Experimental Rupture of Atherosclerotic Lesions Increases Distal Vascular Resistance
A Limiting Factor to the Success of Infarct Angioplasty

Andrew J. Taylor, Alex Bobik, Michael C. Berndt, Debra Ramsay, Garry Jennings

Abstract—Rupture of atherosclerotic lesions, resulting in localized thrombi and marked falls in distal blood flow, is a pivotal event in unstable coronary syndromes. We tested the hypothesis that after lesion rupture, vasoconstrictor mechanisms are major contributors to a marked rise in distal microvascular resistance, which is responsible for much of the interruption in blood flow. Cholesterol-fed rabbits underwent endothelial denudation of their left iliac arteries to induce angiographically severe, fatty, American Heart Association type IV–like atherosclerotic lesions. After lesion disruption with a stiff wire, we measured distal blood flow and pressure, capillary patency in the distal vascular bed, and the response to the vasodilators adenosine, nitroprusside, and glyceryl trinitrate. Morphology of the lesions and of the associated thrombi was also examined to assess lumen restriction at the site of rupture. Disruption of atherosclerotic lesions reduced mean flow from 5.04±1.21 to 1.23±0.37 mL/min (P<0.005), and calculated distal vascular resistance rose rapidly, from 17.5±2.9 to 37.9±6.4 mm Hg · min/mL (P<0.005). Lesion rupture did not significantly affect capillary patency in the distal muscle vascular bed, and although nonocclusive thrombi were present at the site of nearly all ruptured lesions, embolized thrombi were rare in capillaries (<1%). The early rise in distal microvascular resistance could be normalized with intra-arterially administered adenosine or the NO donor nitroprusside, but not glyceryl trinitrate, an organonitrate possessing large muscular artery–selective vasodilator characteristics. Thus, rupture of atherosclerotic lesions induces rapid and marked increases in distal vascular resistance, which is the consequence of severe microvascular vasoconstriction. Therapeutic targeting of the microvasculature should improve reperfusion in acute coronary syndromes. (Arterioscler Thromb Vasc Biol. 2002;22:153-160.)

Key Words: atherosclerosis ■ plaque rupture ■ thrombosis ■ blood flow ■ vasodilators

The abrupt closure of coronary arteries by occlusive thrombi is the main cause of acute myocardial infarction (AMI). The initiating event in most cases is the rupture of an unstable atherosclerotic lesion.1 Atherosclerotic lesions most susceptible to rupture and thrombosis are typically heavily infiltrated with lipid-laden foam cells and possess a thin fibrous cap.2 Activated metalloproteinases secreted by macrophage-derived foam cells also contribute to weaken their subendothelial matrix,3,4 increasing the susceptibility of the lesion to fissure and exposing its highly thrombogenic contents.

Strategies aimed at restoring blood flow down the infarct-related artery, such as thrombolysis5,6 and percutaneous transluminal coronary angioplasty,7,8 improve survival after AMI; however, overall in-hospital mortality rates from AMI remain at ≈12%.9 In many instances, flow down the culprit artery remains poor despite restoration of the patency at the lesion site, a phenomenon commonly referred to as “no reflow.” This no-reflow phenomenon is an important prognostic indicator after AMI,10 with impaired reflow being noted in up to 40% of the cases11,12 despite successful reopening of the infarct-related artery. Multiple mechanisms have been suggested to contribute to this impaired reflow, including microvascular obstruction,13 neutrophil activation,14 the production of oxygen-derived free radicals, and the loss of antioxidant enzymes.15 These latter effects can greatly impair endothelial cell functions in the distal microvasculature. In the resistance arteries and smaller vessels, high levels of locally generated superoxide anions and platelet activation can also contribute to the changing properties of endothelial cells, resulting in the perturbation of vasomotor function,16 enhanced expression of cell surface adhesion molecules,17 and even apoptosis.18 Such mechanisms may further exacerbate a hyperreactive vasculature, responding to local and circulating vasoactive agents liberated during atherosclerotic lesion rupture and thrombosis, possibly causing profound microvascular constriction. The recent finding that the frequency of no-reflow phenomenon is reduced in patients treated with adenosine at the time of infarct angioplasty also suggests a significant role for vasoconstriction.19

