A peer-reviewed version of this preprint was published in PeerJ on 6 January 2015.

Quaranta M, Erez O, Mastrolia SA, Koifman A, Leron E, Eshkoli T, Mazor M, Holcberg G. 2015. The physiologic and therapeutic role of heparin in implantation and placentation. PeerJ 3:e691
https://doi.org/10.7717/peerj.691

The physiologic and therapeutic role of Heparin in implantation and placentation

Implantation, trophoblast development and placentation are crucial processes in the establishment and development of normal pregnancy. Abnormalities of these processes can lead to pregnancy complications named the great obstetrical syndromes (preeclampsia, intrauterine growth restriction, fetal demise, premature prelabor rupture or membranes, preterm labor, and recurrent pregnancy loss). There is mounting evidence regarding the physiological and therapeutic role of heparins in the establishment of normal gestation and as a modality for treatment and prevention of pregnancy complications. In this review we will summarize the properties and the physiological contribute of heparins to the success of implantation and placentation and normal pregnancy.

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The physiologic and therapeutic role of Heparin in implantation and placentation

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1. Introduction

The use of Heparins have increased constantly since their discovery, and this is due to the number of properties and effects shared by these molecules. In addition to their anticoagulant and anti-inflammatory effect, that justifies their employment in the prevention and treatment of pregnancy complications, these molecules have a physiologic role during gestation and especially during implantation and placentation; which is a critical process in the establishment and success of pregnancy[1]. In this review we will present the role of heparin in implantation, placentation, and we will discuss their role in the immunologic balance among the mother and the fetus.

2. The physiological role of heparin

Heparin is one of the oldest drugs currently in widespread clinical use. Its discovery in 1916 predates the establishment of the Food and Drug Administration of the United States, although it did not enter clinical trials until 1935. It was originally isolated from canine liver cells[2], hence its name (hepar or "ήπαρ" is Greek for "liver").

It is principally employed for its anticoagulation properties. Moreover, its true physiological role in the body remains uncertain, since blood anticoagulation is achieved mostly by heparan sulfate proteoglycans derived from endothelial cells[3]. Heparin is usually stored within the mast cells secretory granules and released only into the vasculature at sites of tissue injury. It has been proposed that, in addition to its anticoagulant properties heparin may play a role in the defense against invading bacteria and other foreign materials[4].

Heparin is a glycosaminoglycan composed of chains of alternating residues of d-glucosamine and uronic acid. It's major anticoagulant effect is accounted for a unique pentasaccharide (GlcNAc/NS(6S)-GlcA-GlcNS(3S,6S)-IdoA(2S)-GlcNS(6S) structure that has a high binding affinity sequence to anti-thrombin III (AT III)[5]; however, *in-vitro* studies suggest that this structure is present only in about one third of heparin molecules[6].

The interaction between heparin and AT-III mediates the majority of the anticoagulant effect of the former. Their binding produces a conformational change in AT-III (Fig. 1) that accelerates up to 1000 fold[7] its ability to inactivate the major coagulation factors, including mainly thrombin (factor IIa), factor Xa, and factor IXa[5].

Heparin increases the inhibitory effect of AT-III on thrombin and Factor Xa activity by distinct mechanisms (Fig. 2). The acceleration of the inhibition of thrombin by AT-III necessitates the binding of this molecule to the heparin polymer proximally to the pentasaccharide units. Heparin has a highly negative charge that is derived from the number of its saccharide units, which contributes to the strong electrostatic interaction of AT-III with thrombin. Thus, heparin's activity against thrombin is size-dependent, and the ternary complex (including thrombin, ATIII and

