HEPARIN CENTENNIAL SYMPOSIUM

Scientific and Clinical Developments and their Impact on the Understanding of the Pathogenesis of Thrombosis and Its Management

Friday, October 28, 2016

COURSE DIRECTOR:
Jawed Fareed, PhD, FAHA

Sponsored by:
Loyola University Chicago
Stritch School of Medicine
Department of Pathology and the Division of Continuing Medical Education
In collaboration with The International Union of Angiology And The North American Thrombosis Forum
Forward

The discovery of heparin in 1916 by Jay McLean, a medical student at Johns Hopkins University, not only provided a universal anti-coagulant but also laid the foundation for the discipline of hemostasis and thrombosis. Much of what is known today regarding bleeding and thrombotic disorders is based on the observations and scientific research on heparin and related drugs. The surgical, interventional, and medical usage of heparin has revolutionized medicine. For nearly one hundred years, new discoveries and innovative findings continue to contribute to the expansion of our knowledge.

Over the last 25 years, several major developments have occurred which have revolutionized the scientific approaches and the clinical use of heparin and related drugs. The introduction of the fractionated heparins and subsequent development of low molecular weight heparins (LMWHs) in the 1980s has added a new dimension to the management of surgical and medical thrombosis. Eventually, with knowledge of the composition and structure of LMWHs, synthetic heparins such as the pentasaccharide were developed by French investigators. Currently, biotechnology based methods are being employed to design and develop heparin related drugs.

The global contaminant crisis in 2008 was a wake-up call to all of the scientists, clinicians and regulatory bodies who use and study heparin. It prompted the establishment of defined guidelines and quality assurance procedures to confirm the structural integrity and activity of heparins. With the introduction of such methods as NMR and MS, the pioneering work of Italian scientists, in particular the Ronzoni Institute (Milan, Italy), led to the establishment of analytical methods to investigate the structure and corresponding functional relevance of the components of heparins. Such methods have since been implied in the quality assurance of heparins and the detection of impurities and contaminants. Through this effort, the heparins available globally have improved purity and are safer to use. We are very pleased to have some of the investigators who advanced and validated these methods present as speakers here today.

The step-wise clinical development of heparin and related drugs from initial indication for surgical anticoagulation onwards to medical usage and prophylactic use for post-surgical thrombosis management played a key role in the expansion of the clinical indications of heparins and LMWHs. As pleiotropic agents, the heparins target multiple sites and accordingly are relevant in the management of diverse thrombotic and vascular disorders. Additional indications include cardiovascular disease, cancer, autoimmune diseases, neurodegenerative diseases and sepsis associated coagulopathy. Much of the pharmacology of heparin remain to be explored.
This international symposium is organized to commemorate the Heparin Centennial and will cover major developments in scientific and clinical arenas. Loyola University Chicago has been engaged in the investigations on heparin for nearly 50 years. The Department of Pathology fostered the development of an integrated program in basic, translational and clinical research and supported the development of the Hemostasis and Thrombosis Laboratories in collaboration with the Department of Pharmacology. Heparin has remained a focused area of research for the Loyola group with many national and international collaboration. This symposium will cover the developments in heparin drug substances, the development of heparin related drugs and the chemistry and biology of these drugs with the application of advanced methods for their studies. This symposium is open to basic scientists, clinicians, allied health professionals, and regulatory groups.

The speakers and discussants in this symposium represent some of the pioneers and leaders in their area of expertise. It is hoped that this symposium will also provide an open platform to exchange ideas and information. The proceeding of this symposium will published and provided to the attendees. This symposium is organized under the auspices of the International Union of Angiology (IUA) and the North American Thrombosis Forum (NATF). Both of these organizations are committed to increasing the awareness of newer anticoagulants in the management of thrombosis.

We are thankful to the Loyola University Chicago CME Division for their expert organization of this symposia. Special thanks are extended to the International Union of Angiology and North American Thrombosis Forum for their sponsorship and support. We are also thankful to Provost Callahan for her encouragement and support in expanding collaborative educational and research activities at national and international levels.

Jawed Fareed, PhD, FAHA

On behalf of the Organization Committee
Dear Participants of the Heparin Symposium and Distinguished Faculty,

It is indeed an honor and pleasure for me to welcome you all to the Loyola University Chicago Health Science Division to participate in this special celebration to recognize 100 years of the discovery of the universal anti-coagulant heparin. We are honored to have all of you at this important event. We hope that this program will provide an informative platform to highlight scientific and clinical developments and their impact on the understanding thrombosis and hemostasis.

This life saving drug has been used clinically for over 75 years and has been crucial in the surgical, interventional and medical management of thrombosis, open heart surgery, vascular interventions and hemodialysis. Despite dramatic developments in anticoagulant therapies, heparins have remained to be the standard of care for these indications.

Over the past 50 years, Loyola University Chicago faculty has developed integrated bench to bedside projects which have played an important role in the optimization of the use of unfractionated heparin in open heart surgery, coronary angioplasty, introduction of low molecular weight heparins, step wise basic and clinical development of synthetic heparin pentasaccharide.

Our faculty and students have also interacted with national and international centers of excellence in conducting bench to bedside research on heparin and related drugs. Collectively, over 1,000 publications and several books have resulted from this dedicated research program. In addition, post graduates programs to train scientists and clinicians have been established. We are thankful to our international colleagues who have worked with our faculty and some of whom are present today in this symposium.

For nearly a century, since its discovery, heparin has continually provided scientific and clinical challenges which required integrated teamwork between clinicians and scientists, academia and industry, international collaboration and the recognition of the need for multi-disciplinary approaches to learn from the knowledge gained from this complex drug. Not only as a crucial anti-coagulant, but the understanding of heparin’s interaction with blood and vascular systems has laid the foundation of modern hemostasis and thrombosis.

The speakers of this symposium represent most of the pioneers who have devoted their lifetimes in advancing and enhancing the use of heparin and related drugs for treatment of thrombosis. I would like to especially thank all of the speakers in this symposium for their lifelong dedication in enhancing the knowledge and developing clinical and educational programs.

Loyola University Chicago and most of the key pharmaceutical industry involved in heparin development have a long tradition of working together in developing academic and research programs. The evolution of heparin, low molecular weight heparins, heparinoids, synthetic and bio-engineered heparins as life-saving drugs, would have not been possible without a close collaboration with pharmaceutical industry. The stepwise development of low molecular weight heparins and pentasaccharide led by the French scientists is a testimony of collaboration between academia, industry and professional organizations. Such industrial collaborations will continue to play a key role in the future advancement of the development of newer drugs to treat vascular diseases.

I would like to thank the IUA and NATF and the program committee for organizing this symposium at our campus. I also wish to thank all of the attendees and participants and do hope that this symposium will not only update the advances in heparins clinical usage and scientific research but, will provide a platform to openly exchange ideas and networking opportunities.

I wish all of you a wonderful stay in this beautiful city and look forward to welcoming you to our University.

Thank you.

Margaret Faut Callahan, CRNA, PhD, FNAP, FAAN
Provost, Health Science Division
Professor, Niehoff School of Nursing
Message from the Dean

Welcome! We are so pleased that you have joined us on the Health Sciences Campus of Loyola University Chicago for the Heparin Centennial Symposium. We hope that your time with us is engaging, stimulating and rewarding. Our Loyola team is pleased to share their reflections on the past 100 years in heparin science; and their work to advance care for patients who rely on this critical medication. We are extremely proud to have them as part of our Stritch School of Medicine faculty.

