Thromboxane Is Produced in Response to Intracoronary Infusions of Complement C5a in Pigs

Cyclooxygenase Blockade Does Not Reduce the Myocardial Ischemia and Leukocyte Accumulation

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Activated polymorphonuclear leukocytes (PMNs) contribute to myocardial injury during ischemia and reperfusion. There is evidence that activation of the complement pathway may be one of the mechanisms of PMN activation during ischemia. Intracoronary infusion of complement C5a during normal perfusion pressure is associated with decreased coronary flow, contractile dysfunction, and PMN accumulation. The mechanisms responsible for these changes have not been identified. Thromboxane A$_2$ (TXA$_2$) is a potential mediator of this myocardial ischemic response. Activated PMNs produce TXA$_2$, a known coronary vasoconstrictor, and TXA$_2$ was shown to be a mediator of the pulmonary hypertensive response to activated complement. The goal of the present study was to determine if an enhanced TXA$_2$ production is associated with the myocardial response to C5a and whether cyclooxygenase blockade would reduce the myocardial ischemia. In open-chest pigs, intracoronary C5a (500 ng) caused reversible reductions in blood flow (50.0% of control), regional contractile function (25.8% of control), leukocyte trapping (1.0x10$^6$ cells/g myocardium or a peak artery—coronary venous difference of 5.3x10$^3$ cells/ml blood), and increased coronary venous TXB$_2$ (the TXA$_2$ breakdown product) from 1.6 pmol/ml to a peak of 6.9 pmol/ml. Cyclooxygenase blockade with aspirin or indomethacin, which prevented TXB$_2$ production, did not alter the response in flow, function, or PMN trapping. Ibuprofen, a known direct inhibitor of PMNs in addition to its cyclooxygenase blockade effect, reduced the response slightly. The pig coronary vascular bed was responsive to the TXA$_2$ agonist U46619, which reduced flow and function without PMN trapping. Mechanical reductions in coronary flow to levels equivalent to those during the C5a infusions did not increase coronary venous TXB$_2$ nor cause PMN trapping but did cause equivalent contractile dysfunction. Incubation of whole blood with C5a at concentrations equivalent to those achieved in vivo did not cause TXB$_2$ production. We conclude that 1) TXA$_2$ is produced in response to intracoronary C5a and 2) cyclooxygenase blockade does not prevent the C5a-induced myocardial ischemia, contractile dysfunction, and PMN trapping. The TXA$_2$ production likely involves a vascular site or a blood cell–vascular interaction. This model system indicates the potential for persistently activated PMNs to cause continued ischemia during myocardial reperfusion. (Circulation Research 1989;65:1220–1232)
PMN-mediated processes in myocardial ischemia. There is evidence that activation of the complement cascade with the generation of species with PMN chemotactic and secretagogue effects may be one of the mechanisms of PMN activation associated with ischemia. Components of the complement pathway are found in ischemic myocardium and correlate with the degree of leukocyte accumulation. In addition, inactivation of the complement system reduces the degree of myocardial damage after experimental acute coronary artery occlusion. Intracoronary infusion of purified activated complement C5a in the presence of normal coronary perfusion pressures is associated with transient decreases in myocardial blood flow, depressed contractile function, and leukocyte accumulation. These latter data suggest that the presence of activated complement components in ischemic or reperfused myocardium may further compromise myocardial perfusion and contractile function.

Activated complement is a potent stimulator of PMN adhesion and aggregation and initiates production and release of oxygen free radicals and production of vasoactive substances. Of particular interest is the finding that PMNs are a source of arachidonic acid via the cyclooxygenase pathway. TXA2 and its analogues are potent coronary vasoconstrictors and have been shown to contribute to the extension of ischemic damage through vasoactive or platelet-aggregating mechanisms and to propagate circulatory shock. TXA2 and its analogues are potent coronary vasoconstrictors and have been shown to contribute to the extension of ischemic damage through vasoactive or platelet-aggregating mechanisms and to propagate circulatory shock. However, the role of TXA2 in mediating the myocardial response to activated complement is unknown.

The goals of the present study were 1) to determine if the arachidonic acid product, TXA2, is produced and released into the coronary circulation coincident with the decrease in myocardial blood flow and contractile function elicited by intracoronary infusions of C5a and 2) to assess the effect of cyclooxygenase blockade in preventing the myocardial ischemia and transient leukocyte accumulation induced by C5a.

