Thrombosis Research 140 (2016) 149–152

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Full Length Article

Murine model of large-vein electrolytic injury induction of thrombosis with slow resolution

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ARTICLE INFO

Article history:
Received 22 November 2015
Received in revised form 17 January 2016
Accepted 27 January 2016
Available online 28 January 2016

ABSTRACT

Deep vein thrombosis (DVT) and its sequela, pulmonary embolism, occur at a rate of 1 per 1000 person/year. Experimental models for evaluation of DVT have many short-comings, such as mechanical occlusion or stenosis to cause thrombosis, rather than the clinical scenario of thrombosis causing occlusion/stenosis. The goal of this study was to develop a model of flow-based large-vein thrombosis with resistance to resolution, to model clinical DVT behavior. Adult male C57Bl/6 mice underwent thrombus induction via an electrolytic injury to the femoral vein (3 V positive current for 90 s), with subsequent intra-vital fluorescence quantitation of platelet and fibrin accumulation through the first 60 min, and final histomorphometric volume evaluation at 1, 7, 14, and 28 days. Platelet accumulation at the injury site was comparable to a milder electrolytic injury, whereas fibrin was greatly augmented by 60 min in the more severe injury model. Thrombi showed persistent presence at 1 and 7 days, with remodeling to a stenotic fibrosis that encroached into the lumen at 14 and 28 days. The thrombotic/fibrotic volume within the femoral vein fell by 23% from 1 to 7 days, but had a residual presence at 28 days that was 31% the 1-day volume. This new model may provide an alternative approach to evaluating DVT persistence and therapeutic inhibition, to develop a better understanding of the clinical progression of DVT to thrombophlebitis.

1. Introduction

Despite high clinical prevalence of and several clearly identified risk factors for deep vein thrombosis (DVT), the initiating cause or causes of thrombus growth remain speculative [1–4]. The incidence of thrombophlebitis (also called post-phlebitic or post-thrombotic syndrome) after diagnosis of DVT also lacks a clear predictive strategy [5–7], highlighting the unknown developmental factor(s) in thrombophlebitis [7]. Up to one-third of DVT patients develop thrombophlebitis within two years, strongly implicating a continuing presence of venous thrombosis or thrombus-initiated vein-wall changes in the progression to thrombophlebitis [7–9]. Therefore, a better understanding of the causes of unresolved venous thrombosis and associated vein-wall remodeling should provide insight into the development of TPB.

Recent experimental investigations of venous thrombosis have turned predominantly to murine models [10–11]. There is no ideal murine model for studying DVT. A commonly used model is done in the vena cava, using either complete [12] or partial ligation; [13] these models are based on the Wessler model of ligation-induced stasis [14]. Major drawbacks to these models are: 1) the placement of a permanent ligature around the vessel mechanically hinders (partial ligation) or abrogates (total ligation) flow and subsequent potential for thrombolytic resolution, a natural process by which DVTs can be overcome in patients; 2) the thrombus develops upstream of the occluding/stenosing site, which does not simulate the downstream thrombotic propagation that occurs with clinical DVTs; 3) the inferior vena cava is the main central vein of the lower half of the body and is not a site where clinical DVT typically originates. Other models of murine DVT use an electrolytic injury, involving iron ion deposition to induce a free radical injury to the vessel surface [15] or within the lumen [16–18], resulting in endothelial injury and subsequent thrombogenesis. The current report describes an extension of this electrolytic injury, using a more severe injury to the femoral vein to induce a substantially massive and downstream-propagating thrombus that maintains structural presence and lumenal encroachment over 4 weeks.

2. Materials and methods

Adult male C57Bl/6 mice, 3–4 months old, were used under an institution-approved IACUC protocol, following national guidelines for the care and use of laboratory animals. Under pentobarbital anesthesia (50 mg/kg, i.p.), the femoral vein was exposed through a groin skin incision. The blunt end of a 75-micron microsurgical needle (Sharpoint, Reading, PA) was gently placed on the vein surface for 30 or 90 s

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http://dx.doi.org/10.1016/j.thromres.2016.01.023
0049-3848/

(Supplemental Fig. 1), with delivery of positive current via a 1.5- or 3-volt battery, respectively; 90 s with 3 V (severe injury) yielded 6 times the electrolytic deposition in comparison to 30 s at 1.5 V (standard injury, as previously described) [15]. Five minutes prior to this thrombus induction, rhodamine 6G (0.5 mg/kg) and anti-fibrin antibody (monoclonal 59D8; clone kindly provided by Dr. Marschall Runge) labeled with Alexa-Fluor-647 (Invitrogen) were injected into the external jugular vein. The femoral vein thrombus site was subsequently imaged with intra-vital microscopy using time-lapse capture over 60 min. Fluorescence quantitation of platelet and fibrin accumulation was determined and normalized off-line, analyzing for intensity of each fluorophore over the region of observed thrombus to provide a quantitative assessment of the accumulation of each thrombotic component (platelets and fibrin) over time. Wounds were closed and animals recovered from anesthesia. Five groups were used: 4 severe injury groups (n = 8/group) with harvests at 1, 7, 14, or 28 days, respectively, and one standard injury group (n = 8) harvested at 1 day. Each harvested vein with thrombus was fixed in 4% buffered formaldehyde, processed with paraffin embedding, and sectioned longitudinally in its entirety for H&E staining and histomorphometric volume reconstruction of the residual thrombus and remodeling vein wall [16].

Data were statistically analyzed with Student's *t*-tests and analysis of variance with posthoc Fisher's LSD tests. A $p < 0.05$ value was used to determine statistical significance.

