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P-selectin/ PSGL-1 Inhibitors *versus* enoxaparin in the resolution of venous thrombosis: a meta-analysis

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Abstract

Background—P-selectin antagonism has been shown to decrease thrombogenesis and inflammation in animal models of deep venous thrombosis (DVT).

Objective—To determine the effectiveness of P-selectin inhibitors *versus* saline and enoxaparin in venous resolution in nonhuman primate models of venous thrombosis.

Methods—Studies reporting vein re-opening, inflammation expressed as Gadolinium enhancement and coagulation parameters were searched in the literature and pooled into a meta-analysis using an inverse variance with random effects.

Results—Five studies were identified comparing P-selectin/ PSGL-1 inhibitors *versus* saline or enoxaparin regarding venous thrombosis resolution. Vein re-opening was significantly higher on P-selectin/ PSGL-1 compounds, when compared to saline (Inverse Variance [IV] 95% CI; 44.37 [17.77–70.96], $p=0.001$, $I^2=97\%$) and similar to enoxaparin (IV 95% CI; 5.03 [–8.88–18.95], $p=0.48$, $I^2=41\%$). Inflammation, reflected as Gadolinium enhancement at magnetic resonance venography (MRV), was significantly decreased in the P-selectin treated group when compared to saline (IV 95% CI; –17.84 [–14.98 – –8.30], $p<0.00001$, $I^2=80\%$). No significant differences on vein wall inflammation were observed between P-selectin/ PSGL-1 inhibitors and enoxaparin treated animals (IV95% CI; –3.59 [–10.67–3.48], $p=0.32$, $I^2=66\%$). In addition, there was no differences in the coagulation parameters (aPTT, TCT, BT, D-Dimer, fibrinogen, platelets) between P-selectin/ PSGL-1 inhibitors and enoxaparin (IV 95% CI; –1.12 [–2.36–0.11], $p=0.07$, $I^2=92\%$), although there was a trend showing less prolongation in TCT with P-selectin /PSGL-1 inhibitors over enoxaparin ($p<0.0001$).

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Conflict of interest
None.

Conclusion—P-selectin antagonism successfully paralleled the low-molecular-weight-heparin enoxaparin, for the treatment of DVT in nonhuman primate models, by decreasing both thrombus burden and inflammation without causing any bleeding complications and increasing coagulation times.

Keywords

P-selectin; inflammation; venous thrombosis; enoxaparin; animal model; meta-analysis

Introduction

Venous thromboembolism (VTE) is a severe multifactorial disease that includes DVT and pulmonary embolism (PE). Nine hundred thousand cases of VTE are estimated per year in the US, with 300,000 deaths annually from PE¹. The ideal treatment of DVT should relieve edema and pain, facilitate thrombus resolution and avoid death by PE in the early stages of treatment. In the long term, the ideal treatment should also prevent recurrence of DVT, post-thrombotic syndrome and chronic pulmonary hypertension that occurs secondarily to PE².

Anticoagulation with heparin is still the standard therapy for VTE. It is effective in preventing fatal PE, decreasing the incidence of recurrent DVT and reducing thrombus extension. Moreover, low-molecular-weight heparin (LMWH) has improved results with the convenience of subcutaneous administration and home therapy³. However, this still requires daily dosing and can lead to bleeding in up to 10%, and even fatal hemorrhage in up to 2% of cases⁴.

The relationship between DVT and vein wall inflammation has been well established¹. Anti-inflammatory therapy directed towards P-selectin⁵⁻⁸ (a leukocyte cellular adhesion molecule) is associated with decreased thrombosis and increased thrombolysis when used at the time of thrombus formation, in various animal venous thrombosis models⁹⁻¹⁴. The objective of this systematic review is to compare the efficacy of P-selectin or its ligand PSGL-1 inhibitors with LMWH for resolution of venous thrombosis in nonhuman primate models and for its anti-inflammatory effects.