Materials and Methods

General Preparation

Domestic pigs (25–30 kg) of either sex were premedicated with ketamine (2.5 mg/kg i.m.) and anesthetized 10–15 minutes later, initially with sodium thiamylal (10–15 mg/kg i.v.) followed by α-chloralose (100 mg/kg i.v.). After intubation with a cuffed endotracheal tube, animals were ventilated with a positive-pressure respirator (Harvard Apparatus, South Natick, Massachusetts) with an end-expiratory pressure of 3–5 cm H2O. Arterial blood was regularly sampled for determination of PO2, PCO2, or pH (Radiometer ABL-3, Copenhagen, Denmark). Blood gas values were kept within the physiological range by adjustments of the ventilatory rate and oxygen supplementation. The anesthetic level was maintained with bolus doses of α-chloralose (250–500 mg i.v.). Animal core temperature (rectal) was monitored and maintained at 37°C with a circulating water heating pad.

Aortic blood pressure was measured with a strain gauge manometer (Statham P23Db, Gould, Cleveland, Ohio) through a polyethylene catheter placed into the aorta via the right femoral artery. Left ventricular (LV) pressure was obtained with a catheter-tipped manometer (model 7F, Millar, Houston, Texas) inserted into the left ventricle via the right carotid artery. This signal was recorded at full scale and high gain for determination of peak LV pressure and LV end-diastolic pressure and differentiated to obtain LV dp/dt.

The heart was exposed through a midline sternotomy and left thoracotomy in the fourth intercostal space and suspended in a pericardial cradle. A section of the left anterior descending coronary artery (LAD) just distal to the bifurcation of the left main artery was dissected free from underlying tissue for catheterization and flowmeter placement. Anticoagulation was achieved with sodium heparin (500 units/kg i.v.) and maintained by hourly supplemental doses of 250 units/kg.

Sonomicrometry

Pairs of lensed ultrasonic dimension crystals (2 mm diameter) were placed into the inner third of the myocardial wall (6–8 mm deep), approximately 1.0–1.5 cm apart and perpendicular to the base-apex chord to allow continuous measurement of regional myocardial contractile function. One crystal pair was placed into the region perfused by the dissected LAD (treatment region). A second pair was placed into the area perfused by the circumflex artery in the basal-lateral region of the ventricular free wall. Dimension signals were calibrated before and after each experiment.

Measurements of Coronary Blood Flow

Coronary blood flow in the LAD was measured with a noncannulating ultrasonic transit-time flow probe (Transonic Systems, Ithaca, New York) placed around the prepared LAD vessel. A modified Teflon intravenous catheter (24-gauge) was inserted into the LAD just distal to the flow probe for intracoronary infusions of activated complement C5a.

In those experiments requiring control of coronary perfusion (n = 2), the LAD was cannulated and perfused through a silastic and polyethylene extracorporeal shunt from a carotid artery to the LAD. Perfusion pressure was measured with a strain gauge manometer (Statham P23Db, Gould) just proximal to the cannula tip through a side arm, and coronary flow was measured with an in-line flow probe (Transonic Systems). The infusions of C5a...
were made directly into the coronary perfusion line. The coronary perfusion setup in this latter case allowed controlled reduction in coronary flow by use of a screw clamp. The flow probe was calibrated at the conclusion of each study with whole blood by use of timed collections.

**Coronary Venous Blood Sampling**

The interventricular vein adjacent to the prepared LAD vessel was ligated and cannulated distally with a small polyethylene and silastic catheter (10 cm long) to allow sampling of coronary venous blood draining the LAD perfusion region. The catheter end was placed 5–10 cm below heart level, and the blood was continuously drained into a small beaker and returned to a jugular vein by use of a roller pump.

Blood samples for complete blood count determination were collected in EDTA and processed using a QBC differential blood cell counter (Beckton-Dickinson Clay Adams, Franklin Lakes, New Jersey). Plasma from blood for determination of thromboxane B2 (TXB2) concentrations was separated by centrifugation at 4°C and at 1,500g for 15 minutes and frozen at −20°C. Blinded measurement of TXB2 was made by a radioimmunoassay technique with a lower limit of resolution of approximately 0.2–0.3 pmol/mL.