Isn't it amazing that – 100 years since discovery - heparin remains such an important treatment! The investigators who will participate in this high-impact conference are many of the leaders who work in this scientific field. Thank you for your past and ongoing work to benefit patients in need.

We wish each and every one of you all best wishes for a robust scientific exchange.

Sincerely,

[Signature]

Linda Brubaker, MD MS
Dean and Chief Diversity Officer
Professor, OG and Urology
Stritch School of Medicine
Loyola University Chicago
AGENDA

**SESSION I** - Chairpersons: Karel Roztocil, MD, PhD
Jawed Fareed, PhD, FAHA

7:30 am  Registration

8:30 am  Welcome and Opening Remarks
*Pieter de Tombe, PhD*

8:40 am  The Discovery of Heparin as a Landmark for the Foundation of Surgical Anticoagulation: A Cardiovascular Surgeon’s Perspective
*Victor Ferraris, MD, PhD*

9:00 am  A Step-wise Evolution of Heparins and Synthetic Pentasaccharide
*Pierre Willaime, MBA*

9:30 am  100 Years After the Discovery of Heparin: Lessons Learned
*Jawed Fareed, PhD, FAHA*

10:00 am  BREAK

**SESSION II** - Chairpersons: Helena Nader, PhD
Shaker Mousa, PhD

10:30 am  Structural Analysis of Heparins and Bioengineered Heparins
*Robert Linhardt, PhD*

10:50 am  Analysis of Heparins Using Integrated Approaches
*Bernd Diehl, PhD*

11:10 am  Validation of Sole Anti-Xa Oligosaccharides as Antithrombotic Agents
*Jeanine Walenga, PhD*

11:30 am  The Discovery of Pentasaccharides and their Impact on the Development of Newer Therapeutic Agents
*Maurice Petitou, PhD*

11:50 am  The Heparin Contaminants: Lessons Learned
*Marco Guerrini, PhD*

12:10 pm  The Ronzoni Institute. Impact on Heparin and GAG Sciences
*Giangiacomo Torri, PhD*
12:30 pm  LUNCH

**SESSION III** - Chairpersons:  Job Harenberg, MD, PhD and Alexander G.G. Turpie, MD

1:30 pm  VTE Treatment with Heparins and Other Glycosaminoglycans
    *Russell Hull, MD, PhD*

1:50 pm  Endothelial Modulators of Heparin: Lessons from Clinical Trials
    *Debra Hoppensteadt, PhD*

2:10 pm  Glycosaminoglycans and Beyond
    *Job Harenberg, MD, PhD*

2:30 pm  VTE Prevention with Heparin and Other GAGs in Major Orthopaedic Surgery
    *Alexander G.G. Turpie, MD*

2:50 pm  Current Consensus on DVT Management: An Update
    *Eduardo Ramacciotti, MD, PhD*

3:10 pm  BREAK

**SESSION IV** - Chairpersons:  Ali Al-Hakim, PhD and Umberto Cornelli, MD, PhD

3:40 pm  Understanding the Structure-Activity of Heparan Sulfate Using a Synthetic Approach
    *Jian Liu, PhD*

4:00 pm  Heparins of Different Origins: Where Do We Stand?
    *Helena Nader, PhD*

4:20 pm  Sheep Heparins as Biosimilar Equivalent of Porcine Heparins
    *Yiming Yao, PhD and Fabian Magalhaes, PhD*

4:40 pm  Sulodexide in Vascular Medicine
    *Giuseppe Maria Andreozzi, MD*

5:00 pm  Regulatory Position on the Development of Bovine Heparins
    *Ali Al-Hakim, PhD*

5:20 pm  Invited Discussion
    *Anna Maria Naggi, PhD*

5:30 pm  Adjournment
HEPARIN CENTENNIAL SYMPOSIUM
“Scientific and Clinical Developments and their Impact on the Understanding of the Pathogenesis of Thrombosis and Its Management”
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ACCREDITATION
The Loyola University Chicago Stritch School of Medicine is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education for physicians.

The Loyola University Chicago Stritch School of Medicine designates this live activity for a maximum of 7.0 AMA PRA Category 1 Credit(s)™. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

Nurses and other health professionals will receive a Certificate of Attendance. For information on applicability and acceptance, please consult your professional licensing board.

PROGRAM DESCRIPTION
This international symposium is organized to update the current knowledge on the clinical and fundamental research developments in the area of heparin and related drugs. This program will focus on the structure, biology, and clinical aspects of heparins including low molecular weight heparins and ultra-low molecular weight heparins. The recent developments in synthetic and bio-engineered heparins will also be updated. The clinical indications for these drugs will be reviewed by leading experts. The program is integrated to include basic scientists, clinicians, regulatory spokesmen, and allied health personnel. The program will also provide an opportunity for open discussions and networking among various groups.

TARGET AUDIENCE
This program is designed for individuals interested in the fundamental research and clinical use of heparins: allied health personnel, pharmacists, nurses, physicians and surgeons, pharmaceutical industry personnel, and post-graduate students.
CURRICULAR GOALS AND OBJECTIVES

Updated knowledge on the recent developments in anticoagulant drugs with a focus on heparin.
Upon completion of this activity, participants should be able to:
- Understand the stepwise development of heparins, the scientific rationale behind their clinical use, and its sustainability;
- Understand the pharmacology and clinical applications of heparins;
- Appreciate the newer developments in the areas related to heparin;
- Appreciate the impact of current advancements on the development of newer heparin related drugs.

Provide an interactive platform to discuss the optimal use of heparin-like drugs among various professionals.
Upon completion of this activity, participants should be able to:
- Indicate the ongoing issues related to heparin usage;
- Identify how heparin and related drugs can be resourced from other sources than porcine tissues;
- Summarize the regulatory requirements for the approval of heparins to assure their safety and efficacy.

Project future usage of heparins in newer clinical indications.
Upon completion of this activity, participants should be able to:
- Know that unfractionated heparin is the only drug for surgical indications with an antagonist;
- Recognize that heparin resourcing from bovine and ovine sources will provide alternate approaches to meet supply and demand;
- Appreciate that besides its anti-coagulant usage, heparins have other important applications in cancer, auto-immune diseases, and other indications;
- Develop newer drugs based on heparin’s structure and functionality.

COMPETENCIES
This educational event will address the following competencies: medical knowledge; practice-based learning and improvement; interpersonal and communication skills; interdisciplinary team skills; professionalism; system-based practices and quality improvement.
LEARNERS BILL OF RIGHTS

Loyola University Chicago Stritch School of Medicine recognizes that you are a life-long learner who has chosen to engage in continuing medical education to identify or fill a gap in knowledge, skill, or performance. As part of Loyola University Chicago Stritch School of Medicine’s duty to you as a learner, you have the right to expect that your continuing medical education experience with Loyola University Chicago Stritch School of Medicine includes:

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  - is driven and based on learning needs, not commercial interests;
  - addresses the stated objectives or purpose; and
  - is evaluated for its effectiveness in meeting the identified educational need.

- **A learning environment** that:
  - supports learners’ ability to meet their individual needs;
  - respects and attends to any special needs of the learners;
  - respects the diversity of groups of learners; and
  - is free of promotional, commercial, and/or sales activities.

