Influence of Anatomical Location on Arterial Thrombosis

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Abstract—Atherosclerosis manifests as a systemic disease with near global involvement of the named segments of the arterial tree. Acute thrombotic arterial occlusion, however, is not equally distributed. To evaluate intra-individual regional differences in arterial thrombogenicity, we compared 111In-platelet deposition in porcine carotid and femoral arteries after a standardized crush injury. Within the unidirectional flow conditions of elastic carotid arteries, platelet deposition was more than 3-fold higher compared with predominantly muscular femoral arteries with triphasic arterial flow. To determine the influence of rheology on platelet deposition after crush injury, carotid arteries were transplanted into the femoral position and compared with the paired native carotid and femoral arteries. Similarly, femoral arteries transposed to the carotid position were compared with the paired native carotid artery. In each of these experiments, arterial transposition to a new anatomic location imparts a predilection for platelet deposition indigenous to the new location. In the controlled environment of two high-shear thrombin–independent and –dependent flow chambers, porcine carotid and femoral arterial substrates were indistinguishable from one another with respect to platelet deposition. Regional differences in arterial hemodynamics may account for substantial differences in thrombosis arising from deep arterial injury. (Arterioscler Thromb Vasc Biol. 2002;22:342-347.)

Key Words: arteries ♦ thrombosis ♦ platelets

Atherosclerosis is an insidious inflammatory fibroproliferative response to injury, which is central in the pathogenesis of myocardial infarction, stroke, and critical limb ischemia.1 When present, atherosclerosis manifests as a systemic disease globally involving the named segments of the arterial tree, whereby a strong correlation exists between atherosclerotic disease found in one vascular territory as compared with another.2–6 Despite the inclusive involvement of atherosclerosis across arterial beds, the incidence of acute platelet-rich thrombotic occlusion secondary to ruptured arterial plaque is not equally distributed. Every year in the United States, 1.5 million individuals have myocardial infarction, whereas the incidence of stroke is 0.6 million.7,8 Although atherosclerosis affects the internal and external carotid arteries equally, thrombotic occlusion of the former is common, whereas occlusion of the latter is very rare.9,10 In contrast to the incidence of myocardial infarction and stroke, a much lower percentage of the population (approximately 0.1 million) has acute limb ischemia.11–13 Indeed, the pathophysiology of acute limb ischemia is embolic rather than thrombosis in situ in half of the cases.12 Potential explanations for this discrepancy between the prevalence of atherosclerotic disease and the incidence of acute thrombotic complications could arise from histologic or biochemical differences in arterial wall or atherosclerotic plaque composition, differences in rate of plaque rupture, or differences in vascular geometry between various arteries. Alternatively, hemodynamic differences could explain this disparate propensity for thrombosis. Arterial flow in coronary beds occurs primarily during diastole, whereas internal carotid flow is in the forward direction throughout the cardiac cycle. In contrast, arterial flow to the high resistance muscular beds of the external carotid and lower extremity is triphasic in nature. It is unlikely that either cellular or plasmatic constituents of whole blood explains the different thrombotic propensity as each of these latter variables are common to the entire arterial tree. Although evident from clinical epidemiology studies, differences in thrombotic propensity comparing one arterial segment to another has not previously been documented experimentally. The next logical step then is to determine whether a difference in thrombotic propensity exists between normal arterial segments of different vascular beds.

We have developed a well-characterized procedure for generating platelet-rich arterial thrombi that uses traumatic (crush) injury of porcine carotid arteries.14 In a cohort of 20 pigs, the thrombotic response to this injury was broad, spanning a 7-fold distribution from lowest to highest responders.15 There was, however, a strong intra-individual correlation comparing platelet deposition in one carotid artery to the other. This correlation between paired arteries provides a unique tool to address regional differences in thrombosis within an individual. To define differences in thrombotic propensity between arterial beds, we compared platelet deposition after standardized arterial injury in carotid and fem-
oral arteries in pigs. We furthermore sought to determine whether this difference in thrombotic propensity could be explained by either arterial wall elements or arterial flow characteristics.

