated animals, the mean percent creatine kinase reductions in both the epicardial and endocardial halves of ECG-designated infarct sites were intermediate between the six saline- and the six CVF-treated animals (Table 2). The mean percent reduction in epicardial creatine kinase in the ibuprofen-treated group (45.0±5.1%) was not significantly different when compared with the saline-treated group (p=0.24). Also, the mean

### Table 1. Myocardial Creatine Kinase Content in Normal and Electrocardiographically Designated Infarcted Sites

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Normal sites</th>
<th>Expected infarct sites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Epi</td>
<td>Endo</td>
</tr>
<tr>
<td>Saline</td>
<td>28.3±1.9</td>
<td>28.6±1.7</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>29.2±2.1</td>
<td>27.9±1.9</td>
</tr>
<tr>
<td>Cobra venom factor</td>
<td>27.2±1.8</td>
<td>26.4±1.6</td>
</tr>
</tbody>
</table>

Data are presented as the group mean±SEM of the averaged creatine kinase content (IU/mg protein) calculated for each animal; the results are based on weighted analysis of variance.

*Epi, endo, epicardial and endocardial half of the transmural myocardial sites, respectively.

Cobra venom factor vs. saline, p=0.057.

Cobra venom factor vs. saline, p=0.042.
percent reduction in endocardial creatine kinase in the ibuprofen-treated group (50.6±4.5%) was not statistically different when compared with the saline-treated group (p=0.22). Although the percent epicardial and endocardial creatine kinase reductions appeared less in the CVF-treated group compared with the ibuprofen group, the differences did not quite achieve statistical significance (p=0.10 and 0.11, respectively).

Histopathology

In control, saline-treated animals, myocardial destruction in the expected infarct sites was extensive and characterized by transmural myofibril disintegration (with sparing of fibers immediately adjacent to the endocardium and epicardium). These injured fibers lacked nuclei, were shrunken, and were intensely eosinophilic. Of importance, this widespread, transmural myocardial injury in these infarct sites was always accompanied by significant PMN infiltration. In contrast to the saline-treated animals, a large proportion of ECG-designated infarct sites from both the CVF-treated and ibuprofen-treated animals were nontransmural and retained considerable myofibril preservation. Many of these fibers had retained nuclear detail although they were swollen and were more eosinophilic than normal. This preservation was most pronounced in areas of myocardium in the endocardial half of the transmural sections.

Blinded histopathological assessment of tissues from all sites in all animals documented that transmural involvement of the myocardium was present in every ECG-designated infarct site (n=15) in the control, saline-treated animals. By contrast, only six of the 15 expected infarct sites in the CVF-treated animals and 10 of 17 in the ibuprofen-treated animals were similarly affected. When the logit of the proportion of expected infarct sites with nontransmural involvement from all animals were compared, both the CVF-treated and the ibuprofen-treated groups were significantly different from the saline control group (Table 3). Although CVF treatment appeared more effective than ibuprofen treatment with respect to histological preservation, the difference between the two groups in the proportion of sites that were classified as nontransmural was not statistically significant.

In addition, and of importance, both CVF and ibuprofen interventions reduced the infiltration and interstitial accumulation of PMNs in the ischemic myocardium. Occasionally, PMNs were present within the vasculature in these infarct sites; however, as compared with infarcted myocardium from control animals, these acute inflammatory cells were reduced in both treatment groups (Table 4). PMN infiltration into the epicardium was regularly observed in most sites (both infarcted and normal) from all animals but was not included in this enumeration; this infiltrate likely reflected the trauma of the surgical procedure and the repeated unipolar ECG mapping required both immediately before and after coronary artery ligation. Of note in light of the histological preservation described above, the myocardial thickness in ECG-designated infarct sites was comparable in all groups. Thus, the extensive myofibril shrinkage in infarct sites from saline-treated animals was compensated by increased interstitial space.

