Critical Review of Mouse Models of Venous Thrombosis

Jose A. Diaz, Andrea T. Obi, Daniel D. Myers, Jr, Shirley K. Wrobleski, Peter K. Henke, Nigel Mackman, Thomas W. Wakefield

Abstract—Deep vein thrombosis and pulmonary embolism are a significant health care concern, representing a major source of mortality and morbidity. In order to understand the pathophysiology of thrombogenesis and thrombus resolution, animal models are necessary. Mouse models of venous thrombosis contribute to our understanding of the initiation, propagation, and resolution of venous thrombus, as well as allow for the evaluation of new pharmaceutical approaches to prophylaxis and treatment of deep vein thrombosis. In this work we review the ferric chloride model, the inferior vena cava ligation model, the inferior vena cava stenosis models, and the electrolytic inferior vena cava model and compare their advantages and disadvantages. (Arterioscler Thromb Vasc Biol. 2012;32:556-562.)

Key Words: thrombosis ■ vascular biology ■ venous thrombosis ■ animal models ■ mouse

Venous thromboembolism, including deep vein thrombosis (DVT) and pulmonary embolism, are a major source of morbidity and mortality worldwide. Recent research estimates the incidence of venous thromboembolism in the United States alone at 900,000 per year, with a quarter of the cases resulting in fatality.1,2 In 2008, The Surgeon General’s Call to Action to Prevent Deep Vein Thrombosis and Pulmonary Embolism, states “the disease disproportionately affects older Americans, and we can expect more suffering and more deaths in the future as the population ages—unless we do something about it.” This document invited multiple stakeholders, including all areas of research and the health care systems, to come together in a coordinated effort to reverse this dramatic projected trend.3 Animal models are fundamental to accomplishing this objective. Mouse models of DVT constitute a unique biological environment in which investigators may evaluate not only the biology behind DVT, but also new pharmaceutical approaches. Here we review the most widely used mouse models to evaluate venous thrombogenesis and thrombus resolution.

General Considerations

Impetus for Development of a Murine Model

Humans stand alone as the singular species with propensity to develop spontaneous DVT. Because Virchow’s original description of a canine model, there has been interest in the development of animal models of thrombosis to mimic the human condition.4 Initial labors were directed toward development of large mammal models.4,5 The transgenic mouse era led toward efforts to translate the existing models into the smaller rodent species. Mice are amenable for the study of venous thrombosis and have the advantages of relatively low cost and the availability of different genetically manipulated strains. However, important differences to humans include body size, life span, genetic differences, and vessel size. Nevertheless, mouse models have increased our understanding of venous thrombosis.

Applications in Mice

Studying the genetic defects in humans and mice is crucial in order to gain insight into venous thrombosis. A fairly complete list of genetic deficiencies involved in human bleeding disorders is known, but our knowledge regarding genetic factors related to the increased propensity toward thrombosis remains incomplete.6 Sequencing of the mouse genome has allowed physiological manipulation of gene addition (transgenic) and gene deletion (knockout) mice, which has greatly increased our understanding of thrombotic processes.7,8

Because, in most cases, it is not possible to obtain human samples from the site of DVT to study the disease, it is advantageous for researchers to use animal models to investigate DVT in a biological setting. The link between inflammation, innate immunity, and venous thrombosis has recently been demonstrated through impaired venous thrombus resolution via a non-MyD88 dependent toll-like receptor 9 pathway in mice undergoing venous ligation.9 Similarly, multiple aspects of inflammation and venous thrombosis have been derived from mouse models.10 The essential role of E-selectin in the pathogenicity of antiphospholipid antibody syndrome was directly demonstrated by a mechanical femoral vein injury in E-selectin knockout mice treated with antiphospholipid antibody syndrome antibodies.11 However, these observations still need to be confirmed in patients.

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The understanding of the biological events associated with venous thrombosis and their manipulation by pharmacological agents is dependent on studying animal models. The relative successes of recombinant tissue plasminogen activator, enoxaparin, hirudin, and argatroban could not have been predicted without information gleaned from early preclinical laboratory experiments with animals, nor the failures of other anticoagulant drugs that appeared promising in in vitro studies but failed to live up to expectations.\textsuperscript{12–16} The use of thrombosis models in rodents plays a crucial role in bridging the bench to bedside transition. For example, primate studies are validating mouse studies that have suggested P-selectin inhibitors are potential therapeutic agents in the prophylaxis and treatment of DVT.\textsuperscript{8,17,18}

