Role of Thromboxane A2 in the Cardiovascular Response to Intracoronary C5a

Gregory L. Stahl, Ezra A. Amsterdam, J. David Symons, and John C. Longhurst

Intracoronary administration of complement component C5a induces transient decreases in coronary blood flow and regional left ventricular segment shortening, associated with intramyocardial granulocyte trapping. We evaluated the influence of a cyclooxygenase inhibitor (acetylsalicylic acid, n=8) or a thromboxane A2/prostaglandin H2 receptor antagonist (SQ29548, n=6) on these C5a-induced cardiovascular responses. Open-chest anesthetized pigs were instrumented to monitor heart rate, arterial blood pressure, left anterior descending coronary blood flow, regional left ventricular segment shortening, and dP/dt. Oxygen content, lactate concentration, leukocyte count, and thromboxane B2, the stable metabolite of thromboxane A2, were measured in arterial and regional coronary venous blood. Repetitive injections of intracoronary C5a (500 ng) given 60 minutes apart showed no tachyphylaxis of the hemodynamic response. However, tachyphylaxis was seen in coronary blood flow changes when injections were spaced 30 minutes apart. An increase in myocardial oxygen extraction and lactate production was observed after intracoronary C5a. Administration of acetylsalicylic acid (50 mg/kg i.v.) attenuated C5a-induced decreases in coronary blood flow (−8±1 vs. −3±1 ml/min) and regional left ventricular segmental shortening (−10±3% vs. −2±1%) and blocked the maximal increase in coronary venous thromboxane B2 (2.0±0.1 vs. 0.2±0.1 pmol/ml plasma). Furthermore, SQ29548 (30 µg/kg/min) reduced C5a-induced changes in coronary blood flow (−13±2 vs. −4±2 ml/min) and segmental shortening (−14±2% vs. −3±1%). Neither cyclooxygenase inhibition nor thromboxane A2/prostaglandin H2 antagonism blocked the decrease in coronary venous granulocyte count. Thus, the deleterious cardiac effects of C5a, which include induction of myocardial ischemia, are dependent on the actions of thromboxane A2 in the pig. Our data also suggest that leukocyte extraction alone in the coronary circulation is not sufficient for the myocardial response to anaphylatoxin administration. (Circulation Research 1990;66:1103–1111)

Myocardial ischemia activates the complement cascade, which promotes further cardiac injury.1–4 Evidence for a role of complement products in myocardial ischemic injury is supported by several lines of experimental and clinical evidence. Complement anaphylatoxin C5a activates granulocytes,5 which may occlude the coronary microcirculation by increased intravascular adherence.6 C5a also constricts vascular smooth muscle5,7 and, thereby, may directly increase coronary artery resistance and decrease coronary flow.6 Our group previously has shown that intracoronary administration of purified porcine C5a in swine produces transient reductions in coronary blood flow and left ventricular (LV) segmental function,6 suggesting myocardial ischemia. It is also known that complement components C1q, C3, C4, and C5 localize in experimentally infarcted myocardium.1–8 Conversely, depletion of complement reduces the extent of infarction in association with absence of complement in the infarcted zone.2,4 Finally, recent findings indicate activation of the complement system in patients with myocardial infarction.9 Taken together, these observations are consistent with an important role of anaphylatoxins in mediating ischemic injury in myocardial infarction.

The mechanisms underlying the deleterious cardiac effects of complement have not been clarified.
Existence of a granulocyte-dependent process is supported by complement’s enhancement of granulocyte adherence, as well as by reduction of cardiac ischemic injury and blunting of the coronary hemodynamic effects of C5a in granulocyte-ficient animals. Activated granulocytes can produce products of arachidonic acid metabolism, such as leukotrienes and thromboxane, which, through their action on vascular smooth muscle, may reduce coronary flow and augment myocardial ischemia. Therefore, we hypothesized that 1) the regional LV dysfunction consequent to C5a administration is a manifestation of ischemia as reflected by myocardial lactate production and 2) intracoronary administration of C5a stimulates production of thromboxane A2 (TXA2), which is responsible for the C5a-induced reduction in coronary blood flow and myocardial function.

