



[haematologica reports]
2005;1(9):59-60

Heparin effects in metastasis and Trousseau's syndrome: anticoagulation is not the primary mechanism

STEVENSON JL
CHOI SH
WAHRENBROCK M
VARKI A
VARKI NM

*Glycobiology Research and Training Center, the Moores Cancer Center, Biomedical Sciences and Biological Sciences Graduate Programs
Departments of Medicine, Pathology, Biology, and Cellular & Molecular Medicine,
University of California, San Diego, La Jolla, CA, USA*

The existence of recurring and successful *International Conference on Thrombosis and Hemostasis Issues in Cancer* arises is due to the well-documented and frequent clinical association between malignancy and excessive thrombosis in humans. Unfractionated heparin (UFH) has been the classic anticoagulant of choice to treat cancer patients with thrombosis, and recent studies have indicated that UFH could be replaced by various low molecular weight heparins (LMWHs), which have several advantages for clinical use. Heparins are also the agent of choice in treating patients with classic Trousseau's Syndrome (spontaneous migratory thrombophlebitis with microangiopathic symptoms, typically associated with mucin-producing carcinomas). Meanwhile, UFH has been shown to reduce tumor metastasis in many murine models. Also, retrospective and post-hoc analyses of clinical studies suggest that UFH and some LMWHs might improve outcomes in human cancer.

Given all these findings, a natural assumption has been that heparins must ameliorate cancer metastasis and Trousseau's syndrome via their well-known activity in blocking the fluid phase coagulation pathway, by potentiating antithrombin inactivation of factor Xa (as well as IIa, in the case of UFH). In support of this notion, some studies have shown that hirudin (a natural leech-derived thrombin inactivator) can also attenuate the metastatic process. However, this assumption belies the frequent failure of anti-coagulation by Vitamin K antagonists (which also reduce factor X and II levels) to ameliorate either tumor progression or Trousseau's Syndrome. Furthermore, the doses of heparin or hirudin used in mouse studies have been far in excess of those that might be clinically tolerated in humans (surprisingly, heparin pharmacokinetics in mice has not been a subject of well-documented studies). Also, heparins are natural compounds that comprise a polydisperse mixture of glycosaminoglycan chains of vari-

able length, sulfation and epimerization, only a small fraction of which can actually potentiate antithrombin action. Finally, heparins have many other potential biological actions that are not directly related to fluid phase coagulation.

One such non-anticoagulant action of heparin is inhibition of P- and L-selectin recognition of mucin-like glycoprotein ligands e.g., appropriately glycosylated and sulfated forms of PSGL-1 on leukocytes and molecules such CD34 and MAdCAM on endothelium. P- and L-selectin are cell adhesion/signaling receptors that normally mediate interactions of leukocytes and activated platelets with one another, and/or with the endothelium lining blood vessels (see Figure 1, left panel). In this regard, we have shown that P- and L-selectin can play critical roles in mouse models of tumor metastasis, and that heparin has limited additional efficacy in mice deficient in these selectins. Also, while heparin effectively inhibits a P- and L-selectin-mediated mouse model of carcinoma mucin-induced Trousseau's syndrome, hirudin treatment had no effect. Meanwhile, we have noted that heparin inhibition of human P- and L-selectin occurs *in vitro* at concentrations that are in the clinically acceptable range. Selectin blockade thus poses a new paradigm to explain how heparin might attenuate hematogenous tumor metastasis and Trousseau's syndrome. This non-anticoagulant action of heparin would affect critical selectin-mediated interactions that occur very early in the cascade of pathological events that follow entry of carcinoma cells or carcinoma mucins into the bloodstream (see Figure 1, right panel).

As for other events in tumor progression that might be affected by heparins (e.g., heparanase action, angiogenesis and integrin function), almost all are expected to be involved only after the initial selectin-mediated interactions have occurred. Thus, effective inhibition of two of the earliest mediators of the metastatic cascade (P-

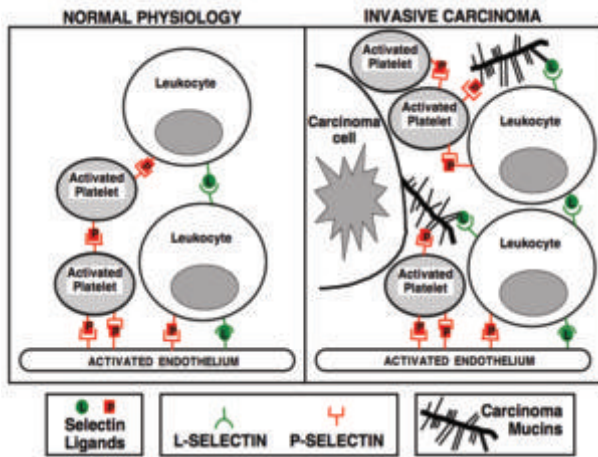


Figure 1. The cartoon shows P- and L-selectin mediated interactions in normal physiology (left panel), and potential P- and/or L-selectin-mediated interactions involving tumor cells, their membrane-associated and secreted mucins, blood cells and endothelial cells (right panel). P- and L-selectin ligands on both normal cells and tumor cells are often comprised of mucin-like molecules bearing the correct glycosylation and sulfation patterns to permit recognition by these selectins. Such selectin-binding mucins can also be secreted into the blood circulation by carcinoma cells. Some clinical formulations of heparins contain subsets of molecules that can inhibit all these selectin-mediated interactions at clinically-tolerable levels, potentially attenuating tumor spread and/or Trousseau's syndrome.

and L-selectin) could make inhibition of subsequent events by heparin less practically important. Even if this is not entirely true, the fact remains that the other known effects of heparin are also beneficial for the control of tumor progression. Based on all these considerations, we have suggested that the failure of vitamin K antagonists to affect tumor progression should be ignored, and that heparin therapy for metastasis prevention in humans should be revisited, with these new paradigms in mind. While anticoagulation may indeed be a major mechanism in rodent experiments involving high doses heparin or hirudin, this is proba-

bly not the case when using heparin in clinically practical doses in humans.

Our current studies are focused on comparing and contrasting the *in vitro* and *in vivo* selectin-inhibitory properties of various heparins and heparinoids in current clinical use in the USA, and at optimizing their dosing in mice to better mimic the human clinical situation. Our data suggest marked differences in the ability of various clinical approved heparins to inhibit P- and L-selectin-mediated processes *in vitro* and *in vivo*, and show the lack of significance of their antithrombotic activity in controlling tumor spread in mice at clinically-relevant doses. This data will prove to be important for designing prospective clinical trials of heparin in preventing metastasis.

Selectin blockade can have beneficial effects not only in carcinoma metastasis and Trousseau's Syndrome, but also in a variety of other pathological circumstances, involving acute or chronic inflammation and in reperfusion injury. Caution is therefore raised about the current trend towards preferring various LMWHs in clinical practice, based on other criteria. Despite equivalent anticoagulation, hitherto unsuspected benefits of selectin inhibition in various clinical circumstances may be unwittingly discarded. Finally, we are working to define heparin fragments with potent selectin-inhibitory properties relative to anticoagulant activity - allowing for clinical inhibition of selectin-mediated pathologies, with only a limited effect on the patient's coagulation state. These data will eventually be important in making more specific suggestions for a neo-adjuvant trial in patients with recently diagnosed carcinomas.

Acknowledgements

We gratefully acknowledge many prior members of the lab as well as expert collaborators for some of the published results described here. Because of space considerations, the literature citations listed are limited to those arising from our own work.

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