Methods

The authors conducted a MEDLINE search for articles published from 1990 to the present. Search terms included P-selectin, enoxaparin, venous thrombosis, primates and experimental models. Reference lists of included articles, relevant articles, and related systematic reviews were screened. The “Related Articles” feature in PubMed was used to identify additional citations. In addition, colleagues and investigators as well as the manufacturers of various LMWH and P-selectin inhibitors preparations were consulted to identify unpublished or missed studies.

Selection

Two reviewers independently screened the titles and abstracts for eligibility. To be eligible for this meta-analysis, articles had to include evaluation of P-selectin/ PSGL-1 in primate models of venous thrombosis, including information of unpublished data.

Data Abstraction

A total of 6 studies were identified that met the inclusion criteria. Data were collected relating to the intervention (P-selectin/ PSGL-1 inhibitors) and its control (saline and/ or enoxaparin) and 2 outcome assessments (vein re-opening and inflammation reflected as gadolinium [Gd] enhancement, both evaluated with magnetic resonance venography [MRV])¹⁰. Briefly, vein-

re-opening was initially evaluated by contrast venography (using un-subtracted imaging and subtracted imaging) and MRV on days 0, 2 and 6 after thrombus formation, with data stored on a computer. In the iliac and the inferior vena cava (IVC) system, vein reopening was quantitated by time-of-flight MRV. Typically, a region of interest was drawn around the flow channel on the affected vein and then this was compared to the contralateral iliac vein. Animals that underwent IVC thrombosis, MRV evaluations at day 2 and day 6 were compared to pre-thrombosis MRV analysis. Inflammation was evaluated by Gd enhancement images. Gd, a heavy metal chelate that prolongs T1 relaxation and extravasates selectively in areas of capillary leakage was used as a non-invasive marker of inflammation. Images of the IVC, right and left iliac veins were acquired after intravenous Gd injection. A region of interest (ROI) was drawn around the area of enhancement and measured in mm². Day 6 was chosen for the evaluation day for this meta-analysis, because all studies included day 6 in the protocols, treatment was occurring on this day and all of the outcomes were documented at this period.

Coagulation parameters (hematology activated partial thromboplastin time [APTT], thrombin clotting time [TCT], bleeding time [BT], D-dimers, fibrinogen and platelet count) were collected at baselines and at different timepoints. P-selectin inhibitors and enoxaparin coagulation parameters were also compared for this meta-analysis at time point 6 days after drug administration.

Statistical analysis

An inverse variance method was used for variables expressed with means and standard deviations. The average effect size across all studies was computed as a weighted mean, whereby the weights were equal to the inverse variance of each study's effect estimator. Larger studies and studies with less random variation were given greater weight than smaller studies. A forest plot was used as a graphical display illustrating the relative strength of treatment effects in multiple quantitative outcomes. A funnel plot for each outcome was constructed to evaluate publication bias. A confidence interval of 95% was constructed, and significance was defined as $p < 0.05$.

Results

Six studies were identified comparing P-selectin/PSGL-1 inhibitors *versus* saline or enoxaparin for treating experimentally induced venous thrombosis in a nonhuman primate model; one study did not provide vein re-opening quantitation by MRV and was excluded from the analysis. The remaining 5 studies (1 involving IVC and 4 assessing the right iliac vein) were pooled. Details of the protocol used in all studies can be found elsewhere^{13, 14}. Interventions and outcomes are listed in table 1. To our knowledge, the Conrad Jobst Vascular Research Laboratories is the only one performing such trials in nonhuman models. Thus, all studies analyzed in this meta-analysis were from a single institution.

Vein re-opening

Vein re-opening was significantly greater with P-selectin/PSGL-1 compounds when compared to saline (IV 95% CI; 44.37 [17.77–70.96], $p = 0.001$, $I^2 = 97\%$, Figure 1). No significant differences were observed between P-selectin/PSGL-1 inhibitors and enoxaparin (IV 95% CI; 5.03 [–8.88–18.95], $p = 0.48$, $I^2 = 41\%$, Figure 2).