Aortic pressure, LV pressure, LV end-diastolic pressure, LV dP/dt, ultrasonic segment lengths, and mean coronary blood flow were recorded on an eight-channel oscillographic recorder (Gould). All measurements were obtained with respiration suspended at end expiration to avoid respiratory fluctuations in segment lengths.

**Preparation of Activated Complement C5a**

Porcine C5a was prepared from zymosan-activated porcine serum containing 10 μM 2-mercaptomethyl-3-guanidinoethylthio-propanoic acid (Calbiochem, La Jolla, California) as a carboxypeptidase inhibitor as previously described by Hugli et al. The purity and quantity of the polypeptide was established by amino acid analysis after acid hydrolysis. The solutions of C5a were formulated by diluting with 1% bovine serum albumin.

**Experimental Design**

Three series of in vivo experiments were performed: 1) measurements of C5a-induced thromboxane production, 2) pharmacological blockade of thromboxane production, and 3) infusions of a thromboxane agonist to test for coronary vascular responsiveness.

**Thromboxane production.** To determine if an enhanced TXA2 production is a part of the myocardial response to intracoronary infusions of C5a, the stable TXA2 breakdown product, TXB2, was measured in arterial and coronary venous blood during and after intracoronary infusions of 500 ng C5a in 12 animals. To rule out the effect of myocardial ischemia alone on thromboxane production, the LAD was cannulated and perfused with a carotid to coronary artery shunt in two additional animals. After determining the control response to C5a, an experimental run was made in which coronary flow was mechanically reduced to the same degree and duration, mimicking the myocardial response observed during the C5a injection. Coronary venous blood was obtained during the mechanically induced decreases in blood flow for TXB2 determinations.

**Pharmacological blockade.** To determine the ability of cyclooxygenase blockade to prevent the myocardial response to C5a, animals were subjected to one of four treatment protocols: 1) Aspirin (10 mg/kg i.v., n=5) was administered to block the production of thromboxane. 2) Indomethacin (5 mg/kg i.v., n=2) was given to confirm the effect of cyclooxygenase blockade on the C5a response. 3) Ibuprofen (10 mg/kg i.v., n=4), a cyclooxygenase inhibitory agent with independent effects on neutrophil function, was administered. 4) In a control group (n=4), the response to C5a was repeated without the addition of any drug treatment to assess any effect of tachyphylaxis and preparation degradation on the C5a response.

**Thromboxane analogue.** To verify that the coronary vascular bed in the porcine preparation used in these experiments was responsive to thromboxane, a TXA2/prostaglandin H2 agonist, U46619 (9-11-methano-epoxy prostaglandin H2, Upjohn, Kalamazoo, Michigan), was infused (7.5 μg i.c. over 1 minute) in three animals while changes in coronary blood flow and coronary venous leukocyte count were monitored. These experiments also provided data on the direct effect of thromboxane on myocardial leukocyte accumulation.

**In vitro studies.** To determine the effects of purified C5a on thromboxane generation of whole blood, 20 ml heparinized porcine arterial blood (n=7) was incubated at 37°C in a shaker bath. Serial samples were taken just before and 7.5 minutes and 15 minutes after the addition of 400 ng C5a. The final concentration of C5a (20 ng/ml) was estimated to be at least twice the concentration achieved with the intracoronary infusions of 500 ng into the LAD by use of the average measured coronary blood flow. In a parallel experiment, 20 ml arterial blood was incubated with arachidonic acid (Sigma Chemical, St. Louis, Missouri) to a final concentration of 1 mM (n=3) or 0.01 mM (n=3). Blood samples were taken at the same time points described above. All samples were then processed for TXB2 determination in an identical manner to those obtained from the in vivo experiments.

**Experimental Protocol**

Control experimental runs were obtained in all animals under steady-state conditions before pharmacological treatment. The protocol for an experimental run consisted of a prestimulus period, a 1-minute C5a-infusion period, and a 5-minute recov-
Figure 1. Graphs showing effects of 500 ng C5a injected over 1 minute in the left anterior descending coronary artery on coronary blood flow (left panel) and percent segment shortening (right panel). The infusion of C5a produced a decrease in coronary blood flow and a regional myocardial contractile dysfunction. *p<0.01.

