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CHAPTER 5

USEFULNESS AND LIMITATIONS OF ANIMAL MODELS OF VENOUS THROMBOSIS

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Summary

Animal models of venous thrombosis are frequently used to study the pathogenesis of venous thrombosis or to assess the effect of a pharmacological intervention on the formation, growth, or lysis of venous thrombosis. We have systematically reviewed published studies containing experimental results obtained in venous thrombosis models in animals. The vast majority of publications concerned pharmacological studies. Most experimental animal models of venous thrombosis are based on similar principles, although animal species, methods of thrombus formation and outcome assessment may vary considerably. Animal models of venous thrombosis appear to play an important role in the study of venous thrombosis and the evaluation of antithrombotic properties of novel anticoagulant agents. However, dose-finding studies in experimental venous thrombosis models in animals and studies comparing the efficacy of different antithrombotic strategies should be interpreted with caution, since the outcome of these studies often inaccurately predicts the effect in clinical studies.
Introduction

Venous thrombosis is a common disorder that is associated with substantial morbidity and even mortality if not adequately diagnosed and treated. Patients with venous thrombosis are usually treated with anticoagulants that prevent the growth of an existing thrombus. Anticoagulants may also be used to prevent the occurrence of venous thrombosis in high-risk situations, such as postoperatively or in trauma patients. Although in recent years major progress in our understanding of coagulation \textit{in vivo} has been made, the pathogenesis of venous thrombosis is only partly understood. Also, despite major improvements in the management of venous thrombosis, prevention and treatment strategies are in some situations insufficiently effective and are associated with important adverse effects, of which bleeding is most important. Therefore, newer antithrombotic agents are being designed and developed for potential clinical use.

To study the pathogenesis of \textit{in vivo} thrombus formation and to evaluate novel preventive or therapeutic strategies, animal models of venous thrombosis have been in use for more than 50 years. In the past decades numerous animal models of venous thrombosis have been developed and have been applied for a variety of objectives and mostly without certain predictive value for the relevance to human venous thrombosis. The Subcommittee on Animal, Cellular, and Molecular Models of Thrombosis and Haemostasis of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis decided to systematically review the various animal models of venous thrombosis and to analyze the contribution of these models to the study of the pathogenesis and treatment of venous thrombosis. This manuscript summarizes the results of this analysis and it draws some conclusions on the appropriate use of animal models for venous thrombosis.

\textit{General aim of animal models for venous thrombosis}

The need for \textit{in vivo} models to study thrombosis and to evaluate interventions that may affect thrombus formation, growth and lysis is obvious. Blood coagulation takes place in an environment of non-anticoagulated blood, in the presence of blood cells and vascular wall components, under flow conditions and in a constantly changing milieu, such as local hypoxia and acidosis. Hence, there is no \textit{ex vivo} model, however sophisticated, that is able to mimic this complex situation.

Animal models of venous thrombosis may be used for four different reasons: Firstly, animal models may be used to study the pathogenesis of venous thrombosis and to establish factors and/or pathways that play a pivotal role in this pathogenesis. Secondly, experimental models can be applied to be able to accurately determine the \textit{in vivo} biological effect of an intervention. Hence, the \textit{in vivo} antithrombotic efficacy or pro-thrombotic effect of compounds with \textit{in vivo} anticoagulant or procoagulant properties, respectively, may be confirmed. Indeed, not every
agent that is able to block coagulation *in vitro* will have antithrombotic properties *in vivo*, hence this confirmation may be essential. Thirdly, animal models of venous thrombosis are used to facilitate dose-finding of novel antithrombotic agents. Lastly, animal models are often applied for comparative pharmacology, i.e. to predict a superior clinical antithrombotic efficacy of one agent over the other. In the following we will analyze to what extent animal models of venous thrombosis may be useful for each of these objectives.

*Animal models of venous thrombosis*

We have performed a literature search in MEDLINE and EMBASE databases from 1966 until July 1999. Terms that were used for the search were both MESH terms and (part of) the textwords “venous thrombosis”, or “vein” adjacent to “thrombosis” or “thrombus”. The search results were then limited to “disease models, animals”, or “animals”. All titles and abstracts of the remaining studies were screened to check whether the study really dealt with an animal model of venous thrombosis. The search was limited to review articles and the references of appropriate reviews were cross-checked for other potentially relevant studies.

