Review

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Pharmacological effects and clinical applications of ultra low molecular weight heparins

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Summary **Heparin, one of the common anticoagulants, is clinically used to prevent and treat venous thromboembolism (VTE). Though it has been the drug of choice for many advanced medical and surgical procedures with a long history, the adverse events, such as bleeding, heparin-induced thrombocytopenia (HIT), allergic reactions, follow. Therefore, low molecular weight heparins (LMWHs) and ultra low molecular weight heparins (ULMWHs), with lower molecular weights, higher anti-FXa activity, longer half-life times and lower incidence of adverse events than unfractionated heparin (UFH), were researched and developed. Fondaparinux, a chemically synthesized ULMWH of pentasaccharide, has the same antithrombin III (AT-III)-binding sequence as found in UFH and LMWH. In addition, AVE5026 and RO-14, another two ULMWHs, are obtained by selective chemical depolymerization. In this paper, we review the preparation process, pharmacological effects and clinical applications of fondaparinux, AVE5026 and RO-14.**

Keywords: Pharmacological effects, clinical applications, ultra low molecular weight heparin, fondaparinux, AVE5026, RO-14

1. Introduction

Heparin was discovered in 1916. Because it plays an essential role in many medical and surgical procedures, heparin has been widely used clinically since 1934 (*1*). Currently, heparin is the drug of choice to prevent and treat venous thromboembolism (VTE). Pharmaceutical heparin is usually obtained from porcine intestines or bovine lungs (*2-5*). The chemical parameters, such as purity, molecular mass distribution and degree of sulfation, must be strictly controlled to ensure appropriate biological activities. There are three forms of heparin drugs: UFH (average molecular weight (MWavg) ~15,000), LMWHs (MWavg 3,500 to 6,000) and ULMWHs (MWavg < 3000).

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Heparin, a linear sulfated polysaccharide, consists of repeating disaccharide subunits of α-1,4 linked uronic acid and D-glucosamine (panel A of Figure 1) (*6*). The uronic acid residue of heparin may be either α-L-iduronic acid (IdoA) or β-D-glucuronic acid (GlcA) and can be unsubstituted or sulfonated at the 2-*O* position. The glucosamine residue may be unmodified (GlcN), *N*-sulfonated (GlcNS) or *N*-acetylated (GlcNA), and can contain variable patterns of *O*-sulfonation at the 3-*O* and/or 6-*O* positions. The major disaccharide sequence of heparin is the trisulfonated L-IdoA(2S)-D-GlcNS(6S). It has been demonstrated that the locations of the sulfo groups, IdoA, and GlcA lead to the anticoagulant activity of heparin.

Due to the ability to bind to antithrombin III (AT-III), heparin has anticoagulant activity. AT-III has a conformational change when heparin interacts with it, exposing the reactive center. Then, this reactive center within AT-III can interact with coagulant enzymes, such as thrombin and factor Xa (FXa). A unique pentasaccharide domain, the residue A to E in panel A of Figure 1, is critical in inducing the conformational change of AT-III. Therefore, both the pentasaccharide domain and the thrombin-binding domain (Figure 2) are acquired

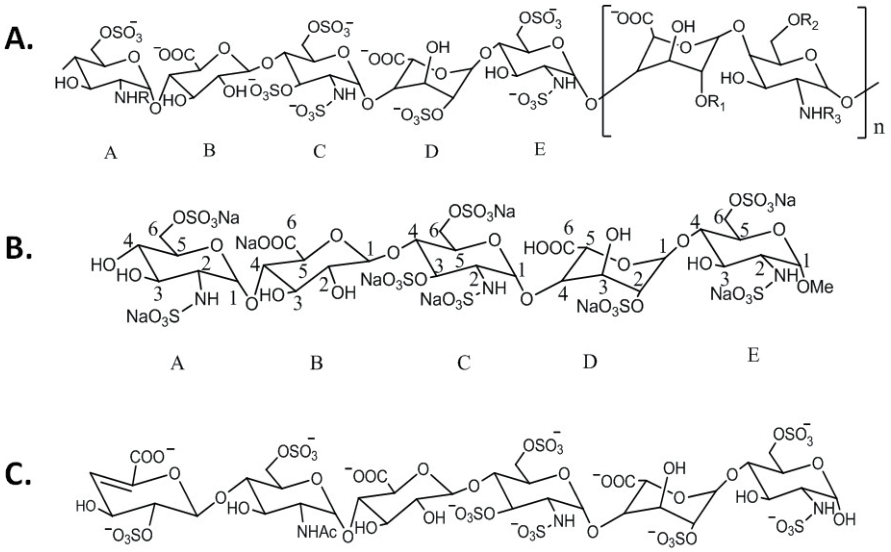

Figure 1. The structure of UFH, fondaparinux and ΔIIa-IIs-Is. A: The unique AT-III-binding pentasaccharide sequence and repeating disaccharide units of heparin. (R_1 = H, SO_3^-; R_2 = H, SO_3^-; R_3 = H, acetyl, SO_3^-). **B:** The structure of fondaparinux. C: The structure of the ΔIIa-IIs-Is.