Materials and Methods

Animals
Four-month-old, pre-estrus, female pigs of the Babcock 4-way cross stock (a mixture of Landrace, Yorkshire, Hampshire, and Duroc breeds) were purchased through the Mayo Clinic’s section of Veterinary Medicine and housed at the Mayo Institute Hills Facility (Rochester, Minn.). The study was approved by the Mayo Clinic Animal Care and Use Committee and conformed to the National Institutes of Health and United States Department of Agriculture guidelines.

Induction of Thrombosis
To assess the regional differences in thrombus formation, platelet deposition was compared after arterial injury to porcine carotid and femoral arteries in 51 pigs. Anesthesia, 111In-platelet labeling, and arterial crush injury were performed as described previously. Briefly, after bilateral carotid and femoral arterial localization, sequential arterial injury of all four vessels was performed by six serial hemostat crushes of 5-second duration, interspersed with a 3-second rest period, visually abutting each subsequent injury to the previous injury site. The thrombus was then allowed to propagate unimpeded for 30 minutes before harvesting. Arterial blood flow was monitored continuously by dual-channel Doppler volumetric transducers positioned downstream of the injury site. At the end of each preparation, injured arterial segments were placed in 3.7% paraformaldehyde solution and then assayed in a scintillation counter for 111In content.

Arterial Transplantation
To assess the influence of arterial blood flow characteristics on thrombus generation, a series of autologous arterial transplantations were performed with native femoral or carotid arteries as vascular conduits (n=12). In the first set of six experiments, the thrombogenicity of a carotid artery transplanted to the femoral position was compared with the contralateral native carotid and femoral arteries. A 2-cm portion of a randomly chosen autologous carotid artery was excised and immediately placed in saline solution containing heparin (50U/Kg) was administered intravenously to maintain the activated partial thromboplastin time at a goal of twice control.

Results

After a standardized injury, thrombus generation was significantly more robust in carotid arteries compared with femoral arteries in 51 pigs. Anesthesia, 111In-platelet labeling, and arterial injury of femoral arteries transplanted to the carotid arterial position were performed with native femoral or carotid arteries as vascular conduits (n=12). In the first set of six experiments, the thrombogenicity of a carotid artery transplanted to the femoral position was compared with the contralateral native carotid and femoral arteries. A 2-cm portion of a randomly chosen autologous carotid artery was excised and immediately placed in saline solution containing heparin (50U/Kg) was administered intravenously to maintain the activated partial thromboplastin time at a goal of twice control. After the arterial anastomoses, each vessel was externally bathed and immediately reimplanted by using the same surgical technique, a 2-cm segment of a randomly chosen autologous femoral artery was interposed into the carotid arterial position.

Anticoagulation with heparin, surgical anastomoses, Duplex ultrasound, Doppler volume flow monitoring, arterial injury, and 111In quantification were performed as before.