heparin) requires at least 18 saccharide units for efficient formation and thrombin inactivation[8, 9]. In contrast, the effect of heparin on the inhibition of factor Xa by ATIII is dependent on the conformational change of this molecule at the heparin-binding site; therefore, the size of heparin has no importance in the inhibition of factor Xa by ATIII. This has therapeutic implications and led to the development of a new generation of heparin derived anticoagulants including low-molecular-weight heparins (LMWH) and fondaparinux. LMWH are obtained as fragments of unfractionated heparin as a result of enzymatic or chemical depolymerization, yielding to molecules of mean weight of 5000 Da (Table 1)[10] while fondaparinux is a synthetic pentasaccharide based on the heparin antithrombin-binding domain[11]. These medications target the anti-factor Xa activity rather than anti-thrombin (IIa) activity of AT-III, aiming to facilitate a more subtle regulation of coagulation with an improved therapeutic index and less side effects. Indeed, each molecule of fondaparinux binds to one molecule of AT-III at a specific site, and with very high affinity. The binding is rapid, non-covalent, and reversible. It induces a critical conformational change in AT-III, exposing a loop containing an arginine residue that binds factor Xa. Exposure of the arginine-containing loop greatly increases the affinity of AT-III for factor Xa, potentiating the natural inhibitory effect of AT-III against factor Xa by a factor of approximately 300[8, 9].

3. The role of heparins in implantation and placentation

3.1 What are the stages of implantation and placentation?

Implantation, a critical step for the establishment of pregnancy, requires a complex molecular and cellular events resulting in uterine growth and differentiation, blastocyst adhesion, invasion, and placental formation. Successful implantation necessitates a receptive endometrium, a normal and functional embryo at the blastocyst stage, and a synchronized dialogue between the mother and the developing embryo[12]. In addition to the well-characterized role of sex steroids, the complexity of blastocyst implantation and placentation is exemplified by the role played by a number of cytokines and growth factors in these processes. Indeed, the process of implantation is orchestrated by hormones like sex steroids, and hCG; growth factors such as TGF-B, HB-EGF, IGF-1; cytokines as Leukemia Inhibitory Factor , Interleukin-6 and Interleukin -11; adhesion molecules including L-selectin and E-cadherin ,the extracellular matrix (ECM) proteins, and prostaglandins[12].

Embryonic implantation is initiated by the recognition and adhesion between the blastocyst surface and the uterine endometrial epithelium. Adhesion occurs when a free-floating blastocyst comes into contact with the endometrium during the 'receptive window' in which it is able to respond to the signals from the blastocyst. This contact is then stabilized in a process known as adhesion in which the trophoblast cells establish contact with the micro protrusions present on the surface of the endometrium known as pinopodes[13]. The last step of implantation is the invasion process, which involves penetration of the embryo through the luminal epithelium into the endometrial stroma; this activity is mainly controlled by the trophoblast.

The trophoblast lineage is the first to differentiate during human development, at the transition between morula and blastocyst. Initially, at day 6 to 7 post-conception, a single layer of mononucleated trophoblast cells surrounds the blastocoel and the inner cell mass. At the site of attachment and direct contact to maternal tissues, trophoblast cells fuse to form a second layer of postmitotic multinucleated syncytiotrophoblast[14]. Once formed, the syncytiotrophoblast grows by means of steady incorporation of new mononucleated trophoblast cells from a proximal subset of stem cells located at the cytotrophoblast layer[15].

Tongues of syncitiotrophoblast cells begin to penetrate the endometrial cells and gradually the embryo is embedded into the stratum compactum of the endometrium. A plug of fibrin initially seals the defect in the uterine surface, but by days 10 to 12 the epithelium is restored[16]. Only at around the 14th day mononucleated cytotrophoblasts break through the syncytiotrophoblast layer and begin to invade the uterine stroma at sites called trophoblastic cell columns. Such cells

constitute the extravillous trophoblast, and have at least two main subpopulations: interstitial trophoblast, comprising all those extravillous trophoblast cells that invade uterine tissues and that are not located inside vessel walls and lumina; and endovascular trophoblast, located inside the media or lining the spiral artery lumina and partly occluding them (sometimes this subtypes is further subdivided into intramural and endovascular trophoblast)[16].

