A Case Study on Anticoagulants with special reference to Heparin

Crest Premedia Solutions (P) Ltd
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1. Query & Objective

1.1 Query:
A Case Study on Anticoagulants with special reference to Heparin

1.2 Objective:
As is well-established, anticoagulants are essential for treatment and prevention of thrombosis. Presently, anticoagulant based drugs are indispensible in numerous medical procedures, disease prophylaxis and cure. Despite its limitations heparin is one of the most commonly used anticoagulant till date. Thus, in the present study an attempt was made to review the literature and pharmaceutical drugs related information available on well-known anticoagulants and emphasise the aspects related to the past, present and future of the oldest known anticoagulant – Heparin.
2. General Approach and Search strategies used

**General Approach:** The above mentioned query was analysed thoroughly, the information associated with the query was acquired via online searches using various data sources, the data obtained were screened and the most relevant search results were selected to answer the query efficiently and have been included in this report.

**Search Strategies used:**
- First and foremost comprehensive understanding of all fundamental aspects related to the query viz., coagulation, blood clots, thrombosis, anticoagulants, Heparin, its therapeutic significance and the various pharmaceutical anticoagulant drugs was developed using information available on public domain and other data sources
- After completion of this preliminary analysis based on the important facets, query related keywords were identified
- Based on the keywords selected, data mining was carried out using public domain and available databases
- The data were screened and the relevant information with respect to the query was accumulated

The list of data sources referred to, keywords and examples of search building using combination of keywords that were used to answer the query are given below:
3. Data Sources, Keywords and Search building approach

3.1 Data sources and screening details

<table>
<thead>
<tr>
<th>Data sources</th>
<th>Number of query related articles screened for report preparation</th>
<th>Number of articles included in the report (selected from the screened results)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AdisInsight</td>
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<td>01</td>
</tr>
<tr>
<td>DrugBank</td>
<td>01</td>
<td>01</td>
</tr>
<tr>
<td>PubMed</td>
<td>83</td>
<td>20</td>
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<tr>
<td>Spresi&lt;sup&gt;web&lt;/sup&gt;</td>
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<td>-</td>
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<tr>
<td>Other relevant websites (patents, drug information, journal websites etc.)</td>
<td>43</td>
<td>12*</td>
</tr>
<tr>
<td>Total</td>
<td>255</td>
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</tr>
</tbody>
</table>

* One article out of these also sourced from Web of Science-WOS accession number is provided

3.2 List of Keywords

<table>
<thead>
<tr>
<th>Anticoagulants</th>
<th>Blood</th>
<th>Blood clot</th>
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<tbody>
<tr>
<td>Coagulation cascade</td>
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<td>Heparin</td>
</tr>
<tr>
<td>Heparinoids</td>
<td>Heparin chemistry</td>
<td>Heparin contaminants</td>
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<td>Heparin history</td>
<td>Heparin manufacturers</td>
<td>Heparin overdose</td>
</tr>
<tr>
<td>Heparin sources</td>
<td>Heparin side effects</td>
<td>Heparin therapy</td>
</tr>
<tr>
<td>Heparin treatment</td>
<td>Low Molecular Weight Heparin</td>
<td>Oral-anticoagulant</td>
</tr>
<tr>
<td>Oral heparin</td>
<td>Protamine sulphate</td>
<td>Thromboembolism</td>
</tr>
<tr>
<td>Thrombosis</td>
<td>Unfractionated heparin</td>
<td>Warfarin</td>
</tr>
</tbody>
</table>
### 3.3 Search building approach:
Examples of search building exercise using combination keywords and Boolean operators on PubMed to narrow down searches

**Example 1: Search terms “heparins” and “sources”**

<table>
<thead>
<tr>
<th>Search</th>
<th>Query</th>
<th>Items found</th>
</tr>
</thead>
<tbody>
<tr>
<td>#3</td>
<td>Search heparins[Title] AND sources[Title]</td>
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</tr>
<tr>
<td>#2</td>
<td>Search heparins[Title/Abstract] AND sources[Title/Abstract]</td>
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<tr>
<td>#1</td>
<td>Search heparins AND sources</td>
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</tbody>
</table>

**Example 2: Search terms “heparin”, “Venous thromboembolism” and “thrombosis”**

<table>
<thead>
<tr>
<th>Search</th>
<th>Query</th>
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</tr>
</thead>
<tbody>
<tr>
<td>#10</td>
<td>Search (Heparin[Title] AND Venous thromboembolism[Title]) Filters: Full text available; published in the last 5 years; Humans; Randomized Controlled Trial</td>
<td>5</td>
</tr>
<tr>
<td>#9</td>
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</tr>
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<td>#7</td>
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</tr>
<tr>
<td>#6</td>
<td>Search (Heparin[Title] AND Venous thromboembolism[Title])</td>
<td>281</td>
</tr>
<tr>
<td>#5</td>
<td>Search (Heparin[Title] AND thrombosis[Title])</td>
<td>1638</td>
</tr>
<tr>
<td>#4</td>
<td>Search (Heparin[Title]) AND (Venous thromboembolism[Title] OR thrombosis[Title])</td>
<td>1913</td>
</tr>
<tr>
<td>#3</td>
<td>Search (Heparin[Title/Abstract]) AND (Venous thromboembolism[Title/Abstract] OR thrombosis[Title/Abstract])</td>
<td>10326</td>
</tr>
<tr>
<td>#2</td>
<td>Search (Heparin) AND (Venous thromboembolism OR thrombosis)</td>
<td>20461</td>
</tr>
<tr>
<td>#1</td>
<td>Search Heparin AND Venous thromboembolism OR thrombosis</td>
<td>174757</td>
</tr>
</tbody>
</table>
4. Coagulation and Anticoagulants – A brief introduction

4.1 The process of blood clotting – An insight:
In vertebrates, the cardiovascular system supplies oxygen and nutrients to all parts of the body and removes carbon dioxide and other wastes from body cells via a specialized fluid called blood. Blood is defined as the fluid that carries nutrients and oxygen through circulation in the blood vessels. In human beings, blood is composed of red (non-nucleated mature erythrocytes) and white (nucleated leukocytes) cells (fig. 4.1). The white cells include granulocytes, neutrophils, eosinophils and basophils, B and T lymphocytes and natural killer cells. The differentiation of these various types of cells from the multipotential hematopoietic stem cells is determined by a combination of growth factors, such as interleukins, stem cell factors, colony stimulating factors, etc. The blood contains also platelets (thrombocytes) and the blood plasma, the non-corpusculate yellowish fraction (Gooch ed., 2011; Redei ed., 2008).

According to Stolerman ed., 2010, platelets (Thrombocytes) are small cytoplasmic bodies derived from megakaryocytes. They circulate in the blood and are mainly involved in hemostasis. Platelets have no nucleus and display a lifespan of 7-10 days. When damage to the endothelium of blood vessels occurs, platelets go through activation, change shape, release granule contents and, finally, aggregate and adhere to the endothelial surface in order to form the blood clot (thrombus). Intact blood vessels and platelets are central to modulate blood's tendency to clot; the disruption of the correct balance between the involved regulators may result in different diseases.