### Acute Versus Chronic DVT in Mice

Mice have a short life span and high metabolic rate, compared to humans. Thus, events occur faster in mice. Clinically, DVT in humans can be defined as acute, subacute and chronic. In mice, acute DVT is defined, in terms of time, from thrombus induction up to 2 to 3 days. Beyond that time point, subacute and chronic DVT occur. Neutrophil migration occurs during the acute phase, and they are the main inflammatory cells in both the vein wall and thrombus. A shifting between neutrophils and monocytes occurs by day 6. After that, monocytes become the predominant inflammatory cells in both vein wall and thrombus\textsuperscript{19} (Figure 1). In addition, fibrotic changes after DVT help us to understand stages of DVT in mice. Collagen deposition and tissue remodeling events, almost absent during acute DVT, are present during the more chronic stages in both vein wall and thrombus. Mouse models having long-term endpoints are suitable for the study of chronic DVT, with remodeling of the vein wall similar to what is observed in the post-thrombotic syndrome.\textsuperscript{4,20,21}

### Analytic Evaluative Methods

The mouse models presented in this review contribute to the understanding of the pathophysiology at various stages of DVT. Large vessel models (mostly involving the IVC) are used to explore both acute and chronic DVT.\textsuperscript{8,9,17,20,22–25} These models can be used to measure various parameters, including thrombus formation, thrombus weights,\textsuperscript{8,9,17,20,22–25} vein wall inflammatory cell counts,\textsuperscript{20} perform immunohistochemistry,\textsuperscript{20} and stain for fibrosis (Masson’s stain,\textsuperscript{9,20} Sirius Red stain). In addition, gene expression,\textsuperscript{20} protein levels (ELISA,\textsuperscript{20,23} western blot), and protein activity (eg, measuring matrix metalloproteinases using zymography)\textsuperscript{23} can be measured. In blood, microparticles, hematology, coagulation, and markers of inflammation (interleukin-6, CCL2, interleukin-1, soluble P- and E-selectin, etc.) can be studied.
Small Vein Models
Mechanical injury,26 endothelial stimulation,22,27–30 and photochemical injury11–37 are all important models to study venous thrombosis. For example, leukocyte and platelet rolling are studied in mesenteric venules stimulated with calcium ionophore A23187 using intravital microscopy.27,38 These studies analyze events that occur within the vein over a short period of time, helping to understand principles of thrombogenesis (acute thrombosis) but not long-term consequences of thrombosis. However they will not be reviewed in the current work because we are focusing on larger vein models.

Large Vein Models
Ferric Chloride Model
The ferric chloride model was initially developed over 20 years ago with the intent to study arterial thrombosis in a rat model.39 Ferric chloride induced thrombosis has been described in both microvascular28 and macrovascular models of mouse venous thrombosis.40 The mouse is anesthetized, the IVC is exposed through a midline incision, and the infrarenal surface cleared to the level of the ilioinguinal veins. A 2-mm × 4-mm filter paper is presoaked in 3.5% FeCl₃ solution and placed on the surface of the vein for 2 to 3 minutes, then removed. Alternatively, microvascular thrombosis may be induced with the application of 10% FeCl₃ to mesenteric venules or internal jugular vein.28,41 The resulting transmural vessel wall injury leads to the formation of occlusive thrombi.4 This model reliably produces thrombi within minutes post injury. The size and speed of thrombus formation is FeCl₃ concentration/time of exposure dependent. In general, the resultant venous thrombus is smaller than those produced by stasis. A disadvantage of this model is the production of a transmural vein wall injury that mimics only a minority of clinical DVT cases (Table).

IVC Ligation Model
This model provides a total stasis environment and results in the most severe vein wall reaction to thrombosis of the models discussed.5,17,20,23 Studies in rats suggest that after IVC ligation a combination of stasis-induced vein wall injury and enhanced tissue factor expression in endothelial cells and leukocytes produce thrombosis.32 In this model, mice are anesthetized and a midline laparotomy is performed. The small bowel is exteriorized and placed on a moistened gauze pad to the animal’s left. The infrarenal IVC is identified and all side branches are ligated with nonreactive 7-0 Prolene suture. Posterior venous branches are cauterized.23 A 7-0 Prolene suture is tied down on the IVC, caudal to the left renal vein. This model has been widely used by our group for the study of venous thrombosis.8,17,20,23 It provides reproducible thrombus weights beginning at 3 hours and extending to 21 days, for most mouse strains. It has proven valuable in the study of interactions between the vein wall and thrombus during the progression from acute to chronic inflammation and remodeling of the vein wall. Disadvantages include the lack of blood flow. A technical pitfall unique to this procedure is the potential to induce initial hypotension. However, compensation by vertebral veins is observed and the survival rate for this model is around 95%, based on our laboratory’s observations. In addition, the IVC cannot reopen because of the ligature. This model cannot reproduce the clinical scenario where a thrombus is nonocclusive, but it can mimic complete occlusion (Figure 2 and Table). As a guideline, data from our laboratories in C57BL/6 mice shows, approximate thrombus weights (IVC + thrombus at harvest), of 33 mg at day 2, 29 mg at day 6, and 18 mg at day 14.