Despite initial, potentially encouraging therapeutic interventions with vasodilators,20 the lack of appropriate animal...
models has made elucidation of the relative contributions of the above-postulated mechanisms to impaired reflow exceptionally difficult. It has also hindered definition of the stages of reversible vascular impairment after AMI and the assessment of therapies to reduce the frequency of impairment in blood flow after apparently successful revascularization procedures. In the present study, we report on the development of an experimental model to study mechanisms that lead to impaired microvascular flow after rupture of an atherosclerotic lesion. Mild balloon catheter injury is used to stimulate the development of a localized, fatty, macrophage-rich, atherosclerotic lesion in the iliac artery of a hypercholesterolemic rabbit. An anterograde approach rather than the commonly used retrograde approach is used to stimulate lesion development, so that the entire vasculature distal to the site of lesion rupture is available for subsequent hemodynamic and morphological evaluations. We demonstrate that after lesion rupture, there is a dramatic time-dependent fall in iliac artery blood flow accompanied by a marked increase in distal vascular resistance. Localized thrombi at the site of the lesion only infrequently accounted for the marked loss in blood flow. The major contributor to the rise in distal vascular resistance appeared to be severe vasoconstriction, because capillary patency was not significantly reduced after lesion rupture and because specific vasodilators could reverse the large rise in distal microvascular resistance.

Methods

Animals and Study Design

All experiments were approved by the Alfred Hospital/Baker Institute Animal Experimentation Committee. A total of 43 cholesterol-fed rabbits, weighing 2.5 to 3.0 kg, underwent mild balloon catheter-induced injury of their left iliac arteries to stimulate fatty atherosclerotic lesion development. The rabbits were a colony derived from an original multicolored strain with Dutch Belted, which was introduced in 1994 (Baker Medical Research Institute, Melbourne, Australia). A 1% cholesterol-rich diet was begun 3 days before iliac artery injury and continued throughout the course of all the experiments.

Four rabbits constituting group A were studied 2 weeks after the initial injury; the remainder, 39 rabbits constituting group B, were studied 4 weeks after the initial injury. Angiography was performed in all group A rabbits and the first 8 group B rabbits to determine the time at which a prominent, partially occlusive, atherosclerotic lesion would be suitable for rupture studies. Plaque rupture was undertaken in 32 group B rabbits. Lesion morphology was assessed in all group A rabbits and the first 13 group B plaque-rupture rabbits. In the remaining 19 group B plaque-rupture rabbits, 8 were administered india ink, and 11 received vasodilator agents. Seven group B rabbits not subjected to plaque rupture and the 4 group A rabbits formed the control group of 11 rabbits.

Initiation of Localized Iliac Artery Intimal Fatty Atherosclerotic Lesions

Each rabbit underwent a balloon catheter injury procedure to denude a segment of the left iliac artery of endothelium. Briefly, the internal carotid artery was cannulated, and an angioplasty wire was negotiated through the aortic bifurcation into the distal iliac artery. Twenty minutes after the initial injury, angiography was performed in all group A rabbits and the first 8 group B rabbits to determine the time at which a prominent, partially occlusive, atherosclerotic lesion would be suitable for rupture studies. Plaque rupture was undertaken in 32 group B rabbits. Lesion morphology was assessed in all group A rabbits and the first 13 group B plaque-rupture rabbits. In the remaining 19 group B plaque-rupture rabbits, 8 were administered india ink, and 11 received vasodilator agents. Seven group B rabbits not subjected to plaque rupture and the 4 group A rabbits formed the control group of 11 rabbits.

Lesion Rupture

Lesion disruption was carried out in 32 group B rabbits. The rabbits were anesthetized, and the carotid artery was again cannulated for arterial access. After initial angiography of the atherosclerotic iliac artery, an over-the-wire balloon catheter was placed just proximal to the lesion. Then a modified angioplasty wire was passed through its lumen to the site of the lesion; the modification involved removing its soft tip, leaving a stiff end to disrupt the lesion. The wire was passed back and forth across the lesion. Blood flow through the vessel was monitored with a Doppler flow probe attached to the vessel distal to the lesion. On completion of each experiment, euthanasia was induced with pentobarbitone sodium (200 mg/kg IV).