At a molecular level, trophoblast adhesion from the stage of implantation onwards is an integrin-dependent process[17, 18] that takes place in a chemokine- and cytokine- rich microenvironment analogous to the blood-vascular interface. Of note, in human, uterine expression of chemokines is hormonally regulated and the blastocyst expresses chemokine receptors. In addition, oxygen tension plays an important role in guiding the differentiation process that leads to cytotrophoblast invasion to the uterus[19, 20].

3.2 What is the role of heparin and heparin derived molecules in the process of implantation?

Heparin and heparin derived molecules influence all stages of implantation. This anticoagulant has an effect on the expression of adhesion molecules, matrix degrading enzymes and trophoblast phenotype and apoptosis (see table 2).

3.2.1 Selectins and Cadherins

Selectins and cadherins families are the main adhesion molecules investigated with regard to the implantation process. Selectins are a group of three carbohydrate-binding proteins that are named following the cell type expressing them (E- endothelium, P- platelets, and L- leucocytes): E-selectin is expressed on the endothelial surface; P-selectin on the surface of activated platelets; and L-selectin on lymphocytes, where it plays an essential role in the homing mechanism of these cells[21, 22]. The selectins adhesion system may constitute an initial step in the implantation process. Indeed, L-selectin is strongly expressed on the blastocyst surface while, during the window of implantation, there is an upregulation in the decidual expression of the selectin oligosaccharide-based ligands, predominantly on endometrial luminal epithelium[23]. This may assist in the blastocyst decidual apposition during the implantation process.

The effect of heparin on selectins during implantation is unclear. Due to its high density in negatively charged sulfates and carboxylates, heparin is able to bind the two binding sites of the natural ligand of selectin molecules (P and L-selectins) (one for the sialyl Lewis X moiety and another for the tyrosine sulfate-rich region of its native ligand P-selectin glycoprotein ligand-1 [PSGL-1]), and the number of sites bonded is dependent on the length of the heparin chain. Evidence in support is presented by the study of Stevenson et al[24] who investigated the effect of

different unfractionated heparin and LMWH on selectin molecules in cancer cell lines. Tinzaparin, with 22% to 36% of fragments greater than 8 kDA, significantly impaired L-selectin binding to its ligand; whereas enoxaparin, with 0% to 18% fragments greater than 8 kDa, did not affect L-selectin expression[24]. Thus, heparins with high proportion of fragments longer than 8kDa may reduce inflammatory cell adhesion and homing, on the other hand they may affect blastocyst adhesion by blocking selectins ligand binding sites.

Cadherins are a group of cell adhesion proteins that mediate Ca^{2+}-dependent cell–cell adhesion, a fundamental process required for blastocyst implantation and embryonal development[25]. E- cadherin plays an important role in maintaining cell adhesion. In cancer cells, the reduction of E-cadherin expression promotes acquisition of invasive phenotype. Interestingly, gestational trophoblastic diseases (choriocarcinoma and complete hydatidiform mole) that are characterized by invasive trophoblast behavior has a lower E-cadherin trophoblastic expression than that of first-trimester placenta[26]. In contrast, the trophoblast expression of E-cadherin is higher in placentas of patients with preeclampsia, than in those of normal pregnant women[27]. The effect of heparin on E-cadherin expression was studied by Erden and coworkers[28], who randomly treated female rats with different heparins (UFH, enoxaparin, and tinzaparin) during the preconceptional period, and examined E-cadherin expression in tissue sections of placenta and decidua from the different groups. The group treated by UFH had a lower E-cadherin placental staining than other study groups. In addition, the decidual staining score of this molecule was lower both in the UFH and Enoxaparin groups in comparison to controls and rats treated with Tinzaparin. Therefore, there is evidence to support the effect of heparins on trophoblast invasiveness through E-cadherin expression, providing a possible mechanism by which heparin could promote trophoblast cell differentiation and motility.

