Individual Propensity for Arterial Thrombosis

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Abstract—Arterial thrombophilia independent of vascular pathology has not been previously defined either experimentally or epidemiologically. To address the existence of an individual propensity to arterial thrombosis, we exploited a previously developed procedure entailing traumatic (crush) injury of paired porcine carotid arteries for generating platelet-rich thrombi. Porcine carotid arteries were injured bilaterally by serial hemostat crushes. Thrombus generation was monitored by local accumulation of autologous 111In-labeled platelets and Doppler blood flow. Within this cohort of animals of similar age and size, the lowest to the highest responders in thrombus mass spanned a 7-fold range, showing no correlation with shear, platelet or leukocyte count, or plasma concentrations of fibrinogen or von Willebrand factor. However, there was strong intra-individual correlation ($r^2=0.80$; $P<0.001$) of thrombus deposition between carotid artery pairs. The wide variation in thrombotic response to a standardized stimulus, not accounted for by shear stress or typical hematological variables, appears to be an intrinsic propensity of the individual. The experimental system for thrombus generation is sufficiently quantitative for assessment of variables determining this propensity. ([Arterioscler Thromb Vasc Biol. 1999;19:883-886.])

Key Words: thrombosis ■ vascular disease ■ risk factors

The morphology of arterial stenosis is poorly predictive of acute thrombosis. Variable thrombotic propensity in the face of lesion constancy, implicit in angiographic findings,1–3 could be chaotic, where trivial differences in starting conditions translate randomly into large differences in outcomes. However, propensity to thrombosis could also arise from biological variability in cellular or humoral control pathways of the hemostasis system and thus, be an intrinsic attribute of the individual.4 Although well established for venous thrombosis,5,6 an arterial thrombophilia independent of vascular pathology has not been detected either experimentally or epidemiologically. The very variability in thrombotic responses to experimental vascular injury in animal models precludes evaluating thrombotic propensity across cohorts of animals. In the course of developing a system for quantitative analysis of platelet thrombosis, we developed a procedure for generating platelet-rich thrombi that uses traumatic (crush) injury of porcine carotid arteries.7 This assay is opportune for addressing the propensity hypothesis because first, in contrast to clinical thrombosis, the analysis is quantitative and the thrombogenic lesion is standardized and intense enough to yield a measurable thrombus on every attempt and second, because these vessels are paired to furnish an internal control.

Methods

Animals

Four-month-old, preestrous female pigs (n=20; mean weight, 30 kg) of the Babcock 4-way-cross stock (a mixture of Landrace, Yorkshire, Hampshire, and Duroc breeds) were purchased through Mayo Veterinary Medicine Department and housed at Mayo Institute Hills farm. The study was approved by the Mayo Clinic Animal Care Committee and conformed to National Institutes of Health and United States Department of Agriculture guidelines.

Induction of Thrombosis

On the day before the experiment, autologous platelets were labeled with 111In and then reinjected into the animal.8 Anesthesia of pigs, arterial and venous catheterizations, bilateral internal carotid artery dissection, crush injury, and clotting assays were performed as described previously.7 In brief, carotid arterial injury comprised 6 serial hemostat crushes of 5-second duration with a 5-second intervening rest period and visually abutting the subsequent injury to the prior injury site. To maximize uniformity, all injuries were performed by a single individual using the same hemostat closed to the second ratchet tooth. The right and left carotid arteries were injured simultaneously, and thrombosis was allowed to evolve for 30 minutes before harvesting the vessel. The injured carotid segments were then placed in 4% paraformaldehyde solution, measured for length of injury and vessel diameter, and assayed in a gamma counter with windows at 173 and 247 keV for 111In. Bilateral carotid arterial blood flow was monitored continuously with dual-channel Doppler transducers, and 111In counts reflecting platelet deposition were registered throughout the experiments with shielded scintillation detectors positioned over each injury site. After the carotid injury, each vessel was bathed with 1% lidocaine solution to prevent vasoconstriction. Characterization of vascular injury by this methodology has been extensively studied for reproducibility.7,9,10 Complete blood counts were obtained on peripheral blood taken before injury and analyzed at the clinical core laboratory. Fibrinogen11 and von Willebrand factor12 assays were performed by previously described methodologies.

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For shear stress calculations, the carotid arteries were assumed to have the geometry of a simple cylinder. Differences in blood hematocrit were negligible between animals, and therefore Newtonian viscosity was inferred. Shear stress ($\gamma$) was calculated from the formula $\gamma = 4Q/\pi R^2$. Blood flow velocity ($Q$) was measured with the Doppler probe throughout the procedure. Carotid diameter was measured at the time of vessel harvest after fixation with paraformaldehyde.

### Results

There was strong intra-individual correlation ($P<0.001$) when the thrombus deposition of 1 carotid artery was compared with the other (Figure 1). Within this cohort of female animals of similar age and size, thrombus masses of the lowest and highest responders spanned a 7-fold range. Furthermore, the range was evenly populated.