Angiography

Rabbits underwent repeat angiography just before lesion disruption (8 group B rabbits) or after 2 weeks of lesion development (4 group A rabbits). Angiographic images were recorded on SVHS film and then analyzed with a computerized frame-grabber and image-processing program (Image Pro Plus). The minimal luminal diameter of each lesion was measured and expressed as a percentage of a reference segment measured at the origin of the left iliac artery.

Blood Flow, Blood Pressure, and Distal Vascular Resistance

Iliac artery blood flow was measured by placing a Doppler flow probe over the left iliac artery just distal to the atherosclerotic lesion in all 32 group B rabbits subjected to plaque disruption. Blood flow was continuously recorded on a Grass recorder, with measurements taken at baseline and after the achievement of a steady state after plaque rupture. To assess the effect of lesion rupture on distal vascular resistance, distal blood flow and blood pressure were recorded simultaneously in the last 24 group B plaque-rupture rabbits and also in the 7 group B control rabbits. This involved inserting the artery at the site of the flow probe at a fine-gauge needle attached to a pressure transducer. Distal vascular resistance was calculated by dividing mean arterial pressure by flow.

Assessment of Capillary Patency and Vasodilator Responses

To determine the effect of plaque disruption on distal capillary patency, india ink was injected into the aortic bifurcation of 8 group B rabbits 10 minutes after plaque disruption and 4 of the group B control rabbits with intact atherosclerotic lesions.21 Three group B control rabbits with intact atherosclerotic lesions were not injected with india ink; these were used for histological comparisons of the microvasculature. The effect of lesion disruption on functional capillary patency was quantified by counting the number of capillaries containing india ink granules in the hindlimb vasculature of rabbits subjected to plaque rupture and control rabbits.

In 11 group B plaque-rupture rabbits, we assessed the effects of intra-arterial glyceryl trinitrate (GTN) and either adenosine or nitroprusside on distal microvascular resistance after lesion disruption. A bolus of 25 μg GTN was initially administered (at baseline) to eliminate any local vasoconstriction at the site of placement of the iliac artery catheter, and distal microvascular flow and blood pressure were continuously recorded as described above. Subsequent to plaque disruption, the rabbits were administered intra-arterially an additional 25 μg GTN, followed by intra-arterial adenosine (500 μg bolus, n=6) or nitroprusside (200 μg bolus, n=5). A period of 5 minutes was allowed between the different drugs to permit the achievement of any new steady-state hemodynamic level. Distal microvascular resistance was then calculated at baseline, after plaque disruption, after GTN, and after either adenosine or nitroprusside. The second intra-arterial dose of GTN, administered before the adenosine or nitroprusside, eliminated any possible local spasm at the site of plaque rupture that could affect distal flow.

Tissue Processing, Atherosclerotic Lesion Histology, and Capillary Patency

Lesion Morphology

Segments of atherosclerotic lesions from the harvested iliac arteries were fixed in formalin and embedded in paraffin; 3-μm cross
sections were used for immunohistochemical studies. Segments of the lesions were also frozen in OCT (Miles Inc) and then stored at −80°C. Sections were stained with hematoxylin and eosin to characterize the general plaque morphology. Oil red O was used to localize neutral lipids in 6-μm cryostat-cut sections. The procedure involved rehydrating the sections, rinsing in 60% isopropanol, and then staining with 0.25% to 0.5% oil red O in aqueous 40% isopropanol.

Assessment of Capillary Patency
Muscle blocks ~5 mm×5 mm in size were obtained from 4 regions of the left and right hindlimbs. We selected the proximal and distal quadriceps femoris muscle as well as the proximal and distal semimembranosus muscle, ie, a total of 8 muscle blocks per rabbit. The tissues were fixed in formalin and embedded in paraffin, and 10-μm sections were cut and stained with periodic acid–Schiff reagent. High-power (×400) fields from each section were examined, making a total of 48 fields per rabbit. Compared with the clear lumen of microvessels of those rabbits not injected with india ink, perfused vessels containing black grains of india ink could be easily identified.