3.2.2 Heparin binding EGF-like growth factor

Heparin-binding EGF-like growth factor (HB-EGF) is a 76–86 amino acid glycosylated protein that was originally cloned from macrophage-like U937 cells. It is a member of the epidermal growth factor (EGF) family that stimulates growth and differentiation. HB-EGF utilizes various molecules as its “receptors”. The primary receptors are in the ErbB (also named HER) system, especially ErbB1 and ErbB4, human tyrosine kinase receptors. HB-EGF is initially synthesized as a transmembrane precursor protein, similar to other members of the EGF family of growth factors. The membrane-anchored form of HB-EGF (pro HB-EGF) is composed of a pro domain followed by heparin-binding, EGF-like, juxtamembrane, transmembrane and cytoplasmic domains. Subsequently, proHB-EGF is cleaved at the cell surface by a protease to yield the

soluble form of HB-EGF (sHB-EGF) using a mechanism known as ectodomain shedding. sHB-EGF is a potent mitogen and chemoattractant for a number of different cell types. Studies of mice expressing non-cleavable HB-EGF have indicated that the major functions of HB-EGF are mediated by the soluble form[29].

Heparin-binding epidermal-growth-factor-like growth factor (HB-EGF) accumulates in the trophoblast[30] throughout the placenta[31]. Multiple roles for heparin binding epidermal growth factor-like growth factor are suggested by its cell specific expression during the human endometrial cycle and early placentation, and high levels expression in the first trimester[32].

The membrane active precursor functions as a justacrine growth factor and cell-surface receptor. It has been demonstrated to promote adhesion of the blastocyst to the uterine wall in a mouse-in-vitro- system[33] suggesting a role for HB-EGF in embryo attachment to the uterine luminal epithelium. As stated above, the majority of HB-EGF's biological functions are mediated by its mature soluble form. A major role in early stages of placentation is represented by cellular differentiation and consequent invasion of the uterine wall and vascular network.

Several changes occur in the expression of adhesion molecules as cytotrophoblast differentiation proceeds, which results in pseudovasculogenesis or the adaptation by cytotrophoblast of a molecular phenotype that mimics endothelium[18]. For example, during extravillous differentiation in vivo, integrin expression is altered from predominantly α6β4 in the villous trophoblast to α1β1 in cytotrophoblasts migrating throughout the decidual stroma[17] or engaging in endovascular invasion[18].

Leach et al[34] demonstrated the role of HB-EGF in regulating the conversion of human cytotophoblasts into invasive phenotype and the motility of these cells. This study demonstrated the ability of HB-EGF to induce 'integrin switching' through intracellular signaling induced by ligation of HER tyrosine kinases, alters integrin gene expression to stimulate cytotrophoblast invasion at a molecular level.

In addition to its effect on the invasive trophoblast phenotype, HB-EGF can affect cell motility. Indeed, cytotrophoblasts motility was specifically increased by each of the EGF family members examined. The expression by cytotrophoblasts of each growth factor, as well as their receptors, suggests the possibility of an autocrine loop that advances cytotrophoblast differentiation to the extravillous phenotype.

The ability of HB-EGF molecule to prevent hypoxic induced apoptosis plays a fundamental role in early stages of placentation. During the entire 1st trimester, the organogenesis period, embryonic development takes place in a low O_2 tension environment. Oxygen concentration is relatively low (18mmHg or 2%) at the human implantation site through the first 10 weeks of gestation due to occlusion of the uterine spiral arteries by extravillous trophoblasts. Oxygen

availability serves as a developmental cue to regulate trophoblast proliferation. Experimental evidence suggests that this environment is essential for both fetal and placental development, and premature exposure to normal oxygen concentrations is associated with increased rate of pregnancy complications such as preeclampsia, IUGR and miscarriage[35].

First trimester human cytotrophoblast cell survival at 2% O2 is dependent on HB-EGF signaling[36]. Indeed, HB-EGF expression is up regulated by hypoxia, and it functions as a mitogen and potent cell survivor factor during stress. The mechanism proposed for this effect of HB-EGF is as follows: sHB-EGF is released by activated metalloproteinases that cleave the extracellular domain of pro- HB-EGF. sHB-EGF binds to HER1 or HER4 through its EGF-like domain and to heparin sulfate proteoglicans (HSPG) through its heparin binding domain, and this is followed by receptor homo- or heterodimerization with other members of the HER family. Subsequent transphosphorylation of HER cytoplasmatic domains at tyrosine residues initiates a downstream signaling that increases proHB-EGF accumulation and inhibits apoptosis. This positive feedback loop upregulates HBEGF secretion to achieve extracellular HB-EGF levels sufficient to maintain cell survival at 2% O2[36].