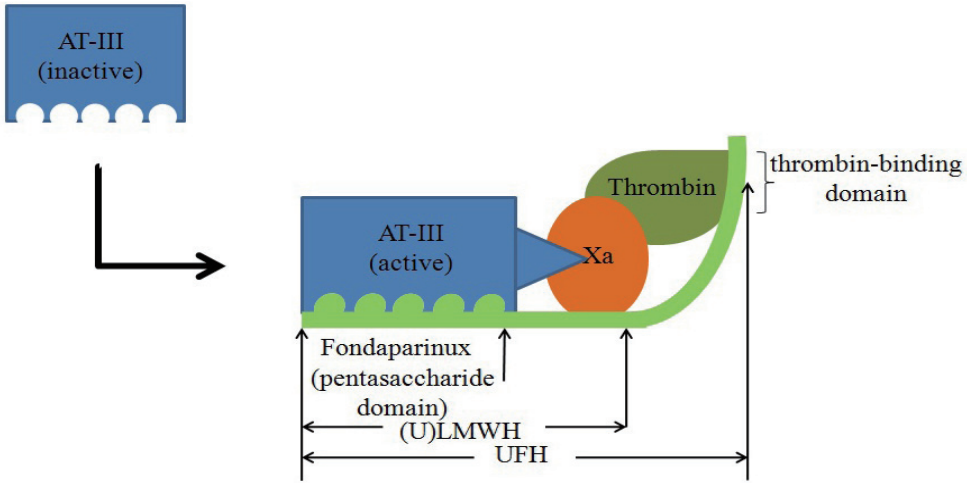

Figure 2. Schematic illustrating the heparin inactivation of FXa and thrombin by antithrombin.

for heparin's antithrombin activity, which means the minimum size of fragment with antithrombin activity is 18 monosaccharides in heparin-based drugs. The schematic of the inactivation of FXa and thrombin by heparin and AT-III is shown in Figure 2.

Though UFH works well as an anticoagulant drug, an adverse bleeding event occurs sometimes when UFH is used for antithrombotic therapy, because all heparin polysaccharides obtained from different preparation processes include saccharide-fragments with high antithrombin activity (Table 1) (*7*). In addition, UFH has other adverse events, such as heparin-induced thrombocytopenia (HIT), allergic reactions, and so on. It is reported that UFH results in a 1%-6% incidence of HIT, a life-threatening complication (*8*). LMWHs, depolymerized products of heparin polysaccharide, have a longer half-life than UFH and can be subcutaneously administered. Because of these advantages, LMWHs have emerged as the most widely prescribed heparins in the US (*9*). However, although the frequency of adverse bleeding events has declined, the antithrombin activity of LMWHs cannot be ignored. For example, the anti-FIIa activity of enoxaparin is 27 IU/mg (Table 1) and this can lead to bleeding in a few patients. Among the three forms of heparin, ULMWHs have the highest anti-FXa activity and the lowest anti-FIIa activity compared to heparin and LMWHs, resulting in the lowest anticoagulant activity and incidence of bleeding (Table 1). In Table 1, we make a comparison of AVE5026, RO-14, fondaparinux, enoxaparin, and UFH.

Fondaparinux, AVE5026 and RO-14 are the three most common ULMWHs. Fondaparinux, a chemically synthesized pentasaccharide, has the same AT-III-binding sequence as the natural heparin polysaccharide. One pentasaccharide domain contained in both UFH (panel A of Figure 1) and fondaparinux (panel B of

Table 1. Comparison of AVE5026, RO-14, fondaparinux, enoxaparin, and UFH

	MeanMW (Da)	Anti-FXa (U mg^{-1})	Anti-FIIa (U mg^{-1})	$t_{1/2}$ (h)	Route	Clearance (primary)	Antidote
UFH	15,000	193	193	0.5-2.5	IV/SC	Cellular metabolism and renal clearance	Protamine
Enoxaparin	4,500	105	27	4.0-4.7	SC	Renal clearance	Protamine may have partial neutralizing effects
AVE5026	2,400	150-200	< 5	16-20	SC	Renal clearance	Protamine may have partial neutralizing effects
RO-14	2,200	80-140	≤ 10	8.1	SC	Renal clearance	Protamine may have partial neutralizing effects
Fondaparinux	1,728	850 ± 27	< 0.1	17	SC	Renal clearance	Recombinant factor VIIa may be effective