Perfused capillaries and muscle fibers were counted with the observer blinded to the identity of the muscle specimens. Only fields with muscle fibers in cross section were used. Perfused capillary and fiber numbers determined in the high-power fields were summed for each block, and then the totals of the 4 blocks for each hindlimb were added. Muscle perfusion was expressed as the ratio of perfused capillaries per muscle fiber and also as total perfused capillaries per square millimeter. Sections were stained with hematoxylin and eosin and examined under high power (×400) for the presence of intra-arteriolar and capillary hyaline and red blood cell aggregates, indicative of thrombotic emboli.22

Immunohistochemistry
A RAM11 antibody (1:100, Dako Corp) was used to assess macrophase accumulation in the atherosclerotic lesion, whereas anti–α-smooth muscle actin (1:500, Dako Corp) was used to localize smooth muscle cells. A monoclonal mouse antibody against human IIb/IIIa receptor (1:500, CRC54; from Dr Alexey Mazurov, Cardiology Research Center, Moscow, Russia),23 which interacts with rabbit platelets, was used to localize platelet aggregates. A biotinylated anti-mouse IgG (1:200, Vector Laboratories) was the secondary antibody. An anti-mouse IgG antibody (1:100, Dako Corp) was used as the primary antibody for negative controls.

RAM11-positive macrophages, and an apparent intact endothelium, indicated by the presence of vWF-positive luminal cells (not shown).

Because rupture of atherosclerotic lesions frequently results in a thrombus at the site of abrasion, we also investigated the extent to which abrading the fatty lesion induced a localized thrombus. Eleven of the 13 rabbits examined developed localized thrombi at the site of lesion disruption, but complete vessel occlusion was observed in only 3. In the remaining rabbits, there was no correlation between the severity of thrombus formation and the degree of blood flow limitation. The composition of thrombi was typically a mixture of platelets, fibrin, foam cells, and lipid particles. Platelet aggregates were present in thrombi attached to the abraded and ruptured lesions (Figure 3). In all cases, the overlying smooth muscle cap had been disrupted, exposing the highly thrombogenic lipid- and macrophase-rich region of the neo-intima. In 10 of 13 lesions examined, the fissure extended down to and, on occasions, beyond the internal elastic lamina.
Effect of Plaque Rupture on Distal Capillary Patency

More than 700 high-power (×400) fields from 15 rabbits were examined (Figure 4). Capillary patency, expressed as the total number of capillaries per muscle fiber, and the total number of perfused capillaries per unit area were unaffected by plaque rupture compared with values in either the contralateral (right) iliac artery or in control arteries (P for differences >0.05, Table). Examination of the muscle sections for evidence of distal embolization of platelets and/or foam cells resulted in the very infrequent detection of collections of red blood cells, platelets, and macrophages, with <1 collection per high-power field (representing <1% of the capillaries).

Vasodilator Responses

We examined the effects of administering intra-arterially 2 NO-generating vasodilators (GTN and nitroprusside) as well as adenosine on the elevated distal microvascular resistance induced by lesion rupture in the remaining 11 group B rabbits (Figure 5). As in the previous 13 rabbits, distal microvascular resistance was markedly elevated after lesion disruption (54.5±7.9 mm Hg · min/mL versus baseline of 23.3±4.5 mm Hg · min/mL, P<0.05). GTN did not significantly affect the elevated resistance (49.1±7.8 versus 54.5±7.9 mm Hg · min/mL, P>0.05), whereas nitroprusside induced a marked reduction in the distal microvascular resistance (15.7±5.1 versus 49.1±7.8 mm Hg · min/mL, P<0.05). Intra-arterial adenosine also reduced the elevated distal vascular resistance to near prelesion rupture values (25.7±1.8 versus 49.1±7.8 mm Hg · min/mL, P<0.05). None of the administered intra-arterial vasodilators had a significant effect on systemic arterial pressure.