Materials and Methods

Experimental Preparation

Adult domestic pigs of either sex, weighing 30–50 kg, were preanesthetized with ketamine (20 mg/kg i.m.), atropine (0.05 mg/kg i.m.), and sodium thiopental (10 mg/kg i.v.). Animals were intubated and ventilated with positive pressure. After intubation, anesthesia was maintained briefly with halothane (1–2%) followed by intravenous α-chloralose for the remainder of the experiment (initial bolus, 100 mg/kg i.v.; 25 mg/kg every 30–60 minutes thereafter).

The chest was opened by a midline sternotomy. Both internal mammary veins were cannulated for administration of anesthesia and drugs. An internal mammary artery was cannulated for measurement of arterial blood pressure and for blood sampling. Arterial blood gases were measured frequently (every 20–30 minutes) (ABL3 Radiometer, Copenhagen, Denmark) and were maintained within physiological limits by adjusting the respirator and administering supplemental oxygen. The right carotid artery was cannulated with a catheter-tipped LV pressure transducer (Millar Instruments, Houston, Texas), which was passed retrogradely into the LV. The right internal jugular vein was cannulated for reinfusion of coronary venous blood. The pericardium was opened, and the heart was suspended in a pericardial cradle.

The proximal left anterior descending coronary artery (LAD) was dissected free from surrounding tissue for placement of an electromagnetic flow transducer (Biotronix, Silver Spring, Maryland). A nonocclusive intra-arterial catheter was placed distal to the flow transducer in the LAD. Topical lidocaine (10 mg) and occasionally papaverine were applied to the area of the LAD during its dissection to reduce spasm. A pair of ultrasonic dimension crystals (2-mm diameter) was positioned in the LV midwall in the hoop axis in the region supplied by the LAD for measurement of local segment function. Their position in the center of the LAD-dependent region was confirmed by injection of patent blue violet dye (Sigma Chemical, St. Louis, Missouri) into the LAD just before killing the animal. Electrocardiogram leads were sutured to the anterior epicardium in the region supplied by the LAD.

The anterior cardiac vein, adjacent to the proximal LAD, was isolated and cannulated. Coronary venous effluent was directed into a reservoir and returned through the internal jugular vein by a servo-controlled pump (Masterflex, Cole-Parmer, Chicago, Illinois) that maintained a constant venous reservoir level. All animals were systemically heparinized (initial bolus, 750 units/kg; 250 units/kg/hr thereafter). Coronary venous return was diverted from the reservoir during, and for 2 minutes after, injection of C5a. During this interval, samples of coronary venous blood were collected through a stopcock attached to extension tubing connecting the cardiac vein cannula and the reservoir. The animal’s circulating blood volume was maintained by intravenous administration of fluid.

Arterial blood pressure, LV pressure and dP/dt, heart rate, LAD blood flow, and anterior LV segment shortening were recorded continuously (Minigraf, Siemens-Elema, Solna, Sweden). The electromagnetic flow transducer signal was calibrated at zero and balanced regularly throughout the study by temporarily occluding the LAD just distal to the transducer. The flowmeter was calibrated at the end of the procedure with the animal’s blood and vessel. End-diastolic coronary blood flow was measured at the zero point of the dP/dt signal just before its initial upsweep or at the peak of the R wave of the electrocardiogram. Mean coronary blood flow was calculated by planimetry of the instantaneous coronary flow tracing. Segment shortening fraction (in percent) was calculated as an estimate of regional function from the formula: [(end-diastolic length minus end-systolic length)/end-diastolic length] ×100. End-diastolic length was measured at the point used for end-diastolic coronary blood flow. End-systolic length was measured 20 msec before peak negative dP/dt. Calculations of pressures, coronary flow, dP/dt, and fractional shortening were made by averaging four or five consecutive cardiac cycles. Measurements were made on recordings obtained at a paper speed of 100 mm/sec.

Lactate concentration in arterial and coronary venous blood samples was measured with a lactate analyzer (model 23L, Yellow Springs Instrument, Yellow Springs, Ohio). Myocardial lactate production was calculated from the formula: [(venous lactate concentration minus arterial lactate concentration)/arterial lactate concentration] ×100. Myocardial oxygen extraction was calculated from the formula: [(arterial oxygen content minus venous oxygen content)/arterial oxygen content] ×100. Coronary venous blood samples were collected in ice-d polypropylene test tubes containing EDTA. Plasma collected after centrifugation was subjected to lipid extraction. Thromboxane concentration in arterial and coronary venous extracts was determined by radioimmunoassay for thromboxane B2 (TXB2; New England Nuclear, Boston, Massachu-
setts). The antibody cross-reacts (<2%) with prostaglandins D₂, E₂, F₂α, A₂, and 6-ketoprostaglandin F₁α. The TXB₂ standard curve was constructed with a lower detection limit of 0.1 pmol TXB₂/ml plasma.

