Old and new applications of non-anticoagulant heparin

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K E Y W O R D S

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A B S T R A C T

The aim of this chapter is to provide an overview of non-anticoagulant effects of heparins and their potential use in new therapeutic applications. Heparin and heparin derivatives have been tested in inflammatory, pulmonary and reproductive diseases, in cardiovascular, nephro- and neuro-tissue protection and repair, but also as agents against angiogenesis, atherosclerosis, metastasis, protozoa and viruses. Targeting and inhibition of specific mediators involved in the inflammatory process, promoting some of the above mentioned pathologies, are reported along with recent studies of heparin conjugates and oral delivery systems. Some reports from the institute of the authors, such as those devoted to glycol-split heparins are also included. Among the members and derivatives of this class, several are undergoing clinical trials as antimetastatic and antimalarial agents and for the treatment of labour pain and severe hereditary anaemia. Other heparins, whose therapeutic targets are non-anticoagulant such as nephropathies, retinopathies and cystic fibrosis are also under investigation.

1. Introduction

This chapter is an overview of the subject starting from the early landmark achievements, and includes papers, extended articles, reviews and books. Heparin and LMWHs are widely used for the prevention and treatment of thrombotic events by inhibiting antithrombin III (AT) and factor Xa through a specific oligosaccharide binding sequence (ATBR) present in only one third of unfractionated heparin chains as has been well-documented in two recent reviews [1,2]. Heparin, the most negatively charged biopolymer has an average of four negative charges for each disaccharide unit, can interact with a wide range of proteins, with interactions that exhibit a range of specificities [3] and induce several associated biological activities. These involve plasma or tissue proteins such as heparin cofactor II (HCII), tissue factor plasminogen inhibitor (TFPI), lipoproteinlipase, growth factors and heparanase. Interestingly, when a heparin ATIII-no-affinity fraction was added to normal plasma containing heparin, a marked increase in anti-factor Xa activity was observed, presumably due to the displacement of heparin with affinity for ATIII from binding plasma proteins [4].

2. Heparin and low molecular weight heparins (LMWH)

2.1. Anti-inflammatory, cardiovascular and tissue protection activities

The early reports of the beneficial effects of heparin in inflammation were attributed to the heparin binding and inhibition of chemokines, complement, growth and angiogenic factors, as reviewed recently [1,5,6,7]. Heparin can also bind to adhesion mediators expressed during inflammation, such as selectins, integrins and their receptors [8,9]. Tissue protection and repair were observed after heparin was inhaled to treat hot smoke inhalation injury in human fire survivors [5]. Topical, ophthalmic and parenteral formulations were also used to treat burns and lesions [10]. In low doses, heparin showed activity in several experimental models of inflammation as well as in the treatment of human chronic pulmonary diseases, by inhalation, or topically in allergic rhinitis [5]. Under various experimental and clinical conditions, such as oedema formation and pulmonary hypertension, heparin reduces leucocyte recruitment at the site of injury or inflammation stimuli, down-regulating cytokines, TNF-α, endotoxins and inhibiting human leukocyte elastase (HLE), as well as heparanase [5]. In response to vascular injuries, excessive repair by artery smooth muscle cells (SMC) can induce vascular disorders such as restenosis and hypoxy-pulmonary hypertension. SMC proliferation was inhibited by heparin in tissue culture and in rat, and rabbit injury models

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[12]. A recent systematic review with meta-analysis showed the beneficial effects of heparin treatment in asthmatic patients [13]. In normal subjects, heparin can inhibit reactive oxygen species (ROS) generation [14], supporting the observed cardiovascular protective effects, and also increases nitric oxide bioavailability through the release of vessel immobilized myeloperoxidase [15]. The role of neutrophil elastase, a highly aggressive endopeptidase, seems to be crucial and is provoked by imbalances of natural inhibitors leading to degradation of connective and tissue components as observed in emphysema, cystic fibrosis, rheumatoid arthritis, psoriasis, perodontitis, mucopolysaccharidosis, wound healing and tumour invasion. Elastase can be inhibited "*in vitro*" by heparin and derivatives [16–18] as well as in emphysema experimental models [19].

2.2. Heparanase inhibition: implication in various pathologies

Heparin can also inhibit the endo-β-D (1-4) glucuronidase, heparanase [20,21] that through cleavage of HS chains of heparan sulfate proteoglycans affects their functions, the integrity and functional state of the extracellular matrix (ECM) and basement membrane of vessel walls. Owing to the ubiquitous presence and multiple roles of HS, such as growth factor storage and activity, cytokines, chemokines and heparanase degrading activity, it is involved in several pathological conditions including inflammation, amyloidosis, diabetic and glomerular nephropathies, cancer metastasis and angiogenesis. Overexpression and enhanced local activity of heparanase were observed particularly in atherosclerosis [22], type 2 diabetes [23], inflammatory bowel disease [24], in synovial fluid from rheumatoid arthritis patients [25], as well as in kidneys from both diabetic nephropathic and glomerular disease patients [26]. The first clinical trial of low dose heparin (in combination) dates back to the 1960s, followed in 1971 by long term high dose heparin in chronic glomerular nephritis [26]. The use of heparin and glycosaminoglycans as potential anti-complement agents in renal dysfunctions has been hypothesized [27].