Flow Chamber
To determine substrate specificity for platelet deposition, to standardize blood flow characteristics, and to isolate the early thrombin independent phase from the subsequent thrombin-dependent phase of platelet deposition, two flow chamber methodologies were employed. The first chamber measures the in vitro thrombin independent early stage of arterial thrombus formation. Porcine carotid and femoral arterial samples was surgically harvested, placed in 2-methylbutane, frozen in liquid nitrogen, and stored at −70°C. On the day before experimentation, 6-μm cryostat arterial cross-sections were mounted on poly-L-lysine–coated glass slides (Sigma) and stored at −20°C until use. Before experimentation, arterial cross-sections were blocked with 2% bovine serum albumin in Tris-buffered saline (0.05 mol/L Tris, 0.1 mol/L NaCl, 0.02% NaN3, pH 7.4) for 45 minutes at room temperature and then rinsed with phosphate-buffered saline. A 0.3-cm wide and 1.5-cm long flow channel was cut into a 3-cm long piece of Scotch double-sided tape (3 mol/L, 1.27 cm×11.4 m, 64-μm thick, confirmed by transmission electron-microscopy). A Plexiglas coverlid (2.3 cm×3.8 cm) containing the inlet and outlet tubing was affixed to the slide by the double-sided tape with the tissue section positioned in the center of the flow channel between the inlet and outlet ports. On the day of experimentation, porcine whole blood was collected in 14.4 U/mL heparin and 100 nMol/L r-hirudin. The chamber containing the arterial sample was placed under a microscope equipped with a video camera and 410-nm light source. This wavelength was chosen for its characteristic absorbance by red cell hemoglobin. After a priming with the buffer, blood was drawn over the vessel section by a syringe pump for 8 minutes at 0.4 mL/min. By assuming laminar flow conditions, the shear rate at the surface of the section was 3350 s⁻¹, as calculated according to the formula 1.03×6 Q(w×h)², where Q is the flow rate in mL/s, and w and h are the width and the height of the flow path in cm, respectively. Platelet accretion on the artery section displaces the red blood cells from the light path. Platelet deposition is therefore measured as a function of increasing light transmission. Each experiment was recorded real-time on VHS, and digital frames were captured every 30 seconds with the NIH Image 1.62 software. Platelet mass was then estimated by calculating the sum of pixel values after subtraction of the background defined and normalized of the data for the variations in the area of the tissue sections analyzed. Platelet deposition was not inhibited by r-hirudin up to a concentration of 20 μmol/L in whole blood, indicating that the initial 60 μm of platelet thrombus formation can occur in a thrombin-independent way.

Because arterial platelet-rich thrombus formation is a thrombin-dominated process, we further sought to compare the thrombin-dependent stage of platelet deposition between these two arteries. Porcine carotid and femoral arteries were surgically harvested, immersed in 2-methylbutane, frozen in liquid nitrogen, and stored at −70°C until use. To provide a consistent injury and equally expose each layer of the arterial wall to flowing blood, thawed arteries were cut into longitudinal segments, sandwiched between 2 sheets of Teflon, and serially compressed with an industrial hydraulic plattin press (1 ton/cm² for 10 seconds, 2 tons/cm² for 10 seconds, and 3 tons/cm² for 30 seconds; Carver Inc). Compressed arterial segments were placed inside a methacrylate Badimon perfusion chamber and were perfused with heparinized (3 U/mL) porcine whole blood containing 111In-labeled platelets for 10 minutes at a flow rate of 5 mL/min (shear rate of 845 s⁻¹) drawn by a peristaltic pump (Drake-Willcock) and then assayed for 111In content. The thrombin dependence of this second chamber was confirmed by comparing platelet deposition after the perfusing of porcine whole blood with and without D-Phe-Pro-Arg-chloromethylketone (100 μmol/L). The addition of D-Phe-Pro-Arg-chloromethylketone reduced platelet deposition by 50%.

Results
arteries (Figure 1). Within this cohort of female animals of similar age and size, platelet deposition occurred over a broad distribution spanning 17- and 12-fold in carotid and femoral arteries, respectively. Within an individual animal, however, platelet deposition was significantly higher in carotid compared with femoral arteries ($P<0.0001$).

Arterial flow characteristics vary greatly in carotid and femoral arteries (Figure 2). Arterial blood flow in the internal carotid artery is monophasic in the forward direction during both systole and diastole. In contrast, blood flow is triphasic in the common femoral artery with initial forward flow followed by flow reversal in early diastole and forward flow again in late diastole reflecting arterial compliance. To determine the contribution of these flow characteristics to arterial thrombosis, platelet deposition was compared in native carotid arteries, carotid arteries transplanted to the femoral position, and native femoral arteries (Figure 3A). After arterial injury, platelet deposition was significantly greater in native carotid arteries compared with either transplanted carotid arteries ($P<0.05$) or native femoral arteries ($P<0.01$). In contrast, there was no difference in comparing transplanted carotid to native femoral arteries. In a similar set of experiments, platelet deposition was compared after arterial injury to native carotid arteries and femoral arteries transplanted to the carotid territory (Figure 3B). There was no significant difference between these two groups of paired vessels. In activity measured in anastomotic segments was negligible relative to the crush injury sites (data not shown).