After instrumentation, the animal was allowed to stabilize for 30 minutes. Blood samples were withdrawn simultaneously from the systemic artery and coronary vein for measurement of blood gases, leukocyte and granulocyte concentrations (QBC II Hematology System, Clay Adams, Franklin Lakes, New Jersey), lactate, and TXB₂ concentrations. Purified porcine C5a (500 ng diluted in 0.5 ml of 1% bovine serum albumin [BSA]) was injected into the LAD cannula over 15 seconds and flushed with heparinized saline over 5 seconds. Coronary venous blood samples were obtained every 30 seconds for 2 minutes after injection of C5a. Other measurements were recorded at the time of maximal changes in coronary blood flow and regional function (typically 30–35 seconds after injection of C5a). Administration of the vehicle (1% BSA) to several animals produced no hemodynamic or metabolic response.

Preparation of C5a

Porcine C5a was prepared from zymosan-activated porcine serum as previously described by Gerard and Hugli. The purity of the polypeptide was established by amino acid analysis after acid hydrolysis. The solutions of C5a were prepared by diluting with 1% BSA.

Experimental Protocols

Repeatability of C5a effects. To determine whether repeated injections of C5a caused tachyphylaxis, 500 ng of the anaphylatoxin was administered twice in two separate groups of animals at intervals of either 30 (n=6) or 60 (n=5) minutes. Hemodynamic and metabolic measurements were made as indicated above. These animals were not used in the subsequent studies.

Cyclooxygenase blockade. Each animal was challenged with C5a (500 ng) while hemodynamic and metabolic parameters were monitored. The effects of cyclooxygenase inhibition on the cardiac actions of C5a were evaluated after administration of 50 mg/kg i.v. acetylsalicylic acid (ASA) to eight animals. ASA was dissolved in 1.0 M sodium hydroxide (0.1 g/ml) and diluted to a 1.6% solution with saline. Starting 10–15 minutes after the initial injection of C5a, ASA was infused over 10–20 minutes. The animal was rechallenged with C5a 60 minutes after the first injection of C5a and at least 30 minutes after ASA infusion. Hemodynamic and metabolic measurements were made as above. Impaired TXB₂ production by C5a injection after administration of ASA was used as an indication of efficacy of blockade.

Thromboxane A₂/PGH₂ receptor antagonism. The effects of TXA₂/prostaglandin H₂ (PGH₂) receptor antagonism were investigated with administration of SQ29548. SQ29548 (30 or 100 µg/kg/min) was dissolved in ethanol and administered at a rate of 0.25 ml/min (begun 3 minutes before injection of C5a and continued for 2 minutes after injection). During the initial response of C5a (i.e., the first injection of C5a), the vehicle for SQ29548 (e.g., ethanol) was delivered at a rate of 0.25 ml/min. Efficacy of blockade was established by intracoronary administration of TXA₂/PGH₂ agonist, U46619 (1 µg), before and after receptor blockade (30 µg/kg/min SQ29548). No hemodynamic or metabolic effects were noted during the infusion of either vehicle or SQ29548. Additionally, three pigs received intracoronary prostaglandin F₂α (PGF₂α), while coronary flow and regional segment shortening were monitored.

Data Analysis

Data are presented as mean±SEM. The maximal C5a-induced responses were compared with control values taken before and after pharmacological intervention by Student's t test for paired data. Differences were considered to be statistically significant at p<0.05.

Results

Assessment of Tachyphylaxis to C5a

The cardiac effects of C5a were transient, dissipating within 2–3 minutes after injection. Absence of tachyphylaxis during a 60-minute period between repetitive injections of C5a was confirmed by demonstrating similar reductions in coronary blood flow and regional segmental shortening after administration of anaphylatoxin in five animals (Table 1). However, when the C5a injections were separated by 30 minutes, we observed a smaller decrease in mean LAD blood flow. Similar reductions in segmental shortening were observed in the 30-minute experiments. Subsequent studies used a 60-minute period between successive injections of C5a to avoid tachyphylaxis.