Fig. 4.1 - White (pale green) and red blood cells (red) also referred to as leucocytes and erythrocytes respectively. Leucocytes are principal cells of the immune system involved in defending the body against both infectious disease and foreign materials and erythrocytes are involved in delivering oxygen to the body (Source: Gargaud ed.; 2011, http://www.springer.com/astronomy/astrobio/b ook/978-3-642-11271-3 or http://www.springerreference.com/docs/html/chapt erdbid/324399.html).

According to Stolerman ed., 2010, platelets (Thrombocytes) are small cytoplasmic bodies derived from megakaryocytes. They circulate in the blood and are mainly involved in hemostasis. Platelets have no nucleus and display a lifespan of 7-10 days. When damage to the endothelium of blood vessels occurs, platelets go through activation, change shape, release granule contents and, finally, aggregate and adhere to the endothelial surface in order to form the blood clot (thrombus). Intact blood vessels and platelets are central to modulate blood's tendency to clot; the disruption of the correct balance between the involved regulators may result in different diseases.

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In case of injuries, a blood clot occurs due to “Hemostasis”, the process which spontaneously arrests the flow of blood from vessels carrying blood under pressure. It is accomplished by contraction of the vessels, adhesion and aggregation of formed blood elements (e.g. Erythrocyte aggregation), and the process of blood coagulation\(^5\). A clot may also occur due to a process called “Thrombosis”, which represents a pathologic state in which there obstruction in blood flow caused by the aggregation of platelets, fibrin, and blood cells. Thrombosis may be caused by mutation in serpin. Antihemophilic factors V, VII, VIII, IX, XI, XII, the von Willebrand disease, tissue plasminogen activator, homocysteine and the activated protein C ratio display a genetic correlation with the incidence of thrombosis (Redei ed., 2003)\(^6\). The blood vessels in this case may be uninjured or with minor injury (fig. 4.2).

Fig. 4.2 - Highly simplified schematic diagram of the process of coagulation and thrombosis. The initiation of blood clot formation occurs following vascular injury and the exposure of tissue factor (TF) to circulating blood. Thrombin exerts a positive feedback loop (\(\text{broken arrows}\)) and additionally activates platelets and other procoagulant proteins. In addition there is variable platelet-leukocyte-endothelial cell (EC) adhesion (Source: Lang ed., 2009, http://www.springer.com/biomed/molecular/book/978-3-540-67136-7 or http://www.springerreference.com/docs/html/chapterdbid/110023.html)

According to Lang ed., 2009, thrombosis is characterized by the formation, development, or existence of a blood clot (with or without downstream embolization) within the arterial or venous vascular system. In the arterial system, this is most commonly due to thrombosis upon an atherosclerotic plaque (atherothrombosis) and in the venous system, it is usually as

a result of deep vein thrombosis (DVT) with possible pulmonary embolism (PE) [collectively referred to as "venous thromboembolism" (VTE)]. "Thromboembolism" refers to the formation of a clot (in a blood vessel) that breaks loose and is carried by the blood stream to plug another vessel. The clot may plug a vessel in the lungs (pulmonary embolism), brain (stroke), gastrointestinal tract, kidneys, or leg.

Offermanns and Rosenthal eds., 2008, noted that in general, arterial thrombi are platelet-rich ("white clots") and form at ruptured atherosclerotic plaques, leading to intraluminal occlusion of arteries that can result in end-organ injury (e.g., myocardial infarction, stroke). In contrast, venous thrombi consist mainly of fibrin and red blood cells ("red clots"), and usually form in low-flow veins of the limbs, producing deep vein thrombosis (DVT); the major threat to life results when lower extremity (and, occasionally, upper extremity) venous thrombi embolize via the right heart chambers into the pulmonary arteries, i.e., pulmonary embolism (PE).

Atherothrombosis is clinically manifested as coronary artery disease (most commonly), stroke or transient ischemic attack, and peripheral arterial disease. Thrombosis is a highly complex process involving simultaneous endothelial activation, the release of proinflammatory cytokines (particularly in the cases of atherothrombosis, e.g. C-reactive protein (CRP)), expression of adhesion molecules (e.g. P-selectin) the initiation and propagation of coagulation with simultaneous platelet activation (with platelet adhesion and aggregation). This ultimately leads to the formation of an endothelium attached platelet/fibrin plug.

Thromboembolism is a significant cause of morbidity (disease) and mortality (death), especially in adults, while, Thrombosis is the leading cause of death in the Western World with an incidence that rises with increasing age. In the case of VTE diagnostic principles include demonstration of occluded pulmonary arteries (using ventilation perfusion scanning or spiral CT), deep veins (ultrasound or venography) or the quantification endogenous fibrinolysis (e.g. D-dimers). For atherothrombosis: cardiac troponins and creatine kinase (both raised with myonecrosis), quantification of vessel luminal narrowing using arterial, stress echocardiography, nuclear cardiology, MRI, positron emission tomography and/or exercise testing (Lang ed., 2009).

Based on the above literature it is evident that thrombosis and thromboembolism are enormously dangerous pathological conditions which may lead to death and require appropriate treatment and care. According to Kreutzer, DeLuca and Caplan eds., 2011, thrombolysis the breakdown or "lysis" of blood clots is the best cure in such cases and is usually induced by using pharmacological agents. Therapeutic principles typically involve antiplatelet therapy for arterial thrombi (e.g. clopidogrel/aspirin), anticoagulation (e.g. vitamin K antagonists, heparins, factor Xa inhibitors, direct thrombin inhibitors) for VTE and in

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specific cases such as atrial fibrillation, fibrinolysis, percutaneous transluminal coronary angioplasty. Thus, in general treatment may involve use of anticoagulants, aspirin, or vasodilators (drugs that relax and widen vessels)\(^{11}\).

4.2 Anticoagulants and their effects on the coagulation cascade:
Anticoagulants are defined as substances that inhibit coagulation by preventing thrombin generation and, ultimately, fibrin formation. They represent one of the two major classes of antithrombotic drugs, the other being anti-platelet agents. Anticoagulants are widely used to treat and prevent thrombosis involving arteries, veins, and intracardiac chambers.

