100 Years of Heparin Discovery: Would Heparins Survive the Challenge?

Shaker A. Mousa, PhD, MBA, FACC, FACB
Professor of Pharmacology, Vice Provost, Executive VP & Chairman

Pharmaceutical Research Institute (PRI)
Albany College of Pharmacy and Health Sciences
Albany, NY USA
<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
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<tbody>
<tr>
<td>1916</td>
<td>Discovery of heparin</td>
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<tr>
<td>1936</td>
<td>Charles and Scott described the preparation of heparin from bovine lung</td>
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<tr>
<td>1936-1940</td>
<td>Clinical use of heparin</td>
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<tr>
<td>1938</td>
<td>Food and Drug Cosmetic Act (safety of drug product)</td>
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<tr>
<td>1939</td>
<td>First NDA on bovine heparin approved</td>
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<tr>
<td>1940s</td>
<td>Additional heparin applications approved</td>
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<tr>
<td>1954</td>
<td>Marks, Truscott and Withcombe published the earliest case series of patients with thrombosis</td>
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<td>1960</td>
<td>Barritt and Jordan published the first randomized trial on the use of heparin</td>
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<td>1962</td>
<td>US Congress passed the Kefauver-Harris amendment requiring pre-marketing proof of effectiveness</td>
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<tr>
<td>1966</td>
<td>FDA contracted with the National Academy of Sciences and National Research Council to validate the efficacy of drugs approved during the period of 1938-1962.</td>
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<tr>
<td>1970</td>
<td>Federal register Notice – heparin labeling was revised. No distinction between bovine and porcine heparin.</td>
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<tr>
<td>1950-1970</td>
<td>Heparin-induced thrombocytopenia was found to be an adverse effect of heparin.</td>
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<tr>
<td>1990</td>
<td>Bovine heparin was withdrawn due to concerns about BSE (Mad Cow Disease).</td>
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<tr>
<td>2008</td>
<td>Porcine Heparin contaminant crisis.</td>
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<tr>
<td>2015</td>
<td>FDA’s interest in reintroducing of bovine mucosal heparin;</td>
</tr>
<tr>
<td>2016</td>
<td>Need for guidance document.</td>
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• Heparin celebrated its first 100 Years since its discovery in 1916 at Johns Hopkins University.
• Heparin and its improved version LMWH have polypharmacological actions at various levels. (1980 – Present)
• Earlier studies focused on the anti-thrombin binding and plasma anti-Xa and anti-IIa pharmacodynamics (PD) for the different LMWHs.
• These diverse pharmacological actions include the release of the vascular TFPI, inhibition of inflammation (NFkB), inhibition of Compliments, inhibition of key matrix-degrading enzymes and heparinases, selectin modulation, inhibition of platelet-cancer cell adhesion, and other mechanisms.
Heparin Depolymerization Processes for LMWH Production

How is the endogenous heparin chain depolymerized?

ATIII = antithrombin III

A pentasaccharide (5 saccharides) is the smallest sequence that has affinity for ATIII
# Molecular and Chemical Characteristics of LMW Heparins Derived from Porcine UFH

<table>
<thead>
<tr>
<th>LMW Heparins</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nadroparin</td>
<td>Presence of 2,5-anhydro-D-mannose at reducing terminus</td>
</tr>
<tr>
<td>Enoxaparin</td>
<td>Presence of 4,5 unsaturated uronic acid at non-reducing terminus</td>
</tr>
<tr>
<td>Dalteparin</td>
<td>Presence of 2,5-anhydro-D-mannose at reducing terminus</td>
</tr>
<tr>
<td>Certoparin</td>
<td>Presence of 2,5-anhydro-D-mannose at reducing terminus</td>
</tr>
<tr>
<td>Tinzaparin</td>
<td>Presence of 4,5-unsaturated uronic acid at non-reducing terminus</td>
</tr>
<tr>
<td>Reviparin</td>
<td>Presence of 2,5-anhydro-D-mannose at reducing terminus</td>
</tr>
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Anticoagulants

Parenteral

- Heparins
  - Unfractionated LMWHs
  - U-LMWHs
  - Oligosaccharides
- Anti-Thrombin
  - Argatroban
  - Desirudin
  - Bivalirudin

Oral

- Vitamin K Antagonists
  - Warfarin
  - Phenprocoumon
  - Acenocoumarol
- Non-Vitamin K antagonist oral anticoagulants
  - Dabigatran
  - Rivaroxaban
  - Apixaban
  - Edoxaban
From Heparin to Heparin Fractions

Unfractionated Heparin (UFH)
- **UFH**
  - 15 kDa
  - Anti-Xa/IIa = 1.0

Low Molecular Weight Heparin
- ~4-7 kDa
- Anti-Xa/IIa = 2-8

Ultra LMWH
- 1-3 kDa
- Anti-Xa/IIa = 10-50

Synthetic Oligosaccharides
- Pentasaccharide
  - <2 kDa
  - Pure Anti-Xa

Only 20% of heparin components are anticoagulants; the other 80% exhibit multiple pharmacological actions which are not yet fully understood.
Some of the commercially available LMWHs
Generic Enoxaparins
Variations in composition & pharmacologic profiles
Lessons learned and Future Directions

• Diversify Sources and Suppliers
• Control supply chain
• Update analytical, QC, and Pharmacopeia methods
• Re-consider process equivalence in developing generics
• Develop an improved understanding of heparin and glycosaminoglycan biology/pharmacology
• Consider non-animal sources of heparin
Heparin is an essential drug for the practice of modern medicine with approximately 100,000 daily doses in the US.

Heparin currently used in the US is prepared entirely from porcine intestine.