Figure 1) is shown from residue A to residue E in Figure 1, and this pentasaccharide domain is critical in inducing the conformational change of AT-III which interacts with it. Therefore, it has good anti-FXa activity. However, because this pentasaccharide is too short to bridge AT-III to thrombin, fondaparinux scarcely has anti-FIIa activity. Fondaparinux has been indicated for primary prevention of VTE in patients undergoing orthopedic or abdominal surgery and for the treatment of VTE (*10*). AVE5026 is a compound from a chemoselective depolymerization of the heparin macromolecule. It was studied for use in prophylaxis of VTE in patients with cancer. Meanwhile, RO-14 exhibited dose-proportional pharmacokinetics and a favorable safety profile. Data from clinical studies of RO-14 have not been published. In addition, bemiparin is approved for use in the prophylaxis of VTE in medical patients and patients undergoing general or orthopedic surgery and for secondary prophylaxis in patients with deep vein thrombosis (DVT) in the US since 1998. Only few articles classify bemiparin as an ULMWH and many others consider it to be the second generation LMWHs. The development of deligoparin was terminated because of a study evaluating its use as an anti-inflammatory treatment in patients with ulcerative colitis failed to meet its end points (*11*). Therefore, we reviewed the preparation process, pharmacological effects, and clinical applications of fondaparinux, AVE5026, and RO-14.

2. Fondaparinux sodium

2.1. *Structure and structure-activity relationships*

Fondaparinux sodium (Arixtra), a chemically-synthesized pentasaccharide, is a specific inhibitor of FXa (*12*) and it went on the market in the USA and Europe in 2002. It is synthesized through a block synthesis in about 55 steps from naturally occurring carbohydrates. When the pentasaccharide skeleton was obtained, *O*-sulfation-hydrogenation-*N*-sulfation became the critical process for synthesis of fondaparinux sodium. Recently, Manikowski and his colleagues reported an alternative way for fondaparinux sodium synthesis based on an efficient and facile one-step *O*- and *N*-sulfation of the appropriate pentasaccharide (*13*). The advantage of this updated approach is minimizing byproduct formation, simplifying the fondaparinux sodium synthesis in comparison to the contemporary methods.

Except for the residue at the reducing end (residue E in panel B of Figure 1), fondaparinux sodium has the same structure as the pentasaccharide sequence contained in all anticoagulant heparin sulfate (HS) isolated from natural sources. The hydroxyl group on position 1 of the reducing end residue in fondaparinux is methylated. The structure and the structure-activity relationships are recounted in detail in Petitou's review (*14*). Briefly, the sulfate groups or carboxylate groups on position 6 of residue A in panel B of Figure 1 (C_A6), C_B5, C_C2, C_C3, C_D5, and C_E2 are essential for the activation of AT-III, whereas the sulfate groups on C_A2, C_D2, and C_E6 only help to increase the biological activity. Meanwhile, though significant anti-FXa activity remains if some carbohydrate units (*e.g.* residues A and D) are replaced with more flexible mimetics, like "open" pyranose analogues and other "open" saccharide analogues, but for others the rigidity is essential. For example, if GlcA (residue E in panel B of Figure 1) in the pentasaccharide is replaced with a flexible "open" pyranose analogue, only 2% of the anti-FXa activity remains. In addition, the extra 3-*O*-sulfate in this GlcA can interact with Arg 46 and Arg 47 in the AT-III, which enhances the interaction between the saccharide and AT-III.

2.2. *Pharmacological effects and clinical applications*

2.2.1. *Prophylaxis and treatment of acute coronary syndromes*

Fondaparinux is currently one of the drugs of choice to prevent and treat VTE and acute coronary syndromes (ACS) (*15*,*16*). Its efficiency in the treatment of patients with non-ST-segment elevation ACS (NSTE-ACS) was also proven (*12*). Compared with LMWH, fondaparinux resulted in a 17% decrease in mortality at 30 days and was associated with a 50% reduction in major bleeding