![Diagram of coagulation cascade and effects of anticoagulants](http://www.springer.com/biomed/molecular+science/book/978-3-540-67136-7)

Fig. 4.3: Overview of coagulation cascade and effects of anticoagulants on the coagulation cascade. Coumarin agents alter the synthesis of four procoagulant zymogens (VII, X, IX, II), shown within circles. The other anticoagulants affect various coagulation factors (dotted arrows). Abbreviations: APC, activated protein C; AT, antithrombin; DTIs, direct thrombin inhibitors; LMWH, low-molecular-weight heparin; NAPc2, nematode anticoagulant protein; TF, tissue factor, TFPI, tissue factor pathway inhibitor; UFH, unfractionated heparin; VIIa, active site-blocked VIIa (Source: Offermanns and Rosenthal eds., 2008, [http://www.springer.com/biomed/pharmaceutical+science/book/978-3-540-38916-3](http://www.springer.com/biomed/pharmaceutical+science/book/978-3-540-38916-3) or [http://www.springerreference.com/docs/html/chapterdbid/137940.html](http://www.springerreference.com/docs/html/chapterdbid/137940.html)).

An overview of coagulation cascade and effects of anticoagulants on the coagulation cascade is depicted in fig. 4.3. As can be seen from the cascade, the common anticoagulant agents include coumarin derivatives (e.g., warfarin, phenprocoumon); heparin, either unfractionated heparin (UFH) or low-molecular-weight heparin (LMWH); danaparoid (heparinoid); fondaparinux (indirect factor Xa-inhibiting pentasaccharide); drotrecogin α (recombinant human activated protein C [APC]); direct thrombin inhibitors (DTIs), including hirudin derivatives (e.g., lepirudin, desirudin, bivalirudin) and small molecule active site inhibitors (e.g., argatroban, ximelagatran). Further, Anticoagulant therapies may be short-term or long term.

Coagulation is regulated by three major inhibitory systems. (i) Antithrombin (AT, formerly, antithrombin III) inhibits circulating thrombin, Xa, IXa, Xla and TF/VII(a). However, AT does not inhibit thrombin bound to fibrin ("clot-bound thrombin") or surface-bound Xa. (ii) The protein C natural anticoagulant pathway is triggered when thrombin binds to a receptor (thrombomodulin, TM) on endothelial cell surfaces: TM-bound thrombin activates protein C to APC, which together with a cofactor (protein S) degrades factors Va and VIIIa, thus downregulating thrombin generation in the TM-rich microcirculation. (iii) Tissue factor pathway inhibitor (TFPI) binds to and inhibits factor Xa; subsequently, TFPI/Xa complexes inhibit VII(a) within VII(a)/TF. The most commonly used anticoagulants - coumarins and heparins - interfere with various steps, involving "propagation" of the coagulation cascade (Offermanns and Rosenthal eds., 2008)\textsuperscript{12}.

### 4.3 List of representative non-heparin anticoagulant products and related complications:

Some of the anticoagulant products that are available in the market for therapeutic purposes are described in the table below (Table 4.1).

<table>
<thead>
<tr>
<th>Company logo</th>
<th>Product name and application</th>
<th>Reference links</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspen pharma</td>
<td>MAREVAN® (Warfarin) Indicated for the prophylaxis and/or treatment of venous thrombosis and its extension and pulmonary embolism. MAREVAN is indicated for the prophylaxis and/or treatment of the thromboembolic complications associated with atrial fibrillation. MAREVAN is not indicated in patients with lone atrial fibrillation who are less than 60 years of age with no risk factors (e.g. previous thromboembolism (TIA, ischemic stroke), diabetes mellitus, hypertension) and an otherwise normal heart. Marevan is indicated for use as an adjunct in the treatment of coronary occlusion.</td>
<td><a href="http://www.aspenpharma.com.au/products/details/index/id/768/product/Marevan">http://www.aspenpharma.com.au/products/details/index/id/768/product/Marevan</a> and <a href="http://www.aspenpharma.com.au/product_info/pI/PI_Marevan.pdf">http://www.aspenpharma.com.au/product_info/pI/PI_Marevan.pdf</a></td>
</tr>
</tbody>
</table>

### Thrombocytopenia

Argatroban is indicated as an anticoagulant in patients with or at risk for heparin-induced thrombocytopenia undergoing percutaneous coronary intervention (PCI).

2) **ARIXTRA® (Fondaparinux)**

- Indicated for the prophylaxis of deep vein thrombosis (DVT), which may lead to pulmonary embolism (PE):
  - in patients undergoing hip, knee fracture surgery, including extended prophylaxis;
  - in patients undergoing abdominal surgery who are at risk for thromboembolic complications.
- Treatment of Acute Deep Vein Thrombosis
  ARIXTRA is indicated for the treatment of acute deep vein thrombosis when administered in conjunction with warfarin sodium.
- Treatment of Acute Pulmonary Embolism
  ARIXTRA is indicated for the treatment of acute pulmonary embolism when administered in conjunction with warfarin sodium when initial therapy is administered in the hospital.

### Table 4.2: List of common non-heparin anticoagulants and associated complications*

<table>
<thead>
<tr>
<th>Name of The Product</th>
<th>Complications</th>
<th>Reference link</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argatroban</td>
<td>• Contraindicated in patients with overt major bleeding, and people hypersensitive to argatroban</td>
<td><a href="http://us.gsk.com/products/assets/us_argatroban.pdf">Link</a></td>
</tr>
</tbody>
</table>

*For heparin based products please refer to table 6.1
| **Arixtra**  
| (Fondaparinux) | ● Bleeding complications and mild local irritations  

* For heparin based products please refer to table 7.1

Since most of the anticoagulants available in the market may induce severe complications in patients (Table 4.2). Several novel anticoagulants and innovative approaches are being explored. Thus, formulations that inhibit thrombin directly or drugs which inhibit initiation of coagulation are under investigation. However, recent market research reports indicate that Merck, Pfizer, BMS anticoagulant drugs have failed in clinical trials. Merck has unveiled that their thrombin receptor antagonist drug failed completely to beat a placebo control in a large Phase III trial in 13,000 at-risk patients. Meanwhile, Pfizer and Bristol-Myers Squibb announced that their anticoagulant hope, apixaban, did not beat heparin (in the form of enoxaparin, Lovenox) for protection against cardiovascular events, about 5,000 patient had participated in this study\(^\text{13}\).

Thus, despite their limitations, the already existing and well-established anticoagulants are being re-examined to minimize their complications and enhance their efficacy. One such anticoagulant is heparin, in the next segment of this report heparin the oldest known anticoagulant which is widely used even today is described and an attempt has been made to highlight the past, present and future of Heparin as an anticoagulant.