As a result HB-EGF has a fundamental role in successful pregnancies. This molecule mediates a vast number of functions beginning from the earliest stages of pregnancy; from adhesion, to implantation and invasion, successful placentation, and protection from hypoxic induced aptoptosis from early stages and up to term. The effect of heparin on this molecule is currently being studied. Di Simone et al[37] demonstrated that LMWH induced an increased decidual expression and secretion of HB-EGF in a dose-dependent manner. In a different study by D'ippolito et al[38] demonstrated that LMWH induces activation of Activator Protein-1 (AP-1), a DNA-binding transcription factor which regulates the expression of HB-EGF. Activated AP-1 translocates to the nucleus and binds the promoter region of HB-EGF gene thus enhancing its protein expression. Hills and Abrahams[39] demonstrate that heparin is capable of activating the EGF receptor in primary villous trophoblast.

Thus, we propose that the accumulating evidence suggests that the beneficial effect of heparin in preventing placental mediated pregnancy complications may derive from its effect on HB-EGF expression and concentration, especially during the first trimester.

3.2.3 Matrix metalloproteinases

In addition to the adhesion molecules, matrix metalloproteinases (MMPs) are an important component in the process of blastocyst implantation. MMPs are a group of matrix degrading enzymes which are secreted as inactive zymogen and must be cleaved to become active[40]. Among the members of the MMP family, MMP-2 and MMP-9 type IV collagenases were suggested to be

involved in trophoblast invasion into endometrial tissues[41]. Indeed, the profile of pro-MMP 2 and 9 secretion differs during the stages of trophoblast invasion and implantation, and differences in these zymogens expression were found between 6-8 and 9-12 weeks of gestation in extravillous cytotrophoblast cells[42]. Di Simone et al investigated the effect of LWMH specifically on placental MMPS[43], and the degrading capacity of the trophoblast cells. This effect is mediated by heparins action on both metalloproteinases (MMPs) and their tissue inhibitors (TIMPs). Heparin increased both the MMPs concentration and activity by affecting their transcription, conversion of the proenzyme into the active form, and reduction of the synthesis of the specific inhibitors TIMPs (both the mRNA and protein levels) in a dose dependent manner[43].

3.3 Immunologic and anti-inflammatory effects of heparins

Immune tolerance of the allogeneic fetus is mandatory for a successful pregnancy outcome[44]. Both innate and adaptive immune responses contribute to a maternal fetal cross-talk that balances the anti- and pro-inflammatory processes in the feto-maternal infterface[45, 46]. Maternal blood is in direct contact with the syncytiotrophoblast at the intervillous space and in the decidual tissue where the extravillous trophoblast anchors the placenta, and further differentiate into endovascular trophoblast that invades spiral arteries and remodels the vessel walls[45, 47, 48]. A successful pregnancy results from the participation of all the components of the immune system including: MHC class I molecules, hormones, complement regulatory proteins, immunoregulatory molecules (i.e. indolamine 2,3-dioxygenase, Fas/Fas- Ligand, IL-10), regulatory T cells (CD4+ CD25+ Foxp3+), regulatory macrophages, and growth factors expressed at the placental–decidual interface[49-56]. These mechanisms act in concert to sustain the maternal tolerance to the semi-allogenic placenta and fetus[57]. In addition to its well-understood anticoagulant activity, heparin also has an impact on the immune system[58-60]. The main known effect of heparin is on the migration and adhesion of leukocytes during an inflammatory response[24].

