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Review Article

Targets of Heparin Anti-Inflammatory Activity, Therapeutics beyond Coagulation

Francesc Cabre[1,2*], Martin Ballarin[2]

[1]Department of Medical and Scientific Research, Menarini Group, Badalona, ESP, Spain; [2]Department of Biochemistry and Molecular Biomedicine, University of Barcelona, Barcelona, ESP, Spain

ABSTRACT

Heparin is one of the drugs used for almost 100 years as anticoagulant and antithrombotic. Structurally is a polysaccharide of natural origin whose anticoagulant activity is mainly due to a fragment of five monosaccharides that enhances the activity of antithrombin, the main inhibitor of the coagulation cascade. Effects, other than anticoagulation, attributable to heparin have recently been described, particularly a relevant anti-inflammatory activity. Although the molecular and cellular interactions of heparin linked to its anti-inflammatory effect are not completely defined, some mediators and cells related to both coagulation and inflammation processes on which heparin can act are known. This brief review describes some of the elements involved in inflammatory processes, whose expression and regulation could be modulated by heparin and therefore be new targets for the design and development of potential anti-inflammatory drugs.

Keywords: Anticoagulation; Antithrombotic; Antithrombin; Monosaccharides; Monocytes

INTRODUCTION

Inflammatory processes, by generating a strong blood extravasation, promote coagulation, and the factors that are activated in blood coagulation, in turn, act as drivers of the inflammatory process. Thus, for example, pro-inflammatory cytokines favor the expression of tissue factor in endothelial cells and monocytes, activating coagulation, and on the other hand, deposit of fibrin is associated with tissue ischemia whose resulting hypoxia is a potent stimulant of inflammatory mediators [1].

Systemic inflammation will invariably lead to activation of the coagulation system, and vice versa, components of the coagulation system can markedly modulate the inflammatory response. Extensive cross-interaction between the two systems at various points is becoming increasingly evident, with key roles of tissue factor, thrombin, components of the protein C pathway, and fibrinolytic activators and inhibitors.

Exploring the anti-inflammatory effects of commonly used anticoagulants, such as heparin, is an emerging concept that is not yet fully covered by current preventive and therapeutic measures.

The purpose of this review is to briefly discuss the potential pathways and mediators through which the anti-inflammatory effect of an anticoagulant with obvious efficacy such as heparin could be explained.

LITERATURE REVIEW

Inflammation and coagulation players

Rather than being a one-way direction of inflammation that leads to coagulation, both systems interact intensively, so clotting can also significantly modulate inflammatory activity. Binding of coagulation proteases (such as thrombin or tissue factor) or anticoagulant proteins (such as activated protein C) to specific cellular receptors on mononuclear cells or endothelial cells can affect cytokine production or inflammatory cell apoptosis. This intercommunication between the two systems is relevant to many disease states, including various manifestations of vascular disease and systemic inflammatory response syndrome, which leads to organ dysfunction and mortality in sepsis or other conditions.

Correspondence to: Francesc Cabre, Department of Medical and Scientific Research, Menarini Group, Badalona, ESP 2, Spain; E-mail: fcabre@ext.menarini.es

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The main interfaces linking coagulation and inflammation are the tissue factor pathway, which plays a central role in initiating inflammation-induced coagulation, thrombin, protein C, and the fibrinolytic system. Pro-inflammatory cytokines and chemokines can affect all of these coagulation mechanisms. Conversely, activated coagulation factors and physiological anticoagulants or components of the fibrinolytic system can modulate inflammation through specific cellular receptors.

The intricate relationship between inflammation and coagulation may not only be relevant to vascular thrombotic disease, but also has important consequences on the pathogenesis of microvascular insufficiency and subsequent multi-organ failure in the setting of severe infection (Table 1) [2].

Table 1: Pathways and mediators through which inflammation effects on coagulation occur and vice versa.