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5. Heparin

5.1 Heparin – Structure, types and detection:
Heparin is a negatively charged mucopolysaccharide consisting of repeated units of uronic acid (glucosamine) and glucuronic acid disulfate (Fig. 5.1). It is secreted into the bloodstream primarily by the liver and it has anticoagulant effects (Redei ed., 2003)\(^\text{14}\). Heparin is used as a non-oral anticoagulant to prevent or treat thromboembolic disorders such as stroke, myocardial infarction, peripheral artery disease, venous thrombosis, pulmonary embolism, and others (Kreutzer, DeLuca and Caplan eds., 2011)\(^\text{15}\).

\[\text{Fig. 5.1: Representative Heparin (non-sulphated) structure (Source: Redei (ed.), 2003; http://www.springer.com/biomed/human+genetics/book/978-1-4020-6753-2 or http://www.springerreference.com/docs/html/978-1-4020-6754-9_7481.html)}\]

Zhang et al., 2011, stated that heparin is a highly sulfated, linear glycosaminoglycan that is abundantly found in mucosal tissues such as the lungs and intestines. The structure of this ~10–20 kDa polysaccharide is predominantly made up of a major repeating disaccharide unit, α-L-IdoA2S (1→4)-α-D-GlcNS6S (where IdoA is idopyranosyluronic acid, S is sulfo and GlcN is 2-deoxy, 2-amino glucopyranose).\(^\text{1}\) The structure and sulfation pattern of the heparin molecule are integral to its therapeutic value\(^\text{16}\).

As described earlier, Heparins are either unfractionated heparin (UFH) or low-molecular-weight heparin (LMWH). LMWH is derived from unfractionated heparin by chemical or enzymatic depolymerization. Compared with unfractionated heparin, LMWH has reduced antifactor IIa activity relative to antifactor Xa activity, and lacks the nonspecific binding affinities of unfractionated heparin. As a result, LMWH preparations have more predictable pharmacokinetic and pharmacodynamic properties (Kulik et al., 2006)\(^\text{17}\).

The activated partial thromboplastin time (aPTT) is usually used to monitor the anticoagulant effect of UFH, with the target aPTT level corresponding to an anti-factor Xa level of 0.35-0.70 U/mL (i.e., a ratio of patient/control aPTT of 1.5-2.5 for many aPTT reagents). However,


\(^{16}\text{Zhang et al., 2011, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3205190/}\

\(^{17}\text{Kulik et al., 2006, http://ats.ctsnetjournals.org/cgi/content/full/81/2/770}\

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prolongation of the aPTT is not sufficiently great to permit monitoring of LMWH therapy by this test\textsuperscript{18}.

Bates and Weitz, 2005, stated that anti–factor Xa assays are used to measure levels of heparin and low-molecular-weight heparin (LMWH). These are chromogenic assays that use a factor Xa substrate onto which a chromophore has been linked. Factor Xa cleaves the chromogenic substrate, releasing a colored compound that can be detected with a spectrophotometer and is directly proportional to the amount of factor Xa present. When a known amount of factor Xa is added to plasma containing heparin (or LMWH), the heparin enhances factor Xa inhibition by antithrombin rendering less factor Xa available to cleave the substrate. By correlating this result with a standard curve produced with known amounts of heparin, one can calculate the heparin concentration in the plasma\textsuperscript{19}.

5.2 Heparin – History:
According to Patil et al., 2009, Heparin’s 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, hence its name (hepar or "ήπαρ" is Greek for "liver")\textsuperscript{20}.

Heparin was accidentally discovered in 1916 by Jay McLean, a second-year medical student working at the Department of Physiology of the Johns Hopkins Medical School in Baltimore, under the direction of the eminent scientist William Howell (Dinis da Gama, 2008)\textsuperscript{21}. It was Howell and Holt (1918) who coined the word heparin\textsuperscript{22}.

The Toronto heparin story began in 1928-29 when Best decided to explore its practical value. At the time only small amounts of heparin, made from dog liver, was available, but it was extremely expensive, toxic and unsafe for humans. Best, thus, had two goals: 1) to find a method to produce large amounts of pure heparin; and 2) study the effects of heparin in animals, and then humans, to control thrombosis. After early work proved encouraging, Best expanded his research team in 1929 to include Drs. Arthur F. Charles, a young organic chemist, and David A. Scott, who was closely involved in insulin production at Connaught. Soon after Charles and Scott began their work, Dr. Gordon Murray, a prominent surgeon at Toronto General Hospital, joined the team to conduct experimental surgery using heparin.

The first task of Charles and Scott was to find a cheaper source of heparin than dog liver. They turned to beef liver, readily available from local slaughterhouses, and were successful in extracting significant amounts of heparin. However, a growing pet food industry drove up...
the price of beef liver, forcing Charles and Scott to try other tissues. They found that beef lung and intestines were also good sources of heparin; the latter more plentiful and cheap. Charles and Scott first reported on this work in the fall of 1933, followed by studies of the still mysterious chemistry of heparin. Between 1933 and 1936, they succeeded in purifying and then crystallizing heparin into a standardized dry form that could be administered in a salt solution. Meanwhile, Murray conducted experimental surgery with various animals using Connaught's more potent heparin. He discovered that heparin definitely cleared up internal blood clots, and also seemed useful for many other dangerous operations where blood coagulated quickly. The first human trials began in May 1935 and soon involved hundreds of complex surgical cases during which Connaught's heparin played an essential and often dramatic life-saving role. By 1937 it was clear that Connaught's heparin was a safe, easily available and effective blood anticoagulant. Best's heparin team had opened the door to such operations as organ transplants and open heart surgery, as well as the artificial kidney that was pioneered by Murray.23

Interest in low-molecular-weight heparins (LMWHs) as potential antithrombotic agents was stimulated by two observations in the mid-1970s and early 1980s. The first was finding that LMWH fractions prepared from unfractionated heparin (UFH) progressively lost their ability to prolong the activated partial thromboplastin time (APTT) while retaining their ability to inhibit Factor Xa. The second was the observation that LMWHs prepared by chemical depolymerization of UFH are antithrombotic in experimental animal models but produce less microvascular bleeding in experimental models for an equivalent antithrombotic effect than the UFH from which they are derived (Hirsh, 1991).24

5.3 Sources of Heparin:
Warda et al., 2003, reported that commercial manufacture of heparin relies on either porcine intestinal or bovine lung tissue as the raw material.25 Linhardt and Gunay, 1999, mentioned that there are several commercially produced LMWHs that are prepared through the controlled depolymerization of pharmaceutical grade heparin. The author discussed the chemistry of the commercial processes used for manufacturing LMWHs. Further they stated that structural differences are found in the LMWHs prepared using different commercial processes. Careful control of process variables has generally resulted in the reproducible preparation of LMWHs that are structurally uniform and of high quality. The specifications, however, remain different for each LMWH.26

5.4 Mode of administration:
Hirsh et al. in 2001 stated that the preferred routes of unfractionated heparin (UFH) administration are continuous intravenous (IV) infusion and subcutaneous (SC) injection. When the SC route is selected, the initial dose must be sufficient to overcome the lower bioavailability associated with this route of administration. An immediate anticoagulant effect requires an IV bolus. Based on these pharmacokinetic limitations, heparin therapy is usually

23 Rutty; http://www.healthheritageresearch.com/Heparin-Conntact9608.html
restricted to the hospital setting, where its effect can be monitored and its dosage adjusted frequently. In contrast, LMWH preparations can be administered in either the in-hospital or out-of-hospital setting because they can be administered subcutaneously (SC) without the need for laboratory monitoring. When long term anticoagulant therapy is indicated, heparin or LMWH administration is usually followed by treatment with oral anticoagulants.