- **Disclosure of:**
  - relevant financial relationships planners, teachers, and authors have with commercial interests related to the content of the activity; and
  - commercial support (funding or in-kind resources) of the activity.
FACULTY

Ali Al-Hakim, PhD
Acting Director
API
Food and Drug Administration
Rockville, Maryland, USA

Giuseppe Maria Andreozzi, MD
Emeritus, Professor of Angiology and Vascular Medicine
University Hospital Padua
Padua, Italy

Umberto F.G. Cornelli, MD, PhD
Chairman
Cornelli Consulting Sas
Milan, Italy

Bernd Diehl, PhD
CEO
Spectral Service AG
Cologne, Germany

Victor A. Ferraris, MD, PhD
Thoracic Surgery
University of Kentucky
Lexington, Kentucky, USA

Marco Guerrini, PhD
Vice-Director
NMR Center
Instituto di Ricerche e Biochimiche G-Ronzoni
Milan, Italy

Job Harenberg, MD, PhD
Professor of Medicine
Ruprecht – Karls University Heidelberg
Mannheim, Germany

Russell Hull, MD, PhD
Professor of Medicine
The University of Calgary
Thrombosis Research Unit
Foothills Hospital
Calgary, Alberta, Canada
Robert Linhardt, PhD
Senior Constellation Chair in Biocatalysis and Metabolic Engineering, and
Professor of Chemistry, Biology and Chemical and Biological Engineering
Rensselaer Polytechnic Institute
Troy, New York, USA

Jian Liu, PhD
John & Deborah McNeill, Jr. Distinguished Professor
University of North Carolina
Eshelman School of Pharmacy
Chapel Hill, North Carolina, USA

Fabian Magalhaes, PhD
Managing Partner
Results Consulting
Sao Paulo, SP, Brazil

Shaker Mousa, PhD
Vice Provost of Research and
Professor of Pharmacology
Pharmaceutical Research Institute
Albany College of Pharmacy and Health Sciences
Rensselaer, New York, USA

Helena Nader, PhD
Dean of Undergraduate Studies
Biochemistry and Molecular Biology
Federal University of Sao Paulo
Sao Paulo, SP, Brazil

Anna Maria Naggi, PhD
Director
Instituto di Ricerche e Biochimiche G-Ronzoni
Milan, Italy

Maurice Petitou, PhD
Private Consultant
France

Eduardo Ramacciotti, MD, PhD
Director
Vascular Surgery
Hospital Israeli Albert Einstein
Santo Andre, SP, Brazil
Karel Roztocil, MD, PhD
Professor
Klinika Kardiologie
Prague, Czech Republic

Giangiacomo Torri, PhD
Senior Scientist and Advisor
Instituto di Ricerche e Biochimiche G-Ronzoni
Milan, Italy

Alexander G.G. Turpie, MD
Professor
HHSC Division
Hamilton General Hospital
Hamilton, Ontario, Canada

Pierre Willaime, MBA
Private Consultant and Advisor
Paris, France

Yiming Yao, PhD
CEO
Ronnsi Pharma Co., Ltd.
JiangSu, China

LOYOLA UNIVERSITY CHICAGO STRITCH SCHOOL OF MEDICINE
LOYOLA UNIVERSITY HEALTH SYSTEM FACULTY

Peter Bacher, MD, PhD (Planning Committee)
Department of Pharmacology
Loyola University Chicago
Stritch School of Medicine
Maywood, Illinois, USA

Vinod Bansal, MD (Planning Committee)
Professor, Department of Medicine, Nephrology
Loyola University Chicago
Stritch School of Medicine
Maywood, Illinois, USA
**Pieter De Tombe, PhD**
Interim Vice Dean for Research and
Professor and Chair, Department of Cell and Molecular Physiology
Loyola University Medical Center
Loyola University Chicago
Stritch School of Medicine
Maywood, Illinois, USA

**Jawed Fareed, PhD, FAHA**
Professor of Pathology & Pharmacology
Director, Hemostasis and Thrombosis Research Laboratories
Loyola University Chicago
Stritch School of Medicine
Maywood, Illinois, USA

**Debra Hoppensteadt, PhD**
Professor of Pathology & Pharmacology
Technical Director of the Hemostasis & Thrombosis Research Laboratories
Loyola University Chicago
Stritch School of Medicine
Maywood, Illinois, USA

**Omer Iqbal, MD (Planning Committee)**
Professor of Pathology
Loyola University Chicago
Stritch School of Medicine
Maywood, Illinois, USA

**Walter Jeske, PhD (Planning Committee)**
Professor, Thoracic & Cardiovascular Surgery and Pathology
Loyola University Chicago
Stritch School of Medicine
Maywood, Illinois, USA

**Jeanine Walenga, PhD**
Professor, Thoracic & Cardiovascular Surgery and Pathology
Co-Director, Hemostasis & Thrombosis Research Laboratory
Loyola University Chicago
Stritch School of Medicine
Maywood, Illinois, USA
Disclosure Information

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Division of CME Administration and Review Committee Disclosure – Pertaining to this activity, administrators and review committee members have no financial relationships with commercial interests & no relationships between commercial interests and first degree relatives.

Keith Muccino, SJ, MD
Karen Bertucci, MEd, CHCP
Anna Thibodeau

Gregory Gruener, MD
Eileen Hall, CHCP
Sponsoring Department Disclosure

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Participants Disclosure

Giuseppe Maria Andreozzi, MD
- Congress lectures and scientific consultancies:  CIC Rome, Italy; Planning Congress Bologna, Italy; Alfa Wassermann, Bologna, Italy; Mediolanum Farmaceutici, Milan, Italy; Staff Italia Incentive & Motivation, Rome, Italy; GC Congressi, Rome, Italy; GC Congressi, Rome, Italy; Alfa Wassermann Praha, Czech Republic; Alfa Wassermann Bratislava, Slovakia; Alfa Wassermann Bucharest, Romania.
- Received consultancy fee as member of Steering committee of SURVEY Study (OsCS CODE: ALFAWAS_III_2010_001) Alfa Wassermann Bologna, Italy
- No relationships between commercial interests and first degree relatives exist
- Speaker does not intend to discuss an unapproved/investigative use of a commercial product/device

Victor A. Ferraris, MD, PhD
- Consultant for Protego Medical
- No relationships between commercial interests and first degree relatives exist
- Speaker does not intend to discuss an unapproved/investigative use of a commercial product/device

Russell Hull, MD, PhD
- Receives grant support for the University of Calgary from Portola
- Receives research support for the University of Calgary from Leo Pharma
- No relationships between commercial interests and first degree relatives exist
- Speaker does not intend to discuss an unapproved/investigative use of a commercial product/device

Jian Liu, PhD
- Major stock holder Glycan Therapeutics, LLC.
- No relationships between commercial interests and first degree relatives exist
- Inventor of synthetic heparin and plans to discuss the use of synthetic heparin.

Eduardo Ramacciotti, MD, PhD
- Receives grant/research support from Aspen Pharma; Portola; Bayer, and Cristalis
- Member of the speaker’s bureau for Aspen Pharma; Bayer; and Pfizer
- Consultant for Bayer
- No relationships between commercial interests and first degree relatives exist
- Speaker does not intend to discuss an unapproved/investigative use of a commercial product/device.
Alexander G.G. Turpie, MD
- Member of the speaker’s bureau for Bayer and Janssen
- Consultant for Bayer
- No relationships between commercial interests and first degree relatives exist
- Speaker may discuss NOAC’s

Yiming Yao, PhD
- Has no financial relationships with commercial interests
- No relationships between commercial interests and first degree relatives exist
- Speakers intend to discuss the development of ovine heparin products

The following conference participants and planning committee members.

