Intravital Fluorescence Microscopy Improves Thrombosis Phenotype Scoring in Mice

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Over the past 15 years, the laboratory mouse has yielded a wealth of information on the pathogenesis of thrombosis. To a large extent, the driving force behind the use of mice to study thrombosis has been the remarkable advances in molecular approaches to selectively modifying gene expression, thereby allowing analyses of the function of specific blood and vascular wall proteins in vivo. Although all murine blood vessels might be considered small, studies of thrombosis in mice have been categorized as macrovascular and microvascular. The former pertains to conductance arteries and large veins, such as the carotid artery, aorta, inferior vena cava, and jugular vein. Studies of these blood vessels are relevant to human diseases such as myocardial infarction, stroke, and deep venous thrombosis. Microvascular thrombosis models have involved analyses of arterioles (diameters approximately 30 to 100 μm) within vascular beds that can be exteriorized en bloc and imaged microscopically as thin sections, such as those of the cremaster muscle and mesentery. Microvascular studies are relevant to pathological thrombosis that occurs during sepsis and other inflammatory disorders.

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For most murine macrovascular arterial thrombosis studies, investigators have used the carotid artery. Arterial injury can be induced by several approaches. The end point of many of these experiments is the time required to form a completely occlusive thrombus, assessed with a vascular flow probe. As with many experiments that correlate genotype and phenotype, the weak link of murine thrombosis studies typically is not the genetic intervention or assessment but rather phenotype scoring. Although it is valid and effective, thrombosis time is a relatively crude end point that has limitations. For example, it can sometimes be challenging to determine the precise time that an artery completely occludes. A typical rate of carotid artery blood flow in the adult anesthetized mouse is approximately 1 mL/minute. Very low levels of flow (eg, <0.05 mL/minute), resulting from formation of a nearly occlusive thrombus, can sometimes be difficult to discern from minor baseline drift in the blood flow tracing, which can make the precise determination of occlusion time challenging. Sometimes an artery does not occlude after vascular injury. Such a phenotype could result from either minimal thrombosis or substantial yet nonocclusive thrombus formation—2 very different thrombotic responses to vascular injury. These limitations can be overcome by including sufficient numbers of animals in experiments, using intermediate end points (eg, percentage of time after injury that blood flow is >25% of baseline [ie, preinjury] flow), and performing experiments in a blinded fashion. Nevertheless, despite recent advances, improved methods for scoring macrovascular thrombosis phenotype in mice are needed, particularly in 2 areas. The first is the development of methods that enable direct visualization of forming thrombi. The second is the development of approaches that allow real-time assessment of fibrin, blood platelets, and other clotting factors and cells in forming thrombi. Several years ago, Falati et al reported an approach to visualizing real-time accretion of specific thrombus components in murine cremaster muscle arterioles. This elegant and powerful approach has subsequently been used to study the roles of several blood clotting factors in microvascular thrombosis. However, the technique has not been applied to larger blood vessels of mice, primarily because larger thrombi attenuate the excitation and emitted signal of shorter-wavelength-activated fluorophores.

In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Cooley reports the use of intravital fluorescence microscopy to study macrovascular thrombosis in mice. The study describes a novel technique for imaging of thrombi that form in response to electrolytic and chemical injury in murine carotid arteries and femoral veins. Through imaging of fluorescently labeled platelets and antibodies to fibrin and other clotting factors, the technique measures the accumulation of specific cell types and clotting factors in thrombi in real time. This approach enables investigators to extract substantially more information from experiments than can be obtained by simply measuring occlusion time. Key to this approach is the use of fluorophores that are activated by longer wavelengths, thereby allowing their excitation and detection within larger thrombi. However, this additional information comes at a cost. Moderately expensive lasers and microscopes must be purchased. Each animal must be injected with labeled cells or antibodies. Careful controls must be performed to enable comparisons between animals. Such an investment may not be feasible for many investigators or necessary for many experiments. Nevertheless, the author is to be commended for this important work, which should provide a more robust phenotypic assessment of genetically modified mice and facilitate studies of pharmacological compounds that inhibit thrombosis. For example, by using fluorescently tagged antibodies against tissue factor and other...
plasma clotting factors, the roles of blood-borne tissue factor and intrinsic clotting factors (eg, factor XI) in macrovascular thrombosis can be studied in greater detail. Intravital fluorescence imaging should also prove useful for studying the roles of leukocytes and microparticles derived from them in macrovascular thrombosis.

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**References**


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