for the treatment of NSTE-ACS (*17*). In addition, in the Fifth Organization to Assess Strategies in Acute Ischemic Syndromes (OASIS-5) study, it was also demonstrated that fondaparinux was similarly efficient compared to enoxaparin in preventing ischaemic events but reduced major bleeding, mortality and morbidity in NSTE-ACS patients regardless of their risk of persistent ischemia (*17*,*18*). Based on OASIS-5 population data, it was shown that fondaparinux was a more cost-effective antithrombotic agent than enoxaparin during both short and long term NSTE-ACS treatment (*19*). Therefore, use of fondaparinux for treatment of patients with NSTE-ACS is superior to that of enoxaparin in terms of prevention of further cardiovascular events and at a lower cost (*20*). Michel and his colleagues also found that using fondaparinux instead of enoxaparin in patients with NSTE-ACS could yield substantial savings at the local as well as the national level in Switzerland (*21*). The use of fondaparinux in the post-coronary artery bypass grafting (CABG) population appears to be safe and is not associated with an increase in bleeding, transfusion or re-operation for bleeding (*22*). However, the risk of catheter thrombosis was higher with fondaparinux than with LMWH or heparin in ACS patients who underwent percutaneous coronary intervention (PCI) (*23*), and because of this problem, fondaparinux was of no benefit in patients undergoing urgent PCI (*24*).

2.2.2. *Prophylaxis and treatment of DVT*

The soluble fibrin (SF) and D-dimer tests might be affected after administration of fondaparinux, complicating the diagnosis of DVT. The D-dimer test on postoperative day 7 is useful for DVT screening in patients treated with fondaparinux. The SF on postoperative day 4, 7, and 14 and D-dimer levels on postoperative day 14 and 21 in patients treated with fondaparinux without DVT were lower than the ones in patients without fondaparinux treatment, while the D-dimer levels on postoperative day 14 and 21 in patients treated with fondaparinux with DVT were higher than that in patients without fondaparinux treatment (*25*). In the fondaparinux treated group, the frequency of DVT and the hemoglobin level were significantly lower than those in the group without fondaparinux treatment, indicating that fondaparinux is useful for the prophylaxis of DVT, but may increase bleeding (*26*).

2.2.3. *Applications in patients with a history of HIT*

Because fondaparinux did not cross-react with HIT-associated antibodies, it had a decreased risk of causing HIT syndrome as compared to enoxaparin (*27*). Savi's group designed a prospective and blinded study in which 39 sera from patients with clinically and serologically confirmed HIT and 15 control sera were collected followed by a particular HIT assay by 3 different specialized laboratories (*28*). The serotonin release assay, heparin-induced platelet agglutination assay, and platelet aggregation assay were performed in these labs independently. The results showed that fondaparinux was nonreactive to HIT sera, suggesting the possibility that this pentasaccharide could be applied to prophylaxis and treatment of thrombosis in patients with a history of HIT.

2.2.4. *Applications in orthopedic surgery patients*

In vitro studies had shown that fondaparinux did not have a negative effect on human osteoblast proliferation by comparing the effects of LMWHs (enoxaparin and dalteparin) and fondaparinux on bone metabolism (*29*,*30*). Papathanasopoulos *et al.* reported that fondaparinux had no adverse effects on either mesenchymal stem cell (MSC) proliferation or osteogenic as well as chondrogenic cell differentiation *in vitro* (*31*). Based on these characteristics, it would be more safe and efficient when fondaparinux was applied in orthopedic surgery patients.

2.2.5. *Applications in heparin allergy patients*

The anti-inflammatory effect of fondaparinux has been demonstrated in intestinal ischemia and reperfusion injury models (*32*). It seems that fondaparinux is characterized by an anti-inflammatory effect manifested by reduction of plasma monocyte chemotactic protein-1 (MCP-1) (*33*), one receptor of fondaparinux (*7*). Palmo-plantal pruritus after application of heparins was an early sign of the immediate type hypersensitivity reaction (*34*). Fondaparinux was found to be a safe alternative for immediate heparin allergy (*34*).

2.2.6. *Applications in renal dysfunction patients*

Fondaparinux is primarily metabolized through the kidney, and thus, is contraindicated in renal-impaired patients (creatinine clearance < 30 mL/min) (*27*). According to post hoc analysis (*17*), the rate of major bleeding during fondaparinux administration was the highest in patients with moderate renal impairment, aged 75 years or more, and body weight less than 50 kg (*35*). Yukizawa's group (*36*) enrolled 85 patients who received subcutaneous fondaparinux 2.5 mg after total hip arthroplasty (THA). Then, the anti-FXa activity was measured on postoperative days 1, 3, 7, and 14. The data obtained in their study indicated that anti-FXa activity levels were significantly higher in patients with renal dysfunction and a poor correlation was observed between the plasma levels of anti-FXa activity and age or body weight. Besides, the patients with normal renal function also showed an increase in anti-FXa activity with repeated administration of fondaparinux.