Thromboxane Production

Control response to C5a (n=12). The intracoronary infusion of 500 ng C5a resulted in a prompt and...
significant decrease in LAD myocardial blood flow and segment shortening (Figure 1). Average blood flow at 45 seconds of injection reached a nadir of 50±5.5% (p<0.01) of the preinjection value of 0.64±0.08 ml/g/min. Blood flow returned to control levels within 1 minute after infusion and was followed by a hyperemic phase reaching peak levels of 145±14.6% of control (p<0.01). Average normalized segment shortening in the LAD region fell to a minimum level of 25.8±11.6% (p<0.01) of the preinjection control value by the end of injection. Percent systolic segment shortening before C5a infusion was 13.3±0.9% of the end-diastolic length (10.9±0.8 mm) and increased slightly but significantly (p<0.01) coincident with the decrease in contractile function in the treatment region (Figure 1). There were no significant changes in heart rate over the course of the experimental run from a preinfusion value of 127±5.7 beats/min. Peak LV pressure fell slightly, but significantly, by less than 10 mm Hg during the C5a infusion (p<0.05). At the time of the nadir in coronary flow (45 seconds of infusion), LV pressure had fallen by 7.1±2.8 mm Hg from the preinjection value (101.0±6.3 mm Hg).

Leukocyte counts in arterial and coronary venous blood during and after the infusion of C5a are depicted in Figure 2, left panel. The intracoronary infusion of C5a resulted in a transient net myocardial accumulation of leukocytes. Leukocyte count in coronary venous blood fell from a control value of 11.1±1.2×10^3/μl to a minimal value of 5.8±0.7×10^3/μl (p<0.01) and recovered to preinfusion and arterial levels within 2 minutes. The leukocyte count in arterial blood was 10.0±1.1×10^3/μl before C5a infusion and did not significantly change during or after the infusion period. The average estimated leukocyte accumulation during the 1-minute infusion was 1.62±0.31×10^6 cells/g and up to 1 minute after infusion was 1.62±0.31×10^6 cells/g perfused myocardium. There were no significant changes in hematocrit in response to C5a injection.

Plasma concentrations of TXB_2 in arterial and coronary venous blood are shown in Figure 2, right panel. The intracoronary infusion of C5a resulted in a significant increase of approximately fourfold in the TXB_2 concentration in coronary venous blood from a control value of 1.6±0.4 pmol/ml to a maximal value at the end of infusion of 6.9±1.4 pmol/ml (p<0.01). Coronary venous TXB_2 concentrations decreased over time but were still above arterial values 2 minutes after the end of C5a infusion. The concentrations of TXB_2 in arterial blood determined just before injection and 2 minutes after injection were not significantly different (p>0.10).

**Ischemia group:** Effect of myocardial ischemia on blood TXB_2 concentrations (n=2). Mechanically induced reductions in coronary blood flow, to the same level as that achieved with the control infusion of C5a and sufficient to lower contractile function, did not result in elevations in coronary venous TXB_2 concentrations. In these two animals, coronary flow was 64.3% of control levels during the C5a infusion and 63.3% of control during the flow restriction. Segment shortening fell to less than 60% of control levels during both interventions. Average TXB_2 concentration in coronary sinus blood (n=2) was 4.2 pmol/ml before the flow restriction and 3.9 pmol/ml during the restricted coronary flow. Coronary venous leukocyte count was also not detectably altered during or after the ischemia, in contrast to the control C5a run. Thus, the increases in coronary...
venous blood thromboxane concentrations and the leukocyte trapping observed with infusions of C5a was not secondary to the myocardial ischemia.

Pharmacological Blockade

Aspirin group: Response to C5a after aspirin treatment \((n=5)\). Average values for the response to C5a in five pigs, before and after aspirin administration are shown in Figures 3 and 4. Before aspirin treatment, C5a resulted in a decrease in coronary blood flow at 45 seconds of infusion to 69.7±9.2% of the preinfusion value. After the administration of aspirin (10 mg/kg), the flow reduction in response to 500 ng C5a was slightly slower in onset, but the nadir (72.5±2.8) was not significantly different \((p>0.1)\) compared with the control response (Figure 3, left panel). A similar pattern was also observed in regional contractile function. Before the administration of aspirin, normalized segment shortening in the treatment area fell to a nadir of 42.2±15.0% of the preinfusion value. In presence of aspirin, the decrease in segment shortening was slower to develop; however, the nadir value (55.3±8.9%) was not significantly different from the control response \((p>0.10)\) (Figure 3, right panel). The C5a-induced maximal increase in myocardial leukocyte extraction (arterial-coronary venous) was also not significantly different \((p>0.10)\) after aspirin treatment (Figure 4, left panel). The leukocyte accumulation during the C5a injection was \(2.0±0.2\times10^6\) cells/g during control and \(1.6±0.3\times10^6\) cells/g after aspirin treatment. The estimated total trapping (up to 1 minute after injection) during control injection was \(3.1±0.6\times10^6\) cells/g and after aspirin treatment was \(2.6±0.5\times10^6\) cells/g. In contrast, the administration of aspirin completely inhibited the C5a-induced increase in TXB2 (Figure 4, right panel). The arterial and coronary venous concentrations of TXB2 remained stable at less than 0.6 pmol/ml.