Cardiovascular Effects of C5a

Significant alterations in LAD blood flow, LV segment shortening, coronary venous oxygen content, and lactate concentrations were observed in this study in the absence of pharmacological intervention.

Table 1. Coronary Blood Flow and Myocardial Segment Shortening After Two Injections of C5a Separated by 30 or 60 Minutes

<table>
<thead>
<tr>
<th></th>
<th>Blood flow (ml/min)</th>
<th>Myocardial segment shortening (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control C5a</td>
<td>Control C5a</td>
</tr>
<tr>
<td>30 minutes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injection 1</td>
<td>27±4</td>
<td>13±4*</td>
</tr>
<tr>
<td>Injection 2</td>
<td>24±4†</td>
<td>18±4*†</td>
</tr>
<tr>
<td>60 minutes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injection 1</td>
<td>75±14</td>
<td>57±9*</td>
</tr>
<tr>
<td>Injection 2</td>
<td>71±21†</td>
<td>52±20*†</td>
</tr>
</tbody>
</table>

Values are mean±SEM.
*Significant difference compared with first injection of C5a.
†No significant difference compared with injection of C5a.
C5a-induced myocardial ischemia using the TXA2/PGH2 receptor antagonist SQ29548.16 Figure 3 illustrates an example of a recording of one pig receiving C5a in the presence of vehicle for SQ29548 (ethanol) or SQ29548 (30 μg/kg/min). C5a induced decreases in LAD blood flow and myocardial segment shortening without altering LV pressure, dP/dt, or systemic arterial pressure (Figure 3A, Table 3). After treatment with SQ29548, attenuation of these cardiac effects was observed (Figure 3B). Figure 4 summarizes the effect of C5a plus the vehicle for SQ29548 and C5a plus SQ29548 (30 μg/kg/min) in six animals. Before SQ29548 administration, C5a reduced coronary blood flow, myocardial segment shortening, and coronary venous granulocyte count. SQ29548 plus C5a resulted in insignificant changes in LAD blood flow and regional segmental shortening. However, SQ29548 did not alter C5a-induced granulocyte trapping in the heart. SQ29548 (30 μg/kg/min) significantly attenuated intracoronary U46619-induced decreases in LAD flow (−13±2 vs. −2±1 ml/min) and regional segmental shortening (−19±1% vs. −3±2%). A high dose of SQ29548 (100 μg/kg/min) caused no further antagonism of the C5a response in two additional animals. Intracoronary injection of PGF2α (1 μg) did not alter LAD blood flow (33±9 vs. 34±8 ml/min) or regional segmental shortening (17±3% vs. 17±3%) in three pigs.

**Discussion**

We have shown that cyclooxygenase blockade and TXA2/PGH2 receptor antagonism attenuates the cardiovascular effects after intracoronary administration of C5a. Additionally, C5a converts normal myocardial lactate extraction to lactate production. These data are consistent with the hypothesis that intracoronary C5a induced myocardial ischemia. Furthermore, the cardiac effects are dependent on the production of TXA2.

Tachyphylaxis of the coronary blood flow response to C5a occurs after repetitive injections when separated by only 30 minutes. Such tachyphylaxis of flow was not observed when the injections of C5a were separated by 60 minutes. This is an important observation since other investigators17,18 have used 30-minute intervals to study the C5a response. Their observations of a reduced response to C5a may have resulted from tachyphylaxis rather than a direct effect caused by enzyme inhibition or receptor antagonism.