2.3. Anticancer activity of heparin and LMWHs

The inhibitory activity of heparin on the growth of transplanted tumour tissues was first reported in 1930 [28]. Heparin and LMWHs have shown "*in vivo*" inhibitory activity in several experimental models. Their anti-metastatic activity seems to be based mainly on interference with the spreading of tumour cells in the blood and inhibition of angiogenesis, selectins as well as heparanase [29] and tissue factor (TF) over-expressed during inflammation and in the presence of aggressive cancers [30]. A recent report indicated an additional chemo-sensitizing activity of heparin through inhibition of P-glycoprotein- mediated multidrug resistance [31]. The results of "*in vitro*", "*in vivo*" studies and clinical trials have been reviewed [29,32–34]. Retrospective evaluation of early clinical trials indicated that heparin can provide survival benefits to cancer patients compared to other anti-thrombotics [32]. The inhibition of clots associated with tumour tissues, by heparin and LMWHs can increase accessibility of anticancer drugs and the efficacy of chemotherapy, but also induces drug resistance in some cases [35,36]. LMWHs are considered by current clinical guidelines to be the drug of choice for antithrombotic treatment in cancer patients. Adjunct therapy in small cell lung cancer patients with or without Bemiparin compared to chemo-radiation showed increased response rates and median survival times [37]. Comparable results were obtained with Dalteparin combined with chemotherapy in non-small cell lung cancer patients when compared with chemotherapy alone [38]. Other positive effects were observed in terminal cancer patients treated with Nadroparin or Dalteparin, as well as with Enoxaparin combined with chemotherapy in pancreatic cancer patients [29]. An

Table 1
Heparin: anecdotal* and clinical trials

Target	References
Acute respiratory distress syndrome*	[5,42]
Allergic rhinitis	[43]
Antimalaric[a]	[44]
Antiphospholipid syndrome	[45]
Asthma and bronchial constriction	[5,13]
Cardioversion of atrial fibrillation	[46]
Cardiopathies	[47]
Chronic obstructive pulmonary diseases	[5]
Cystic fibrosis	[18,48]
Glomerulonephritis	[26]
Hyperlipemias	[49]
Inflammatory bowel diseases	[5,29,50]
Mucolytic agent (Inhaled)	[51]
Nervous system protection by radiation	[52]
Rheumatoid arthritis	[5,53]
Severe sepsis	[54]
Tissue repair and wound healing	[5]
Vasoprotection	[55]

[a] Heparin, inhibiting and reversing cytoadherence and rosetting of *Plasmodium falciparium* infected erythrocytes "in vitro". Tested from 1967 in severe malaria clinical trials, after some overall promising outcomes, were discontinued due to severe intracranial bleeding [44]

updated systematic review and meta-analysis of randomized trials on survival of cancer patients treated with LMWHs has been reported [39]. A retrospective study on small cell lung cancer patients treated with heparin showed overall beneficial effects even when using different commercial heparin preparations [38]. Their intrinsic heterogeneity, despite their comparable anticoagulant activity, was probably one of the major factors behind some irreproducible or conflicting results. In this context the seminal report of J. Folkman *et al.* "Angiogenesis inhibition and tumour regression caused by heparin or a heparin fragments in the presence of cortisone" [40]. Following a preliminary selection from commercial heparins for their anti-angiogenic activity, the most active heparin found was "Panheparin" from Abbot that was discontinued soon after. Difficulties in reproducing the results using different commercial heparins interrupted this line of research [42]. Beneficial effects observed in anecdotal and clinical trials are reported in Table 1.

2.4. Other experimentally investigated activities

After an early report [56] on the "*in vitro*" inhibition of *Herpes simplex* virus, in the mid-1980s, the potential for heparin and sulfated polysaccharides, to inhibit HIV *in vitro* [57], were investigated. Other reports showed the broad spectrum of "*in vitro*" activity of heparin on a variety of RNA and DNA viruses [58,59]. As for other therapeutic applications, anticoagulant activity limited doses, poor pharmacokinetics and the poor oral absorption of heparin were the major drawbacks to further developments. Heparin can exert neuroprotective effects in some models of neurodegenerative diseases inhibiting apoptotic processes [60]. Table 2 shows some other "*in vitro*" and "*in vivo*" activities of heparin that have been investigated.

2.5. LMWHs, ultra LMWHs and related oligosaccharides as nephro- and neuroprotective agents

Experimental and clinical studies in patients with proteinuric glomerulonephritis showed benefit by oral treatment with sulodexide, a mixture of LMW heparin and dermatan sulfate in a