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Oversulfated chondroitin sulfate interaction with heparin-binding proteins: New insights into adverse reactions from contaminated heparins

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ABSTRACT

An oversulfated chondroitin sulfate (OSCS) was identified as a contaminant to pharmaceutical heparin and severe anaphylactoid reactions were ascribed to this contaminant. An examination of the biochemistry underlying both the anticoagulant activity and the toxic effects of oversulfated chondroitin sulfate was undertaken. This study demonstrates that the anticoagulant activity of this oversulfated chondroitin sulfate is primarily dependent on heparin cofactor II mediated inhibition of thrombin. Heparin and oversulfated chondroitin sulfate binding to coagulation, kinin–kallikrein and complement proteins were studied by surface plasmon resonance. While oversulfated chondroitin sulfate binds tightly to antithrombin III, unlike heparin, OSCS does not induce antithrombin III to undergo the conformational change required for its inactivation of thrombin and factor Xa. In contrast to heparin, oversulfated chondroitin sulfate tightly binds factor XIIa suggesting a biochemical mechanism for the factor XIIa-based enhancement of vasoactive bradykinin production.

1. Introduction

Heparin is a polydisperse mixture of linear polysaccharides that has been used clinically as an anticoagulant for over 75 years (Fig. 1A) [1–3]. Heparin is unique as one of the oldest drugs currently still in widespread clinical use, predating the US-FDA [4,5] and is one of the first biopolymeric drugs, and one of the few carbohydrate drugs [3]. Heparin is isolated by extraction from animal tissues rich in mast cells, such as porcine intestines [6]. The chondroitin sulfates (CSs) are a second, widely distributed and closely related glycosaminoglycan (GAG) family consisting of CS-A (Fig. 1C), CS-B, CS-C, CS-D and CS-E [7]. Medicinal and natural products chemists have actively investigated chemically modified GAGs having improved or unique pharmacological properties, including the widely used low molecular weight heparins [2,6]. One such semi-synthetic GAG, prepared by sulfonation of CS-A, is oversulfated CS (OSCS) [8] (Fig. 1B). OSCS displays structural similarity to heparin and enhanced anticoagulant activity when compared with CS [8].

A rapid onset, acute side effect, caused by an anaphylactoid response [9], resulted in a spike in adverse events associated with contaminated lots of heparin. Analysis of these lots revealed the presence of OSCS [10]. Both the isolated contaminant and independently synthesized OSCS activated the kinin–kallikrein pathway in human plasma, leading to bradykinin formation [9]. OSCS also induced the generation of complement (C) proteins C3a and C5a. The activation of the kinin–kallikrein and complement pathways is linked through fluid-phase activation of factor XII (FXII) in the coagulation cascade. The functional activity of OSCS on FXIIa was demonstrated using FXIIa-depleted plasma [9]. The kallikrein–kinin pathway starts with human plasma FXII. When exposed to a negatively charge surface such as damaged endothelial cells, FXII, PK and high molecular weight kininogen (HMWK) assemble into a ternary complex capable of forming bradykinin (Fig. 2A). FXII is activated to FXIIa, which is able to cleave prekallikrein (PK) into kallikrein (KK). This results in the production of the potent vasoactive mediator bradykinin and the complement-derived anaphylatoxins. Thus, the kinin–kallikrein, complement and coagulation pathways suggested an explanation for the anaphylactoid response observed in patients intravenously

Abbreviations: CS, chondroitin sulfate; GAG, glycosaminoglycan; OSCS, oversulfated chondroitin sulfate; AT III, antithrombin III; HC II, heparin cofactor II; PK, prekallikrein; KK, kallikrein; SPR, surface plasmon resonance; APTT, activated partial thromboplastin time; PT, prothrombin time; TT, thrombin time; NHP, normal human plasma; PF4, platelet factor 4; HMWK, high molecular weight kininogen.