Discussion

Rupture of atherosclerotic lesions frequently initiates the development of a localized thrombus at the site of rupture and a rapid diminution of blood flow. When it occurs in the coronary artery, it can result in myocardial infarction. The sequence of thrombotic and hemodynamic consequences observed in our newly developed model of lesion rupture is markedly similar to that described for human lesions. Rupture of the experimentally induced fibrofatty-like atherosclerotic lesions in the hypercholesterolemic rabbits was accompanied by marked reductions in distal flow and the development of
platelet-rich thrombi at the site of rupture. The rapid elevation of distal vascular resistance, largely responsible for the fall in blood flow, was due to vasoconstriction of the microvasculature. After plaque disruption, intra-arterial adenosine and nitroprusside were highly effective in normalizing vascular resistance. There was little evidence to suggest that distal microvascular embolization contributed significantly to this elevation in distal vascular resistance after plaque disruption; embolized platelet aggregates, foam cells, and other circulating cell aggregates were very infrequent in distal capillaries. These results strongly support the assertion that after plaque disruption, vasoconstriction of the distal microvasculature is mostly responsible for the rapid elevation in distal microvascular resistance, which results in distal flow reduction.

Distal microvascular constriction may be mediated by the release of a number of potent vasoactive substances liberated from the disrupted plaque and/or aggregating platelets. Endothelin-1 is present in the atherosclerotic lesions of rabbits and in human coronary arteries and is released during coronary angioplasty. Also, the vasocostricotor urotensin-II, with a potency an order of magnitude greater than that of endothelin-1, is frequently present in human coronary lesions. Platelet-derived factors released during platelet activation and thrombus formation, such as serotonin and thromboxane A2, can also contribute to the microvascular constriction. In addition, an inability of an impaired NO system in the endothelium of vessels and platelets to attenuate such vasoconstrictor actions in hypercholesterolemic and atherosclerotic subjects may further exacerbate the severity of constriction.

In our experimental model of plaque rupture, the NO donor nitroprusside and adenosine were highly effective in reversing the elevated microvascular resistance caused by plaque rupture, whereas GTN was largely ineffective. Adenosine and nitroprusside have previously been shown to be effective in dilating the rabbit hindquarter vasculature. Our finding that GTN was relatively ineffective in normalizing the distal microvascular resistance is consistent with its known ability to dilate larger muscular arteries in preference to the microvasculature. In small groups of patients undergoing infarct angioplasty, the administration of intracoronary adenosine and also of nicorandil has been shown to improve but not eradicate impaired reflow. The administration of intracoronary nitroprusside has also recently been shown to ameliorate reflow rate no reflow, complicating elective angioplasty. Incomplete clinical responses to adenosine and nicorandil, which act via ATP-sensitive potassium channels, and nitroprusside, whose action is dependent on the nonenzymatic release of NO, suggest that a number of contributory vasoconstrictor mechanisms may be participating. The present study indicates that early after lesion rupture, microvascular constriction is very likely the most prominent mechanism responsible for the flow reduction. Previous findings that the recovery of coronary resistance vessel function is delayed after angioplasty support the assertion that atherosclerotic plaque rupture has direct effects on microvascular resistance.

In the canine model of coronary thrombosis described by Folts et al., the combination of external constriction and localized trauma to nonatheromatous epicardial vessels induces cyclical flow variations and thrombosis. However, any examination of potential contributors to distal vasoconstriction as outlined above clearly requires a preexisting atheromatous plaque that can be ruptured. Previous studies have used balloon injury together with cholesterol feeding to induce an atherosclerotic lesion; however, they have mostly used a retrograde approach for arterial access, thus disturbing or even partially destroying the distal vasculature. Although Rekhter et al. used an antegrade approach to create atherosclerotic lesions in the rabbit aorta, its high blood flow and the minimal lesion encroachment on the lumen mitigate against any significant localized thrombus forming at the site of lesion rupture. Also, none of the previous models
of atherosclerosis have demonstrated, either angiographically or histologically, the progression of fatty neointimal lesions to severe atherosclerotic lesions. The lesions we created were angiographically severe, mimicking much more closely the atherosclerotic human coronary artery.2 As a result, we were able to demonstrate consistent reductions in distal blood flow and significant local thrombosis after plaque disruption. This new model of atherosclerosis allows the isolation of the complete rabbit hindlimb vasculature to investigate the effects of plaque rupture and thrombosis on distal microvascular structure and function. Lesion development occurs rapidly over 1 month, and by use of a mechanical, not pharmacological, method to induce plaque rupture, we can control the timing and location of plaque rupture and thrombosis. Because ischemia to the rabbit hindlimb is well tolerated,41 prolonged periods of study of the effects of lesion rupture are possible.