Complement Component Levels

Plasma levels of functional C3 were reduced 100% 3 hours after coronary artery ligation in the

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**TABLE 2.** Percent Reduction in Myocardial Creatine Kinase Content in Electrocardiographically Designated Infarct Sites

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Percent creatine kinase reduction</th>
<th>95% confidence interval</th>
<th>Percent creatine kinase reduction</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epicardium</td>
<td>Endocardium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>Mean±SEM</td>
<td>54.1±5.4</td>
<td>42.5–65.7</td>
<td>59.0±4.7</td>
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<tr>
<td>Ibuprofen</td>
<td></td>
<td>45.0±5.1</td>
<td>34.1–56.0</td>
<td>50.6±4.5</td>
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<tr>
<td>Cobra venom factor</td>
<td>31.7±5.6*</td>
<td>19.7–43.7</td>
<td></td>
<td>39.3±4.8†</td>
</tr>
</tbody>
</table>

Mean creatine kinase content from the normal and expected infarct sites for each animal was used to calculate the percent creatine kinase reduction for that animal. These values were used to calculate the mean±SEM percent creatine kinase reduction for each group; the results are based on weighted analysis.

*Cobra venom factor vs. saline, p=0.011.
†Cobra venom factor vs. saline, p=0.008.

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**TABLE 3.** Estimated Proportion of Electrocardiographically Designated Infarct Sites Histologically Classified as Nontransmural

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Proportion (nontransmural)</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>0.14*</td>
<td>0.06–0.30</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>0.47†</td>
<td>0.30–0.64</td>
</tr>
<tr>
<td>Cobra venom factor</td>
<td>0.53‡</td>
<td>0.36–0.70</td>
</tr>
</tbody>
</table>

All electrocardiographically designated infarct sites were classified as either transmural or nontransmural after blinded histopathological examination (see "Materials and Methods"). The proportion of expected infarct sites classified as nontransmural was determined for each group; the results are based on weighted analysis.

*This proportion differs from the observed proportion of 0.0 because of the way a zero value is handled.
†Ibuprofen vs. saline, p=0.008.
‡Cobra venom factor vs. saline, p=0.003.
TABLE 4. Effect of Ibuprofen and Cobra Venom Factor Administration on Myocardial Neutrophil (PMN) Sequestration and Transmural Thickness 24 Hours After Coronary Artery Ligation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PMN/mm²</th>
<th>Transmural thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Expected</td>
<td>Expected</td>
</tr>
<tr>
<td></td>
<td>sites</td>
<td>infarct sites</td>
</tr>
<tr>
<td>Control</td>
<td>36±4</td>
<td>7±1</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>24±4†</td>
<td>6±1</td>
</tr>
<tr>
<td>Cobra venom factor</td>
<td>24±4‡</td>
<td>7±1</td>
</tr>
</tbody>
</table>

Data are presented as the group mean±SEM of averaged values calculated for each animal; the results are based on weighted analysis.

†Ibuprofen vs. saline, p=0.059.
‡Cobra venom factor vs. saline, p=0.062.

CVF-treated animals (i.e., 2.5 hours after initiation of CVF administration) and remained undetectable for 24 hours (Figure 3). There also were substantial reductions in the plasma levels of hemolytically active C5 (i.e., >85% reduction at 6 hours) (Figure 3). In addition, C6 levels fell in concert with C5 until 5 hours after ligation. Plasma levels of both C3 and C5 remained decreased at 24 hours, whereas C6 levels were returning to normal. There were no detectable changes in the plasma levels of C3, C5, and C6 in the saline- and ibuprofen-treated groups during the 24-hour period of the study. Finally, C4 levels were unchanged in all treatment groups.

Immunohistochemistry

The distribution of C3, C4, and C5 in the infarcted baboon myocardium from control animals was consistent with our previous study.22 Briefly, these complement components were found within damaged myocardial fibers in expected infarct sites. The distribution of these components within myofibrils was dissimilar in that C3 and C5 were present in ischemic myocardial fibers in a granular and diffuse pattern, whereas C4 in the same fibers was found in only a diffuse pattern. In addition, C3 and C5 but not C4 were localized in small muscular arteries in the infarct sites.

The administration of ibuprofen had no effect on the distribution of complement within the infarcted baboon myocardium as compared with the infarcted myocardium of the saline-treated animals. Thus, C3, C4, and C5 were all present in infarct sites in both injured myocardial fibers and in small muscular arteries, the vascular distribution being restricted to only C3 and C5.

In contrast to both the ibuprofen- and saline-treated animals, the administration of CVF 30 minutes after coronary artery ligation abrogated both C3 and C5 localization in infarcted baboon myocardium (Figure 4). Of interest, C4 localization in these CVF-treated animals was unchanged, or somewhat enhanced, relative to the saline- and ibuprofen-treated animals.