We observed a significant increase in coronary venous plasma TXB2 concentration after administration of C5a. These data suggest that TXA2 could potentially mediate the cardiovascular responses to this anaphylatoxin. Interruption of the cyclooxygenase pathway allowed us to determine the influence of cyclooxygenase metabolites on the C5a-induced cardiovascular effects in the pig. We used ASA, a known inhibitor of the cyclooxygenase pathway.19 It is possible that the method of dissolving ASA (in NaOH)
converted it to sodium salicylate. However, sodium salicylate also is a cyclooxygenase inhibitor. Furthermore, efficacy of blockade was demonstrated by the observation that coronary venous plasma TXB₂ concentrations were inhibited after administration of C₅a. Interestingly, the peak increase in TXB₂ occurred 30 seconds after C₅a administration, the same time at which the maximum decrease in coronary blood flow and segmental shortening were observed. These data indicate that a significant portion of the cardiac actions of intracoronary porcine C₅a in the pig are mediated by a cyclooxygenase metabolite. However, they are in disagreement with previously published preliminary data. A possible explanation for this discrepant finding may be the dose and temporal administration of ASA. By measuring immunoreactive TXB₂ in the coronary venous effluent, we have shown that administration of 50 mg/kg ASA effectively blocks cyclooxygenase after 30 minutes. Lower doses or shorter intervals after the administration of ASA may not effectively inhibit cyclooxygenase in the pig. Our observations are consistent with an important role of the cyclooxygenase pathway in mediating the C₅a-myocardial response.

However, cyclooxygenase inhibition with ASA is nonspecific and antagonizes the synthesis of many vasoactive cyclooxygenase metabolites. Prostaglandins, such as PGF₂α and PGH₂, can promote vasoconstriction. Therefore, we used the TXA₂/PGH₂ antagonist, SQ29548, to investigate the role of TXA₂ in our model. SQ29548 has been shown to antagonize the vasoconstrictor effects of PGF₂α. The hemodynamic data collected during PGF₂α infusion, demonstrating the absence of any coronary vascular or myocardial response, suggest that this metabolite does not mediate significant cardiac effects after administration of C₅a. Although we cannot exclude the possibility that an interaction between TXA₂ and prostaglandins

**TABLE 3.** Hemodynamic Values of Animals Receiving Complement Component C₅a in the Presence of Acetylsalicylic Acid, SQ29548, and Their Respective Vehicles

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>C₅a+vehicle</th>
<th>Control</th>
<th>C₅a+agent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>HR (beats/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASA</td>
<td>118±9</td>
<td>116±9</td>
<td>151±16</td>
<td>152±16</td>
</tr>
<tr>
<td>SQ29548</td>
<td>126±3</td>
<td>125±3</td>
<td>128±5</td>
<td>128±5</td>
</tr>
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<td></td>
<td></td>
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<td></td>
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<tr>
<td>MAP (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASA</td>
<td>80±5</td>
<td>78±5</td>
<td>82±5</td>
<td>82±4</td>
</tr>
<tr>
<td>SQ29548</td>
<td>77±6</td>
<td>76±6</td>
<td>74±6</td>
<td>70±6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>LV dP/dt (mm Hg/sec)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>ASA</td>
<td>1,113±107</td>
<td>1,103±135</td>
<td>1,444±474</td>
<td>1,428±474</td>
</tr>
<tr>
<td>SQ29548</td>
<td>854±75</td>
<td>846±72</td>
<td>783±91</td>
<td>771±89</td>
</tr>
</tbody>
</table>

Values are mean±SEM. n=6 for both groups. HR, heart rate; ASA, 50 mg/kg acetylsalicylic acid; SQ29548, 30 μg/kg/min thromboxane A₂/prostaglandin H₂ receptor antagonist; MAP, mean arterial pressure; LV, left ventricular. No alterations in HR, MAP, or LV dP/dt were observed after C₅a administration in any of the groups.
influences the effects of C5a on the heart, this possibility seems likely. Our SQ29548 data are in agreement with preliminary data from others, who have shown that C5a-induced cardiac dysfunction can be attenuated with BM13505, a TXA2/PGHa antagonist. Thus, it appears that TXA2 and possibly PGHa play an important role in C5a-induced cardiac dysfunction. The cellular source of thromboxane in this study is unknown at present.

TXA2, an eicosanoid metabolite of arachidonic acid produced through the cycloxygenase pathway, is one of several mediators through which leukocytes might act on ischemic myocardium. Granulocytes can synthesize and release TXA2, which can exacerbate ischemia-induced cardiac injury through its coronary vasoconstrictive and platelet aggregating actions. Cycloxygenase blockade and TXA2 receptor antagonism also inhibit leukocyte infiltration and limit ischemic cardiac damage in experimental myocardial infarction. In addition, inhibition of thromboxane synthetase reduces the extent of experimental infarction. Thus, there is ample evidence indicating that eicosanoids are involved in infarct-related injury of the myocardium.