IVC Stenosis Model
This model was initially developed to study early acute thrombosis and results in a thrombus of similar morphology to the ligation model over time. Currently, the model is used to study acute and chronic DVT.24,43 In the original description of this model, a midline incision is performed and the small bowel exteriorized. No branches are ligated. A neurosurgical vascular clip is applied to the infrarenal IVC for 15 seconds twice, 30 seconds apart to damage the endothelium. A 5-0 Prolene is placed longitudinally along the ventral surface of the IVC and a 4-0 silk suture is tied around both the IVC and Prolene suture. The Prolene suture is then removed, allowing blood flow to resume.44 This model combines external compression with a reduction in blood flow to produce laminar thrombus at early time points and has been used to study thrombogenesis kinetics and the efficacy of therapeutic agents.44,45 A modification of this original technique was recently introduced where no external compression on the IVC is performed, lateral branches are ligated, posterior branches remain open, and a 7-0 Prolene is tied around the IVC and onto a 30 G needle. Then the needle is removed creating a ≈90% stenosis.22 Other investigators do not ligate the lateral branches. A significant disadvantage of this model is the large variation in the size of the thrombus with an absence of thrombi in some mice. In addition, the thrombus may lead to complete vessel occlusion. This model is most suited to analyzing the initiation of thrombosis (Table).

Electrolytic Vein Model
The electrolytic method is an alternative to the stenosis model.46 The use of electrolysis to generate venous thrombosis in a murine model was first described by Cooley et al utilizing the femoral vein and who recently characterized a new model of venous thrombosis.47,48 However, the small size of this vessel and thrombus limit the sample size for molecular analysis.46 The electrolytic IVC model (EIM) involves the identical anesthesia and operative approach as the ligation model. Venous side branches are ligated with 7 to 0 Prolene, but posterior branches remain open. A 25-gauge needle, attached to a 30-gauge silver-coated copper wire is inserted into the subcutaneous tissue (cathode) and caudal IVC (anode). A direct current of 25 μAmp is applied over 15 minutes generating free radicals, which results in endothelial cell activation. A small area of endothelial denudation was observed at the needle entry point, which can contribute to thrombus formation. A laminar thrombus is formed with maintenance of a flow channel. Thrombus weights remain consistent with acceptable standard deviations to detect differences in experimental groups with a sample size as small as 5 mice, from 6 hours to 14 days post injury.46,49,50 Importantly, the electrolysis does not affect the intravascular
Table. Mouse Models of Deep Vein Thrombosis

<table>
<thead>
<tr>
<th>Model</th>
<th>Ferric Chloride Model</th>
<th>IVC Ligation or Stasis Model</th>
<th>IVC Stenosis Model</th>
<th>Electrolytic IVC Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brief method description</td>
<td>A 2 × 4-mm filter paper is pre-soaked in 3.5%–10% FeCl₃ solution and placed on the surface of the venules, jugular or saphenous vein for 2–3 min.</td>
<td>A nonreactive suture ligature is placed around the IVC just below the renal veins to produce complete blood stasis.</td>
<td>A 7-0 Prolene is tied around the IVC and onto a 30 G needle. Then the needle is removed. This modification from the original model has been recently introduced.</td>
<td>A needle placed within the IVC with electrical current forms a thrombus in constant presence of blood flow.</td>
</tr>
<tr>
<td>Branches</td>
<td>Lateral branches are ligated and posterior branches cauterized.</td>
<td>Branches remain open.</td>
<td>Lateral branches are ligated and posterior branches remain open.</td>
<td>Lateral branches are ligated and posterior branches remain open.</td>
</tr>
<tr>
<td>Advantages</td>
<td>This model reliably produces occlusive thrombi within minutes postinjury and enables the study of vein thrombosis by simply measuring occlusion time.</td>
<td>The IVC yields quantifiable amounts of vein wall tissue and thrombus. It has proven useful for evaluating interactions between the vein wall and the occlusive thrombus for assessing the progression from acute to chronic thrombosis.</td>
<td>Reduced flow combined with temporary endothelial compression, produces a laminar thrombus allowing the study of cellular kinetics. This model has been widely used.</td>
<td>Produces consistent thrombus size, enough sample quantity per mice to be able to study thrombogenesis, thrombus resolution and also new agents for DVT prophylaxis and treatment.</td>
</tr>
<tr>
<td>Disadvantages</td>
<td>Produces a trans-mural vein wall injury that mimics a small population of clinical DVT.</td>
<td>Lack of blood flow inhibits the maximal effect of administered systemic therapeutic agents on the thrombus and vein wall.</td>
<td>The degree of stenosis is inconsistent leading to a constant variable in thrombus sizes. Some thrombus grows occluding the stenosis area and literally transforming the basis of this model into a stasis model. Poor suitability to study drugs.</td>
<td>Requires 15 min for the electrolytic process. Possible vein wall injury at the site of the needle.</td>
</tr>
<tr>
<td>Model used mainly to study</td>
<td>Acute vein thrombosis.</td>
<td>Acute and chronic vein thrombosis mimicking the occlusive clinical scenario.</td>
<td>Acute and chronic vein thrombosis mimicking the nonocclusive clinical scenario.</td>
<td>Acute and chronic vein thrombosis mimicking the nonocclusive clinical scenario.</td>
</tr>
<tr>
<td>Variation in thrombus size</td>
<td>End point is to study vein occlusion.</td>
<td>No large variation.</td>
<td>Large variation in thrombus size.</td>
<td>No large variation.</td>
</tr>
<tr>
<td>References</td>
<td>30, 41, 42</td>
<td>9, 18, 21, 22, 26</td>
<td>27, 45–47</td>
<td>48, 51, 52</td>
</tr>
<tr>
<td>Observation and recommendations</td>
<td>The trans-mural damage is the initiation of some clinical cases of DVT (DVT posttrauma or burn injury). This model could be used on those particular scenarios.</td>
<td>Compared to the other models, this particular model cannot reproduce the clinical scenario where a thrombus has reopened after DVT in patients, but it can mimic complete occlusion.</td>
<td>This model can reproduce the clinical scenario where a thrombus has reopened after DVT in patients, but it cannot mimic complete occlusion.</td>
<td></td>
</tr>
</tbody>
</table>