Inflammation procoagulant properties	**Pro-inflammatory and anti-inflammatory properties of coagulation**
Cytokines	**Pro-inflammatory**
• TNFα	• Thrombin
• Suppresses t-PA. • Stimulates release of PAI-1. • Inhibits EPCR and thrombomodulin expression. • Induces TF expression in monocytes and endothelial cells.	• Promotes cytokine synthesis. • Expresses Selectin P in endothelial cells. • Chemotactic for PMN leukocytes. • Stimulates the production of PAF. • Promotes neutrophil-monocyte adhesion.
• IL-1	• Tissue factor/factor VIIa complex
• Inhibits EPCR and thrombomodulin expression.	• Promotes cytokine synthesis. • Induces adhesion molecules expression in macrophages. • Stimulates neutrophil infiltration.
• IL-6	• Fibrinogen/fibrin
• Induces TF expression in monocytes and endothelial cells. • Increases the number of platelets. • Increases platelet thrombogenicity.	• Increases neutrophil and monocyte adhesion. • Promotes cytokine synthesis and MCP-1.
Leukocytes	**Anti-inflammatory**
• Neutrophils	• Antithrombin
• Destroy antithrombin and C1 inhibitor. • Splitting TFPI and thrombomodulin.	• Blocks the expression of NF-κB. • Increases prostacyclin formation. • Decreases leukocyte activation/adhesion.
• Monocytes	• TFPI
• Release of microvesicles (TF source). • Factor X activation.	• Decreases cytokine expression. • Decreases leukocyte activation.
Proteins present in the acute phase	• aPC
• PCR	• Decreases NF-κB mRNA levels, causing. - Decrease of cytokine formation. - Decrease of TF expression. - Decreased of adhesion molecules cell expression. - Prevention of apoptosis.
• Induces TF expression. • Promotes activation of the complement system. - Activates neutrophils. - Promotes neutrophil chemotaxis. - Promotes cytokines. - Exposes phospholipids on the cell surface. - Inactivates S protein.	• Thrombomodulin
• Iα 1-antitrypsin	• Inhibits the adhesion of leukocytes to endothelial cells. • Blocks cytokine synthesis.
• Inhibits aPC • Split the TFPI	

Note: aPC: Activated Protein C; CRP: C-Reactive Protein; EPCR: Endothelial Protein C Receptor; IL: Interleukin; MCP-1: Monocyte Chemotactic Protein 1; NF-κB. Nuclear Factor enhancer of the kappa light chains of activated B; PAF: Platelet Activating Factor; PAI-1: Plasminogen Activator Inhibitor-1; PMN: Polymorphonuclear Leukocytes; Protein S: Vitamin K-dependent glycoprotein that only acts as a cofactor for CRP to act on

clotting factors V and VIII; TF: Tissue Factor; TFPI: Tissue Factor Pathway Inhibitor; TNF α: Tumor Necrosis Factor α; t-PA: tissue Plasminogen Activator

Activation of coagulation and fibrin as a consequence of inflammation can be considered critical to contain inflammatory activity at the site of injury or infection. However, inflammation-induced coagulation can also contribute significantly to the disease, as it is evidenced by the coagulopathy associated with a serious infection, such as sepsis, and also by the fact that thrombus formation in a ruptured atherosclerotic plaque, containing abundant inflammatory cells, is the primary cause of acute arterial thrombotic events. The main mediators activating inflammation-induced coagulation are pro-inflammatory cytokines. Several studies demonstrated, the importance of IL-6 in initiating coagulation activation and the role of TNF-α and IL-1 in the regulation of physiological anticoagulationat [2].

Heparin

As a first meaning, heparin refers to Unfractionated Heparin (UFH), the first anticoagulant agent to be discovered and isolated for medical use, and one of the oldest drugs still widely used therapeutics. It remains on the WHO list of essential medicines, the safest and most effective medicines needed in a healthcare system, and as the desirable option for anticoagulation when an effect is needed in a short time thanks to the rapid onset of action when administered intravenously [3].

Heparin is a mixture of chains of sulfated polymorphic polysaccharides (glycosaminoglycans) of varying lengths and weights obtained after purification of vertebrate organs, with a structure similar to heparan sulphate. In the body, it is produced by basophils and mast cells, which are present in intensely vascularized tissues (lung, intestinal mucosa, liver, kidney, etc.) since, as a natural anticoagulant, it helps to keep the blood flowing in areas where circulation is highly branched and where there are capillaries (small size), preventing small clots from occurring in these capillary territories [4-6].

Anticoagulant and antithrombotic activity of heparin

Heparin is a powerful anticoagulant, not only activating of Antithrombin III (AT), but also acting on the most of the links of the intrinsic and extrinsic pathways of blood coagulation: Prekallikrein, factors XIIa, XIa, IXa, Xa, IIa (thrombin) and XIIIa, preventing the formation of fibrin, as well as inhibiting platelet aggregation and degranulation. AT primarily blocks thrombin and FXa, two key players in the coagulation cascade. Once anti-thrombin binds to thrombin or FXa, they form a complex that is rapidly degraded by the circulation [4,6]. However, heparin concentration in the blood is very low and only under certain physiological conditions does it have significant anticoagulant effects [7].

