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Rational Design of Anticoagulant Drugs Using Oligosaccharide Chemistry

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Abstract: For a long time, heparin and low molecular weight heparins have been the drugs of choice for the management of thrombosis. Discovery of the antithrombin binding domain in heparin, a critical element in the anticoagulant activity of this polysaccharide, allowed a rational approach based on medicinal carbohydrate chemistry in the design of new anticoagulants. The fully synthetic pentasaccharide fondaparinux that selectively targets blood coagulation factor Xa was first to be developed as a drug. Fondaparinux was followed by various heparin mimicking oligosaccharides prepared with a view to replace polydisperse heparin and low molecular weight heparins by structurally-defined anticoagulants with no unwanted side-effects.

Keywords: Anticoagulant · Fondaparinux · Heparin · Idraparinux · Oligosaccharide

1. Introduction

Heparin was discovered in 1916, and has been used as a blood anticoagulant in clinics since 1935.[1] Pharmaceutical heparin is a complex polysaccharide of the glycosaminoglycan family, obtained from the natural heparin proteoglycan of pig intestine. It is largely composed of regular disaccharide sequences of α-1,4-linked 2-*O*-sulfate-L-iduronic acid and 6-*O*-sulfate-*N*-sulfate-D-glucosamine (Fig. 1 left). However, heparin also contains residues of α-L-iduronic acid, β-D-glucuronic acid, and 2-*N*-acetyl-α-D-glucosamine, these latter being occasionally 6-*O*-sulphated. The full structure of heparin chains remains unknown. In clinics, heparin is mainly used for the prevention and the treatment of deep vein thrombosis and pulmonary embolism, for the management of acute coronary syndrome, and during extracorporeal circulation in cardiac surgery. The blood anticoagulant activity of heparin is linked to its ability to boost the activity of the natural anticoagulant plasma protein antithrombin (AT, formerly AT III). Heparin-bound AT undergoes a conformational change allowing its recognition by several blood coagulation factors (enzymes) that are trapped through the formation of a covalent complex with AT (a suicide inhibitor). Two of these factors, thrombin (factor IIa) and factor Xa, have played a crucial role in the development of an improved version of heparin: low molecular weight heparin (LMWH).

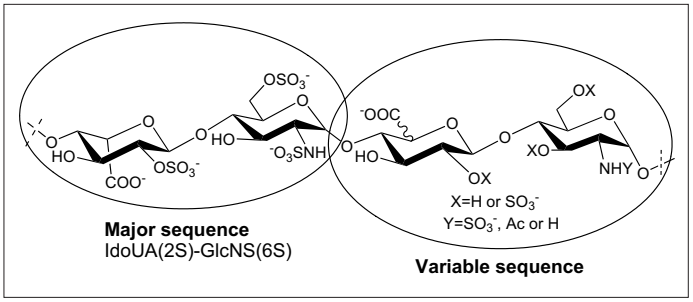

Fig. 1. General structure of heparin.

2. Low Molecular Weight Heparin

Following the hypothesis that factor Xa was the key target for heparin,[2] and the observation that short heparin chains retained their ability to inhibit factor Xa,[3] in the early 1980s LMWHs were developed as new drugs. LMWHs are heterogeneous polysaccharidic mixtures with an average molecular weight of 5 kDa prepared by controlled, chemical or enzymatic, depolymerization of heparin. Inhibition of factor IIa by heparin requires a long chain that interacts simultaneously with antithrombin and with factor IIa itself in a ternary complex. In contrast, inhibition of factor Xa only requires a short chain able to activate antithrombin. LMWHs target factor Xa rather than thrombin, have an improved pharmacokinetics and globally an improved therapeutic index compared to standard heparin. They currently represent a several-billion dollar market.[4]

