

Heparin as an Immunosuppressant: Therapeutic Potential beyond Anticoagulation

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Glycosaminoglycans (GAGs) are components of proteoglycan (PG) molecules found on the cell surface and within the extracellular matrix. PG consists of a range of core proteins that are covalently linked to one or more GAG chains. These chains are chemically defined as heparan sulfate (HS), chondroitin sulfate, dermatan sulfate, or keratan sulfate and range in molecular weight up to 100 kDa. HS is generally the most abundant GAG on endothelial cell surfaces. Both the core protein and the GAG component of a PG can contribute to the molecule's function [1].

A number of growth factors and proinflammatory cytokines have been shown to bind to GAGs; these include acidic and basic fibroblast growth factors, interferon- γ , most chemokines, and several of the interleukins. Whilst the functional implications of these interactions are not fully understood, proteoglycans can protect many small proteins from degradation, act as cytokine storage sites, or aid presentation to specific cell-signaling receptors. Thus these molecules are thought to play an important role in regulation of wide variety of biological processes including inflammation and tumor metastasis.

Heparin (a highly sulphated variant of HS) is synthesized and stored exclusively by mast cells and co-released with histamine. It has been used in the clinic as an anticoagulant for more than 60 years. It is now known that an anionic, pentasaccharide sequence in heparin binds with high affinity to a series of basic amino acids (Arg129, Lys125, Arg46 and Arg47) in antithrombin III (ATIII) [2].

Low molecular weight heparins (LMWH) are now used more frequently than unfractionated heparins as a result of their better pharmacokinetic profiles; the three main LMWH currently in clinical use are enoxaparin (Lovenox), dalteparin sodium (Fragmin) and tinzaparin sodium (Innohep). It is becoming clear that heparin has an array of therapeutic properties in addition to its anticoagulant activity.

Heparin binding proteins such as chemokines, cytokines, growth factors and some extracellular matrix-degrading enzymes are involved in the inflammatory process. Heparin is proposed to have a regulatory role in limiting inflammation by modifying the properties of bound proteins. Chemokines, which plays an important role in leukocyte recruitment, bind to GAG chains of proteoglycans present on the surface of epithelial and vascular endothelial cells and within the extracellular matrix. This interaction occurs between anionic GAG domains and consensus basic amino acid motifs in the chemokine sequence, including XBBXB and XBBBXXB, where B is a basic amino acid residue and X is an intervening residue [3]. The fundamental importance of the interaction between HS and chemokines for the induction of inflammation has been highlighted recently by our demonstration that non HS-binding, mutant forms of the chemokines CCL5, CCL7 and CXCL12 can function as powerful, blood-borne anti-inflammatory agents [4-7].

Although therapeutically it is not possible to modify endogenous chemokines, our group and others have shown that chemokines can be displaced from HS on the endothelial cell surface by competitive binding. Clinically achievable concentrations of heparin (100 ng/ml; ~ 0.05 U/ml) can displace chemokines, immobilized onto HS *in vitro* [8], producing a soluble heparin-chemokine complex which can be either biologically inert or anti-inflammatory. Furthermore,

chemokines can be stripped from the apical surface of endothelium *in vivo* by intravenous injection of heparin [9]. Heparin has also been shown to prolong cardiac allograft survival in rats and in xenograft models.

Previous studies by our group have shown that soluble, heparin-like GAGs are able to antagonize the biological activity of IFN- γ *in vitro* [10]. Furthermore, mixture of heparin and CCL2 prior to incubation with mononuclear leukocytes effectively antagonized the tyrosine phosphorylation of PI 3-kinase and cellular migration produced by CCL2 alone [11]. There is also a precedent for heparin to increase the affinity of FGF molecules for their high affinity receptors on GAG-deficient cells [12]. Heparin and the related molecule fucoidin (a polysaccharide containing sulphated L-fucose) can also inhibit selectin-mediated rolling of leukocytes across the surface of activated endothelium by blocking selectins. *In vivo* imaging has shown that modulation of the function of fucosylated selectin ligands can inhibit such leukocyte rolling in inflammation *in vivo* [5].

There is compelling evidence that the pattern of breast cancer metastasis is determined by interactions between CXCR4 on breast cancer cells and CXCL12 within normal tissue. Work from our group showed that unfractionated heparin and the low-molecular-weight heparin tinzaparin inhibited receptor ligation and the response of CXCR4, expressing human breast cancer cell lines to CXCL12. Both heparin and two clinically relevant dose regimens of tinzaparin reduced haematogenous metastatic spread of human breast cancer cells to the lung in a murine model [9]. Heparinoids also have the potential to inhibit the activity of a wide range of factors that can enhance cancer growth. This includes not only chemokines but also vascular endothelial growth factor, tissue factor, fibroblast growth factors, IFN- γ , histamine, complement, proteases, and heparanases. The potential to inhibit simultaneously this multiplicity of tumor growth-promoting cytokines may explain the reduction in metastatic lesions area observed in heparin-treated animals. Short-term prophylactic doses of tinzaparin at the time of tumor cell release into the vasculature can prevent the seeding and subsequent growth of distant metastases. Hence, the administration of tinzaparin at the time of cancer surgery might have benefits in addition to the prevention of thromboembolic disease.

The mechanism of leukocyte recruitment to developing site of inflammation is very similar to tumor metastasis and heparin has been successfully used in the treatment of both these conditions. However,

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the clinical use of heparin is limited by its anticoagulant activity. Whilst anticoagulation with heparin can provide an additional benefit during surgery, the associated risk of haemorrhage requires careful monitoring followed by protamine reversal at the end of the procedure. Due to the important therapeutic potential, effort has been directed towards generation of non-anti-coagulant heparin, LMWH or heparin like polyanions (heparinoids). Despite the potential of heparin and its derivatives as anti-inflammatory or anti-metastatic agent, the specific heparin ligand structure involved in these roles is not yet fully defined.

Theoretically there are several ways of blocking the protein-GAG interactions. The chemical or enzymatic synthesis of the target protein-specific glycan structure or a mimetic is an option used for synthesis of ATIII-specific heparin pentasaccharide. However, this approach has so far not been successful for blocking other protein-glycan interactions. Another approach is the use of blocking antibodies. The major limitation of this approach is the low immunogenicity of the GAG structure. In addition, binding of some proteins (e.g. chemokines) to GAGs results in significant structural changes of both the chemokines and GAGs resulting in the antibody being unable to bind the target glycan. Although, phage display antibodies against GAGs have been developed [13], there are no therapeutic antibodies targeting proteins specific GAG sequence available so far. Small peptides that mimic the heparin binding domains of cytokines have been used to modulate the inflammatory responses in animal models of inflammation [14]. Recently, a new approach has used the intrinsic specificity of GAG binding protein for its ligand by engineering the proteins with increased GAG binding affinity (e.g. CCL2) [15].

Heparin and its derivatives clearly have some therapeutic potential as an anti-inflammatory agent, but a better understanding of heparin's actions and specificity is essential before its true potential can be realized.

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