Inflammation

Inflammation, reflected as Gd enhancement at MRV, was significantly less in the P-selectin/PSGL-1 inhibitors treated group when compared to saline (IV 95% CI; –17.84 [–14.98 – –8.30], $p < 0.00001$, $I^2 = 80\%$, Figure 3). No significant differences were observed between P-

selectin inhibitors and enoxaparin treated animals (IV95% CI; $-3.59 [-10.67-3.48]$, $p=0.32$, $I^2=66\%$, Figure 4).

Coagulation parameters

No differences in coagulation parameters (aPTT, TCT, BT, D-Dimer, Fibrinogen and Platelets) between P-selectin/PSGL-1 inhibitors and enoxaparin were noted (IV 95% CI; $-1.12 [-2.36-0.11]$, $p=0.07$, $I^2=92\%$, Figure 5). However there was a trend for less prolongation in TCT with P-selectin/PSGL-1 inhibitors over enoxaparin ($p<0.0001$). Inverted funnel plots for both outcomes revealed no publication bias (not shown).

Discussion

For a medical disorder affecting almost a million Americans each year, causing approximately 300,000 deaths, and severe long-term complications, the causes of venous thrombosis remain relatively the same as postulated 150 years ago. Virchow explained the development of thrombus based on stasis, changes in the vessel wall and thrombogenic changes in the blood, and those concepts are still valid 16¹⁷. Thus, the treatment of venous thromboembolism is centered on blood anti-coagulation/fibrinolysis, avoidance of stasis and limiting vein wall damage. Unfractionated heparin (UFH), discovered in 1916 and its derivative, low molecular weight heparins (LMWH) are still the most widely used drugs in the management of venous thrombotic disorders. A broad reduction of mortality and morbidity of VTE was observed after the introduction of heparin therapy 18. Oral antagonists of vitamin K, introduced in the late 50's, and primarily developed as rat poison, are still widely used worldwide, despite displaying relative high bleeding risks.

Limitations of existing antithrombotic drugs have prompted a search for novel agents. From Fondaparinux, a synthetic pentasaccharide already approved for VTE treatment and prophylaxis, to small anti-Xa molecules, with oral bioavailability, there are a large group of new agents, targeting coagulation factors that are in different stages of development. Some of them are already available for clinical practice. From bench to bedside, the rationale for these drugs is relatively simple: increase anticoagulation while decreasing the risk of bleeding. The delicate balance in modulating fibrin formation leads to endpoints where researchers seek the best efficacy with the best safety ratios. The ideal anti-coagulant would promote 100% clot reduction and zero bleeding. This compound yet does not exist. Heparin and LMWH can still promote bleeding complications and are not ideal for out-of-hospital use¹⁹.

In the early 1970's, through the ground-breaking theories of Gwendylen Stewart, a relationship between thrombosis and inflammation was suggested^{20, 21}. Stasis is still a very important factor in venous thrombogenesis, but inflammation is crucial. Inflammation increases tissue factor (TF), the trigger for the coagulation cascade. It also increases platelet reactivity, fibrinogen, and leads to exposure of phosphatidylserine, a molecule present on microparticles (MP) surface and related to de-encryption of MP's TF¹⁶. Inflammation also decreases thrombomodulin and inhibits fibrinolysis. But inflammation also is essential for thrombus resolution. Heparin has both anti-coagulant and anti-inflammatory effects. Selective inhibition of inflammation leads to thrombus modulation.

P-Selectin Inhibitors

Cell adhesion molecules (CAMs) allow leukocyte transmigration, and selectins (P and E-selectin) are integrally involved in thrombosis^{1, 16, 22, 23}. Selectins are the first up-regulated glycoproteins on activated endothelial cells and platelets²⁴⁻²⁶. The cell adhesion molecule P-selectin has been found up-regulated in the vein wall as early as 6 hours after thrombus induction, whereas E-selectin has been found up-regulated at day 6 after thrombosis^{24, 27-29}.