Following this strategy a total number of 5746 articles were selected. Figure 1 shows that during the last 10 years an impressive increase in the number of published studies employing a model for venous thrombosis has occurred. The number of published articles between 1996 and 1999 has increased almost 7-fold as compared with the number of articles between 1975 and 1980. When a selection of the retrieved articles was made by using the subheadings “administration and dosage”, or “therapeutic use”, or “pharmacokinetics” it was clear that the vast majority of studies (91%) concerned any form of pharmacological intervention to prevent or treat venous thrombosis, whereas only a relatively small number of studies addressed the mechanism of thrombus formation or blood coagulation *in vivo* (Figure 1).

A large number of different animal species is used to study venous thrombosis. In fact, 18 different animal species were represented in our literature search. Animal species most frequently employed for venous thrombosis models were dogs, rats and rabbits, together responsible for 77% of the published articles (Figure 2). Although it is not always clear which factors account for the choice of the animal species, characteristics of the model probably play a major role. For example, transgenic studies can only be done in mice so far and for experiments requiring complicated instrumentation it is obvious that larger animals are preferred.
Figure 1. Number of published studies using an experimental venous thrombosis model. The lighter part of the bar represents studies concerning any form of pharmacological intervention to prevent or treat thrombosis, whereas the darker part of the bar depicts the other studies (mostly on the pathogenesis of thrombosis).

Figure 2. Animal species used for experimental venous thrombosis models. The contribution of each species to the total number of publications on animal models of venous thrombosis is shown. The group “others” includes 10 different animal species, including goats, cats, hamster, cattle, and horses.
The method of thrombus formation is also variable and follows Virchow's triad, including various means of inducing vascular wall damage, stasis of blood, and local activation of coagulation (Table 1). Although there is ample variation in these methods, most techniques may be considered as a variation of the Wessler model. Stanford Wessler described in 1952 a model of venous thrombosis following local stasis of blood and the local injection of serum. It is interesting to note that Wessler developed his model primarily to study the pathogenesis of thrombosis and the effect of procoagulant substances on thrombus growth, whereas since then most investigators have used the model to study the effect of antithrombotic interventions. Table 1 also lists the most frequently used means to establish the effect on thrombus formation, growth or lysis. It is clear that most methods will result in a valid measure of the effect on the thrombus, although objective measurements (such as the use of radioactive fibrinogen or flow measurement) may be more precise and may avoid the introduction of bias in the experiments.

### methods of thrombus formation in venous thrombosis models
- **endothelial damage**
  - physical damage (e.g. electricity, vessel contusion)
  - chemical damage (e.g. ferric chloride, caustic substances)
- **stasis**
- **local activation of blood coagulation**
  - injection of clot/clotting blood
  - procoagulant substances (e.g. activated prothrombin complex concentrates)
  - injection of anti-phospholipid antibodies

### methods of assessment of thrombus formation, growth, or lysis
- visual score
- thrombus weight
- accretion of $^{125}$I-labeled fibrinogen
- flow measurement
- subjective scores

**Table 1** Thrombus formation has been induced by various methods achieving vascular wall damage, stasis of blood, and local activation of coagulation
Usefulness and limitations of venous thrombosis models

Animal models of venous thrombosis to study the (patho)physiology of blood coagulation in vivo

Although the published reports in which animal models of venous thrombosis are used to study the (patho)physiology of coagulation in vivo represent a relatively small fraction of all published studies, it is clear that this application of animal models is very useful. There are ample illustrations of the importance of venous thrombosis models, not only as a valuable confirmation of in vitro results but also to yield important observations that might not have been made otherwise. Examples include the role of activated protein C in the prevention of thrombosis, demonstration of the role of factor XI-dependent TAFI activation in thrombolysis, the important relationship between inflammatory and procoagulant mediators in the development of venous thrombosis, the regulatory role of PAI-1 in thrombolysis and thrombus accretion, and the response of the vessel wall to thrombosis.