Hemodynamic Alterations

Although the mean heart rate decreased significantly with time after coronary artery ligation in all three groups (p<0.01), there were no significant differences at any time in the mean heart rate between the three treatment groups (Table 5). Mean arterial pressures showed no significant changes over time, and there were no differences between treatment groups. Initial pulmonary artery wedge pressures were similar for the saline, ibuprofen, and CVF groups (6±2, 6±2, and 8±3 mm Hg, respectively) and did not change significantly over time or between treatment groups.

Discussion

This study documents that CVF and ibuprofen administered 30 minutes after coronary artery ligation in the baboon significantly reduced the amount of ischemic tissue injury 24 hours later. Using a similar baboon infarction model, we had previously
FIGURE 4. Microphotographs showing direct immunohistochemical localization of complement (C3, C4, and C5) in adjacent frozen sections of an electrocardiographically designated infarct site obtained from a cobra venom factor-treated baboon. Panel A: C3 localization. Panel B: C4 localization. Panel C: C5 localization. Panel D: Normal goat Fab′-horseradish peroxidase; immunohistochemical control. Note that in contrast to C3 and C5 localization, C4 was widely distributed in myocardial fibers in this infarct site. No complement components were observed in those fibers immediately adjacent to the endocardium (arrows). Bar represents 100 μm.
demonstrated that the administration of CVF 24 hours before coronary artery ligation significantly decreased ischemic myocardial tissue injury 24 hours later. In the present study, CVF administration was initiated 30 minutes after coronary artery ligation. This intervention resulted in 1) the complete loss of functional C3 activity in the circulating plasma within 3 hours of coronary occlusion, 2) appreciable reductions in the circulating levels of functionally active C5 and C6 within 2-4 hours, 3) an abrogation of C3 and C5 deposition in the ischemic myocardium, 4) a reduction in PMN accumulation in designated infarct sites, and 5) significant reductions in the loss of creatine kinase in designated infarct sites. A comparison of the relative beneficial effects of CVF administered before or 30 minutes after (present study) coronary artery ligation indicated they were comparable. Therefore, complement-dependent events contributing to the subsequent ischemic myocardial injury at 24 hours apparently do not occur within the first 3 hours after coronary artery occlusion.

Ibuprofen was also observed to reduce the extent of myocardial injury 24 hours after coronary ligation in the baboon; however, at least with respect to creatine kinase depletion, the degree of amelioration was not as pronounced as that provided by CVF intervention (Tables 1 and 2). The tissue-sparing effect mediated by ibuprofen was most apparent in the histopathology studies that indicated that it was as effective as CVF in decreasing the amount of myocardial necrosis in ECG-designated infarct sites (Table 3). Ibuprofen administration has also been reported to provide beneficial tissue-sparing effects after acute myocardial ischemic tissue injury in murine, feline, and canine models.

The precise mechanisms for the protective actions of CVF and ibuprofen in sparing the ischemic myocardium presently are not known but likely are a result of multiple effects. One possibility that has been excluded in several studies including the present report (Table 5) is that CVF or ibuprofen do not alter myocardial oxygen demand. However, one common feature shared by both therapeutic agents is their ability to reduce PMN infiltration into the ischemic and infarcted myocardium (Table 4). Such a reduction in the extent of PMN infiltration could decrease tissue injury preventing the release of PMN-derived lysosomal enzymes and oxygen-derivable free radicals or by preventing PMN-mediated alterations in the rheology of the cardiac microcirculation within the ischemic myocardium through capillary plugging. CVF would have the additive effect of abrogating PMN adherence and activation via ablating the membrane accumulation of C3b; in contrast, ibuprofen intervention would not affect such an interaction (i.e., there was substantial C3 accumulation in the ischemic myocardium of these animals). Nevertheless, ibuprofen has been documented to significantly reduce receptor-mediated PMN responses, including lysosomal enzyme secretion and production of superoxide anion.