![Figure 3](image-url) - Graphs showing effects of 10 mg/kg i.v. aspirin (ASA) on the response to 500 ng C5a on the coronary blood flow (left panel) and segment shortening (right panel). The administration of ASA did not greatly influence the C5a-induced decrease in blood flow and contractile function.

![Figure 4](image-url) - Graphs showing effects of 10 mg/kg i.v. aspirin (ASA) on the response to 500 ng C5a on myocardial leukocyte extraction (left panel) and thromboxane production (right panel). ASA had no effect on the C5a-induced myocardial extraction of leukocytes but blocked the increased formation of thromboxane. Control group data from Figure 2, right.
throughout the experimental run. There were no significant alterations in either heart rate or LV pressure during or after C5a administration in either the control or the aspirin experimental runs.

Indomethacin group: Response to C5a after indomethacin (n=2). The absence of major effects of cyclooxygenase blockade on the myocardial response to C5a was verified in two animals by administering indomethacin (5 mg/kg i.v.) after determining the control response to C5a. Before indomethacin administration, intracoronary C5a (500 ng) reduced coronary blood flow to 52.5% of preinfusion values and regional segment shortening to 0.8% of preinfusion values. After cyclooxygenase blockade with indomethacin, the responses were similar with average flow decreasing to 60.2% and segment shortening falling to 7% of preinfusion levels. Indomethacin also had no effect on the C5a-induced fall in the coronary venous leukocyte count, which fell by $8.2 \times 10^3$ cells/μl in the control run and $7.4 \times 10^3$ cells/μl after indomethacin administration. Before indomethacin administration, C5a increased TXB2 concentrations in the coronary venous blood from 0.6 pmol/ml to 12.6 pmol/ml. Similar to the aspirin group, indomethacin completely blocked this response; plasma concentrations in arterial and coronary venous blood remained less than 0.6 pmol/ml throughout the experimental run.

Ibuprofen group: Response to C5a (n=4). Average values for four animals that were subjected to the ibuprofen protocol are shown in Figures 5 and 6. During the control C5a infusion, coronary blood flow decreased to 47.9±11.1% and treatment region segment shortening decreased to 29.7±21.3% of the preinfusion level at 45 seconds. After the administration of ibuprofen (10 mg/kg), the decrease in blood flow and segment shortening in response to the same dose of C5a was slightly but significantly blunted. At 45 seconds of infusion, coronary flow was 82.9±6.4% and segment shortening 84.4±9.8%
of the preinfusion levels; these values were significantly different from the control run ($p<0.05$). The maximal decrease in coronary flow and segment shortening elicited by C5a after ibuprofen administration was delayed and also reduced compared with the control run ($p<0.05$). The effects of ibuprofen on the response in myocardial leukocyte extraction and thromboxane production are depicted in Figure 6. Ibuprofen did not alter the transient increase in myocardial leukocyte extraction in response to C5a, with similar maximal values of approximately $4.0 \times 10^3$ cells/$\mu l$ in both control and ibuprofen experimental runs. Similar to the other cyclooxygenase inhibitors, ibuprofen administration completely eliminated the C5a-induced increase in coronary venous blood TXB2 concentrations ($p<0.05$). Concentrations remained within 0.3 pmol/ml of the preinfusion point throughout the experimental run.

Untreated group: Control responses to two injections of C5a ($n=4$). The myocardial response to two injections of 500 ng C5a given 1 hour apart in the absence of pharmacological blockade is depicted in Figure 7. There was no significant difference ($p>0.1$) in either the decrease in coronary blood flow or percent segment shortening between the two C5a challenges. Thus, there was no tachyphylaxis to C5a in the normal protocol we used.