3. First Synthetic Pentasaccharides

It was only in the early 1980s that the specific AT-binding domain (ABD) in heparin could be identified.[5] The structure of the corresponding pentasaccharide was determined following the controlled enzymatic (heparinase) and chemical (nitrous acid) depolymerization of heparin and the size fractionation of mixtures of oligosaccharides displaying AT-binding (Fig. 2). Remarkably, this sequence contains atypical monosaccharide units like *N*-acetyl-α-D-glucosamine, β-D-glucuronic acid, non-sulphated α-L-iduronic acid, and on top, a remarkable 3-*O*-sulfated-α-D-glucosamine unit, that is now considered as the hallmark of the AT-binding site.[6]

The first total syntheses[7–9] of the AT-binding sequence were undertaken to confirm the structural work.[10] They also set the stage for several years of work in the field of synthetic anticoagulants.[11,12]

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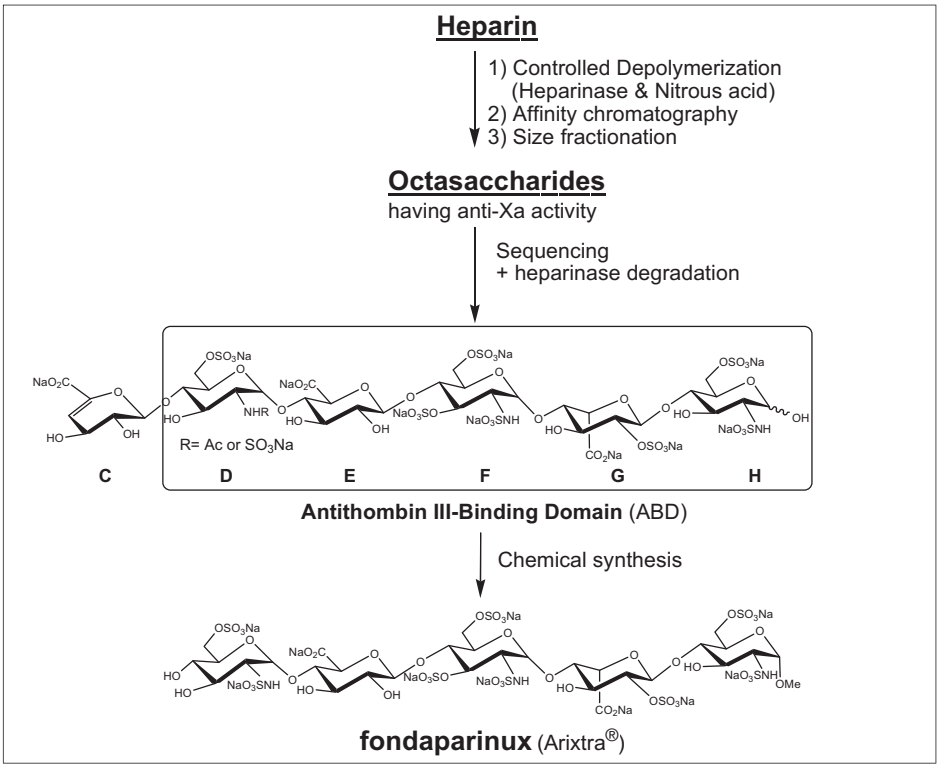
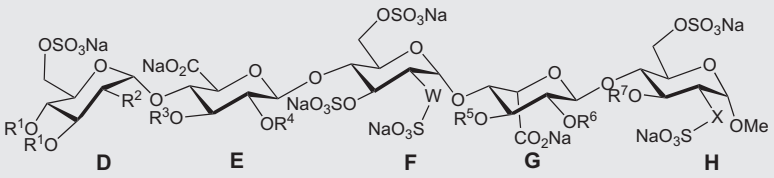
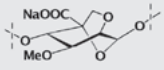
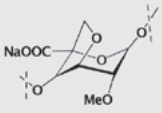
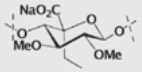
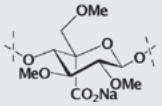


Fig. 2. Discovery of the antithrombin binding domain in heparin and synthetic pentasaccharide fondaparinux.

Table 1. Fondaparinux and key analogues.