To determine the contribution of arterial wall elements to arterial thrombosis, carotid and femoral arterial platelet depo-positions were compared in a series of flow chamber experiments. There was no significant difference in platelet deposition on carotid and femoral arteries as measured by either thrombin-independent (Figure 4A) or thrombin-dependent (Figure 4B) flow chambers.

**Discussion**

We have observed a striking intra-individual difference in platelet deposition after crush injury to porcine carotid and femoral arteries. Within this cohort of female animals of similar age and size, platelet deposition spanned a broad range in response to a uniform crush injury regardless of the vessel injured. Within the individual animal, however, this injury yielded a significantly greater platelet deposition in carotid compared with femoral arteries ($P<0.0001$).

Possible explanations for this observed discrepancy may include differences in arterial flow characteristics, arterial histology, biochemistry, or geometry. We have previously shown a strong intra-individual correlation of thrombus deposition between carotid pairs after crush arterial injury. This correlation provides an animal model to address variables affecting both inter- and intra-individual propensity for arterial thrombosis, whereby one of the arterial pairs provides an internal control for the contralateral vessel. To determine the contribution of arterial flow characteristics to thrombus deposition, a series of autologous arterial transplantations were performed. Arterial transposition to a new anatomic location imparts a predilection for platelet deposition inherent to the new position rather than the vessel transposed. Substantial differences in thrombosis arising from deep arterial injury between carotid and femoral arteries therefore may be accounted for by regional differences in arterial hemodynam-
ics. Flow characteristics vary considerably between these two arterial locations. Flow in the internal carotid artery is primarily unidirectional with forward flow throughout the cardiac cycle caused by the low-resistance circle of Willis downstream.\textsuperscript{18} In contrast, the femoral artery empties into the high-resistance vascular bed of the hindlimb. Flow in this high-resistance arterial bed is undulating with forward flow in early systole and late diastole interposed by flow reversal in early diastole.\textsuperscript{19} Platelet accretion to a growing arterial thrombus arises from a series of steps beginning with glycoprotein Ib-IX-mediated rolling and adhesion.\textsuperscript{20} Glycoprotein \(\alpha_\text{IIb}\beta_\text{3}\) then undergoes an activation-dependent conformational change becoming competent to bind fibrinogen thereby cross-linking adjacent platelets.\textsuperscript{21} Aggregate stabilization increases with time relative to the extent of platelet granule secretion.\textsuperscript{22–24} The triphasic flow to the high resistance vascular bed of the femoral artery may undermine, strip, and embolize fragile platelet aggregates before they can be stabilized. Unperturbed forward flow as seen in the internal carotid artery may provide a more stable environment for platelet accretion.

Figure 3. Platelet deposition in transplanted and native arteries. After crush injury, \(^{111}\)In platelet deposition was significantly greater in native carotid (NC) arteries compared with carotid arteries transplanted to the femoral position (CF) and native femoral (NF) arteries (A; \(P<0.05\) and \(P<0.01\), respectively). In contrast, there was no significant difference in platelet deposition in femoral arteries transplanted to the carotid position (FC) compared with native carotid arteries (B; 2-tailed paired Student’s test).

Figure 4. Carotid and femoral arterial platelet deposition in thrombin-independent (A) and thrombin-dependent (B) chambers.