The anti-inflammatory effects of heparin are derived from several mechanisms: 1) the molecular structure of heparin is so that upon its bounding to the endothelial cells of blood vessels it creates a negatively charged surface that is facing the vessel lumen. These negatively charge molecules repulse the negatively charge leukocytes and prevent their adhesion to the endothelium (heparan sulfate molecules that are expressed on leukocytes surface are responsible for the negative charge of these cells); 2) heparin is a large molecule that can bind a substantial number of proteins which play an important role in inflammation including selectins (L- selectin[61] and P-selectin molecules[62]) and integrins. The B2-integrin adhesion molecule CD11b/CD18, also known as

Macrophage antigen 1 (MAC1), is a member of a subfamily of related cell-surface glycoproteins that coordinate adhesive functions including leukocyte migration[63]. Mac1 is expressed on myeloid cells and binds to molecules as intercellular adhesion molecule 1 (ICAM1), fibrinogen, iC3b, and factor Xa. The heparin-Mac1 bond interferes with myeloid cell adhesion and transmigration[64]. Heparin also binds to platelet/endothelial cell adhesion molecule 1 (PECAM1), a member of the Ig superfamily, expressed on a variety of cells such as platelets, endothelia, monocytes, neutrophils, T-cell subsets and granulocyte/macrophage precursors. This molecule is involved in homotypic and heterotypic cellular adhesion and plays a role in the transmigration of inflammatory cells through the endothelial wall. Heparin is capable of binding PECAM1 and interfering with its action[65], reducing by that the effectiveness of the inflammatory response.

The anti-inflammatory properties of LMWH have been demonstrated within *in vivo* models. Indeed, Wang et al[66] investigated the effects of LMWH on dextran sulfate sodium (DSS)-induced colitis in a mice model. The authors reported that mice which were treated with LMWH had a significant decrease in the expression of both IL-1β and of IL-10 mRNA, leading to a down regulation of inflammatory cytokines production. Of interest, LMWH also imitate the function of Syndecan-1 (a protein that is inversely correlated to the mRNA expression of IL-1β in the intestinal mucosa of DSS-induced colitis), a protein which plays an important role in promoting wound repair, maintaining cell morphogenesis, and mediating inflammatory responses[67] by aiding the clearance of pro-inflammatory chemokines. In addition Li et al[68] found that treatment with UFH can attenuate inflammatory responses of lypopolisaccharide induced acute lung injury in rats. The mechanisms by which UFH exerts its anti-inflammatory effect seem to correlate with its inhibition of IL-1ß and IL-6 production via inactivation of the NF-κB pathways.

In humans the anti-inflammatory activity of heparin has been evidenced by small clinical trials in patients suffering from a range of inflammatory diseases[69], including rheumatoid arthritis and bronchial asthma. Remission of disease has been described in nine of ten patients with refractory ulcerative colitis treated with combined heparin and sulphasalazine[69]. A subjective improvement of asthma symptoms using intravenous heparin is described[70, 71], while other studies with inhaled heparin demonstrated reduced bronchoconstrictive responses in patients with exercise-induced asthma[72, 73].

The clinical rationale for the use of heparin in the treatment of inflammatory diseases may be based on the fact that many of the molecular mechanisms involved in tumor metastasis are the same responsible for cell recruitment in inflammation; and heparin has been successful in treating both conditions[74].

4. Conclusion

Heparins play a role in embryonic implantation and placentation and contribute to the development of a normal pregnancy. This effect is gained through the interaction of heparins with coagulation factors, anticoagulation proteins, their effect on the expression of adhesion molecules, matrix degrading enzymes and trophoblast phenotype and apoptosis, all important components in the process of embryonic implantation and placentation.

Moreover, in addition to their physiologic effects, Heparins can be considered as molecules with still some stories to tell. Indeed, their main function is as anticoagulant medication. However, there is increasing evidence, as described in the present review, suggesting that these drugs may have an anti- inflammatory effect and they affect the activation of the immunologic system (principally acting on leukocyte migration and adhesion processes).

The understanding of these concepts may assist us in tailoring the use of heparins for the prevention and treatment of pregnancy complications in a more targeted manner.