Animal models of venous thrombosis to translate in vitro anti- or procoagulant effects into in vivo anti- or prothrombotic effects

Most agents that have an anticoagulant effect in vitro will show antithrombotic properties in vivo. However, there is sometimes no clear relationship between the potency of the in vitro or ex vivo anticoagulant effect and the antithrombotic efficacy. This may for example be illustrated by the results with low molecular weight heparins or some novel direct thrombin inhibitors and for the use of potent antiplatelet agents in the treatment of venous thrombosis. Likewise, procoagulant substances may have a thrombogenic potential, but also here the in vivo effect is not always predictable. Moreover, some agents may have a clear effect on the prolongation of clotting times but have no effect on coagulation in vivo.

Dose-finding studies in animal models of venous thrombosis

Dose-finding studies in animals may facilitate the assessment of the proper dose of a therapeutic agent in humans. To assess whether dose-finding studies in animal models of venous thrombosis are helpful in determining the dose of antithrombotic agents in humans, we have analyzed all studies containing results of a dose-effect relationship in our literature search. We limited our search to antithrombotic treatment with heparin, low molecular weight heparins, or hirudin, since for these agents the adequate antithrombotic dose in human studies has been assessed, thereby allowing us to compare the optimal dose from the animal studies with the optimal dose in humans.

Using the MESH terms and textwords “heparin”, “low molecular weight heparin”, and “hirudin” our literature search of animal models of venous thrombosis was limited to 714 articles. Further selecting these articles by the textword “dose” and the MESH heading “dose-response relationship” resulted in 134 studies, of which the abstracts were screened. Ultimately,
73 studies were selected for analysis. Of these studies, 43 reported on a dose-effect relationship of heparin, 25 studies mentioned a dose-response of low molecular weight heparins and in 14 articles dose-response effect of hirudin was described. Table 2 shows the optimal dose of the various agents for treatment and prevention of venous thrombosis in animal models of venous thrombosis. The difference between the optimal dose of the compounds in the animal models of venous thrombosis may be significantly different from the ultimately established dose in humans. Interestingly, from this selection of agents it may seem that the optimal dose in animal models of venous thrombosis is always higher (up to a factor 3) than the established dose in humans. Obviously, also other factors may have accounted for the eventual selection of the dose in humans (such as the rate of bleeding complications). On the other hand, bleeding was often taken into account to establish the optimal dose in animal models of venous thrombosis (although the proper assessment of bleeding potential of various agents in animal models is complicated but falls beyond the scope of this article). From the table it is also clear that there is a wide variation in the optimal dose of the various agents in the different venous thrombosis models. No systematic relationship between the characteristics of the model or a specific animal species and the sensitivity to any intervention could be established. However, it seems clear that it is very difficult to use the results from a single dose-finding study in an animal model of venous thrombosis to predict the optimal dose of an antithrombotic compound in humans.

<table>
<thead>
<tr>
<th></th>
<th>optimal dose in animal model of venous thrombosis</th>
<th>recommended dose in clinical practice</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>heparin</strong></td>
<td>50 U/kg/hr [2.5-400 U/kg/hr]</td>
<td>15-20 U/kg/hr</td>
</tr>
<tr>
<td><strong>nadroparin</strong></td>
<td>15 anti-Xa U/kg/hr [7.5-80 anti-Xa U/kg/hr]</td>
<td>5-9 anti-Xa U/kg/hr</td>
</tr>
<tr>
<td><strong>dalteparin</strong></td>
<td>32 anti-Xa U/kg/hr [5-70 anti-Xa U/kg/hr]</td>
<td>8-10 anti-Xa U/kg/hr</td>
</tr>
<tr>
<td><strong>enoxaparin</strong></td>
<td>50 mg/kg [20-100 mg/kg]</td>
<td>20-40 mg/kg</td>
</tr>
<tr>
<td><strong>recombinant hirudin</strong></td>
<td>0.4 mg/kg/hr [0.1-1.4 mg/kg/hr]</td>
<td>0.15 mg/kg/hr</td>
</tr>
</tbody>
</table>