Thromboxane Agonist

Effect of the TXA2 agonist U46619 on coronary flow and leukocyte trapping ($n=3$). The intracoronary administration of U46619 (7.5 $\mu g$) resulted in a 33.2% decrease in coronary blood flow at 15 seconds of infusion and a 35.3% decrease in treatment area segment shortening at 30 seconds of infusion. This response was short-lived, with blood flow returning to within 87.4% of the control value by 45 seconds of the 1-minute infusion period (Figure 8). Heart rate and mean aortic pressure did not significantly change during or after the drug infusion. In addition, arterial and coronary venous blood leukocyte counts remained unchanged throughout the U46619 injection and subsequent recovery period.

In Vitro Studies

Effect of C5a on thromboxane production in blood. To provide information on the source of the elevated coronary venous blood TXB2 levels in response to intracoronary infusions of C5a, porcine arterial whole blood was incubated ($n=7$) at 37°C with C5a at a final concentration of 20 ng/ml. In serial samples taken 7.5 and 15 minutes after C5a addition, TXB2 concentrations were $2.2 \pm 0.5$ pmol/ml and $2.0 \pm 0.5$ pmol/ml; they were not significantly different from the preincubation value of $1.7 \pm 0.4$ pmol/ml. In contrast, the addition of arachidonic acid to arterial blood (final concentration, 1 mM; $n=3$) elevated TXB2 levels to $16.4 \pm 3.3$ pmol/ml at 7.5 minutes and $25.1 \pm 0.9$ pmol/ml at 15 minutes of incubation compared with the basal level of $1.4 \pm 0.6$ pmol/ml. Similar results were obtained with blood incubated at the lower concentration of arachidonic acid (final concentration, 0.01 mM; $n=3$). In these samples, TXB2 levels rose to $5.2 \pm 2.3$ pmol/ml at 7.5 minutes and $7.3 \pm 3.5$ pmol/ml at 15 minutes from the basal levels of $1.5 \pm 0.5$ pmol/ml.

Discussion

These data demonstrate that the intracoronary injection of C5a results in significant decreases in coronary blood flow, a decrease in regional myocardial segment function, and leukocyte trapping accompanied by a transient net myocardial TXA2 production. However, the inhibition of thromboxane generation by cyclooxygenase blockade does not alter the depression in myocardial flow and function or the myocardial leukocyte trapping in response to C5a. The administration of ibuprofen slightly but significantly reduced the decrease in
Effects of Activated Complement on Other Vascular Beds

Much of the work addressing the effects of complement activation on organ perfusion has been conducted on the pulmonary circulation. Initial studies by Craddock and coworkers determined that the pulmonary dysfunction, leukopenia, and pulmonary leukostasis often seen during hemodialysis were due to the activation of the alternate pathway of complement on contact of blood with cellophane membranes. The responsible component was identified as C5a and was shown to promote granulocyte aggregation and adhesion in vitro, as well as in vivo. Intravenous infusions of complement-activated plasma has now been demonstrated in several studies to result in increases in pulmonary vascular resistance, a fall in arterial oxygen tension, and a transient leukopenia with the accumulation of leukocytes in the pulmonary bed. Similar vascular resistance changes have been reported in the femoral circulation of swine. Although the increase in pulmonary vascular resistance was initially attributed to the mechanical effect of leukostatic plugs in the pulmonary capillaries, it was later demonstrated that these two phenomenon could be dissociated. Cyclooxygenase inhibitory agents such as sulfinpyrazone, indomethacin, or aspirin could markedly reduce the increase in resistance without altering the degree of leukopenia in response to intravenous injections of complement-activated plasma. Furthermore, infusions of activated plasma resulted in increases in blood concentrations of the pulmonary vasoconstrictor TXA2 that were abolished by cyclooxygenase inhibition. These data clearly demonstrated that a cyclooxygenase-dependent species with vasoactive properties, most likely TXA2, was predominantly responsible for the changes in vascular resistance.