3. Results

The acute evaluation of fluorescence for platelets and fibrin (out to 1 h) showed highly consistent findings between the 4 replicate groups of 8 mice undergoing the severe electrolytic injury (Supplemental Fig. 2a and b, respectively). None of the developing thrombi showed occlusion of flow in the vein at any time. Pooling the 60-minute time course data for these 4 groups (group size, n = 32), the platelet accumulation was relatively comparable to that for the standard model of electrolytic injury (1.5 V for 30 s; data obtained from previous study data, randomly selecting a subset of n = 10), but appeared to have a more rapid accumulation in the first few minutes, with a more sustained presence beyond ~30 min, though this difference did not achieve statistical significance (Fig. 1a). In contrast, fibrin development was substantially increased over the lesser injury (Fig. 1b). The normalized amount of fibrin was approximately 6 times greater with the 6-fold increase in electrolytic application (90 s at 3 V versus 30 s at 1.5 V) ($p < 0.001$ at 60 min). The extent of the thrombus was also greatly augmented (Supplemental Video 1), in comparison to the lesser electrolytic injury (Supplemental Video 2).

Histomorphometric determination of thrombus volumes was done at each harvest time point (1, 7, 14, or 28 days; n = 8 per time point). The severe injury resulted in a >6-fold larger thrombus at one day postoperatively, in comparison to the standard injury ($p < 0.001$). There was a linear trend toward moderate reduction of thrombus size over time in the severe injury series (Fig. 2). Statistical differences were seen for comparisons of the 1-day vs. 14- and 28-day data and for the 28-day vs. 7- and 14-day data ($p < 0.05$). The overall proportionate decreases in size from 1 day to 7, 14, and 28 days were 23%, 47%, and 69%, respectively.

The histologic features changed from 1 to 28 days, displaying clearly identifiable thrombus material of fibrin and/or platelets at 1 day, with interspersed pockets of red blood cell aggregates (Fig. 3a). This began transitioning to a more solidified clot with cell infiltration by 7 days (Fig. 3b), and a transformation to a cell-rich, fibrotically remodeled vein wall by 14–28 days (Fig. 3c, d).

4. Discussion

The more severe electrolytic injury to the mouse femoral vein results in a large and sustained thrombus which undergoes remodeling into a fibrotic encroachment upon the vessel wall. Unlike the original model of inserting a needle into the vein lumen [16–17] to apply electrolytic injury, this new model uses surface-applied electrolysis, at approximately 6 times the electric-current delivery of a previous model [15]. In a previous study [17], the intraluminal electrolytic injury had a poorly sustained thrombus, falling to under 1% of its 1-day volume after 28 days; in contrast, the current model maintained a volume presence of the thrombus of ~31% of the 1-day volume at the 28-day time point. A somewhat similar electrolytic injury has been described in the infrarenal vena cava (IVC) [18], with a resultant large and sustained thrombus, showing a 42% reduction by 14 days, similar to the 47% reduction of the model of the present report. There are unique features to each of these models; an advantage of the herein-described model

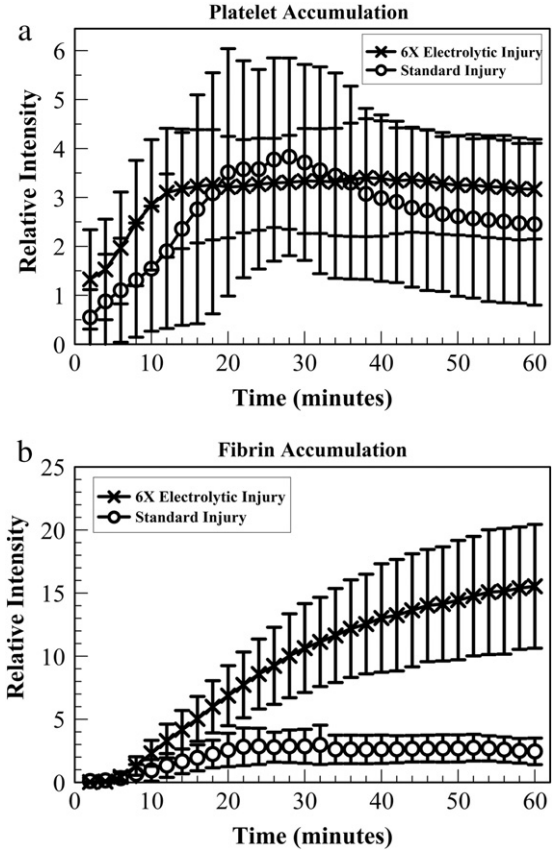

Fig. 1. Graphs of relative normalized intensities of fluorescence at the thrombus site over time for platelet (a) and anti-fibrin (b) accumulation.

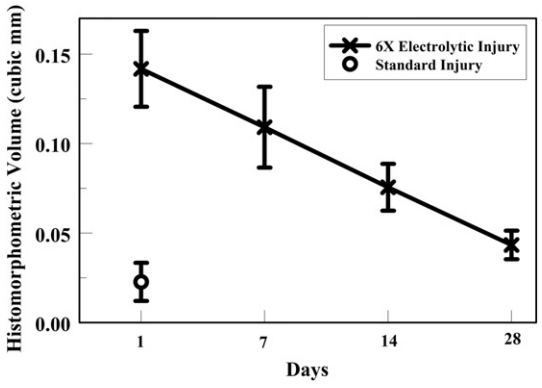

Fig. 2. Histomorphometric volume determination of thrombus size at 1, 7, 14, and 28 days after thrombus induction.