5.5 Mechanism of action:
According to Khan ed., 2011, the anticoagulant activity of heparin requires a cofactor, antithrombin III. A pentasaccharide sequence randomly distributed along one third of the heparin chains mediates the interaction between heparin and antithrombin. The heparin-antithrombin complex inactivates thrombin and thus prevents thrombin-induced activation of factors V and VII (Fig.3)\(^28\). In particular, the unique pentasaccharide sequence present in heparin having the structure, \(\text{GlcNAc6S} \rightarrow \text{GlcA} \rightarrow \text{GlcNS3S6S} \rightarrow \text{IdoA2S} \rightarrow \text{GlcNS6S} \rightarrow\) (where GlcA is D-glucopyranosyluronic acid and Ac is acetyl), is responsible for its specific binding to the serine protease inhibitor antithrombin III (AT) resulting in its conformational activation and leading to the inhibition of major coagulation cascade proteases, including thrombin (factor (F) IIa) and FXa\(^29\).

5.6 The major applications of Heparin are listed below\(^30, 31\):
- For prophylaxis and treatment of venous thrombosis
- For prevention of post-operative deep venous thrombosis, pulmonary embolism, venous thromboembolism
- For prevention of clotting in arterial and cardiac surgery
- In cardiology for prevention of embolisms in patients with atrial fibrillation
- It is used as an adjunct antithrombin therapy in patients with unstable angina and/or non-Q wave myocardial infarctions (i.e. non-ST elevated acute coronary artery syndrome) who are on platelet glycoprotein (IIb/IIIa) receptor inhibitors
- For prevention of clotting during dialysis and surgical procedures
- For maintaining the patency of intravenous injection devices
- For preventing in vitro coagulation during blood transfusions and in blood samples drawn for laboratory testing

There is a considerable body of experimental evidence that heparin is superior as an anticoagulant to any prothrombin depressing drugs\(^32\), thus it has numerous therapeutic applications. The next section of the report discusses a few Heparin products manufacturers.

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\(^{29}\) Zhang et al., 2011; [http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3205190/](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3205190/)

\(^{30}\) Heparin (DurgBank); [http://www.drugbank.ca/drugs/DB01109](http://www.drugbank.ca/drugs/DB01109)


\(^{32}\) Engelberg, 1959; [http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1577959/](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1577959/)
6. Heparin manufacturing companies

6.1 List of representative Heparin based products:
Several pharmaceutical companies manufacture heparin for various clinical uses; table 6.1 gives a list of representative marketed heparin based products.

Table 6.1: List of representative Heparin based products

<table>
<thead>
<tr>
<th>Company logo</th>
<th>Product name and application</th>
<th>Reference links</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pfizer inc.</td>
<td>1) Heparin Sodium Injection (Heparin)</td>
<td><a href="http://labeling.pfizer.com/ShowLabeling.aspx?id=665">http://labeling.pfizer.com/ShowLabeling.aspx?id=665</a></td>
</tr>
<tr>
<td></td>
<td>• Prophylaxis and treatment of venous thrombosis and pulmonary embolism</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Prophylaxis and treatment of thromboembolic complications associated with atrial fibrillation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Treatment of acute and chronic consumption coagulopathies (disseminated intravascular coagulation)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Prevention of clotting in arterial and cardiac surgery</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Prophylaxis and treatment of peripheral arterial embolism</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Anticoagulant use in blood transfusions, extracorporeal circulation, and dialysis procedures</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2) Fragmin®</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Dalataparin) Injection is indicated for the prophylaxis of ischemic complications in unstable angina and non-Q-wave myocardial infarction, when concurrently administered with aspirin therapy (as described in clinical trials, Prophylaxis of Ischemic Complications in Unstable Angina and Non-Q-Wave Myocardial Infarction)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FRAGMIN is also indicated for the prophylaxis of deep vein thrombosis (DVT), which may lead to pulmonary embolism (PE):</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• In patients undergoing abdominal surgery who are at risk for thromboembolic complications</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• In medical patients who are at risk for thromboembolic complications due to Severely restricted mobility during acute illness</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FRAGMIN is also indicated for the extended treatment of symptomatic venous</td>
<td></td>
</tr>
<tr>
<td><strong>Thromboembolism (VTE) (proximal DVT and/or PE), to reduce the recurrence of VTE in patients with cancer</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SANOFI</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sanofi</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lovenox®</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Enoxaparin) is a low molecular weight heparin [LMWH] indicated for:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Prophylaxis of deep vein thrombosis (DVT) in abdominal surgery, hip replacement surgery, knee replacement surgery, or medical patients with severely restricted mobility during acute illness.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Inpatient treatment of acute DVT with or without pulmonary embolism.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Outpatient treatment of acute DVT without pulmonary embolism.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Prophylaxis of ischemic complications of unstable angina and non-Q-wave myocardial infarction [MI]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Treatment of acute ST-segment elevation myocardial infarction [STEMI] managed medically or with subsequent percutaneous coronary intervention.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><a href="http://products.sanofi.us/lovenox/lovenox.html">http://products.sanofi.us/lovenox/lovenox.html</a></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Leo Pharma</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1) Heparin LEO®</strong></td>
</tr>
<tr>
<td>2) Innohep®</td>
</tr>
<tr>
<td>(tinzaparin sodium)LMWH is indicated for the prevention of postoperative venous thromboembolism in patients undergoing orthopaedic surgery and in patients undergoing general surgery who are at high risk of developing postoperative venous thromboembolism</td>
</tr>
<tr>
<td>INNOHEP is indicated for the treatment of deep vein thrombosis and/or pulmonary embolism. INNOHEP is indicated for the prevention of clotting in indwelling intravenous lines for haemodialysis and extracorporeal circulation in patients without high bleeding risk.</td>
</tr>
<tr>
<td><a href="http://www.leopharma.ca/C1256AD9004FA5C9/sysOakFil/innohep%20PM%20Feb03/$File/INH-PM-E,Feb03.pdf">http://www.leopharma.ca/C1256AD9004FA5C9/sysOakFil/innohep%20PM%20Feb03/$File/INH-PM-E,Feb03.pdf</a></td>
</tr>
</tbody>
</table>
6.2 Heparin Coated Dialyzers and their manufacturers:
Heparin is the most commonly used anticoagulant in hemodialysis procedure which requires access to the circulatory system. As unfractionated heparin (UFH) is negatively charged, UFH can adhere to dialyzer surfaces. As such several groups have used 20,000 IU of heparin in the priming solution, rinsing through the extracorporeal circuit for 0.5–1.0 h, and then rinsing with normal saline, to then provide intermittent hemodialysis in patients at risk of bleeding, without any additional anticoagulant. Subsequently several manufacturers have developed heparin-bonded dialyzers (Duraflo®, Baxter, Deerborne, Illinois, USA, AN69®, Hospal, Lyon, France), which have allowed successful intermittent hemodialysis without additional anticoagulation33.