DVT indicates deep vein thrombosis; IVC, inferior vena cava.

This model produces reliable and consistent thrombus weights over a wide range of endpoints, while also allowing for the study of anti-thrombotic and thrombolytic agents. Disadvantages include a longer operative time and damage to the vein wall at the needle insertion site. The EIM produces thrombi that do not occlude the IVC during acute and chronic DVT (unpublished data) because of the constant presence of flow, so it more closely resembles the clinical scenario where thrombus partially occludes a deep vein. As a guideline, data from our laboratories in C57BL/6 mice show an average thrombus weight (IVC+thrombus at harvest) of 15 mg at 2 days, 12 mg at 6 days, and 8 mg at 14 days.
Anatomic Vein Variations in Mice

The vein system in both human and mice varies enormously. The models that used the IVC to study thrombosis face the problem of what to do with the branches. Lateral and posterior branches are apparent in the area of the IVC where the thrombus forms. The flow dynamic within the IVC confers these branches an important role, potentially limiting the size of the thrombus in formation. In the IVC ligation model, the thrombus grows backwards, from cranial to caudal, from the ligature to the first open branch (lateral or posterior) and then stops growing. Thus, the importance of the lateral and posterior branches are critical and because there are mice with no lateral branches, the only alternative is to ligate the branches that influence thrombus size. In the EIM model, the thrombus grows caudal to cranial, following the flow. The importance of the lateral branches is critical as in the ligation model but not the posterior branches likely because in the EIM the thrombus remains associated with the anterior wall of the IVC based on our laboratory’s observations. Thus, in the EIM, the ligation of the side branches is critical and the thrombus grows until the flow from the renal veins limit the thrombus extension.

Discussion

Animal models of venous thrombosis are necessary in order to understand the mechanisms of DVT and adequately test pharmaceuticals for treatment of this disease. As an example, we explored the concept that P-selectin inhibition would help to prevent or treat DVT. We conducted experiments using mice, rats, and currently baboons to test inhibitors of P-selectin.8,17,25,51–54 Our findings suggest that blocking P-selectin limits thrombus formation and accentuates thrombus resolution, protecting the vein wall against fibrosis in all 3 species.8,17,25,51–55

An ideal mouse model of DVT should be technically simple and fast to perform, easily reproducible, generate a large sample for molecular analysis, form thrombus within a deep vein, and provide consistent thrombus size. However, there is no ideal “DVT animal model” due to the simple reason that animals do not develop spontaneous DVT. Thus, investigators are forced to artificially trigger a thrombus within a vein. In this review we have given a detailed overview of the advantages and disadvantages of the large vein models of venous thrombosis. The ferric chloride model enables the study of vein thrombosis from experiments that can be obtained by simply measuring time to occlusion. The IVC ligation and stenosis models and the EIM allow the study of actual tissue samples from the area where vein thrombosis occurs (vein wall and thrombus) and also blood analysis (plasma, serum). In addition, all 3 models allow the investigation of acute and chronic DVT. The ligation model, in particular, is useful for studying those clinical scenarios where complete occlusion and no recanalization occurs causing a severe response in the vein wall. The flow models (stenosis and EIM) are useful to mimic nonocclusive clinical scenarios, either partial occlusion and/or recanalization.

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Disclosures

None.

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