Venous stasis and ischemia results in the up-regulation of P-selectin³⁰ which localizes prothrombotic MP to the area of stasis³¹ and promotes DVT formation³².

This systematic review showed a significant difference in the effect on vein re-opening or inflammation between P-selectin inhibitors and saline in nonhuman primate model of venous thrombosis. In addition, no significant differences in both outcomes were observed when compared to enoxaparin, but trends existed in favor of P-selectin inhibitors.

There are several strengths to this review, including the rigorous search strategy and the assessment of publication bias. The same group under the same protocol conducted all the reported studies, allowing consistency in pooling results in this meta-analysis. No differences regarding coagulation parameters were observed between both compounds, although there was a trend towards improvements in TCT with P-selectin/PSGL-1 inhibitors over enoxaparin 6 days after drugs were administered. Moreover, since bleeding was not reported in any of the studies (major and minor bleeding events did not occur in such controlled animal model studies), it was excluded as an outcome. A limitation was the lack of concealment. Studies were randomized, but not blinded, due to their experimental nature.

P-selectin/ PSGL-1 inhibition is a treatment whose target does not involve the inhibition of the coagulation cascade, but the inhibition of inflammation. Different compounds have already been tested in our laboratory, with results similar to enoxaparin. P-selectin inhibition is a promising therapeutic approach because it:

1. Reduces inflammation (with a potential decrease in chronic venous insufficiency)
2. Reduces thrombosis
3. Reduces bleeding potential
4. Can possibly be used as a multi-modal therapeutic agent with current standard of care
5. Can be made into oral formulations, that have already been tested¹⁴, allowing prolonged use.

In conclusion, this review suggests that P-selectin inhibition should be further evaluated for the treatment and prevention of VTE. Future studies evaluating the association of P-selectin and enoxaparin are warranted.

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Figure 1.

Forest plot of comparison: P-selectin inhibitors vs. saline, outcome: vein re-opening. Note that the mean difference in inverse variance was 44.37 in favor of P-selectin inhibitors.

Figure 2.
Forest plot of comparison: P-selectin inhibitors vs. enoxaparin, outcome: Vein re-opening

Figure 3.
 Forest plot of comparison: P-selectin inhibitors vs. saline, outcome: Gd Enhancement (Inflammation)

Figure 4.
 Forest plot of comparison: P-selectin inhibitors vs. enoxaparin, outcome: Gd Enhancement (Inflammation)

Figure 5.
Forest plot of comparison: P-selectin inhibitor vs. enoxaparin, outcome: coagulation tests 6 days post thrombosis.

Table 1

Interventions including study medication/ doses and outcomes

Study	N (total)	Study drug	Comparator	via	Drug therapy initiation	MRV
1. Wakefield, T.W. et al, 2000	12	rPSGL-1- Ig 4 mg/ Kg	Saline	IV	6 hours prior to thrombosis	Yes
2. Myers Jr, D.D. et al, 2001	8	rPSGL-1 500 µg- 1 mg/ kg	Saline	IV	6 hours prior to thrombosis	Yes
3. Myers Jr, D.D. et al, 2002	8	rPSGL-1- Ig 4 mg/ Kg	Saline/ enoxaparin 1.5 mg/ Kg	IV	48 hours after thrombosis	Yes
4. Myers Jr, D.D. et al, 2007	9	PSI-697 30 mg/ Kg	Saline/ enoxaparin 1.5 mg/ Kg	oral	24 hours prior to thrombosis	Yes
5. Meier, T.R. et al, 2008	9	PSI-421 1 mg/ kg	Saline/ enoxaparin 0.57 mg/ Kg	oral	48 hours prior to thrombosis	Yes