7. Heparin Therapy – Complications and Controversies

7.1 Heparin overdose:
Both the anticoagulant effects and the adverse effects of heparin increase at higher doses. In some cases overdose has proved to be fatal. It was reported in March 2010, that a 2 year old toddler died due to overdose of heparin. It was alleged at the time that the manufacturer of the heparin involved had failed to distinctly differentiate between adult- and infant-strength heparin. The vials were of similar shape and labelling, possibly leading to overdose via administration of adult strength heparin\textsuperscript{34}. Another limitation of heparin arises due to non-oral route of administration. Because of the need for administration by injection, heparin often is not continued long term and also reduces patient compliance and acceptability. Section 7.2 and Table 7.1 highlight some of the complications related to heparin based products.

7.2 Adverse effects\textsuperscript{35, 36, 37, 38, 39, 40}.
Most adverse consequences of heparin derive from its anticoagulant effect these include:
- Bleeding
- Immune mediated skin lesions
- Allergic reactions
- Elevation of transaminase
- Hyperkalemia
- Alopecia (chronic use)
- Skeletal defects - Osteoporosis with prolonged use, likely because heparin both decreases bone formation by osteoblasts and increases bone resorption by osteoclasts
- Heparin-induced thrombocytopenia (“HIT”)
\textsuperscript{#}, which causes a severe reduction in the number of platelets, the blood clotting cells.

\textsuperscript{#}As many as 3-5\% of postoperative patients who receive UFH for 2 weeks develop heparin-induced thrombocytopenia (HIT). This hypercoagulable state is caused by IgG antibodies that recognize complexes between heparin and platelet factor 4 (a platelet α-granule protein). Paradoxically, patients with HIT remain at high risk for thrombosis.

\textsuperscript{34} Gibb, 2010; \texttt{http://www.lawyersandsettlements.com/articles/heparin/heparin-overdose-infant-13889.html}
\textsuperscript{36} Maldonado et al., 2012; \texttt{http://www.ncbi.nlm.nih.gov/pubmed/22998541}
\textsuperscript{37} Bengalorkar et al., 2011; \texttt{http://www.ncbi.nlm.nih.gov/pubmed/22025855}
\textsuperscript{38} Guevara et al., 1993; \texttt{http://www.ncbi.nlm.nih.gov/pubmed/8468111}
\textsuperscript{39} Gervin, 1975; \texttt{http://www.ncbi.nlm.nih.gov/pubmed/1170648}
\textsuperscript{40} Nelson-Pierrcy, 1997; \texttt{http://www.ncbi.nlm.nih.gov/pubmed/9488788}
### Table 7.1: List of common heparin anticoagulants and associated complications

<table>
<thead>
<tr>
<th>Name of The Product</th>
<th>Complications</th>
<th>Reference link</th>
</tr>
</thead>
</table>
| **Lovenox (Enoxaparin)** | • Increased risk of spinal/epidural hematoma, thrombocytopenia, Haemorrhage  
• Elevation in Serum Amniotransferase  
• Response of hypersensitivity to enoxaparin | [http://products.sanofi.us/lovenox/lovenox.html#section-8.6](http://products.sanofi.us/lovenox/lovenox.html#section-8.6) |
| **Fragmin (Daltaparin)** | • Risk of haemorrhage  
| **Innohep (Tinzaparin)** | • Risk of HIT  
• Acute or sub-acute septic Endocarditic  
• Active bleeding from a local lesion such as an acute ulcer  
• Allergy and hypersensitivity to severe hypertension | [http://www.leo-pharma.ca/C1256AD9004FA5C9/sysOakFil/innhep%20PM%20Feb03/$File/INH-PM-E,Feb03.pdf](http://www.leo-pharma.ca/C1256AD9004FA5C9/sysOakFil/innhep%20PM%20Feb03/$File/INH-PM-E,Feb03.pdf) |

### 7.3 Controversies - Contamination and recalls:

In 2007, a retrospective cohort study found that syringes prefilled with heparin flush solution caused an outbreak of *Serratia marcescens* bloodstream infection at an outpatient treatment center in Texas (Su, 2009)\(^4^1\). A total of 11 countries recalled heparin products because of severe side effects, which included 62 deaths reported to the FDA on or after January 1, 2008, in the USA (Dietz et al., 2009)\(^4^2\). Thus, a worldwide recall of heparin occurred. Scientists determined that a contaminant known as oversulfated chondroitin sulfate was responsible for the numerous deaths and adverse events. This contaminant was first traced to a chemical plant in Changzhou, China (Hedlund et al., 2012)\(^4^3\). Table 7.2 includes the details on the two contaminants discussed here.

---


Table 7.2: Heparin products contaminants

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Manifestation</th>
<th>Reference link</th>
</tr>
</thead>
</table>
| OSCS (oversulfated chondroitin sulphate) | Causes severe allergic reactions, sudden drop in blood pressure, difficulty in breathing and abdominal distress | http://www.nigms.nih.gov/News/Results/FDANIGMS04232008.htm  
http://www.lawyersandsettlements.com/case/heparin.html |
| Serratia marcescens                 | Bacterial Infection which could result in life-threatening injuries and/or Death | http://www.ncbi.nlm.nih.gov/pubmed/21492963 |

7.4 Heparin impurities:
Considering the animal source of pharmaceutical heparin, the numbers of potential impurities are relatively large compared with a wholly synthetic therapeutic agent. The range of possible biological contaminants includes viruses, bacterial endotoxins, transmissible spongiform encephalopathy (TSE) agents, lipids, proteins and DNA. During the preparation of pharmaceutical-grade heparin from animal tissues, impurities such as solvents, heavy metals and extraneous cations can be introduced44.

44 Beni et al., 2011: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3015169/
8. Approaches to encounter heparin products limitations

8.1 Combating Overdose issues:
Complications related to overdose of heparin may be resolved by establishing and maintaining optimal dosing can be accomplished by monitoring the results of a simple blood test, called “partial thromboplastin time” or PTT\(^45\). Use of antagonists such as Protamine sulphate for the treatment of heparin overdose is also widely accepted\(^46\).

In response to potential heparin related medication errors some companies have created distinct packaging and labelling alternatives for heparin products (Table 8.1).