*Table 2* Comparison of the optimal dose of various anticoagulant agents in different studies using animal models of venous thrombosis (median values and upper and lower limits) with the recommended use of these agents in humans, based on clinical observations.
Comparative pharmacology in animal models of venous thrombosis

Animal models of venous thrombosis are often used to compare the efficacy of various antithrombotic strategies. It is indeed tempting to compare the antithrombotic effects of a novel agent with conventional treatment as part of the pre-clinical evaluation of such a compound. However, it is not clear whether the results of such a comparison accurately predict a superior efficacy of the agent in clinical practice. To get an impression of the predictive value of the outcome of comparative pharmacological studies in animal models of venous thrombosis we have chosen to analyze the results of such studies in two different area’s: (1) the antithrombotic efficacy of low molecular weight heparin or hirudin as compared with unfractionated heparin, and (2) pharmacological interventions to achieve thrombolysis of venous thrombosis. For both subjects adequate data from clinical studies are available to establish the accuracy of the results obtained in animal studies.

From our literature search of articles with animal models of venous thrombosis we have selected studies containing data of both heparin and low molecular weight heparin or both heparin and hirudin (using the MESH terms and keywords “heparin”, “heparin, low molecular weight”, “LMWH”, or “hirudin”). The abstract of the 334 selected studies were screened to establish whether the article contained original data on a direct comparison between heparin and low molecular weight heparin or between heparin and hirudin. This screening resulted in 86 remaining articles, of which 14 reported on the comparative efficacy of low molecular weight heparin and unfractionated heparin on experimental thrombosis. Another 11 articles showed results of a comparison of heparin with hirudin in experimental models of venous thrombosis. The antithrombotic properties of the compounds were compared at equipotent anticoagulant doses. The majority of the studies comparing low molecular weight heparin with unfractionated heparin (9 out of 14) showed a superior antithrombotic efficacy of low molecular weight heparin, 4 studies showed a similar efficacy of both agents and 1 study showed that unfractionated heparin had better antithrombotic properties. In the animal studies comparing hirudin with heparin, 9 of 11 studies showed a superior efficacy of hirudin over heparin and 2 studies demonstrated a similar efficacy. We now know from large scale clinical studies and meta-analyses that low molecular weight heparin is as effective as unfractionated heparin in the treatment of venous thrombosis. Initial clinical studies comparing hirudin with heparin in the treatment of venous thrombosis show no benefit of one therapy over the other, although prevention of thrombosis may be more effective with hirudin. Taken together, the results from most of the animal models might have been somewhat too optimistic.

Studies comparing the efficacy of various thrombolytic agents on lysis of venous thrombosis in animal models show conflicting results (e.g. t-PA induces a better antithrombotic effect and less bleeding than streptokinase), which are also not in agreement with subsequent clinical
observations\textsuperscript{114-116}. Also observations of enhanced lysis of venous thrombosis with low molecular weight heparin could not be confirmed in clinical studies\textsuperscript{58,117}.

\textbf{Conclusion}

Animal models of venous thrombosis appear to be indispensable to advance our knowledge on venous thrombosis and the optimal management of this disease. There are numerous variations in the design and execution of experimental venous thrombosis in animals, although most models are based on similar principles. Animal models for venous thrombosis appear to be particularly useful for studying the (patho)physiology of blood coagulation \textit{in vivo} and the pathogenesis of venous thrombosis. Models also serve an important role in the assessment of the \textit{in vitro} antithrombotic effect of novel anticoagulant agents. Dose-finding results from animal models of venous thrombosis should be considered with caution, since results may vary significantly and the outcome of these studies may considerably differ from the ultimately effective dose in humans. Also, species differences may play an important role here. Similarly, studies comparing the efficacy of different antithrombotic strategies in animal models of venous thrombosis may not always accurately predict a clinical benefit in human studies. It might at least be advisable to test antithrombotic strategies in different models and different species before drawing any conclusion on its (relative) efficacy as an antithrombotic agent.
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