Cpd#	D	E	F	G	H	Anti-fXa activity [U/mg]
1[15]	R^1 = H R^2 = $NHSO_3Na$	R^3 = R^4 = H	W = NH	R^5 = H R^6 = SO_3Na	R^7 = H X = NH	700
2[18]	R^1 = H R^2 = $NHSO_3Na$	R^3 = R^4 = H	W = NH	R^5 = H R^6 = SO_3Na	R^7 = SO_3Na X = NH	1,250
3[19]	R^1 = H R^2 = $NHSO_3Na$	R^3 = R^4 = H	W = NH	R^5 = H R^6 = SO_3Na	R^7 = SO_3Na X = O	1,250
4[20]	R^1 = H R^2 = $NHSO_3Na$	R^3 = R^4 = H	W = NH	R^5 = Me R^6 = SO_3Na	R^7 = SO_3Na X = O	1,250
5[21]	R^1 = Me R^2 = OSO_3Na	R^3 = R^4 = Me	W = O	R^5 = Me R^6 = SO_3Na	R^7 = SO_3Na X = O	1,323
6[22]	R^1 = R^2 = Me	R^3 = R^4 = Me	W = O	R^5 = R^6 = Me	R^7 = SO_3Na X = O	1,611
7[20-22]	R^1 = R^2 = Me	R^3 = R^4 = Me	W = O		R^7 = SO_3Na X = O	1,073± 61
8[20-22]	R^1 = R^2 = Me	R^3 = R^4 = Me	W = O		R^7 = SO_3Na X = O	43 ± 3
9[20-22]	R^1 = R^2 = Me		W = O		R^7 = SO_3Na X = O	115 ± 3

From a chemistry standpoint this first synthesis was a challenge as it required the development of new methods in the chemistry of uronic acids (glucuronic and iduronic), sulfation (esterification and amidification), and the creation of five interglycosidic bonds. Concerning this latter point, the timely work of Paulsen and his co-workers[13] on the synthesis of α-D-glucosaminides provided a good part of the solution. Later, the development of the imidate glycosylation method was similarly critical for the industrial scale-up of the synthesis.[14] The *O*-methylated analogue, fondaparinux (code numbers SR 90107, Org 31540) was prepared with better yields[15] and is a better drug.[16] Like its non-methylated analogue this compound binds antithrombin specifically and catalyses inhibition of factor Xa. Fondaparinux (**1**, Table 1) has been approved for clinical use in 2002 under the brand name of Arixtra®. Its sales should approach $ 0.5 billion in 2010 and generics should be launched soon.[17]

4. Pentasaccharide Analogues

Following a rational approach, numerous analogs were prepared to better understand the structure–activity relationship (SAR) of the interaction with AT and to develop new, more potent, fondaparinux-like compounds (Table 1). The first interesting finding was the addition of a 3-*O*-sulfo group to residue H (Table 1, **2**). This additional group reinforces the interaction with antithrombin, a property which has been largely exploited in the design of new antithrombotic pentasaccharides. Replacements of *N*-sulfo by *O*-sulfo (**3**), and of OH by OMe (**5**, **6**), fully compatible with the biological activity, have also been particularly exploited because of the resulting dramatic simplification of the chemistry. All the above findings were exploited in the design of idraparinux (**6**, Table 1).[18,19,22,23] This pentasaccharide was found to display a long half life and was developed for the prevention of stroke in patient suffering from atrial fibrillation, a condition that requires life-long treatment where idraprinux was administered once a week. The compound reached phase III clinical trial at which stage it was replaced by its neutralizable version (see below).