Ali Al-Hakim, PhD                    Walter Jeske, PhD
Peter Bacher, MD, PhD                Robert Linhardt, PhD
Vinod Bansal, MD                     Fabian Magalhaes, PhD
Umberto F.G. Cornelli, MD, PhD       Shaker Mousa, PhD
Pieter De Tombe, PhD                 Helena Nader, PhD
Bernd Diehl, PhD                     Anna Maria Naggi, PhD
Erin Erickson-Healy                 Maurice Petitou, PhD
Jawed Fareed, PhD, FAHA              Karel Roztocil, MD, PhD
Marco Guerrini, PhD                  Giangiacomo Torri, PhD
Job Harenberg, MD, PhD               Jeanine Walenga, PhD
Debra Hoppensteadt, PhD             Pierre Willaime, MBA
Omer Iqbal, MD

- Have no financial relationships with commercial interests
- No relationships between commercial interests and first degree relatives exist
- Speaker’s do not intend to discuss an unapproved/investigative use of a commercial product/device
The Discovery of Heparin as a Landmark for the Foundation of Surgical Anticoagulation: A Cardiovascular Surgeon’s Perspective

Victor Ferraris, MD, PhD

There is significant controversy regarding the distribution of credit for the discovery of heparin. This symposium celebrates the 100th anniversary of Jay McLean’s efforts to characterize a liver phosphatide with anticoagulant properties while working in the laboratory of William Henry Howell at the Johns Hopkins Medical School in Baltimore. This liver phosphatide was later given the name of heparin. It took more than 25 years after these initial experiments for the clinical impact of heparin to become apparent.

In the immediate post-WWII years, Congress began 3 decades of generous funding for medical research and education through the National Institutes of Health and the Public Health Service. By 1950 surgeons interested in cardiac operations recognized the need for a heart-lung machine that would allow repair of the majority of congenital cardiac malformations and acquired valvular heart disease. The heart-lung machine required three components: an oxygenator, an anticoagulation agent and a reversal agent for the anticoagulant. At least 5 different investigational designs for a heart-lung machine appeared between 1950 and 1955. John Gibbon at Jefferson Medical College in Philadelphia worked on this problem the longest. His machine made use of the DeBakey roller pump, developed by Michael DeBakey while he was in medical school. Gibbon’s machine used a film oxygenator and he received engineering and financial assistance from the IBM Company.

By the 1960’s, the heart-lung machine evolved to include the DeBakey roller pump coupled to an efficient bubble oxygenator. This combination used heparin and the more modern reversal agent protamine to provide safe and routine cardiopulmonary bypass (CPB). The development of cardiopulmonary bypass (CPB) in the 1960’s was so successful, that rigorous evidence based clinical trials did not play a part in the initial phases of development. Recognizing the observational nature of the evidence base to support the use of modern CPB, the Evidence Based Workforce of the Society of Thoracic Surgeons undertook a project to develop a series of practice guidelines that reflect available evidence for the safe and effective use of CPB in the modern era. A mainstay of the current practice using CPB is anticoagulation with heparin. This presentation will briefly outline the historical development of CPB using heparin anticoagulation and will outline the evidence-based guidelines for the management of heparin during CPB.
A Step-wise Evolution of Heparins and Synthetic Pentasaccharide

Pierre Willaime, MBA

During the 1950s, heparin was of limited use in France because of possible side effects (pyrogens). Three manufacturers shared the French market; Choay was the smallest. These were also years of great progress in surgery.

**Serendipity Phase**

The 1960s saw three demands from physicians who were unhappy with their professional experience: Pr. C. Dubost (1959), a cardiovascular surgeon, Dr. C. Raby (1965), a Colonel and head of the Army Blood Transfusion Centers, and Pr. V.V. Kakkar (1969) an abdominal surgeon. All asked the same sequence of questions: “I have a problem; I also have a possible solution; Would Choay help?” The response had been yes three times.

This led to two consequences; for one, a very significant growth of the heparin franchise for Choay. It also led to two very different approaches to heparin therapy: one, C. Raby’s approach, which was medical, curative, personalized, high dose, and long term, the second, V.V. Kakkar’s approach, which was surgical, preventive, standardized, low dose, and short term.

**Rationality Phase**

This situation lead Jean Choay, the Scientific Manager, to consider heparin as a research project, and it was with this perspective that he went to the Philadelphia ISTH meeting in 1977. He returned with the decision to initiate two research projects, both with a view to offer an improved heparin more focused on anti-Xa activity, with fewer side effects and longer half-life.

One project was to fractionate heparin to a smaller active molecule. The second project was to take the bet that this active molecule could be small enough to be synthetized. Jean Choay reorganized the in-house competence and decided to establish close partnerships with different academic institutions in domains where Choay had no competence. (P. Sinay – Orléans Uty – for sugar synthesis; J. Fareed – Loyola – for pharmacology and B. Casu – G. Ronzoni – for structural studies) Progress moved fast: 1980 saw the first fraction clinical trials by Pr. Kakkar; 1981 the first article on heparin structure, and in 1983 Choay obtained both a natural hexasaccharide and a synthetic pentasaccharid. In March of 1985, Choay was given the first LMWH AMM; the second AMM, for Lovenox, came in April 1987. Arixtra, the synthetic pentasaccharide, was introduced only in 2002.

LMWHs have grown very significantly, and growth seems still present. Clinical trials are numerous. Heparins are still drawing attention and interest.
The year 2016 marks one hundred years since the discovery of the universally used anticoagulant drug heparin. Over this period of time heparin has remained one of the most challenging and rewarding drugs for scientists and clinicians. Despite the development of numerous synthetic and biotechnology based anticoagulant drugs, heparin and its derivatives have remained the standards of care for thrombotic and cardiovascular indications. The chemistry, biology and clinical behavior of this drug is intriguing and has fascinated both the scientific and clinical communities for many decades.

In 1916, a medical student at Johns Hopkins University, Jay McLean, serendipitously discovered that dog liver homogenates contained lipid soluble substances which had anticoagulant properties. As this finding was not in accordance with what his assignment called for, his then mentor, William Howell, was displeased. Soon after this finding, McLean left Johns Hopkins and another student by the name of Holt found an anticoagulant substance in aqueous extracts of dog liver. The focus of research in Howell’s laboratory shifted towards anticoagulants and, after Holt’s findings, Howell took an interest in this project and called the anticoagulant substance Heparin (from the Greek word for liver). Several years later, Howell and his group presented their findings at the American Physiological Society meeting. In 1926, Howell presented further refinement of the process to isolate the anticoagulant substance from liver extracts. While it is difficult to establish who first identified heparin, McLean is generally credited as its discoverer.

The clinical use of heparin began in the late 1920s. The initial batches of heparin, when used clinically, produced side effects such as nausea, vomiting, and headache. This prompted further purification of this agent by various groups. The American pharmaceutical group Hynson, Westcott and Dunning produced commercial amounts of heparin. At the same time, several investigators in other countries started working to further refine heparin production. This work was pioneered by the Canadian group led by Charles Best, then Chair of the Physiology department at the University of Toronto, and was carried out at the Toronto-based Connaught Laboratories. The group developed methods to extract heparin from bovine liver and later from bovine lungs leading to the development of commercial grade heparin for clinical use.