### Table 8.1: Representative companies providing safer heparin packaging solutions

<table>
<thead>
<tr>
<th>Logo</th>
<th>Product details</th>
<th>Reference link</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="logo.png" alt="Sagent Pharmaceutical" /></td>
<td><strong>HEPARIN with Prevent IV Measures(^TM)</strong> Packaging and Labeling helps promote immediate drug and dose recognition for every medical professional who handles a medication – from the pharmacy to the patient’s bedside and at every point in between.</td>
<td><a href="http://www.sagentpharma.com/heparin-sodium-injection-usp.html">http://www.sagentpharma.com/heparin-sodium-injection-usp.html</a></td>
</tr>
<tr>
<td><img src="logo.png" alt="Wockhardt UK Limited" /></td>
<td>Several heparin products e.g. Heparin sodium Entire Heparin range will also be available in Wockhardt’s new safer packaging design, which has been reviewed and commended by the NPSA</td>
<td>[<a href="http://www.wockhardt.co.uk/siteimages/News">http://www.wockhardt.co.uk/siteimages/News</a> Document/New%20Heparin%20Descriptions.pdf](<a href="http://www.wockhardt.co.uk/siteimages/News">http://www.wockhardt.co.uk/siteimages/News</a> Document/New%20Heparin%20Descriptions.pdf)</td>
</tr>
</tbody>
</table>

8.2 Oral heparin for increased patient compliance and acceptability:
According to Paliwal et al., 2012, oral heparin may serve as an alternative to both parenteral heparin as well as presently available oral anticoagulants such as warfarin\(^47\). As described in below, several attempts are being made in obtaining a suitable and stable oral heparin formulation (table 8.2, patent and fig. 8.1 given below).

---

\(^45\) Smythe et al., 2001: [http://171.67.112.51/content/115/1/148.full.pdf](http://171.67.112.51/content/115/1/148.full.pdf)


Table 8.2: A company with oral heparin formulation in its product pipeline

<table>
<thead>
<tr>
<th>Logo</th>
<th>Product</th>
<th>Reference link</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emisphere technologies</td>
<td>Oral Heparin: Oral heparin could represent a significant opportunity for Emisphere and, in theory, would meet unmet market needs. However, realistically, at this stage in its development, it presents significant challenges.</td>
<td><a href="http://www.emisphere.com/oral_heparin.html">http://www.emisphere.com/oral_heparin.html</a></td>
</tr>
</tbody>
</table>

Further, as the titles depicted in fig. 8.1 imply, several pipeline studies are in process for exploring prospects of successfully establishing oral heparin formulations as pharmaceutical products.

In 2007 a USA patent was granted in relation to solid heparin tablet the details are given below:

**Title:** Anticoagulant composition

**Patent number:** US7202087

**Issue date:** Apr 10, 2007

**Inventors:** Bengt Herslöf, Per Tingvall

**Original assignee:** LTP Lipid Technologies Provider AB


**Abstract:** A solid heparin tablet composition has a melting point of 25° C. or higher and is a continuous lipid component containing one or more polar lipids, one or more non-polar lipids, optionally one or several of water and mono- to trivalent alcohol in an amount of up to 15% by weight of the composition, and native heparin or fractionated heparin. Also described is a
corresponding tablet, processes for production of the composition and the tablet, and a method of preventing or treating conditions amenable to preventive or therapeutic treatment by administration of the tablet.

**Data source:** United States Patent and Trademark Office (USPTO)

### 8.3 Flask synthesis approach to prevent presence of contaminants and impurities:
Based on a news article titled “Flask synthesis promises untainted heparin” published online by Chemistry World in 2008, US researchers have created milligrams of pure heparin using enzymes and chemicals - a practical laboratory synthesis that could avoid the contamination issues surrounding the blood-thinning drug, which currently has to be sourced from animal tissue. Robert Linhardt and his colleagues at Rensselaer Polytechnic Institute in Troy believe they will be able to scale up the process sufficiently to carry out clinical trials on their artificial heparin in as little as five years. Linhardt presented the work at the American Chemical Society's Fall 2008 meeting in Philadelphia⁴⁸.

It is evident from the data presented in this report that anticoagulants such as heparin are highly significant in treatment of several diseases and as is emphasized by the literature insight provided in the next section several aspects related to anticoagulants are under investigations.

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⁴⁸ Bhattacharya, 2008; [http://www.rsc.org/chemistryworld/News/2008/August/19080803.asp](http://www.rsc.org/chemistryworld/News/2008/August/19080803.asp)
9. Summary

In vertebrates, the cardiovascular system supplies oxygen and nutrients to all parts of the body and removes carbon dioxide and other wastes from body cells via a specialized fluid called blood.

A blood clot occurs due to a natural physiological process "Hemostasis", the process which spontaneously arrests the flow of blood from vessels carrying blood under pressure. It is accomplished by contraction of the vessels, adhesion and aggregation of formed blood elements (e.g. Erythrocyte aggregation), and the process of blood coagulation.

A blood clot/thrombus may also occur due to a process called “Thrombosis”, which represents a pathologic state in which there is formation of thrombus from the elements of circulating blood.

“Thromboembolism" refers to the formation of a clot (in a blood vessel) that breaks loose and is carried by the blood stream to plug another vessel.

Thrombolysis is the breakdown or "lysis" of blood clots and is usually induced by using pharmacological agents. Therapeutic principles typically involve Antiplatelet therapy and anticoagulation therapy.

Anticoagulants are defined as substances that inhibit coagulation by preventing thrombin generation and, ultimately, fibrin formation.

Coagulation is regulated by three major inhibitory systems. (i) Antithrombin (AT, formerly, antithrombin III) inhibits circulating thrombin, Xa, IXa, XIa and TF/VII(a). (ii) The protein C natural anticoagulant pathway is triggered when thrombin binds to a receptor (thrombomodulin, TM) on endothelial cell surfaces. (iii) Tissue factor pathway inhibitor (TFPI) binds to and inhibits factor Xa; subsequently, TFPI/Xa complexes inhibit VII(a) within VII(a)/TF.

Several anticoagulant pharmaceutical products are available in the markets presently (described earlier). However, most of these anticoagulants available may induce severe complications in patients.

Numerous innovative agents that can inhibit thrombin directly have been or are in the process of being introduced as pharmaceutical drugs. However, these innovative agents have their own limitations.

Thus, despite their limitations, the already existing and well-established anticoagulants are being re-explored to minimize complications one such anticoagulant is heparin. Fig. 9.1 gives the schematic representation of heparin research till date (all aspects have been described in details earlier in the report).
Thus, it may be stated that anticoagulants are essential for treatment and prevention of thrombosis. Presently, anticoagulant based drugs are indispensible in numerous medical procedures, disease prophylaxis, treatment and cure. Despite its limitations heparin is one of the most commonly used anticoagulants till date. Further, it presents a suitable candidate for further research and development to create an “ideal” anticoagulant formulation with universal acceptance in future.
10. Appendix: SpringerLink (www.springerlink.com)
Literature insight – A few citations related to anticoagulant research (2000-2012)

- Ibeagha-Awemu et al. in 2012, based on their results stated that sodium heparin should be the preferred anticoagulant for use in the reliable quantification of the surface expression of mCD14. Furthermore, measurement of mCD14 is best carried out in whole blood samples, both for neutrophils and monocytes\(^49\).

- In 2011, Dettoni et al. reported that the "discontinue drug, and delay surgery" strategy is not suitable for patients on anticoagulant (warfarin) therapy affected by a hip fracture\(^50\).