Our data have potentially significant clinical implications. Evidence is available suggesting a deleterious role of TXA2 in the propagation of myocardial ischemic injury. Furthermore, additional evidence in experimental models of myocardial ischemia indicate a myocardial protective effect of complement depletion either before or after induction of ischemia. In humans, both C5a and TXB2 plasma concentrations increase during myocardial ischemia. In addition, deposition of complement fragments in infarcted myocardium in humans has been observed. Thus, C5a may initiate TXA2 synthesis, propagating ischemic damage through vasoconstriction and granulocyte infiltration. In addition, our data suggest that the anaphylatoxin may be capable of eliciting deleterious effects over repeated short intervals. This finding may have relevance to myocardial infarction that evolves in a protracted or "stuttering" manner. However, our data do not address the effect of C5a during sustained exposure to the myocardium.

Reduction of coronary flow and cardiac function after C5a is consistent with, but does not prove the occurrence of, myocardial ischemia. The potential for an ischemic response is further suggested by the significant fall in coronary venous oxygen content, indicating an imbalance between myocardial oxygen supply and demand. Confirmation that C5a induces myocardial ischemia is provided by demonstrating a deterioration of myocardial lactate metabolism during administration of the anaphylatoxin (Figure 1). Normal myocardial metabolism in the control state, reflected by lactate extraction in all animals, was converted to production. Diminished extraction of lactate in association with cardiac dysfunction also is a sign of myocardial ischemia, albeit not as severe as lactate production. These metabolic derangements were related closely to the impairment of coronary blood flow and cardiac function. The association of myocardial lactate production with these C5a-
induced cardiac derangements confirmed our hypothesis that the anaphylatoxin produces myocardial ischemia.

Since cyclooxygenase inhibition and TXA₂/PGΗ₂ receptor antagonism did not totally eliminate the effects of C5a on coronary flow and myocardial function, we must consider other potential mediators of the cardiac action of anaphylatoxin, including leukotrienes and superoxide radicals. Leukotrienes C₄, D₄, and E₄ are potent coronary vasoconstrictors. C5a produces coronary vasoconstriction and impaired LV function in isolated guinea pig hearts. These effects are attenuated by specific leukotriene blockade. C5a also causes formation and release of toxic oxygen species from granulocytes. These radicals have been implicated in postischemic myocardial tissue injury. Finally, evidence recently has been reported for a direct nongranulocytic mechanism of C5a-induced ischemic damage. Therefore, it is possible that other inflammatory mediators are involved in this model and warrant further investigation.

C5a induces granulocyte adherence to endothelium by the type 3 complement receptor. This adherence is partly dependent on activation of the CD11/CD18 glycoprotein family of adherence proteins. Intracoronary C5a injection in our study was accompanied by significant trapping of granulocytes in the myocardial circulation. Neither ASA nor SQ29548 attenuated the C5a-induced granulocyte trapping. Therefore, our data suggest that a cyclooxygenase metabolite, including TXA₂, does not mediate the myocardial granulocyte trapping. Our data also show that venous granulocyte trapping alone is not sufficient for the C5a-induced cardiac response. However, we believe the roles of adhesion molecules and receptors in this model warrant further investigation.

In summary, this study demonstrates that TXA₂ plays an important role in the cardiac dysfunction after intracoronary administration of C5a. A decrease in coronary flow and cardiac function, associated with abnormal myocardial metabolism, is representative of an ischemic response. Furthermore, the persistence of myocardial granulocyte extraction in the presence of attenuated hemodynamic effects indicates that granulocyte plugging in the microcirculation is not responsible for the entire C5a response.

Addendum: Our results that ASA reduces the coronary and myocardial responses to C5a are at variance with a recent report by Ito et al (Circulation Research 1989;65:1220–1232). Differences in the amount of ASA (10 versus 50 mg/kg, Ito et al versus present study) or time of C5a challenge after ASA treatment (10 versus 30 minutes) may explain their findings. However, efficacy of cyclooxygenase blockade was established by Ito et al. In addition, significant protective effects with ibuprofen were seen in their C5a model. Therefore, we believe that both studies can be interpreted as demonstrating an important role of the cyclooxygenase pathway in mediating the C5a-induced cardiovascular response.

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