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Fig. 1. Antithrombin III after conformational change induced by heparin binding. Reproduced with permission from Whisstoch JC, Pike RN, Jin L, Skinner R, et al. J Mol Biol. 2000; 301:128

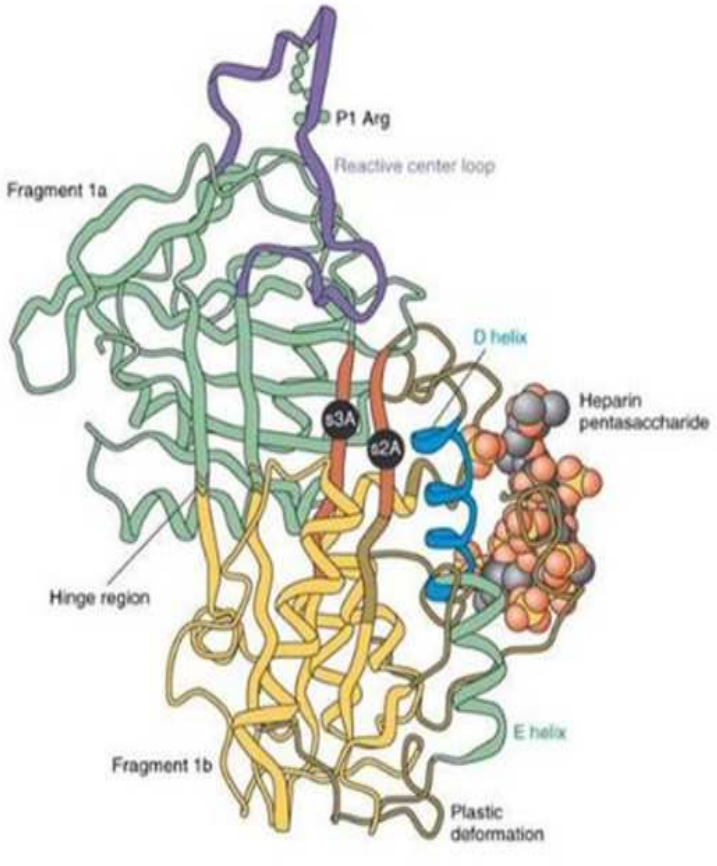

Fig. 2. Mechanisms of interaction between heparin, antithrombin, thrombin and factor Xa. Source: Fauci AS, Kasper DL, Braunwald E, Hauser SL, Longo DL, Jameson JL, Loscalzo J: Harrison's Principles of Internal Meicine, 17th Edition