2.2.7. *Applications in pregnancy*

Different from LMWHs, fondaparinux sodium could cross the placenta though the mechanism of crossing is not clear (*37*). Data on the use of fondaparinux in pregnancy are limited to animal models and a few case reports. In a study of Knoll's group, it was found that fondaparinux did not cause hypersensitive skin reactions and was not associated with bleeding or other complications in the mother and child (*38*). The other several separately reported cases also did not show adverse events to the mother or child (*39-43*). The limited data show that fondaparinux appears efficacious in pregnancy, but bleeding risk is not absent, so care is required when used as a second-line therapy (*44*).

2.3. *The advantages and disadvantages of fondaparinux*

One of the main advantages of fondaparinux is that its dosage does not need to be adjusted based on age or weight, because of its pharmacokinetics, namely, its specific binding to anti-thrombin and near 100% bioavailability (*27*). Fondaparinux with subcutaneous administration has a critically high bioactivity and is mainly excreted by the kidneys with a half-life of 17-21 h (*45*). The recommended dose of fondaparinux sodium in the US and European Union (EU) is 2.5 mg once daily (QD) as a subcutaneous injection, administered postoperatively. Although fondaparinux has been safely administered in many patients, it still has some disadvantages. First, unlike UFH, fondaparinux has no antidote. This is a limitation for patients who are a risk for bleeding. Second, fondaparinux has a long half-life and accumulates in patients with renal dysfunction and even in normal renal function patients (*36*), and its subcutaneous absorption may be unpredictable in a hemodynamically unstable patient. Last, some immune mediators also affect the absorption and metabolism or the activity of fondaparinux, so the dose of fondaparinux should be adjusted when these immune mediators are pre-administered. For example, interleukin-10 (IL-10) decreases the elimination rate of fondaparinux, suggesting that pre-treatment with IL-10 may allow reducing the fondaparinux dose (*46*).

2.4. *Adverse events*

The higher prophylactic efficacy of fondaparinux is associated with a higher risk of bleeding complications (*47,48*), although fondaparinux is supposed to only minimally enhance bleeding and not affect platelet functions (*49*). Bleeding was more prominent in the fondaparinux group compared to the enoxaparin group at an equipotent dose of anti-FXa activity (*50*).

In 2013, Orostegui *et al.* reported a case of liver toxicity likely due to fondaparinux administered to a child (*51*). The mechanism of fondaparinux hepatotoxicity may not be related to drug metabolites since fondaparinux does not undergo hepatic metabolism and is recovered in the urine as the unchanged compound, and it also does not interact with other drugs administered concomitantly, under physiological conditions (*52*). It is hypothesized that inflammatory mediators may have contributed to sensitizing hepatocytes to injurious effects of an unknown compound.

3. AVE5026 (Semuloparin)

AVE5026, a novel and hemi-synthetic ULMWH, is in clinical development for the prevention of VTE (*56*). It possesses a higher anti-FXa activity with a residual anti-FIIa activity (< 5 IU/mg), and has a MWavg of 2.4 kDa (Table 1) and a unique AT-III-binding oligosaccharide ΔIIa-IIs-Is (panel C of Figure 1). It has the AT-III-binding sequence and the 4,5-unsaturated uronic acid-2-*O*-sulfated ester residue. The anti-FXa activity of this characteristic hexasaccharide was found to be 740 IU/mg and it is the major constituent of the AT-III-binding hexasaccharide fraction. AVE5026 is the saccharide fragments of a chemoselective depolymerization of the heparin macromolecules by 2-tert-butylimino-2-diethylamino-1,3-dimethyl-perhydro-1,2,3-diaza-phosphorine (BEMP). The reaction principle is a *β*-eliminative reaction. The hemi-synthetic pathway has six steps (Figure 3) (*53*).

3.1 *The dose-range study of AVE5026*

A dose-range study of AVE5026 for the prevention of VTE in patients after total knee replacement surgery was completed in 2009 (*54*). In this parallel-group, double-blind and double-dummy study, 690 patients were enrolled randomly, and 678 of them were treated with once-daily doses of AVE5026 (5, 10, 20, 40, or 60 mg) or enoxaparin 40 mg. The primary efficacy end point was VTE until post-operative day 11, defined as DVT detected by bilateral venography, symptomatic DVT, non-fatal pulmonary embolism (PE) and VTE-related death. The primary safety outcome was the incidence of major bleeding. The primary efficacy outcome was assessed in 464 patients. There was a significant dose-response across the AVE5026 groups for VTE prevention ($p < 0.0001$) and for proximal DVT ($p = 0.0002$). The incidence of VTE ranged from 5.3% to 44.1% compared to 35.8% in the enoxaparin group. Also, a significant dose-response for AVE5026 was seen for major bleeding ($p = 0.0231$) and any bleeding ($p = 0.0003$). Six patients experienced major bleeding in all the groups treated with the AVE5026 and four of them belonged to the 60 mg group. Meanwhile, none experienced major bleeding in the enoxaparin group. In addition, the risk of VTE was reduced by 58% [95% confidence interval (CI), 26-76], 61% (95%

