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REVIEW ARTICLE

Covalent antithrombin–heparin complexes

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Abstract Unfractionated heparin (UFH) and low molecular weight heparin (LMWH) have been utilized as primary anticoagulants for thrombosis prophylaxis and treatment. However, a number of biophysical and safety limitations have led to development of new anticoagulants. Covalent antithrombin–heparin (ATH) complexes may address many of these issues. Early ATH products were prepared that had increased intravenous half-lives relative to UFH but lacked any improvement in anti-factor Xa activity or had no catalytic activity or reactivity against thrombin. However, a recent conjugate developed by Chan *et al*. has displayed a number of superior properties. Chan *et al*. ATH has an increased direct thrombin inhibition rate and can catalyze coagulant enzyme inhibition by exogenous antithrombin with very high specific activity. Unlike UFH, clot-bound thrombin is readily inhibited by ATH and, at similar antithrombotic efficacy, the ATH has improved bleeding profiles compared to heparins. Given the preclinical findings, Chan *et al*. ATH may warrant clinical trial testing for control of clot propagation.

Contents

Abbreviations: UFH, unfractionated heparin; LMWH, low molecular weight heparin; ATH, covalent antithrombin–heparin; AT, antithrombin; serpin, serine protease inhibitor; HAH, high affinity heparin; TAT, thrombin–antithrombin; GAG, glycosaminoglycan.

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Coagulation and its control

Coagulation is an important process in blood hemostasis. During vascular injury, the coagulation system acts to generate a fibrin–platelet clot and induce repair processes at the damaged site [1–8]. While both these effects are important to vascular integrity, their regulation is also critical. Uncontrolled coagulation has been associated with a pathological state known as thrombosis [9]. This condition is characterized by the development and sustained presence of a clot in the blood vessel(s) [9]. Consequently, this outcome causes blood hemostasis to be compromised and predisposes the patient to complications such as myocardial infarction, stroke, necrosis or pulmonary embolism [10–14].

In vivo, antithombin (AT) is recognized to be the primary regulator of blood coagulation [15]. A member of the serine protease inhibitor (serpin) family, this 58 kDa plasma glycoprotein is synthesized by the liver and circulates at a plasma concentration of approximately 2.4 μM [16]. The regulatory importance of AT stems from its capacity to irreversibly inactivate numerous proteases involved in coagulation. These targets include factors IIa, Xa, VIIa, IXa, XIa and XIIa [17–21]. By inhibiting these coagulation proteases, AT functions to limit protease concentrations from exceeding physiologically relevant levels for repair [22].

The discovery of heparin by Robert Maclean in 1916 signaled a change in the treatment of thrombosis [23]. This finding revealed a molecule with the capacity to prevent blood clotting. Analysis into the characteristics of unfractionated heparin (UFH) showed that its anticoagulant nature was attributable to the catalytic cofactor role it served both AT and heparin cofactor II [24,25]. In particular, UFH binding acts to accelerate the rates for both inhibitor proteins [24,25]. Although UFH accelerates reactions with either AT or heparin cofactor II, the use of UFH to treat thrombotic complications in clinical practice is based primarily on UFH's catalytic role with AT.

Limitations of heparin

Although heparin is a widely used drug, a number of limitations associated with its pharmacokinetic and biophysical properties have hampered heparin's clinical application. Pharmacokinetically, UFH has been shown to have a short dose-dependent, intravenous half-life [26]. This limitation partially arises from the fact that UFH exhibits non-specific protein binding in circulation. In effect, AT competes with basic plasma and cell-surface-associated proteins for heparin binding [27]. Consequently, since the concentration and distribution of UFH-binding proteins vary amongst individuals [28], non-selective protein binding by UFH also results in an unpredictable anticoagulant effect [28]. Due to the relatively small molecular size, both UFH and low molecular weight heparin (LMWH) can also readily pass through tissue layers, thus contributing to its loss from circulation and inability to sequester in vascular spaces [29]. Regarding biophysical properties, UFH lacks the ability to inhibit the surface-bound coagulation factors such as with fibrin-bound thrombin [30,31] and phospholipid-bound factor Xa [32,33]. This is an important limitation of heparin since clinical evidence indicates that clot-bound thrombin activity is a primary factor in clot propagation [31–33]. In fact, it is assumed that early recurrence of unstable coronary artery syndromes after discontinuation of heparin is due to this activity [34]. Another biophysical limitation is bleeding complications that arise from the use of high heparin concentrations [35]. In the case of LMWH, catalysis of thrombin inhibition by AT is impaired due to short chain length [36] and binding of thrombin to fibrin surfaces also leads to LMWH resistance [37].