In the present study, C5a infusions into the coronary bed resulted in transient increases in vascular resistance, decreases in regional contractile function, and leukocyte accumulation. The increases in coronary venous blood concentrations of TXB2 (Figure 2) are similar to those found with C5a infusion in the pulmonary vascular bed. However, in contrast to the findings in the pulmonary model, cyclooxygenase blockade in the heart was without effect on the myocardial vascular response to activated complement (Figures 3-6); the magnitude of the decrease in coronary blood flow and regional segment shortening was similar before and after administration of aspirin or indomethacin at doses sufficient to eliminate the TXB2 release.

The reasons for the difference between the results provided here and those obtained in previous studies in the pulmonary vascular bed are not clear. The porcine coronary vascular bed is responsive to TXA2 analogues such as U46619, which elicit a marked increase in vascular resistance (Figure 8).
Thus, it appears that the coronary bed can respond to elevated blood concentrations of thromboxane as well as produce thromboxane in response to C5a. However, the observation that blockade of this pathway does not alter the myocardial response to C5a suggests several possibilities: 1) Thromboxane concentrations obtained with C5a are insufficient to contribute to the vascular resistance changes. 2) The thromboxane released in response to C5a occurs at a site where it cannot act on the vascular receptor. 3) Production of other vasoconstrictors is sufficient for the response without thromboxane. An additional possibility is that the simultaneous release of a cyclooxygenase-dependent species with opposite vascular effects of thromboxane may negate the vasoconstrictor influence of thromboxane. It has been shown that increases in the stable metabolite of prostaglandin I$_2$, 6-ketoprostaglandin F$_{1alpha}$ accompany the increased thromboxane production during intravenous infusions of complement-activated plasma in sheep. Although prostaglandin I$_2$ has been shown to be a coronary vasodilator, it is unknown if an enhanced prostaglandin I$_2$ production is also part of the myocardial response to activated complement. Another potential mechanism of activated complement-mediated vasoconstriction in the heart may involve arachidonic acid metabolites of the 5-lipoxygenase pathway. Leukotrienes such as leukotriene C$_4$ or leukotriene D$_4$ are potent coronary vasoconstrictors and are produced by activated PMNs. Moreover, some lipoxygenase products are thought to be formed in increased quantities on inhibition of cyclooxygenase. Thus, it is conceivable that the increased production of a vasoconstrictor, that is, leukotriene C$_4$, upon cyclooxygenase blockade in the present study partly masked the contribution of a thromboxane-mediated effect. The potential significance of this mechanism in the coronary circulation is unknown.

**Effect of TXA$_2$ on Leukocyte Trapping**

There is evidence that TXA$_2$ increases granulocyte adhesiveness and aggregation and, thus, may contribute to their accumulation in vascular beds on activation. This pathway does not appear to be a major component of the leukocyte trapping observed in the present study. The transient myocardial accumulation of leukocytes in response to C5a was not significantly altered by the blockade of thromboxane production (Figures 4 and 6). In addition, although the intracoronary administration of the TXA$_2$/prostaglandin H$_2$ analogue, U46619, resulted in a marked decrease in coronary blood flow and myocardial function, this drug was without effect on coronary venous leukocyte levels (Figure 8). These data are in agreement with studies in the pulmonary vascular bed in which the degree of pulmonary leukocyte accumulation was not affected by cyclooxygenase blockade.  

**Source of TXA$_2$ in Response to C5a**

In the present study, intracoronary C5a was associated with increases in the coronary venous concentrations of TXB$_2$, in the absence of alterations in arterial blood concentrations (Figure 2, right panel). The exact source of this increased TXA$_2$ production is not clear, but three possible sources seem reasonable: 1) C5a may result in the release of TXA$_2$ from blood cellular components. 2) C5a may act directly with some cell type present in the heart, which then releases TXA$_2$. 3) C5a might promote a cell to cell interaction between a formed blood element (i.e., neutrophils) and a cell type present in the heart (e.g., myocyte or endothelial cell), which then releases TXA$_2$. The synthesis and release of TXA$_2$ from platelets present in blood in response to a wide variety of stimuli has been well described. It has also been shown more recently that activated neutrophils are also a source of TXA$_2$. The production of TXA$_2$ is not restricted to blood components. The production of TXA$_2$ from organ-specific vascular tissue in some species has been demonstrated and suggested to be primarily released from endothelial cells as opposed to smooth muscle cells.