One contrasting effect between CVF and ibuprofen observed in the present study was that though both agents reduced the extent of histological tissue injury, it appeared that CVF tended to be more effective (p<0.01) in reducing creatine kinase depletion from the ECG-designated infarct sites (Tables 1 and 2). One possible explanation for these apparent disparate indexes of tissue injury was the observation that C5 was localized in the ischemic myocardium of the ibuprofen-treated but not CVF-treated animals. The accumulation of C5 in ischemic myocardium suggests membrane assembly of the C5b-9 MAC; this was also supported by the accumulation of C8 in the saline-treated and ibuprofen-treated but not CVF-treated animals (data not shown). Furthermore, recent studies of human infarcted myocardial tissue demonstrated the presence of MAC neoantigen in the absence of the S protein. This latter observation virtually documents the accumulation of cytolytically active MAC in ischemic but not normal, myocardial tissue. Thus, the cytolytically active MAC could induce the production of transmembrane channels in the sarcolemma with a resultant influx of divalent cations, including Ca++, leading to a more rapid disruption of myocyte function and release of cytosolic constituents, including crea-

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**Table 5. Heart Rate and Mean Arterial Blood Pressure After Coronary Artery Ligation in the Baboon**

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>0</th>
<th>15</th>
<th>60</th>
<th>120</th>
<th>240</th>
<th>360</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>100±3</td>
<td>89±7</td>
<td>85±6</td>
<td>81±6</td>
<td>73±6</td>
<td>72±7†</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>111±10</td>
<td>99±5</td>
<td>97±5</td>
<td>88±4</td>
<td>85±4</td>
<td>82±5†</td>
</tr>
<tr>
<td>Cobra venom factor</td>
<td>101±6</td>
<td>99±7</td>
<td>94±5</td>
<td>86±4</td>
<td>81±4</td>
<td>74±5†</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>77±6*</td>
<td>77±5</td>
<td>85±6</td>
<td>81±5</td>
<td>75±6</td>
<td>75±10</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>86±6</td>
<td>84±6</td>
<td>89±6</td>
<td>82±6</td>
<td>87±2</td>
<td>86±2</td>
</tr>
<tr>
<td>Cobra venom factor</td>
<td>69±9</td>
<td>75±6</td>
<td>79±3</td>
<td>65±3</td>
<td>63±4</td>
<td>61±5</td>
</tr>
</tbody>
</table>

All values represent mean±SEM.
†Significantly different (p<0.01) from preocclusion values.
tine kinase. In the present study, CVF treatment abrogated C5 localization and would therefore prevent the formation of the MAC and thereby reduce creatine kinase release; in contrast, the assembly of cytolytically active MAC likely occurred in the ibuprofen-treated animals. Therefore, we speculate that the localization of cytolytically active MAC in the ibuprofen-treated animals could have been responsible for a more rapid and greater depletion of myocardial creatine kinase in the ECG-designated infarct sites than was observed in the CVF-treated animals.

Although not directly applicable to the current model of permanent coronary artery occlusion, one could anticipate that these detrimental actions of complement-derived phlogistic mediators also would be significantly augmented during coronary reperfusion. For example, alterations in the rheology of the myocardial microcirculation due to capillary plugging by complement-activated PMN may be responsible at least in part for the no-reflow phenomenon observed after reperfusion. This may be of particular relevance in view of recent observations in acute myocardial infarction patients where increased plasma levels of both C3a and C5a occur after the initiation of reperfusion therapy with recombinant tissue plasminogen activator.

In summary, sufficient evidence now has been accumulated to favor possible therapeutic intervention to reduce or abrogate complement and/or PMN recruitment and activation during myocardial ischemia and coronary reperfusion to reduce tissue injury and extend the time available for reperfusion therapy to be effective. Although during acute myocardial ischemia, appropriate anti-inflammatory intervention will reduce tissue injury in the short-term, it is presently unclear whether long-term benefits would be realized. For example, although ibuprofen reduces ischemic myocardial tissue injury, interfering with the inflammatory process also could adversely affect the reparative process, resulting in subsequent scar thinning. In this regard and in keeping with previous considerations, one possible avenue for future consideration would be to temporally define the detrimental period during myocardial ischemia in which the acute inflammatory process modulates tissue injury. If anti-inflammatory therapy were then applied only during this time frame, perhaps the first 24–48 hours after the onset of acute myocardial ischemia, it might allow the subsequent inflammatory process to facilitate a normal reparative process.

References


**KEY WORDS**
complement • ibuprofen • inflammation • experimental myocardial infarction • polymorphonuclear cells
Complement and neutrophil activation in the pathogenesis of ischemic myocardial injury. 
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