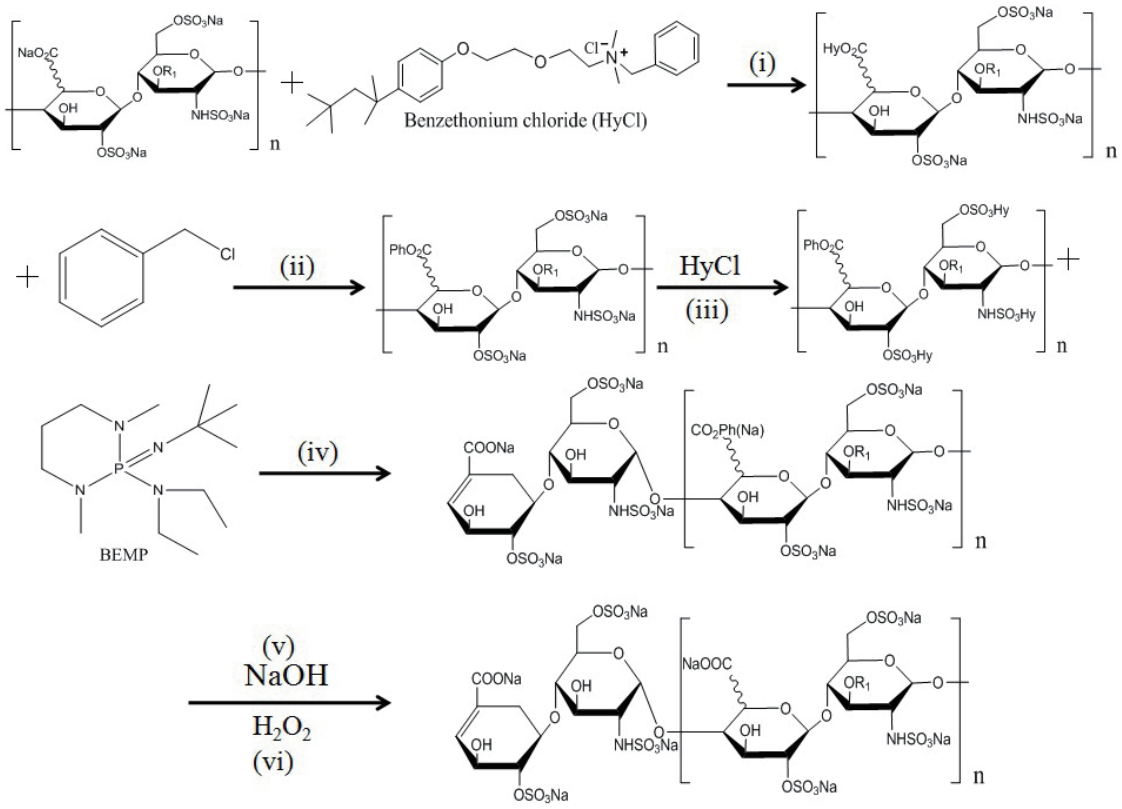

Figure 3.The hemi-synthetic pathway of AVE5026. The hemi-synthetic pathway has 6 steps: (*i*) transalification of heparin sodium with benzethonium salts; (*ii*) esterification of heparin benzethonium salts by benzyl chloride; (*iii*) transalification of the heparin benzyl ester by benzethonium salts; (*iv*) depolymerization of the heparin benzyl ester, benzethonium salts by BEMP; (*v*) saponification of benzyl esters; (*vi*) purification to obtain AVE5026.

CI, 30-79) and 85% (95% CI, 60-94) in the AVE5026 20 mg, 40 mg, and 60 mg groups, respectively. It was demonstrated that AVE5026 at high doses (≥ 20mg) was significantly more effective at reducing confirmed VTE compared to enoxaparin. The safety and efficacy results of this study suggested that a dose range of AVE5026 between 20 mg and 40 mg presented an adequate benefit-to-risk ratio.