The introduction of heparin as an anticoagulant attracted many chemists and biologists to further study this agent. In 1929 a Swedish scientist, Erik Jorpes, visited the department of Physiology in Toronto to work with Dr. Best. He became interested in the study of heparin and upon returning to Sweden initiated his own research program. The composition of heparin proved to be rather complex and difficult to investigate. It became a challenge to many people. Jorpes also prompted clinicians to use this drug. A Swedish surgeon Crafoord was the first to use it clinically in 1938. The use of heparin was expanded at the Banting Institute in Canada when Gordon Murray used heparin in the first surgical indication. Simultaneously, Canadian physiologist Louis Jacques identified heparin to be a carbohydrate like substance. Several international scientists worked with the Canadian and Swedish groups to understand the chemistry and biology of heparin. These included groups at the University of Chicago and in Brazil. Carl Dietrich from São Paulo, who was working with Jacques, separated heparin into its components and eventually characterized these components. The Chicago group also separated heparin on chromatographic columns to show...
its components. Industry became very interested in producing heparin from various sources and the stock yards in Chicago provided a large source of hog mucosa to be used as raw material for heparin production. The Swedish and the French later developed methods to fractionate heparin based on molecular weight. This led to the development of Low Molecular Weight Heparins, a class of drugs which has revolutionized the management of venous thrombosis.

The fractionation of heparin and the subsequent characterization of its various components resulted in the identification of small molecular weight chains called oligosaccharides. This led to the development of synthetic pentasaccharides, one of which is now used clinically.

The development of heparin as an anticoagulant was a landmark project which not only provided an anticoagulant for clinical use, but also spurred investigations into the pathogenesis and treatment of thrombotic disorders. The foundations for our current understanding of hemostasis and thrombosis have been developed on the basis of our awareness of how heparin and related drugs modulate these processes. More recently, the isolation of heparins from other mammalian sources including sheep (ovine) and cow (bovine) tissues is being pursued. Furthermore, the concept of blended heparins has also emerged. Synthetic, biosynthetic and hybrid approaches to produce heparin and heparin-related drugs are currently being investigated.

During the manufacturing of heparin several other glycosaminoglycans such as dermatan sulfate, heparin sulfate and chondroitin sulfate are also obtained. While structurally similar these agents do not have a strong anticoagulant activity and can be used for other therapeutic purposes. From these agents such drugs as sulodexide and danaparoid are obtained. Chondroitin sulfate is widely used as an anti-inflammatory agent in the management of joint diseases. Chemically modified heparins and heparin coating on surfaces are also important uses of this anticoagulant drug.

The development of heparin and related drugs has also faced some challenges in terms of impurities, standardization, intentional contamination and adulteration and interchangeability. Some of these issues have been resolved while others will eventually be sorted out. With the introduction of newer oral anticoagulant drugs it was initially thought that heparin and related drugs will be eventually replaced by these agents for most indications. Since heparin is a pleotropic agent with multiple pharmacologic actions it is unlikely to be replaced and will continued to be used for expanded indications.

Our understanding of the clinical utility of heparin and heparin related drugs will continue to evolve for some time. Although considered to be an old drug, the advances in cellular and molecular sciences will continue to provide us newer information on this universal anticoagulant to improve its production, to refine the drug products and to identify additional indications. Thus heparin, low molecular weight heparins, and synthetic or biosynthetic oligosaccharides will continue to impact the management of thrombotic and vascular disorders for years to come.
Heparin, a highly sulfated polysaccharide anticoagulant, commands a worldwide market of ~$7B. Currently, heparin is prepared by extraction from porcine intestine or alternatively from bovine lung or bovine intestine or ovine intestine. These products are complex polycomponent drugs and their structural characteristics are difficult to analyze. The application of NMR and MS analysis to understanding the differences in the structures of heparins from different sources will be discussed. Low molecular weight (LMW) heparins, prepared through the controlled chemical or enzymatic depolymerization of heparin, have become increasingly important drugs primarily based on their improved pharmacological properties. The application of sophisticated top-down and bottom-up strategies for their analysis will also be discussed. These methods are useful in comparing LMW heparins from different manufacturers and from different heparin source materials.

Recently, our laboratory has been actively developing a bioengineered heparin to avoid problems and limitations associated with the animal-sourced product. Our approach relies on fermentation to produce heparosan followed by chemoenzymatic synthesis chemical relying on Golgi-derived biosynthetic enzymes, including sulfotransferases and epimerase. We are also exploring metabolic engineering for heparin production. Such biotechnological processes should allow the cGMP preparation of these critical drugs, affording improved products reducing impurities, contaminants and adulterants that resulted in the 2008 heparin contamination crisis. An update on the progress of this research will be provided.
Analysis of Heparins Using Integrated Approaches
Bernd Diehl, PhD

$^1$H NMR spectroscopy was used to distinguish pure porcine heparin and porcine heparin blended with bovine species and to quantify the degree of such adulteration. For multivariate modelling several statistical methods such as partial least squares regression (PLS), ridge regression (RR), stepwise regression with variable selection (SR), stepwise principal component regression (SPCR) were utilized for modeling NMR data of in-house prepared blends (n=80). The models were exhaustively validated using independent test and prediction sets. PLS and RR showed the best performance for estimating heparin falsification regarding its animal origin with the limit of detection (LOD) and root mean square error of validation (RMSEV) below 2% w/w and 1% w/w, respectively. Reproducibility expressed in coefficients of variation was estimated to be below 10% starting from approximately 5% w/w of bovine adulteration. Acceptable calibration model was obtained by SPCR, by its application range was limited, whereas SR is least recommended for heparin matrix. The developed method was found to be applicable also to heparinoid matrix (not purified heparin). In this case root mean square of prediction (RMSEP) and LOD were approximately 7% w/w and 8% w/w, respectively. The simple and cheap NMR method is recommended for screening of heparin animal origin in parallel with official NMR test of heparin authenticity and purity.
Validation of Sole Anti-Xa Oligosaccharides as Antithrombotic Agents

Jeanine Walenga, PhD

The recognition of the pleotropic pharmacological profile of heparin has led to many innovative discoveries for not only the understanding of the mechanisms involved in thrombogenesis and its control but also in the development of new targets to develop antithrombotic drugs. The discovery of antithrombin (AT) as a naturally occurring serpin with affinity to heparin related glycosaminoglycans led to the identification of AT binding oligosaccharide consensus sequences in heparin. Coupled with the fractionation of heparin, the isolation of low molecular weight oligosaccharides such as octasaccharides and hexasaccharides by the French group laid the foundation for synthetic oligosaccharides. The first synthesis was a unique pentasaccharide with a defined sequence composed of an irregular region of three saccharide units containing a glucosamine 3-O sulfated and N-sulfated, and a 6-O sulfated glucosamine, with a regular disaccharide region containing an important N-sulfated glucosamine. Each saccharide, in sequence and composition, was a functional unit required for high binding of heparin to AT. Pentasaccharide provided a unique tool to understand the molecular interactions between heparin and AT and as such proved to be the prime determinant of the validation of the hypothesis that sole inhibition of FXa (anti-FXa) is capable of producing antithrombotic activity. This action was effected by indirectly inhibiting the generation of thrombin. The synthesis of pentasaccharide eventually led to the currently marketed fondaparinux (Arixtra®), widely used in the management of thrombotic cardiovascular disorders and as an alternate anticoagulant in the management of patients with heparin-induced thrombocytopenia (HIT). After the successful clinical introduction of the original pentasaccharide (Arixtra®), several generic versions became available. Since then developments became modest due to low revenue return and other factors, additional approaches were not supported, including the formation of complexes of pentasaccharide with AT, and today heparin-derived oligosaccharides remain under-developed. However, it is the concept of the heparin pentasaccharide with sole anti-FXa activity which led to the development of the currently marketed oral FXa inhibitor drugs. There remains a significant research focus in developing oligosaccharides with high affinity to endogenous protein modulators which, it is hoped, will continue to provide therapeutic agents targeted to a wide variety of diseases.
The Discovery of Pentasaccharides and their Impact on the Development of Newer Therapeutic Agents