In the present study, the incubation of porcine blood in vitro with purified C5a at approximately twice the concentration achieved with intracoronary infusions in vivo did not result in thromboxane production, in contrast to additions of arachidonic acid. These data indicate that, although blood cellular components are potential sources of TXA$_2$, they alone are not responsible for the increase in TXA$_2$ production in response to C5a in the pig coronary circulation. An organ or tissue component appears to be required for TXA$_2$ production. This conclusion is in agreement with a previous study on the source of complement-induced TXA$_2$ production in the pulmonary vascular bed. It was suggested in this study that C5a-activated leukocytes may stimulate vascular endothelial cell release of TXA$_2$. This process may involve the generation of oxygen free radicals or hydrogen peroxide, which may act to increase the production of TXA$_2$ from some cell type. It has recently been shown that scavenging hydrogen peroxide with dimethylthiourea attenuated the pulmonary vasoconstriction and thromboxane production in sheep in response to complement activation with heparin and protamine.

The failure of mechanically induced transient myocardial ischemia to result in detectable increases in coronary venous blood TXB$_2$ concentrations excludes the possibility that the observed increase with C5a administration was secondary to the ischemic response.

**Effect of Ibuprofen**

Similar to the results obtained with aspirin and indomethacin, the administration of ibuprofen
block the C5a-induced increase in coronary venous blood TXB2 levels (Figure 6). However, in contrast to these other cyclooxygenase inhibitors, ibuprofen did have a small but significant effect on the decrease in coronary blood flow and segment shortening elicited by C5a (Figure 5). The reason behind the apparent discrepancy is presently unclear. However, similar findings have been described in models of acute pulmonary injury produced by thrombin-induced microembolism and infusions of zymosan-activated plasma in which neutrophil activation and sequestration also occur. In these studies, ibuprofen was much more effective in preventing the increases in pulmonary vascular resistance and vascular permeability normally seen with acute lung injury. In acute myocardial injury resulting from periods of ischemia, ibuprofen has been shown to have myocardial protective properties and to reduce the accumulation of neutrophils. These properties are not shared by other cyclooxygenase inhibitors such as aspirin and indomethacin. The basis for these beneficial effects of ibuprofen are not clear. However, there is evidence that alterations in neutrophil function including reductions in neutrophil adherence to vascular endothelium, aggregation, superoxide production, and PMN lysosomal enzyme release may be involved. The contribution of these mechanisms to the C5a-induced myocardial ischemia in the present study is unknown. However, similar degrees of leukocyte accumulation in response to C5a before and after ibuprofen (Figure 6, right panel) suggest that neutrophil trapping alone is not responsible for the decrease in blood flow and that the beneficial effect of ibuprofen in this model is not mediated by this mechanism.

### Leukocyte Trapping: Relevancy to Myocardial Ischemia

C5a-receptor stimulation on neutrophils causes increased vascular adherence and trapping in the microcirculation, agglutination, and secretagogue activation. The degree of neutrophil accumulation in response to 500 ng C5a (1.0–3.0×10^6 cells/g) is the same order of magnitude seen during experimental myocardial infarction. Thus, the pig model we used may be relevant to the leukocyte-mediated increases in vascular resistance seen during experimental myocardial ischemia and reperfusion when leukocyte activation would be maintained. Cyclooxygenase blockade did not significantly decrease leukocyte accumulation, and U46619 did not cause leukocyte trapping; these findings indicated that thromboxane was likely not an important mechanism of leukocyte accumulation. It is important to note the difference in time course for leukocyte accumulation between the C5a model of leukocyte activation and in vivo ischemia; with C5a the accumulation occurs over 1–2 minutes whereas accumulation to the same degree in vivo occurs over several hours during ischemia or after 40 minutes of ischemia with reperfusion.

The conclusions of the present study are fourfold: 1) TXA2 is produced in response to intracoronary infusions of C5a. 2) Cyclooxygenase blockade does not diminish the C5a-induced increases in vascular resistance, myocardial dysfunction, or leukocyte accumulation. 3) TXA2 production is independent of the myocardial ischemia itself and likely involves a vascular site of production or blood cell–vascular interaction. 4) Unlike aspirin, the administration of ibuprofen, slightly attenuates the increases in coronary resistance and depressed function in response to intracoronary C5a. This model system indicates the potential for persistently activated PMNs to cause continued ischemia and dysfunction during myocardial reperfusion, and further investigation into the mechanisms involved is warranted.

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### References


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