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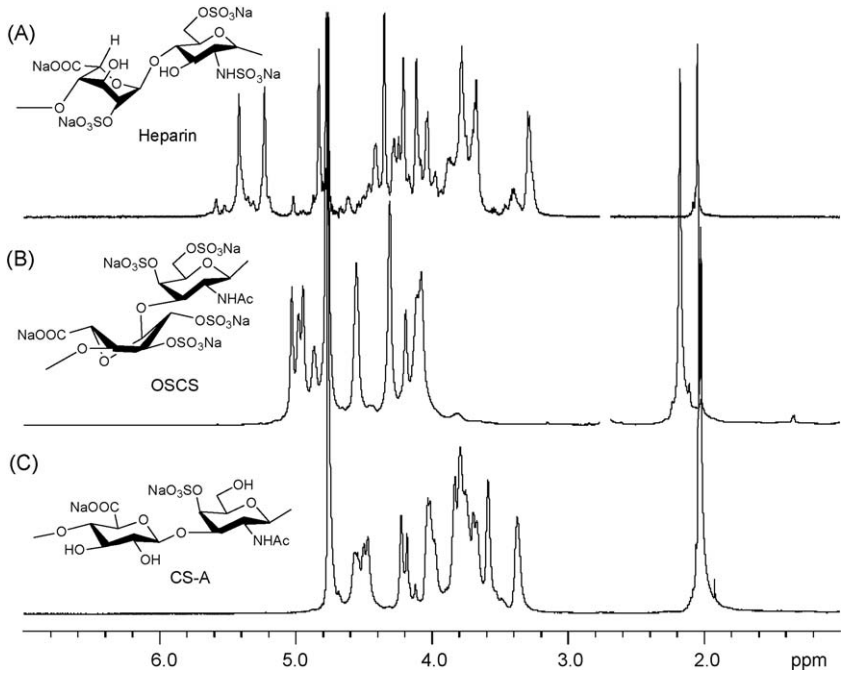

Fig. 1. Structures and conformations of the major repeating units and the 600 MHz ^{1}H NMR spectra of (A) heparin, (B) OSCS and (C) CSA.

administered OSCS contaminated heparin. Pigs and humans are sensitive to the effects of OSCS in a similar manner [9].

Heparin is known to be involved in the regulation of the coagulation cascade through its binding and activation of the serine protease inhibitors (serpins) antithrombin III (ATIII) and heparin cofactor II (HCII) [11]. OSCS can also affect the fibrinolytic system, activating plasminogen, possibly explaining the bleeding effects associated with OSCS contaminated heparin [12]. Serpins, activated by heparin, can rapidly inhibit activated blood coagulation enzymes including thrombin (FIIa), FXa and FXIIa [11] and the

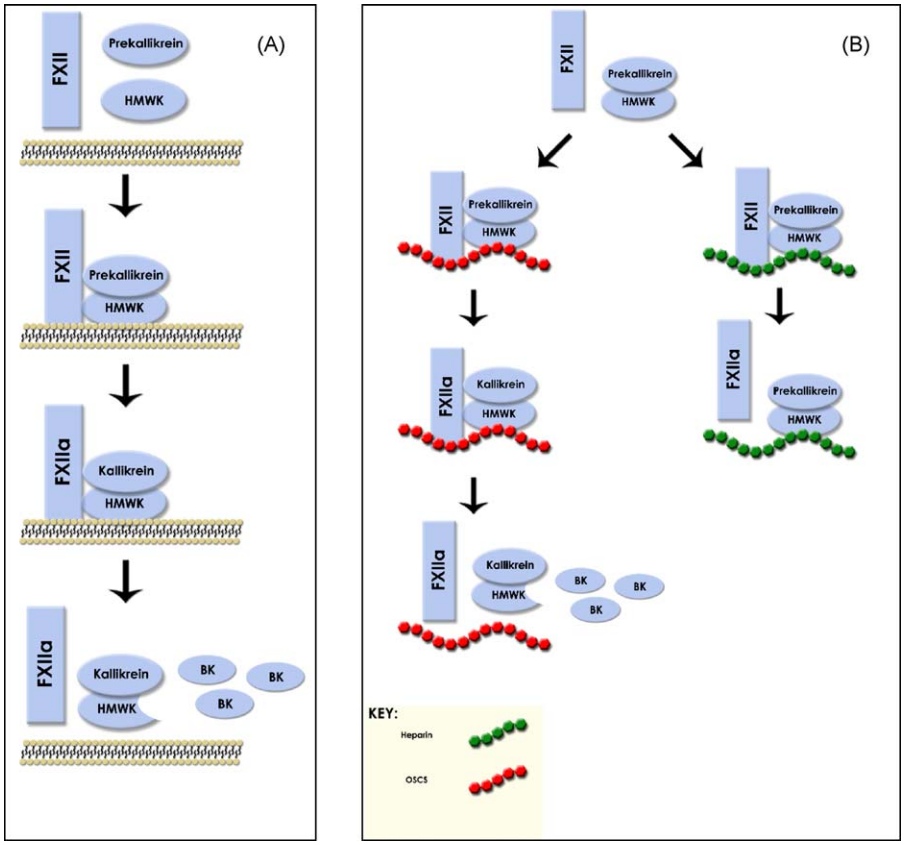

Fig. 2. (A) Physiological role of FXIIa on bradykinin production at the surface of a membrane. (B) Proposed mechanism of OSCS enhancement of bradykinin production on contact activation of FXII, PK and HMWK. Heparin fails to enhance bradykinin production.