Cellular rolling and adhesion, as occurs with both leukocytes and platelets, are governed in part by the kinetic and mechanical properties of receptor-ligand binding.\textsuperscript{25–29} Both selectin- and glycoprotein Ib–binding have been shown to have a shear threshold dependence whereby resistance to detachment increases proportional to increasing shear. These shear requirements are important not only for adhesion promotion but also maintenance. With a rapid reduction in shear or with shear reversal, as would be anticipated in triphasic flow conditions, receptor substrate interactions cease. These cell receptor properties may in part promote platelet aggregate destabilization under triphasic flow conditions as observed in this study.

A striking histologic difference is readily apparent when comparing the femoral and carotid arteries by light microscopy. The carotid arterial media, rich in elastin, is histologically quite distinct from the predominantly muscular media of the femoral artery.\textsuperscript{30} Within the controlled environment and uniform non-pulsatile blood flow of the thrombin-independent flow chamber, platelet deposition was equivalent for carotid and femoral arterial substrates. Thrombogenic variances between these two vastly different arteries, therefore, are not easily explained by differences in arterial biochemistry or histology. Although regional differences in
vessel wall thromboplastin might be anticipated, once separated from flowing blood by the growing thrombus mass, biochemical factors within the vessel wall would be unlikely to have significant influence on newly acr'etating platelets. Furthermore, differences in platelet deposition do not likely arise from whole blood constituents. These variables, including platelets, leukocytes, red blood cells, or coagulation factors, are common to the entire arterial circulation. While it is possible arterial substrate interaction could differ with each of these variables, both the in vivo arterial transposition experiments and thrombin-dependent flow chamber experiments excluded these as contributing factors.

With this and previous studies, we have observed a marked inter-individual variability of thrombotic response to a reproducible arterial injury.\(^{15}\) This variability presents a challenging obstacle when assessing variables contributing to arterial thrombosis propensity. Within an individual, however, a strong correlation exists comparing the thrombotic response of paired arteries. Therefore, as there are no current tools for assessing the thrombotic propensity of an individual animal before arterial injury, when testing hypotheses of this nature, it remains prudent to perform intra-individual comparisons (with paired t-tests) whenever possible. Two thirds of the animals in Figure 3A appear to be either moderate or high responders with regard to thrombosis predisposition whereas the animals in Figure 3B are either moderate or low responders. Use of the intraindividual correlation showed a significant difference between arterial segments in Figure 3A whereas no difference was observed between vessel segments in Figure 3B. Indeed, there was a slight trend favoring an increased thrombotic tendency in the transposed femoral segments relative to the native carotid segments (600±500 versus 270±165 platelets × 10⁷/cm², respectively; \(P = 0.24\)) in the latter experiments. Although a potential difference cannot be excluded if high responders only were tested, this latter trend prevents finding an overall statistical difference even if the data points for Figure 3B were doubled. Given the sample size, the possibility of a potential difference therefore cannot be entirely excluded. The intent of this investigation, however, was to determine a thrombotic difference between arterial segments of different anatomic locations. Proof of equivalence would require a much larger sample size given these preliminary data.

Although the hypothesis generated for this study resulted from clinical observations and published literature of atherosclerotic disease, the results of these experiments may not be generalized to diseased vessels. To test the hypothesis that flow characteristics contribute to arterial thrombus generation, healthy pre-estrus pigs were chosen to insure that arterial segments were normal and that robust triphasic flow conditions were present in the hind limb. Arterial stenoses imposed by progressing atherosclerotic plaque change the femoral arterial flow characteristics from triphasic to monophasic, thereby prohibiting this type of experiment. Although the extent of underlying femoral or internal carotid atherosclerotic disease before plaque rupture and thrombus formation has not been well characterized, epidemiologic data suggests that most coronary thrombi occur at sites of insignificant stenosis.\(^{31,32}\) The contribution of atherosclerosis to these regional differences in thrombogenicity remains an important issue for future study.

In conclusion, substantial regional differences in arterial hemodynamics may contribute to the observed differential platelet deposition in response to deep arterial injury. These data may help explain regional differences in the incidence of acute occlusive arterial thrombosis observed clinically.

References


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