Maurice Petitou, PhD

The structural characterization of the antithrombin binding sequence in heparin was soon followed by the full chemical synthesis of the corresponding pentasaccharide. This compound (fondaparinux) displayed selective binding to antithrombin (Kd 50 nM) and showed a remarkable ability for activation of this proteinase inhibitor against blood coagulation factor Xa. Tested in clinical trials, fondaparinux was found to display a remarkable effect in the prevention and the treatment of venous and arterial thrombosis. First considered as a prototype, it was finally submitted to exhaustive clinical testing and registered as the drug Arixtra®, the first in class of selective factor Xa inhibitors. Idraparinux, a long lasting analogue of fondaparinux (half-life five days vs. 17 hours for fondaparinux) was also tested successfully in clinical trials before being replaced by idrabiotaparinux, an equivalent version, but neutralizable by avidin. Once a week injection of idrabiotaparinux compared favorably with vitamin K antagonists, both in terms of efficacy and safety, for the treatment of thromboembolic events in patients with pulmonary embolism with or without deep vein thrombosis, and the anticoagulant activity could be instantaneously reversed by infusion of avidin. Other sophisticated anticoagulants with different pharmacological profiles, inhibiting not only factor Xa but also thrombin, have been designed around the structure of fondaparinux; several of them have been tested successfully in clinical trials before being dropped, mostly for economical or marketing reasons. In addition to providing a new drug (Arixtra®), the studied performed with fondaparinux and its derivatives have validated selective inhibition of factor Xa as a pharmacological target for the development of new antithrombotic agents, thus paving the way for the new oral anticoagulants.
The Heparin Contaminants: Lessons Learned
Marco Guerrini, PhD

NMR spectroscopy, which does not demand any sample manipulation, provides both qualitative and quantitative structural information of heparin and low molecular weight heparins (LMWH). Following the heparin crisis, all of the major Pharmacopoeias introduced NMR for the purposes of identification and characterization of the active principle, and also allowed NMR to be used to demonstrate the degree of “sameness” between branded and generic LMWHs. However, a simple proton spectrum, such as that requested by Pharmacopoeias for heparin, cannot fully guarantee the quality of the sample, both in term of structural properties and contamination. HSQC NMR experiment was demonstrated to be one of the most successful approach to the analysis and quantification of mono- and disaccharide composition of heparin and LMWH, through integrations of diagnostic signals.

In spite of structural similarity, the combination of NMR and statistical analysis of an ensemble of spectra of validated “bona fide” samples forming a library can differentiate samples having structural peculiarities alien to those represented by the library. These techniques have a potential application in the regulatory environment, allowing branded and generic products to be compared.
The Ronzoni Institute. Impact on Heparin and GAG Sciences
Giangiacomo Torri, PhD

I will show the scientific contribution that the Foundation "Ronzoni Institute" gave in heparin research in the last 40 years through studies of structure activity relationships.

The first important scientific contribution was the characterization of NMR spectra of beef lung heparin achieved by the collaboration of B Casu with A Perlin (Mc Gill-Montreal) and G Gatti (CNR-Milan). Major contributions, mostly in collaboration with international groups, have been:

- The rational thought that the unusual conformational properties of iduronic acid residues are determinant in biological activities of glycosaminoglycans,
- NMR studies on structure of the active site of heparin for antithrombin,
- Collaboration in the development of a new synthetic commercial drug,
- Determination of the "active" conformations of heparin oligomers and assessment of their 3D structures in complexes with AT and FGF.

More recently, we worked on the development of NMR and MS techniques for the structural characterization of LMWHs and heparin oligosaccharides. The application of these techniques allowed the detection in commercial heparin preparations of a contaminant associated with adverse clinical events, identification of structural peculiarities of beef mucosal heparins respect to pig mucosal heparins as well as the comparison of structural peculiarities of commercial and generic LMWHs. Moreover, the combination of NMR and chemometric methods allowed the detection of structural variation in test samples, the detection of alien materials as well as identification of both native and aberrant structures with unprecedented sensitivity.

Ronzoni provided also important contribution in chemical approaches for structural modification of GAGs, such as the synthesis of non-radioactive heparin derivatives for pharmacokinetics studies or the synthesis of heparin derivatives with antitumor and antimetastatic activities.

It is relevant to point out the role that many meaningful collaborations played in the international visibility of Ronzoni researches. It is difficult to mention all these collaborations. Among them the long collaboration with J. Choay led us to many scientific results as well as a human unique experience.
VTE Treatment with Heparins and Other Glycosaminoglycans
Russell Hull, MD, PhD

Heparin, one of the oldest drugs still in widespread clinical use, is a naturally occurring
glycosaminoglycan whose main function is to inhibit the coagulation of blood. It was
discovered almost a century ago and took many years to move from the laboratory to the
bedside. In 1963, a plaque was unveiled in Johns Hopkins to commemorate the ‘major
collection [of McLean] to the discovery of heparin in 1916 in collaboration with Professor

**Treatment Objectives:**
- Prevent death and disability from PE, pulmonary hypertension and peripheral venous
disease
- Prevent recurrence of VTE and development of postthrombotic syndrome

**Need for Drug Dose Monitoring an Achilles’ heel:**
- Several studies suggest that when using UFH for initial treatment of DVT the rapid
achievement of an activated partial thromboplastin time within the therapeutic range
(2.0-3.0 times the control) within 24 hours will reduce the rate of recurrent DVT.
- VKA treatment should be adjusted to maintain the INR between 2.0 and 3.0 (target
INR 2.5).
  - Taken from several studies showing the risk of bleeding in relation to different
INR ranges

Regulatory affairs approval of Low-Molecular-Weight Heparin (LMWH) therapy has
made IV heparin therapy obsolete in the most patients. Subcutaneous LMWH therapy allows
outpatient therapy in the majority of the patients with deep vein thrombosis or sub-massive
PE.

**Thrombocytopenia:**
LMWH is associated with a lower frequency of thrombocytopenia.

**Recommendations for Treating VTE:**
- Initial treatment is with intravenous UFH, LMWH, or fondaparinux for at least 5 days,
discontinued when the stable INR is in the therapeutic range.
- Patients with cancer history or who are pregnant would recommend LMWH as initial
treatment as an alternative to VKA therapy.

**Antithrombotic Therapy for VTE Disease in 2016:**
- For VTE without cancer, dabigatran, rivaroxaban, apixaban, or edoxaban is suggested
over VKA therapy. VKA therapy is suggested over LMWH.
  - Moderate quality, but weak evidence
- For VTE with cancer, LMWH is suggested over VKA, dabigatra, rivaroxaba, apixaba, or
edoxaban.
  - Moderate quality, but weak evidence
- For VTE treated with anticoagulants, an inferior vena cava filter is not recommended.
  - Moderate quality, but strong evidence
**Recommendations for Treating VTE for Patients with Cancer:**

- **Recommendation 4.1.** LMWH is preferred over UFH for the initial 5 to 10 days of anticoagulation for the patient with cancer with newly diagnosed VTE who does not have severe renal impairment (defined as creatinine clearance <30 mL/min).

- **Recommendation 4.2.** For long-term anticoagulation, LMWH for at least 6 months is preferred because of improved efficacy over vitamin K antagonists (VKAs). VKAs are an acceptable alternative for long-term therapy if LMWH is not available.

- **Recommendation 4.3.** Anticoagulation with LMWH or VKAs beyond the initial 6 months may be considered for select patients with active cancer, such as those with metastatic disease or those receiving chemotherapy.