This variability of thrombotic response could not be accounted for by shear stress, which spanned a 6-fold range in this cohort of animals (Figure 2). Despite the pairwise correlation in platelet deposition, there was no intra-individual correlation in shear stress (Figure 2A). These differences in shear stress were accounted for by a broad distribution of preinjury or postinjury arterial flow, or both, among animals and often within an individual carotid pair. These differences were not correctable by bathing the vessel with lidocaine and hence, were not the consequence of local vasospasm. Likewise, among all samples, there was no correlation between shear stress and platelet deposition (Figure 2B). Because intra-individual carotid arterial flow was frequently discordant, possible minor contributions of shear stress to thrombosis could be evaluated. Plotting platelet deposition in the vessel with the higher shear of each pair against that in the vessel with lower shear (Figure 3) adds little ($r^2=0.82$) to the global right-left correlation ($r^2=0.80$).

Several hematological variables previously identified as epidemiological risk factors for atherosclerosis were analyzed for their contribution to thrombogenicity. These variables, which include von Willebrand factor, fibrinogen, white blood count, and platelet count, were assayed from peripheral blood drawn before the injury (Figure 4, top and bottom left panels). Although there was moderate variability with respect to each of these parameters within this cohort, no single parameter was correlated with platelet deposition. Likewise, platelet deposition was not correlated with either vessel diameter or length of injury (Figure 4 bottom, middle and right panels). Arterial diameter was measured after vessel harvest and fixation and therefore, was not affected by local vasoreactivity. Because each animal received 6 crushes per carotid artery, the length of injury was determined by the progression of the hemostat for each crush. This variance in injury approximation could theoretically change platelet accretion by varying the blood rheology at the injury site. Spacing of injuries (ie, length), however, had no effect on platelet deposition.
Discussion

Although explicit pathogenesis of arterial thrombotic disease remains undefined, experimental and epidemiological pathobiology studies have yielded a long and growing list of risk factors.14 Helpful in risk stratification, these factors neither alone nor in combination have power to identify the individual predilection for either atherosclerosis or thrombosis.15–17 Extreme values of some variables, including cholesterol and homocysteine, are strongly associated with atherosclerosis, but only a minority of patients with clinically manifest disease possess such values.18–20 Indeed, the typical patient with vascular occlusive disease has a clustering of risk factors, with no single factor carrying disproportionate weight. Known risk factors, therefore, are not essential links in a process having a common outcome, ie, platelet thrombosis.21,22 From an experimental standpoint, the issue hinges, on one hand, on the creation of arterial lesions having sufficient reproducibility to yield a signal above the lesion-dependent noise, and on the other, the existence of the anticipated biological variability. We now provide direct evidence for a basal predisposition to arterial thrombosis, independent of shear, in response to a standardized lesion. The thrombotic potential was analyzed within a cohort of presumably low-risk animals: preestrus female, juvenile pigs that should, by inference from human epidemiology, have the lowest risk for thrombosis of an entire population. However, these young, healthy, preestrus pigs exhibited a 7-fold variability in thrombotic response to a reproducible arterial injury not explained by environmental variables, which were minimized across the cohort. Despite this interindividual variability, the thrombotic response within each individual was remarkably constant ($r^2 = 0.80$).

Three compartments for variables contributing to arterial thrombosis include the injured vessel wall, hemoraul clotting factors, and circulating platelets. Activities of the autonomic nervous system, including catecholamines, can affect each of the 3 compartments.23 Shear and rheology, known to influence platelet thrombosis in vitro,24 appear to have limited impact on thrombosis, at least within the range encountered in the injured carotid arteries. Although the vessel wall is a rich source of thromboplastin, once separated from flowing blood by the thrombus mass, biochemical factors within the vessel wall would be unlikely to have significant influence on newly accreting platelets.

Arterial thrombosis induced in pigs has been shown to be thrombin sensitive where specific inhibition of either thrombin or prothrombinase both blocks thrombosis and results in rapid dissipation of preformed thrombi.9 Humoral factors, therefore, are instrumental in both thrombus propagation and maintenance. Most of these factors, however, vary <10% between individuals and therefore would not be expected to explain the 7-fold variability seen within this cohort of animals. Possible exceptions could include the existence of an unknown humoral factor or an unexpected threshold response to previously defined factors. Fibrinogen and von Willebrand factor, whose concentrations are exceptionally labile, appear not to impact this process. Basal tissue factor pathway inhibitor and activated protein C, not measured in this study, are examples of factors that in principle could.

Irrespective of whether they participate in atherogenesis, platelets are the foundation of the final common pathway of thrombogenesis associated with atherosclerosis. Thus, platelets, as surveillance cells anticipated to have biologically variable excitability, might be expected to determine whether a given lesion will yield a thrombotic event. Platelet variability cannot be accounted for by quantity, because platelet counts taken from peripheral blood samples vary little between individuals. Lasne et al4 have recently documented that platelet responses to a defined stimulus vary by at least 6-fold within a cohort of normal donors and that the response was found qualitatively to be a propensity of the individual. Substantial variability in intrinsic platelet excitability predicts the existence of a cohort of individuals with an inherent hypercoagulability, in whom environmental stimuli produce an exaggerated arterial thrombotic response. We now provide technology and an animal model to address these issues.

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References