Importantly, it was demonstrated with the conformationnally constrained compounds (**7**–**9**, Table 1), that L-iduronic acid adopts the unique $^2S_0$ conformation when heparin binds to antithrombin.[24,25] The nature of the interaction of heparin and antithrombin could also be investigated through crystallography studies only possible with the use of synthetic homogeneous compounds.[26]

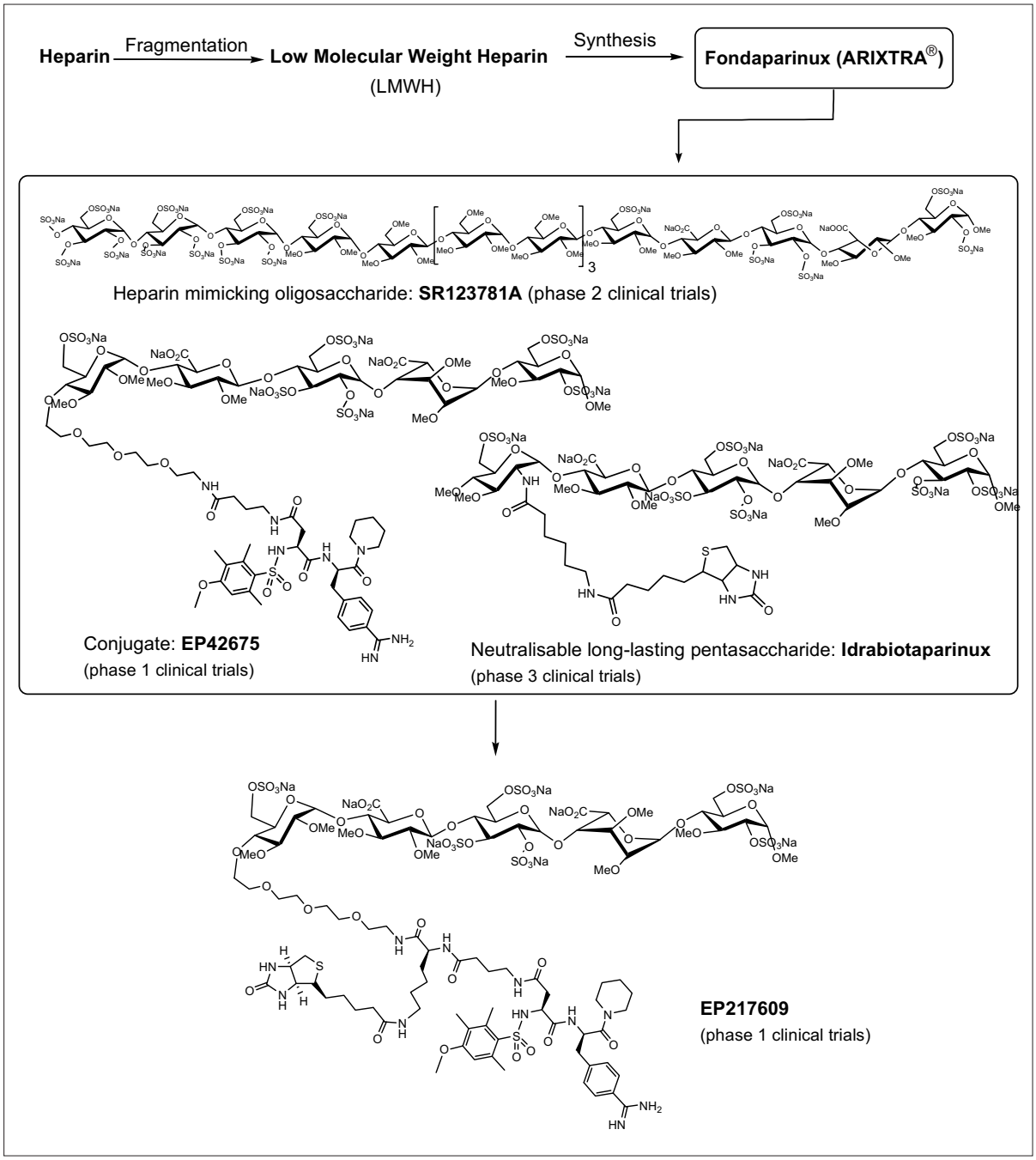


Fig. 3. From heparin to rationally designed synthetic heparin mimetics.