**Low-Molecular-Weight Heparin Treatment:**

- Long-term LMWH is equally as effective as standard therapy for patients without cancer, but more effective for patients with cancer.
  - Lower incidence of recurrent VTE for LMWH than VKA groups
  - Lower incidence of major bleeding for patients

- Better recanalization in LMWH groups than standard treatment.
Endothelial modulation by drugs is an important mechanism by which many of the anticoagulant and anti-ischemic agents produce their therapeutic effects. The endothelial modulation may result in the anticoagulant, profibrinolytic, and vasodilatory effects. Heparin and low molecular weight heparins (LMWH) represent multifunctional polyelectrolytes which may modulate the endothelial processes by direct and indirect mechanisms. Intravenous administration of heparin and LMWH has been shown to markedly increase the tissue factor pathway inhibitor (TFPI) levels, which correlate with the circulating levels of these agents. Subcutaneous administration of both heparin and LMWH also result in a sustained release of TFPI. Interestingly, heparin fractionated on antithrombin columns into non-anticoagulant and anticoagulant forms mediates TFPI release, thus the non-anticoagulant forms of heparin are also capable of releasing this mediator. The heparin pentasaccharide, while a very strong inhibitor of antithrombin, produces relatively low release of TFPI. Thus, there is a molecular weight dependence of the release of TFPI by heparins. Heparins have also been shown to increase the release of tissue plasminogen activator (TPa) from the vascular sites. These effects are also antithrombin-HCII independent. Hypersulfated forms of heparin produce stronger release of both TPa and TFPI. In several clinical trials, measurement of TFPI and TPa have been shown to be associated with the administration of heparin and LMWH. Besides the release of TPa and TFPI, heparins are also capable of increasing nitric oxide (NO) levels, which are mediated by endothelial cells. Unlike heparins, the newer anticoagulant drugs, including parenteral antithrombin agents such as argatroban and dabigatran do not produce any release of TFPI and TPa. More recently, a heparinoid named sulodexide has been found to enhance the release of TFPI and TPa after oral administration. Based on the analysis of the plasma samples from patients treated with heparin and LMWH, it is suggested that TFPI release plays a very important role in the mediation of not only the anticoagulant effects but also the anti-inflammatory actions of heparins. Thus, heparins can be differentiated from other anticoagulant drugs, such as newer oral anticoagulants, parenteral antithrombin agents, and antiplatelet agents based on this property as these drugs are devoid of this effect. Our clinical and basic observation suggests that the release of TFPI plays an important role in the mediation of heparin’s therapeutic effects, which is a unique property of this pleiotropic agent.
Glycosaminoglycans and Beyond

Job Harenberg, MD, PhD

The effect of glycosaminoglycans on the blood coagulation system depends on its affinity to bind to antithrombin and results in inhibition of the activity of many serine proteases involved in this process. The non-anticoagulant actions of glycosaminoglycans are independent of the antithrombin binding site and play a major role in their biological activity. The analyses of the interactions of glycosaminoglycans with coagulation proteases as well as with non-anticoagulant proteins are currently improved by very specialized analytical methods. The synthesis of oligosaccharides of original and modified heparin-like products improves the understanding of specific interactions with proteins. The ultimate goal of these investigations is the development of defined glycosaminoglycans for treatment of non-thrombotic diseases.
VTE Prevention with Heparin and Other GAGs in Major Orthopaedic Surgery
Alexander G.G. Turpie, MD

My first job after qualifying in Medicine in 1962 was as a House Surgeon in Orthopaedics at the Glasgow Royal infirmary. My morning duties included drawing blood, doing blood pressures and checking for phlebitis as it was recognized that pulmonary embolism was a major cause of death in young trauma victims. In 1968, Sir John Charnley stated that venous thromboembolism (VTE) was the most common post-operative complication following hip surgery and the single largest cause of death. In the early 1970’s, V V Kakkar demonstrated that low doses of unfractionated heparin reduced post-operative VTE in surgical patients and in 1975 showed that it prevented fatal post-operative pulmonary embolism. Since then, numerous studies have confirmed the benefit of low dose heparin for VTE prophylaxis in a variety of clinical settings. In 1970, I joined the Thrombosis Programme at McMaster University, Hamilton, Canada under the direction of J Hirsh. The programme included basic scientists and clinicians dedicated to the study of diagnosis, prevention and treatment of thrombosis.

In 1986, we published the results of one of the first clinical trials of Low Molecular Weight Heparin (LMWH) for the prevention of VTE in orthopaedic patients. This 100 patient study spawned numerous trials of LMWH across the spectrum of thrombosis which today remains the standard treatment for many thrombotic disorders including treatment of VTE and acute coronary syndromes (ACS)

_A randomized controlled trial of a low-molecular-weight heparin (enoxaparin) to prevent deep-vein thrombosis in patients undergoing elective hip surgery._


Further fractionation of heparin led to the development of fondaparinux, the indirect Factor Xa inhibitor. We demonstrated that it was slightly more effective than LMWH in preventing VTE in orthopaedic patients.

_Fondaparinux vs enoxaparin for the prevention of venous thromboembolism in major orthopedic surgery: a meta-analysis of 4 randomized double-blind studies._

Turpie AG1, Bauer KA, Eriksson BI, Lassen MR _Arch Intern Med._ 2002 Sep 9;162(16):1833-40.

The evaluation of orally active targeted anticoagulants in the early 2000’s has resulted in an revolution in anticoagulant therapy. We demonstrated in the effectiveness of rivaroxaban a series of clinical trials in orthopaedic surgery

_BAY 59-7939: an oral, direct factor Xa inhibitor for the prevention of venous thromboembolism in patients after total knee replacement. A phase II dose-ranging study._

_SESSION III – 1:30 – 3:10 PM_

Rivaroxaban for the prevention of venous thromboembolism after hip or knee arthroplasty. Pooled analysis of four studies.


We are now embarked on a series of Phase IV trials to demonstrate the effectiveness of rivaroxaban in routine clinical practice.

Rationale and design of XAMOS: noninterventional study of rivaroxaban for prophylaxis of venous thromboembolism after major hip and knee surgery.

Turpie AG¹, Schmidt AC, Kreutz R, Lassen MR, Jamal W, Mantovani L, Haas S.


Thus, I have had the privilege, along with many colleagues worldwide, of being involved in the evolution of antithrombotic therapy from the old standards of heparin and vitamin K antagonists through the introduction of LMWH and now the orally active drugs that target single coagulation factors.
Current Consensus on DVT Management: An Update
Eduardo Ramacciotti, MD, PhD

Venous thromboembolism (VTE), the formation of blood clots in the vein and pulmonary arteries is a prevalent and a leading cause of death and disability. Each year, there are approximately 10 million cases of VTE worldwide. This presentation will review and update the recommendations of 2 evidence-based guidelines, the 2016 Antithrombotic Therapy for VTE Disease: CHEST Guideline, from the American College of Chest Physicians and the International Union of Angiology guidelines for VTE management.

These guidelines provides the most up-to-date prophylactic and treatment options for patients with VTE. The guideline presents stronger recommendations and weaker suggestions for treatment based on the best available evidence, and identifies gaps in our knowledge and areas for future research.
Heparan sulfate is a low sulfated form of heparin and is widely expressed on the cell surface and in the extracellular matrix. The sulfated saccharide sequences play important roles in regulating the biological function of heparan sulfate. However, synthesis of structurally defined heparin and heparan sulfate oligosaccharides using a purely chemical approach remains to be challenging. A chemoenzymatic method has been recently developed to prepare synthetic heparin and heparan sulfate. The methods only involves the use of five different enzymes, including sulfotransferases, an epimerase and glycosyltransferases. Using these enzymes, a highly diverse heparan sulfate oligosaccharide library have been prepared. In addition, this method has been successfully utilized to synthesize ultra-low molecular weight heparin and low-molecular weight heparins, to improve the pharmacology of heparin-based anticoagulant drugs. The enzyme-based synthetic approach has synthesized more than 50 oligosaccharides with different sulfation patterns and size. The availability of heparan sulfate oligosaccharide library offers a unique tool to study the function and activity relationship of heparan sulfate in biological systems.
Heparins of Different Origins: Where Do We Stand?