Heparin inhibits coagulation, *in vivo* and *in vitro*, mainly through the activation of AT by binding to the active site, through a characteristic pentasaccharide sequence, then modifies AT conformation increasing its affinity for serine proteases, and enhancing the activity of this main natural inhibitor of coagulation, which in healthy individuals accounts for the largest share of all natural anticoagulants [4,6]. Although heparin strongly inhibits plasma thrombin; the heparin/AT complex is unable to inhibit fibrin-bound thrombin. Approximately one-third of heparin molecules contain the high-affinity pentasaccharide required for anticoagulant activity. Only heparin chains composed of at least 18 saccharide units, corresponding to a molecular weight of 5400, are long enough to perform this binding function. With an average molecular weight of 15,000, all heparin chains are long enough to facilitate this binding [8,9].

A second way in which heparin exerts an anticoagulant effect is through its activity as a catalyst for thrombin inactivation by heparin cofactor II. This anticoagulant effect is specific to thrombin and requires much higher doses of heparin than those necessary to activate AT. Finally, the third anticoagulant effect of heparin is the modulation of factor Xa generation, by binding to factor IXa. This effect is not considered clinically significant because it requires a much higher dose of heparin than is necessary to achieve therapeutic efficacy and maintain safety [8].

Since heparin accelerates the mode of action of AT by approximately 1000 times, it works as a very effective anticoagulant drug. The effects and pharmacological properties vary with the size of the molecules. In general, heparins used in pharmaceutical specialties are found in a wide range of molecular weights (5000-30000), with the average being 15000.

Anti-inflammatory activity of heparin

Although heparin was discovered and developed as an anticoagulant, approximately 70% of the polysaccharide chain in UFH was found to be non-binding to AT, this fraction that was designated as "inactive heparin" or "low-affinity material. Thus, over the years of clinical practice, patients treated with UFH have received these "inactive" heparin fragments. The question that arises is whether these fragments can contribute positively to the overall therapeutic effects of UFH. Some answer seems to exist, since it has been observed that the "inactive" fraction of heparin can generate activities that appear to be protective of tissues. Therefore, and in retrospect, the beneficial properties, other that anticoagulation, of heparin may have contributed to the success of heparin [6,10,11].

Heparin has inhibitory and activating functions in molecular and cellular mechanisms. In addition to its potent effect by inhibiting coagulation, heparin exerts a number of effects on mediators and cells involved in several processes [11-13].

Interacts and degrades platelets; inhibits angiogenesis; inhibits heparanase, an enzyme that acts by degrading heparan sulfate into shorter-chain oligosaccharides; blocates P and L selectins, transmembrane receptors involved in intercellular adhesions; inhibits metastasis; interacts with proteins of extracellular matrix and improves the remodeling; interacts with integrins and growth factors; and is involved in inflammatory processes and regulates inflammation.

It is known that more than 400 substances, pro-inflammatory mediators and adhesion molecules involved in the recruitment of inflammatory cells, have in their structure regions of binding to heparin, so that when heparin binds to these inflammatory proteins, it alters their function. These effects could be involved in the experimental and clinical evidence of an anti-inflammatory action of heparin [6,11]. However, while heparin is thought to exert many of its non-anticoagulant actions, particularly anti-inflammatory actions, by binding to proteins associated with the inflammatory response, including, but not limited to, cytokines, growth factors, adhesion molecules, cytotoxic enzymes, and tissue degraders such as elastase and metalloproteinases; in most cases, the exact structural characteristics that mediate these anti-inflammatory effects are not fully understood.

Since endogenous heparin is stored in mast cell granules, it would seem reasonable to assume that therapeutic heparin could exhibit immunomodulatory properties, as mast cells play an important role in inflammatory and allergic diseases, as they contribute to increased vascular permeability and allergic and anaphylactic reactions.

Mast cell heparin, with certain structural differences when compared to clinical-grade heparin, has been reported to have pro-inflammatory properties *via* the stimulation of bradykinin, similar to that described for chondroitin sulfate. However, heparin-like glycosaminoglycans present on the surface of endothelial cells and administered heparin appear to have the opposite effect [11].

The mechanisms by which heparin and its derivatives may express their anti-inflammatory properties reflect their multifaceted effects on biological processes. Anti-inflammatory properties can be roughly divided into two modes of action: Modulation by binding to soluble plasma ligands and modulation by binding to receptors or macromolecules bound to the cell surface, with potential effects on signaling pathways. By these ways, heparin is able to interfere with several (if not all) stages of leukocyte transmigration and extravasation in the target tissue [11].