3.2 *The phase III trials of AVE5026*

There have been 6 phase III trials during the development of AVE5026 for VTE prophylaxis. In addition, another trial, the seventh one, was initiated but terminated early in acutely ill medical patients. According to the sponsor's reports about these clinical studies, AVE5026 was successful against placebo (evaluation of AVE5026 in the prevention of VTE in cancer patients undergoing chemotherapy (SAVE-ONCO); evaluation of AVE5026 as compared to placebo for the extended prophylaxis of VTE in patients undergoing hip fracture surgery (SAVE-HIP3)). AVE5026 did not meet the primary efficacy endpoint, any VTE or any death caused by either VTE or other reasons (all-causes of death), in 3 of the 4 completed enoxaparin-controlled trials, including both superiority and non-inferiority study designs. These were conducted in patients undergoing orthopedic surgery, including the study comparing the efficacy and safety of AVE5026 with enoxaparin for the prevention of VTE in patients undergoing elective knee replacement surgery (SAVE-KNEE) and the study comparing the efficacy and safety of AVE5026 with enoxaparin for the prevention of VTE in patients undergoing hip fracture surgery (SAVE-HIP2), and the study comparing the efficacy and safety of AVE5026 with enoxaparin for the prevention of VTE in patients undergoing major abdominal surgery (SAVE-ABDO). One of the 4 enoxaparin-controlled trials (the study comparing the efficacy and safety of AVE5026 with enoxaparin for the prevention of VTE in patients undergoing elective hip replacement surgery (SAVE-HIP1)) met the primary efficacy endpoint (any VTE or all-causes of death), but did not meet the secondary efficacy endpoint (major VTE or all-causes of death). The proportion of patients was larger in the enoxaparin group (10.6%) than in the AVE5026 group (4.5%) for the safety population in this study.

3.2.1 *The study of SAVE-ONCO*

The efficacy and safety of AVE5026 for prevention of VTE in patients receiving chemotherapy for cancer was being evaluated in the study of SAVE-ONCO, a double-blind, multicenter trial (*55*). In this study, 3,212 patients who were undergoing chemotherapy for locally advanced or metastatic cancers of lung,

pancreas, stomach, colon/rectum, bladder, or ovary were enrolled. Patients were randomized 1:1 to receive either AVE5026 20 mg QD subcutaneously (SC) or placebo for a minimum of 3 months while receiving chemotherapy. In the results, the median treatment duration was 3.5 months. VTE occurred in 20 of 1,608 patients (1.2%) receiving AVE5026, as compared to 55 of 1,604 (3.4%) receiving placebo (hazard ratio (the ratio of 1.2% to 3.4%), 0.36; 95% CI, 0.21 to 0.60; $p < 0.001$), with consistent efficacy among subgroups defined according to the origin and stage of cancer and the baseline risk of VTE. The incidence of clinically relevant bleeding was 2.8% and 2.0% in the AVE5026 and placebo groups, respectively (hazard ratio, 1.40; 95% CI, 0.89 to 2.21). Major bleeding occurred in 19 of 1589 patients (1.2%) receiving AVE5026 and 18 of 1583 (1.1%) receiving placebo (hazard ratio, 1.05; 95% CI, 0.55 to 1.99). Incidences of all other adverse events were similar in the two study groups. In conclusion, this study showed that AVE5026, as compared to placebo, reduced the incidence of VTE in patients with locally advanced or metastatic cancer, with no apparent increase in major bleeding.

3.2.2 *The study of SAVE-ABDO*

Regarding other trial with AVE5026 for VTE prophylaxis in patients with cancer, in the study of SAVE-ABDO, 81% (2451) of the primary efficacy population were patients with cancer undergoing oncologic surgery. SAVE-ABDO was a randomized active-controlled trial in patients undergoing major abdominal surgery. A total of 4,413 patients were randomized 1:1 to receive either AVE5026 20 mg QD SC or enoxaparin 40 mg QD SC for a duration of 7-10 days after surgery. A US Food and Drug Administration (FDA) exploratory analysis in the subgroup of patients with cancer showed a numerically higher proportion of subjects with VTE events in the AVE5026 group compared to the enoxaparin group (7.1% *vs.* 5.9%, respectively; Odds Ratio 1.23 (0.89, 1.69)). This trial failed to meet its primary efficacy endpoint of any VTE or all-causes of death in a noninferiority comparison of AVE5026 versus enoxaparin (Odds Ratio 1.16, with the upper bound of the 95% CI (1.59) failing to meet the pre-specified noninferiority margin of 1.25).

A total of 7,616 patients have been exposed to AVE5026 across 21 clinical trials, including one phase II clinical dose-finding study, seven phase III clinical efficacy/safety studies of AVE5026 for VTE prophylaxis (6,826 patients, except for the patients in a phase III clinical study which was initiated but terminated early) and thirteen phase I clinical pharmacology studies (354 exposed to AVE5026, including 255 healthy subjects). The totality of safety data from these studies suggested that the safety profile of AVE5026, including bleeding adverse events, was similar to that of enoxaparin.