When data are extrapolated from animal atherosclerotic models, a number of limitations need to be considered. The rabbit hindlimb has a large capacity for collateral circulation after occlusion of more distal arterial subbranches, although this is not associated with a change in vascular reactivity.33 In addition, differences exist between peripheral and coronary blood flow regarding the regulators of microvascular tone and also the effect of myocardial contractions on intramyocardial resistance vessels. To overcome some of these shortcomings, we aimed to produce atherosclerotic plaques that resembled, in many aspects, human coronary lesions. Many prior models of atherosclerosis in animals do not necessarily resemble the labile lesions found in humans and are merely an accumulation of foam cells and extracellular lipid deposition, with no luminal encroachment or fibrous cap development. Such

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### Hindlimb Muscle Capillary Patency After Plaque Rupture

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<thead>
<tr>
<th></th>
<th>Plaque-Rupture Rabbits*</th>
<th>Control Rabbits*</th>
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<tbody>
<tr>
<td></td>
<td>Left Hindlimb</td>
<td>Right Hindlimb</td>
</tr>
<tr>
<td>Total No. of perfused capillaries counted (per section)</td>
<td>202±11</td>
<td>187±12</td>
</tr>
<tr>
<td>Total No. of perfused capillaries (per mm²)</td>
<td>110±6</td>
<td>102±6</td>
</tr>
<tr>
<td>Ratio of perfused capillaries per muscle fiber</td>
<td>0.71±0.04</td>
<td>0.66±0.04</td>
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</tbody>
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*Values are mean±SE.
*Data are from 8 rabbits with plaque rupture and 4 control rabbits.
†P>0.05 between groups by ANOVA.
lesions, which more closely resemble fatty streaks or “intimal xanthomas,” are unlikely to induce the deleterious events observed in humans. Recently, their significance for studying the pathogenesis of human atherosclerosis has been questioned. In the present study, the induced fibrofatty-like atherosclerotic lesions appeared to have progressed beyond those frequently seen in experimental animals. Although these lesions generally lacked a lipid core, there was marked luminal encroachment and smooth muscle cell cap development, with heavy macrophage infiltration accompanied by intracellular and extracellular lipid deposition, resembling somewhat the American Heart Association–classified type IV and V lesions.2 Our achieved aim was that the experimental lesions, like the human lesions, should precipitate local thrombus formation and flow reduction after their disruption. Approaches to prevent the catastrophic consequences of atherosclerotic plaque rupture have mostly, if not entirely, focused on thrombolytic therapy and, more recently, on angioplasty to restore vessel patency and blood flow to the microvasculature. Despite the very clear merits of these treatments, incomplete myocardial reperfusion or no reflow is a major limitation to the success of these therapies. Our attention now turns to the microvasculature as an important site of obstruction and, therefore, a potential focus for intervention. Our findings implicate concomitant rapid constrictor effects on the vasculature distal to the site of rupture, causing a rapid rise in distal vascular resistance, the time course and characteristics of which are consistent with severe but pharmacologically reversible microvascular constriction. This sequence of events is most likely initiated by vasoconstrictors released from the ruptured lesions, together with those released from the developing thrombi. Precise definition of these effects in a hypercholesterolemic model such as the one described in the present study is critical to our pursuit of complete vascular reperfusion in acute coronary syndromes.

Acknowledgments
This work was supported by a National Health and Medical Research Council institutional grant for the Baker Medical Research Institute and a Center for Clinical Excellence grant for the Alfred and Baker Medical Unit. Dr Andrew Taylor was supported by an Australian National Heart Foundation Medical Postgraduate Scholarship.

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doi: 10.1161/hq0102.101128

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