The supply chain for heparin is fragile.

- The supply chain was contaminated in 2007-8 resulting in over 180 deaths in the US.
- Since heparin is sourced from a single species diseases in pigs can threaten its supply.
- There are a limited number of pigs from which heparin can be prepared limiting its future availability.
- Since most heparin comes from a single country its supply is considered unstable.
100 More Years for Expanded Uses of Heparin

- Improved preparation and synthesis of heparins from various sources
- New heparin mimetic
- New therapeutic applications for heparin/heparan sulfate
- Structure-activity relationships of heparin, LMWHs and heparin oligosaccharides for inflammation, Vascular, cancer and anticoagulant activities
- Oral Delivery of heparins and other GAGs
Heparin Beyond Anti-Coagulation

- Growth Factor
- Anti-Cancer
- Anti-Inflammatory
- Anti-Coagulant
- Thrombus
- TFPI
- NO
- PGI₂
- TNF-α
- CRP
- Matrix Proteins
Isolation and characterization of Heparin from Camel Organs

Quantification of isolated GAGs by Carbazole assay

<table>
<thead>
<tr>
<th>Camel Organ</th>
<th>Heparin (mg / gram tissue)</th>
</tr>
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<tbody>
<tr>
<td>Intestine</td>
<td>2.26 ± 0.02</td>
</tr>
<tr>
<td>Liver</td>
<td>0.1 ± 0.01</td>
</tr>
<tr>
<td>Lung</td>
<td>0.80 ± 0.0054</td>
</tr>
</tbody>
</table>
Analysis of Isolated GAGs Properties by PAGE

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Weight Average</th>
<th>Polydispersity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestine</td>
<td>15,308</td>
<td>1.45</td>
</tr>
<tr>
<td>Liver</td>
<td>13,052</td>
<td>1.40</td>
</tr>
<tr>
<td>Lung</td>
<td>16,570</td>
<td>1.42</td>
</tr>
<tr>
<td>Lung</td>
<td>16,812</td>
<td>1.43</td>
</tr>
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Heparin is biosynthesized in the Golgi of cells.
Chemo-enzymatic synthesis of heparins

100,000-L fermentation affording 1 metric ton (10g/L) heparosan, chemically modified and enzymatically transformed with 4 enzymes (1-10 kg/each) and PAPS (1 kg with 5000x recycling) to afford 2 metric tons heparin run 50 times/y to meet annual world supply (100 tons) at $18,000/kg

Expanded Uses of Heparins

- Heparin and its derived LMWHs used in thromboembolic disorders in *hundreds of millions of patients*

- Polypharmacological activities

- Heparin derivatives has great potential for treatment of many complex diseases - Sickle Cell Disease, fibrosis, inflammatory diseases, and cancer.

- The only option in the prevention and treatment of VTE during Pregnancy
How it works: Multiple pathways targeted by UFH/LMWH

LMWH has a powerful multi-modal mechanism of action – but use in other indications is prevented by the narrow safety margin (risk of bleeding)
Heparins in Sickle Cell Disease
Clinical Proof of Concept

Randomized, double-blind, placebo controlled, multi-center Phase 2 clinical study with tinzaparin

- 250 patients
- Lead investigator Mohamed Qari, King Abdulaziz Medical University, Jeddah, Saudi Arabia

Results:

- Tinzaparin significantly shortened duration of hospitalization and of VOC crisis by ~40%
- Significant and faster resolution of pain, p < 0.01

Qari et al., 2007, *Thrombosis and Haemostasis*
S-NACH Innovation: Open 1 Pentasaccharide Ring **Eliminates** Bleeding Risk – **But Maintains All Other Activities**

Conventional LMWH

- Anti-adhesive P, L, E selectins
- Anti-coagulation IIa, Xa
- Anti-angiogenic VEGF, FGF, HGF
- Endothelial relaxation ↑NO
- Anti-thrombotic Factor VIIa
- Anti-fibrotic Block TGF-β

S-NACH

- Anti-adhesive P, L, E selectins
- Anti-coagulation IIa, Xa
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- Endothelial relaxation ↑NO
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Heparin and Cancer - Summary

- 90% of cancer deaths are due to metastasis
- Surgery exacerbates metastasis

**Unique opportunity:** short-term administration as adjunct to surgery to improve survival – by preventing metastasis

- Complimentary to other therapies

**Proofs of Concept**

- *Multiple clinical trials with LMWHs in many cancers show improvement in cancer-associated thrombosis and perhaps in survival* depending on the tumor types and the duration of treatment

- *Numerous animal studies show S-NACH has superior efficacy and safety vs. LMWHs in multiple cancer models*

Heparin and Heparinomimetics Beyond Newer Anticoagulants

- Pleotropic with multiple targets with broad applications.
  
- Resourcing of heparin and heparinoids including sulodexide and danaparoid.
  
- Synthetic and biosynthetic heparin related drugs including pentasaccharide.
  
- Orally bioavailable heparins and heparinoids (sulodexide).
  
- Blended heparins
Innovations with Heparins and Related Drugs

- Anticancer effects of heparin
- Fertility and reproductive biology
- Endogenous glycosylation and biologic regulation
- Molecular and structural analysis utilizing advanced methods and techniques
- Immune and inflammation modulation, Vascular hemostasis, and Complement modulations
Heparin in Anti-Coagulation and Beyond

Would Heparin Survive the Challenge?

Growth Factor

TFPI

Anti-Cancer

PGI₂

NO

Anti-Inflammatory

Selectins

HEPARIN

Anti-Coagulant

TNF-α

CRP

Thrombus

Matrix

Proteins

Heparinases