Table 2 summarizes some molecular (action on inflammatory mediators) and cellular effects, evidenced in experimental studies, that could be correlated with the potential anti-inflammatory properties of heparin [6,11-16].

Table 2: Molecular and cellular effects (experimental studies) related to the potential anti-inflammatory properties of heparin.

Mediator cell	Action of heparin	Potential ant-inflammatory properties of heparin
TNFα	• Reduces levels. • Inhibition of synthesis in monocytes.	Effect on pro-inflammatory cytokines.
IL-6	Reduces levels.	
IL-10	Increases levels.	
Matrix metalloproteinases	Limits synthesis	
NO	Promotes NO formation in endothelial cells.	• NO inhibits platelet aggregation, neutrophil activation, and endothelial cell adhesion. • Vasodilator.
IL-1	Limits release.	Effect on pro-inflammatory cytokines.
IL-8	Reduces chemotactic responses.	
Leukocytes	• Binds on P and L selectins. • Blocks generation and activity of superoxide. • Induces chemokine activation • Modulates heparanase activity and expression. • Attenuates B11b-dependent leukocyte adhesion (integrin).	• Inhibits adhesion to the endothelial surface and subsequent migration to tissue. • Prevents harmful oxidative stress. • Inhibits chemoattractant activation of leukocytes. • Attenuates transendothelial migration of immune cells.
Lymphocytes	• Decreases the cytotoxic activity of T cells. • Impairs T cell adhesion and migration. • Suppresses the activity of natural killer cells.	Attenuates the function of T lymphocytes.

Neutrophils	• Limits granulocyte activation. • Inhibits the production of granulocyte enzymes (lactoferrin and myeloperoxidase). • Attenuates infiltration. • Attenuates neutrophil-mediated phagocytosis. • Inhibits leukocyte integrin MAC-1. • Inhibits the formation of neutrophil superoxide. • Reduces neutrophil chemotaxis in transendothelial migration.	• Prevents adhesion to vascular endothelial cells. • Prevents harmful oxidative stress.
Eosinophils	Inhibits chemoattractant mediators.	Inhibits recruitment into tissues (infiltration).
Monocytes	Decreases monocyte adhesion and infiltration.	
Mast cells	Attenuates mast cell activation.	

DISCUSSION

Some patients with pathologies exhibiting an inflammatory and/or immunological component (asthma, allergic rhinitis, inflammatory bowel disease, eye disorders, cystic fibrosis, burns, etc.) seem to benefit from the administration of heparin due to its anti-inflammatory properties, although the evidence is not always convincing. In several cardiovascular conditions with a clear inflammatory component (acute coronary syndrome, cardiopulmonary bypass and thrombophlebitis) the use of heparin appears to be beneficial. In organ preservation and transplantation, where ischemia and reperfusion processes are evident, heparin is used to reduce vascular thrombosis and ischemia-reperfusion injury. However, the exact benefit or risk of heparin treatment during the different stages of these processes remains a matter of debate [11,13].

Finally, a recent indication for heparin is the use as an anticoagulant in patients with severe acute respiratory distress syndrome due to SARS-CoV-2 (COVID-19) infection. In particular, in the inflammatory and prothrombotic state of SARS-CoV-2 infection, an elevation of coagulation potential may require higher doses of heparin than the standard dose [17,18]. In COVID-19, not only the anticoagulant effects, but also the anti-inflammatory and antiviral effects of heparin and its derivatives may be beneficial, as UFH and heparin derivatives are assumed to inhibit viral and protozoal infections by preventing the interaction between pathogenic proteins and heparan sulfate on the cell surface [19-21].

CONCLUSION

Based on the existence of a broad spectrum of potential pharmacological activity observed for heparin, both UFHs and Low Molecular Weight Heparins (LMWHs) have been used beyond as antithrombotic therapeutic agents. This includes applications such as surface coating of biomedical devices, the treatment of hemodynamic disorders, the modulation of growth factors, and as an adjunct to chemotherapy and anti-inflammatory drugs. The modulatory effects of heparin do not necessarily depend on its anticoagulant activity and an increasing number of reported effects may contribute to the non-anticoagulant properties of heparin. In fact, heparin is now known to be useful in the treatment of a number of inflammatory diseases where anticoagulant effects are not always necessary.

In conclusion, on the one hand, the evident, although not fully understood, inflammatory modulation functions of heparin in synergy with its anticoagulant activity generate an exceptional set of potential therapeutic uses and understanding the interactions between heparin and the specific mediators involved in the inflammatory response may facilitate the discovery and development of a series of new anti-inflammatory drugs lacking anticoagulant activity.

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