- Shukala and Bhatia in 2010 stated that outcome of patients with hepatic venous outflow tract obstruction (HVOTO) has improved with newer treatments, including anticoagulants, radiological interventions and liver transplant. In their study consecutive patients with HVOTO, treated with oral anticoagulation and supportive medical therapy but no radiological or surgical intervention, were followed up for at least 12 months. Diagnosis of HVOTO was based on color Doppler, and either angiography or magnetic resonance venography. Warfarin dose was adjusted to maintain international normalized ratio (INR) between 2.0 and 3.0. Patients with secondary HVOTO and those with baseline INR \(\geq 2.0\) were excluded. They concluded that more than half of patients with HVOTO show response with only supportive medical therapy and anticoagulants. This occurs more often in patients with low CP score. Some patients may have delayed response\(^51\).

- M118 is a novel LMWH with low polydispersity and pronounced anti-Xa and anti-thrombin (IIa) activity as compared to current LMWHs was studied by Chakrabarti et al., in 2009. To determine if M118 is effective in preventing thrombosis in the setting of a vascular plaque, apolipoprotein E knockout mice fed a high fat diet were injected with M118, enoxaparin, unfractionated heparin, or saline control and examined for arterial thrombosis using a rose bengal laser induced carotid artery injury model\(^52\).

- Gao et al. 2008, introduced Fucoidan (FC), an effective anticoagulant constituent extracted from brown algae into silk fibroin (SF) for improving its blood compatibility. The testing results indicated that the introduction of FC increased the roughness,

\(^{49}\) Ibeagha-Awemu et. al., (2012), The influence of different anticoagulants and sample preparation methods on measurement of mCD14 on bovine monocytes and polymorphonuclear neutrophil leukocytes, BMC Research Notes, 5: 93.

\(^{50}\) Dettoni et. al., (2011), Influence of timing and oral anticoagulant/antiplatelet therapy on outcomes of patients affected by hip fractures, European Journal of Trauma and Emergency Surgery, 37: 511-518.


\(^{52}\) Chakrabarti et. al., (2009), M118, a novel low-molecular weight heparin with decreased polydispersity leads to enhanced anticoagulant activity and thrombotic occlusion in ApoE knockout mice, Journal of Thrombosis and Thrombolysis, 28: 394-400.
hydrophilicity and sulfate component of the film surface without impeding the formation of β-sheet conformation in SF. More important, FC brought excellent anticoagulant activity and better endothelial cell affinity to SF. The SF/FC blend film was hopeful to be used as blood-contacting biomaterials.

- Based on their study in 2007, Grabovac et al., concluded that the co-administration of papain with heparin represents a new approach in improvement of absorption and bioavailability of orally administered heparin.

- Rost et al., 2006, in their study Site-Directed Mutagenesis of VKORC1, the Target Protein of Coumarin-Type Anticoagulants, supported the hypothesis of different binding sites for vitamin K epoxide and Coumarins and underlines the crucial roles of the coumarin binding motif TYA and the thioredoxin motif CXXC:

- From their study Schryver et al., 2005, concluded that as found in the literature on nonadherence in general, age of ≥ 65 years and a higher dose of aspirin (300 mg versus 30 mg) were independently associated with non–adherence with aspirin treatment that was prescribed for secondary prevention after cerebral ischaemia of arterial origin. Older patients may require extra encouragement to continue antithrombotic treatment. Lower doses of aspirin may improve treatment adherence.

- Park et al., 2004, in the abstract on their study entitled “Anticoagulant activity of heterochitosans and their oligosaccharide sulfates” described that for their study three kinds of partially deacetylated heterochitosans, 90% deacetylated chitosan, 75% deacetylated chitosan and 50% deacetylated chitosan, were prepared from crab chitin by N-deacetylation with 40% sodium hydroxide solution for different durations. Nine kinds of heterochitooligosaccharides (hetero-COSs) with relatively high molecular weights (5,000–10,000 Da; 90-HMWCOSs, 75-HMWCOSs, and 50-HMWCOSs), medium molecular weights (1,000–5,000 Da; 90-MMWCOSs, 75-MMWCOSs, and 50-MMWCOSs), and low molecular weights (below 1,000 Da; 90-LMWCOSs, 75-LMWCOSs, and 50-LMWCOSs) were prepared using an ultrafiltration membrane reactor system, respectively. In addition, their sulfated derivatives were prepared by a method using a trimethylamine-sulfur trioxide, and the anticoagulant properties of the heterochitosans and their COS sulfates with different chain lengths and degrees of deacetylation were investigated. Clotting times in thrombin-time assay were prolonged in the presence of various concentrations of the heterochitosans and their COS sulfates using normal human plasma. The 90% deacetylated chitosan sulfate exhibited the highest anticoagulant activity among all the heterochitosans and their COS sulfates.

55 Rost et. al., (2006), Site-Directed Mutagenesis of VKORC1, the target of coumarin-type anticoagulants, 35th Hemophilia Symposium, VII., VIIe., 242-244.
• Kleindienst et al., in 2003, interpreted from their 3 year period based analysis of records of patients admitted for elective neuro-surgery (ES), head injury (HI) or spontaneous intracranial haemorrhage (ICH) that in neurosurgical patients, antithrombotic prophylaxis with certoparin was determined to be safe and efficacious when contraindications are carefully considered and a 12-hour time interval before and after surgery was guaranteed. This retrospective analysis should encourage a prospective trial of early LMWH prophylaxis.

• Bemt et al., 2002, reported that oral anticoagulant therapy is initiated in most hospitals in The Netherlands by clinicians who routinely dose oral anticoagulants (without using an algorithm). This may explain the low proportion of patients leaving the hospital stabilized. To test this hypothesis this study compared the dosing of acenocoumarol in orthopedic and surgical patients using an algorithm with routine dosing. According to the authors, the algorithm provides a safe dosing schedule for elderly postoperative patients who use low molecular weight heparin and NSAIDs concomitantly and are thus at high risk for bleeding complications.

• Kim et al. 2001, carried out investigations in which a series of batch, fed-batch, and continuous cultures was carried out to analyze the effects of methanol on the fermentation characteristics of recombinant *Hansenula polymorpha* for the production of hirudin, an anticoagulant. Hirudin expression efficiencies were greatly influenced by the methanol concentrations in continuous and fed-batch culture modes.

• Tilanus et al., 2000, studied the relationship between anticoagulant medication and massive intraocular hemorrhage in age-related macular degeneration. According to the authors a massive intraocular hemorrhage in the course of age-related macular degeneration (AMD) is a devastating event. In the study the authors set out to determine the role of anticoagulant therapy prescribed for vascular or cardiac indications in the development of a massive hemorrhage by conducting a retrospective case-controlled study was conducted of 50 cases of age-related macular degeneration complicated by massive subretinal and vitreous hemorrhage, the control group consisted of 50 cases of AMD with small subretinal hemorrhage.

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