4. RO-14

RO-14, a derivative of bemiparin (*57*), developed by Laboratorios Farmaceúticos Rovi, S.A. of Spain, is obtained by selective chemical depolymerization of UFH in a non-aqueous medium. The reaction mechanism is β-elimination, as well. The anti-FXa activity of RO-14 is between 80 and 140 IU/mg, and the anti-FIIa activity is lower than or equal to 10 IU/mg (Table 1). The ratio of anti-FXa to anti-FIIa is higher than 20 (*58*). Its molecular weight is between 1.8 kDa and 3.0 kDa and the MWavg is about 2.2 kDa.

So far, only two articles were published about the research of RO-14 (except reviews and conference reports), one is about RO-14 reducing the endothelial angiogenic features elicited by leukemia, lung cancer or breast cancer cells (*59*), and the other is a phase I clinical study about RO-14 (*58*). Vignoli and his colleagues have evaluated whether RO-14 may retain the antiangiogenic properties observed with LMWH. In this study, they investigated the capacity of RO-14 to inhibit the angiogenic features of the endothelium stimulated by leukemic, breast cancer, and small cell lung cancer cells, or by standard proangiogenic factors in an *in vitro* system of interaction of cancer cells with microvascular endothelial cells. They found that RO-14 had an antiangiogenic activity, suggesting RO-14 can be applied in cancer treatment as an adjuvant drug.

The phase I clinical study was a two-stage, single-center, open-label, randomized study. Eighteen volunteers were enrolled in this study. Thirteen of the volunteers participated in one stage which assessed 6 ascending dose levels of RO-14 (1,750, 2,450, 3,500, 4,550, 5,600, 6,650 IU anti-FXa), and 12 of the volunteers participated in the other stage which assessed 6 additional strengths of RO-14 (7,700, 10,150, 12,600, 15,050, 17,500, and 19,950 IU anti-FXa). Blood samples were collected in tubes containing citrate sodium 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 14, 18, and 24 h after drug administration for pharmacodynamic analysis. Safety was assessed by spontaneous/elicited adverse events, medical examination and laboratory tests. In this study, all doses were well tolerated and there were no bleeding events. The anti-FXa activity at the lowest and the highest dose levels were 0.16 (± 0.02) IU/mL and 1.67 (± 0.15) IU/mL, respectively. At the highest dose levels, the $t_{1/2}$ was 8.05 h. The mean T_{max} was 2.86 (± 0.39) h at all dose levels. RO-14 showed proportional and linear pharmacodynamics. There were no clinically significant changes in the platelet count, activated partial thromboplastin time (APTT), prothrombin time (PT), thrombin time (TT), fibrinogen, and antithrombin. This phase I clinical study demonstrates that RO-14 has a high anti-FXa activity for prophylaxis or treatment of VTE and a good safety profile, linear pharmacodynamics and a long elimination half-life.

5. Conclusion

Here, we reviewed the preparation processes, pharmacological effects and clinical applications of fondaparinux, AVE5026 and RO-14. Though fondaparinux is a chemically synthesized pentasaccharide and both AVE5026 and RO-14 are obtained by degradation of UFH through a β-elimination reaction, all of them have lower molecular weight, higher anti-FXa activity and a longer half-life time than enoxaparin (Table 1). The US FDA has recently approved the generic forms of LMWHs and ULMWHs, which underscores the rapid growth in the development of heparin-based drugs. Among fondaparinux, AVE5026, and RO-14, fondaparinux was the only one that has gone on the market, and has been wildly applied in prophylaxis and treatment of DVT. Fondaparinux does not have the same contamination risks associated with animal-sourced UFH and LMWH, is subcutaneously bioavailable and has reduced risks of HIT and osteoporosis. However, fondaparinux is contraindicated in renal-impaired patients and lacks an antidote. Therefore, better ULMWHs are being developed by many groups and companies. AVE5026 and RO-14 have a high anti-FXa activity for prophylaxis or treatment of VTE according to limited data. However, Sanofi-Aventis, the development company of AVE5026, revoked the listing application of AVE5026 for prophylaxis of VTE in patients receiving chemotherapy for locally advanced or metastatic pancreatic or lung cancers or for locally advanced or metastatic solid tumors with a VTE risk score ≥ 3. The FDA advisory committee denied the application, because the absolute efficiency is low although the relatively efficiency is statistically significant. Despite this, we believe that research steps for ULMWHs will still be going on and new ULMWHs with high anti-FXa activity and low incidence of adverse events will be developed in the future.

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