5. Longer Oligosaccharides

AT-mediated inhibition of factor Xa only represents part of the anticoagulant activity of heparin that can also inhibit thrombin as well as several other coagulation factors. As already mentioned, inhibition of thrombin (factor IIa) not only requires activation of antithrombin by the pentasaccharide sequence, but it also requires that heparin and antithrombin bind to the same heparin molecule in a ternary complex.[27] Two approaches were developed to obtain new synthetic chemical entities able to inhibit factor Xa and thrombin, and so to more closely mimic heparin. In one of them a non-carbohydrate linker connects an antithrombin binding domain (ABD) and a thrombin binding domain (TBD).[28] In the second one both domains are present on a single oligosaccharide molecule.[29,30] Thus, SR123781A,[31] a synthetic hexadecasaccharide comprising an ABD and a TBD was obtained from glucose through a convergent synthesis. This heparin mimetic reached phase II-III clinical trials in the prevention of venous thromboembolism (VTE) and in acute coronary syndrome.[32]

6. Conjugates of Pentasaccharides and Non-carbohydrate Entities

To obtain drugs displaying different anticoagulant profiles it was tempting to create a new family of anticoagulants where indirect factor Xa inhibition is combined with direct thrombin inhibition. This was possible by coupling an antithrombin binding pentasaccharide to a NAPAP analogue. Thus, (N-(4-methoxy-2,3,6-trimethylphenylsulfonyl)-glycyl-(D)-4-amidinophenyl-alanyl-piperidine) was conjugated to a pentasaccharide to give EP42675[33] (Fig. 3). In animal models of thrombosis, this compound demonstrated very interesting antithrombotic properties.[34]

7. Neutralizable Anticoagulants

Because of the long half life (120 h in man), the bleeding risk, and the therapeutic scheme associated with the use of idraparinux (once a week administration

for eventually life-long treatments), it was highly desirable to make this drug neutralizable to rub out the anticoagulant effect in case of an emergency. To this end a biotin moiety was covalently linked to the structure of the compound to give idrabiotaparinux (Fig. 3). Biotin is well known to form a very strong complex with avidin (K_D in the order of 10^{-15} M) which allowed a fast clearance of idrabiotaparinux after injection of avidin, an egg-derived protein endowed with a very short half-life.[35]

Similarly, a biotin moiety was introduced in the structure of EP42675 to allow its immediate neutralization. The corresponding compound, EP217609,[36] currently in phase I clinical trials, will be developed for extracorporeal circulation during cardiac surgery, a very demanding situation requiring a potent and reversible anticoagulant. Despite several attempts, heparin and its antidote protamine have not been surpassed in this setting since the 1950s.

8. Conclusion

Anticoagulants are clinically used in a wide range of conditions, from very short treatments in acute situations to life-long treatments in patient having a chronic risk of thrombotic complications. During decades injectable heparin and low molecular weight heparin, with the help of oral anti-vitamin K (AVK) for long term treatment, have been used to cover the whole spectrum of clinical indications. These drugs are either of animal origin, thus at risk of contamination,[37,38] or difficult to manage, like AVK, and there is much room to improve anticoagulant treatments.

Pentasaccharide fondaparinux was designed first, for short daily treatment in the prevention of thrombosis (*e.g.* after orthopedic surgery). This paved the way for the development of other more sophisticated drugs like idraparinux/idrabiotaparinux, injected once a week, and therefore usable for the long term prevention of stroke in atrial fibrillation patients. At the opposite end of the spectrum, potent neutralizable anticoagulants were synthesized, for acute situations requiring a short treatment followed by an immediate neutralization.

The clinical trials carried out with fondaparinux definitively validated factor Xa as a target for antithrombotic therapy. This finding triggered a huge research effort in the medicinal chemistry community to identify orally available factor Xa inhibitors. As a result, today small molecule drugs issued from classical heterocyclic medicinal chemistry and targeting factor Xa are ready to enter the market.[39]

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