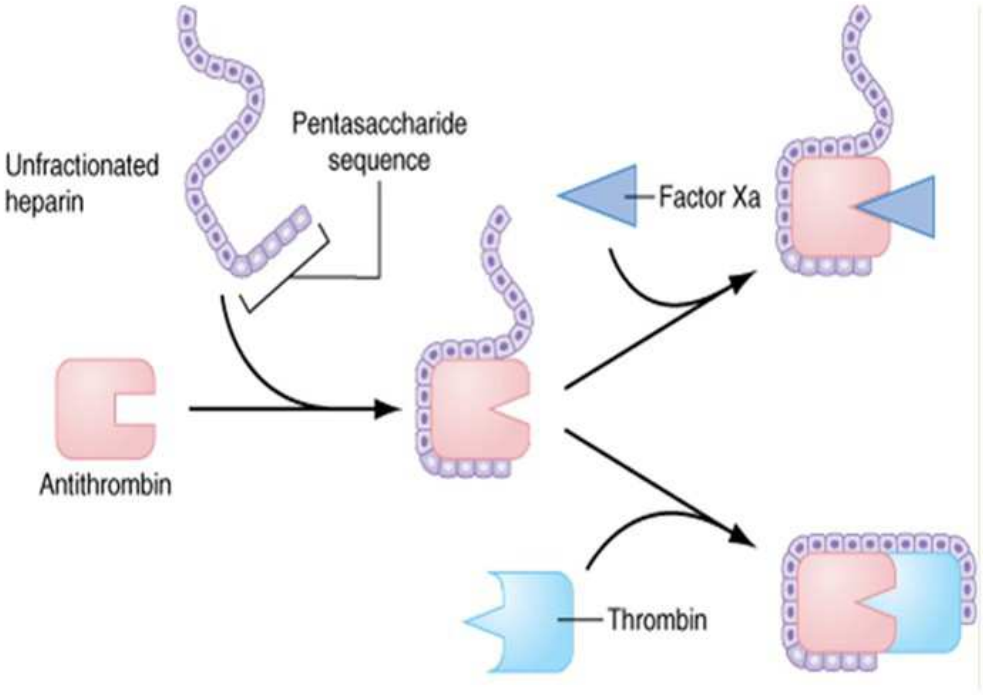

Table 1 (on next page)

Table 1.

Comparison among low molecular weight heparin preparations (From Weitz JI. Low-molecular-weight heparins. N Engl J Med 1997;337:688-98. With permission)

Table 1. Comparison among low molecular weight heparin preparations (From WEITZ JI. Low-molecular-weight heparins. N Engl J Med 1997;337:688-98. With permission)

PREPARATION	METHOD OF PREPARATION	MEAN MOLECULAR WEIGHT	ANTI-XA:ANTI-IIA RATIO*
Ardeparin (Normiflo)	Peroxidative depolymerization	6000	1.9
Dalteparin (Fragmin)	Nitrous acid depolymerization	6000	2.7
Enoxaparin (Lovenox)	Benzylation and alkaline depolymerization	4200	3.8
Nadroparin (Fraxiparine)	Nitrous acid depolymerization	4500	3.6
Reviparin (Clivarine)	Nitrous acid depolymerization, chromatographic purification	4000	3.5
Tinzaparin (Innohep)	Heparinase digestion	4500	1.9

*The ratios were calculated by dividing the anti–factor Xa (anti-Xa) activity by the antithrombin (anti-IIa) activity. The ratios are based on information provided by the manufacturers.

Table 2(on next page)

Table 2

Overview of molecules involved in the process of implantation, trophoblast development and placentation, and effect of heparin on these molecules

Table 2. Overview of molecules involved in the process of implantation, trophoblast development and placentation, and effect of heparin on these molecules

Molecule	**Site of expression**	**Activity**	**Effect of Heparin**
Anti-thrombin III	Maternal circulation Trophoblast	Inactivation of coagulation factors, including mainly thrombin (factor IIa), factor Xa, and factor IXa	Conformational change in AT-III that accelerates its ability to inactivate the coagulation factors
Selectins (E- P- and L-selectins)	E-selectin endothelium, P-selectin platelets, and L-selectin leucocytes and blastocyst surface.	Cell adhesion and homing	Interference with inflammatory cells adhesion and homing but probable interference with blastocyst decidual adhesion
Cadherins	Trophoblast, placenta, decidua	Cell adhesion (invasive phenotype acquired in case of reduction of expression)	Reduction of expression
Heparin-binding EGF-like growth factor (HB-EGF)	Trophoblast and placenta	1)potent mitogen and chemoattractant in its soluble form promoter of adhesion of the blastocyst to the uterine wall in a mouse-in-vitro-system 2)regulation of the conversion of human cytotophoblasts into invasive phenotype and influence on the motility of these cells 3)prevention of hypoxic induced apoptosis	Increased decidual expression and secretion of HB-EGF
Matrix metalloproteinases (MMPs)	Soluble form	Involvement in trophoblast invasion into endometrial tissues	Increased expression
Tissue inhibitors of metalloproteinases (TIMPs)	Soluble form	Inhibition of metalloproteinases and their function	Reduction of expression
Macrophage antigen 1 (Mac1)	Surface of myeloid cells	Coordination of adhesive functions of leukocyte and their migration	Interference with myeloid cell adhesion and transmigration
Platelet/endothelial cell adhesion molecule 1 (PECAM1)	Surface of platelets, endothelia, monocytes, neutrophils, T-cell subsets and granulocyte/macrophage precursors	transmigration of inflammatory cells through the endothelial wall	Interference with inflammatory cells transmigration