Helena Nader, PhD

Heparin was first isolated in 1916 and ever since played a significant role in thrombosis management. Its anticoagulant effect results from the interaction with specific proteins from the coagulation cascade such as antithrombin and heparin cofactor II. Its discovery had a tremendous impact on health once major surgical procedures, especially those requiring cardiopulmonary bypass, were made possible. Since those early days, heparin became the key drug for open-heart surgery as well as dialysis. Among the natural occurring anionic polymers, heparin is the compound with the highest charge density which allows it to bind to basic patches on protein surfaces, impacting physiological processes, such as fibrinolysis, inflammation, angiogenesis, cell adhesion, migration, invasion, proliferation and recognition, among others. Thus, heparin is as a pleiotropic drug that shows peculiar structural characteristics according to the tissue and species of origin. Pharmaceutical heparins, however, are mainly obtained from bovine and porcine intestinal mucosa, and to some extent from bovine lung and ovine intestinal mucosa. In the last two decades, in Europe and USA, the main source of heparin is porcine origin. Nonetheless, in a recent study we have demonstrated that there are no differences between the use of bovine and porcine heparin during open-heart surgery. As mentioned, heparin shows many other relevant physiological effects besides coagulation and after 100 years of its discovery and several decades of medical use, it becomes clear that there are still structural peculiarities and biological functions to be revealed but the pharmaceutical agent, in regards to its role in open-heart surgery, does not seem too dependent on the tissue of origin. Heparin as both pharmaceutical agent and biomolecule has a well-built place in the future since new findings keep arising from the ongoing research.
Sheep Heparins as Biosimilar Equivalent of Porcine Heparins
Yiming Yao, PhD, and Fabian Magalhaes, PhD

Heparin is the most widely used anticoagulant drug for surgical and interventional indications. Unfractionated heparin and low molecular weight heparin are widely used for the management of thrombotic and cardiovascular disorders. Most of the heparins are derived from pig mucosa (porcine mucosa) however heparin from other sources has also been manufactured from other mammalian sources such as bovine (cow) and ovine (sheep). Although most of the heparins used globally are obtained of porcine origin, bovine and ovine heparins have also been used in unregulated markets. The North American and European community primarily use heparins derived from porcine sources which are primarily of Chinese origin. Worldwide there are 1.4 billion cattle, 1.9 billion sheep and goats and 980 million pigs. Both cow and sheep provide alternate sources of heparin which are not widely used due to regulatory reasons. The FDA is currently considering the introduction of bovine unfractionated heparin. Ovine heparin and ovine enoxaprin provide an additional source of this important drug and are currently under development. Ovine heparin and ovine enoxaprin are found to be comparable to the porcine heparin and porcine enoxaprin in both in vitro assay and structural characterization. The specific activity of sheep heparin is around 180 USP u/mg which is comparable to porcine heparin. The ovine enoxaparin generated by depolymerization of ovine heparin meets all USP specifications. Extensive studies on the structural characterization and functional properties have been found to be similar in both sheep and porcine heparin. In particular enoxaparin derived from sheep heparin is comparable to enoxaparin derived from porcine heparin. Sheep heparin provides an alternate source of heparin and low molecular weight heparins which is particularly suitable for the Muslim world for religious reasons. It is affordable and cost effective to produce sheep heparin and enoxaparin. Currently sheep heparin is under pre-clinical development and will soon undergo clinical validation. Thus sheep heparin will fill an unmet need not only for the Muslim world but will also provide an alternate source of heparin.
Sulodexide (natural glycosaminoglycan with antithrombotic and profibrinolytic activities) can be administered orally or parenterally, with a very low risk of bleeding. It exerts its actions through the complexation with Antithrombin and Heparin Cofactor II, and has favorable effects on endothelial dysfunction. The main evidences in the field of vascular medicine concern the prevention of recurrence of venous thromboembolism, the treatment of venous ulcers and chronic venous disease, and treatment of peripheral arterial disease (intermittent claudication).

**Prevention of r-VTE.** Last November, have been published the results of the SURVET study, randomized double-blind controlled trial, aimed to verify efficacy and safety of SDX in the prevention of r-VTE after VKA discontinuation, in patients with a first-ever unprovoked venous VTE. 617 patients have been enrolled, 308 treated group (sulodexide 2 cps 250 LSU bid) and 309 control group (placebo 2 cps bid). Elastic stockings were recommended in both treatment groups. After 24 months r-VTE occurred in 15 and 30 patients in treated and control groups, respectively (Hazard ratio: 0.49; 95% CI, 0.27 to 0.92 [P = 0.02] and HR adjusted for confounders 0.45; 95% CI, 0.24 to 0.84; [P = 0.01]). No episodes of major bleeding occurred in either treatment group. Clinically relevant non-major bleeding occurred in 2 patients in each group. These results highlight the importance of Sulodexide as a treatment option in the prevention of r-VTE, especially for those with high risk of thrombosis and bleeding, and for elderly patients.

**Venous Ulcers and CVD.** In this field we have many evidences showing that sulodexide, compared to placebo, induces a better healing of VU, with significant reduction of surface, higher rate of complete wound healing, and in a significantly shorter time. Moreover, another study showed that sulodexide, in addition to standard treatment, in a period of 54 months after first-ever DVT, significantly reduces of post-thrombotic syndrome.

**PAD and Claudication.** Between 1989 and 1996, many studies on PAD have been published. All were included in an important meta-analysis, which demonstrate significant increase in pain free walking distance, lowering of fibrinogen, viscosity and triglycerides, and significant increase of HDL-Cholesterol.

In the following years, to verify these results it was designed a study on intermittent claudication (absolute walking distance of 100 to 300 m.) 141 patients were enrolled in the active group and 143 in the control group. The first received for 20 days 60 LSU of sulodexide intramuscularly, and 50 LSU orally bid in the following 6 months. The primary endpoint was the doubling baseline walking distance. 23.8% of patients in the active group and 9.1 in the control group have doubled the ICD, while the percentage of patients who doubled the ACD was 25.9 in the sulodexide group and 6.3 in the control group, respectively (p <0.001). The NNT was 7 for ICD, and 5 for ACD. Although the study was not designed to compare the rate of CV events, their rate was lower in the sulodexide group than in controls.
Regulatory Position on the Development of Bovine Heparins
Ali Al-Hakim, PhD

My talk will focus on FDA Perspectives and Recommendations for Heparin Applications and Proposal for Reintroduction of Bovine Heparin to the US Market. The presentation will include items related to heparin manufacturing process (critical control points and good manufacturing practice/strategy), risk prioritization and assessment, updated specifications, regulatory aspects of heparin applications and the proposal to reintroduce bovine heparin to the US market. Discussion will explain current status of FDA-approved porcine heparin applications and related experience with the porcine heparin supply chain. Regulatory history of bovine heparin New Drug Applications (NDAs) and current understanding of bovine spongiform encephalopathy (BSE) risk will also be discussed.
In memory of Erwin Coyne, PhD

Lifetime devotion to Heparins.
His legacy lives on
Loyola University Chicago
Health Sciences Division
Center for Translational Research and Education