Non-anticoagulant Activities of Glycosaminoglycans: A Promise for the Future Clinical Applications

Jawed Fareed, PhD
Professor of Pathology and Pharmacology,
Loyola University Chicago
Jaques – Heparin is a polyelectrolyte with polytherapeutic profile
Molecular Heterogeneity of Heparin

Both functional and molecular heterogeneity is observed.
Non Anticoagulant Effects of Heparins and Related Agents

1. Down Regulation of Inflammatory Mediators
2. Modulation of Adhesion Molecules
3. Effect on Heparanase and its Regulation
4. Release of Endogenous Mediators
5. Regulation of Lipids
6. Profibrinolytic Effects
7. Interaction with Growth Factors
8. Cellular Interactions
9. Gene Regulation
<table>
<thead>
<tr>
<th>Condition</th>
<th>Potential Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer</td>
<td>- TFPI release, anti-inflammatory actions, angiogenesis, growth factor interactions, endothelial modulation. (Several Reports)</td>
</tr>
<tr>
<td>Arthritis</td>
<td>- Collagen/Elastase modulation, angiostatic effects, inhibition of cell accumulation. (Anecdotal Reports)</td>
</tr>
<tr>
<td>Transplant Rejection</td>
<td>- Prolongs allograft survival in animal models. (Clinical Reports)</td>
</tr>
<tr>
<td>Asthma</td>
<td>- Improved lung function, anti-inflammatory effects in airways, down regulation of cytokines and cytotoxic cell products. (Controlled Clinical Trials)</td>
</tr>
<tr>
<td>ARDS</td>
<td>- Improved lung function, down regulation of inflammatory mediators same as asthma. (Controlled Clinical Trials)</td>
</tr>
</tbody>
</table>
### Potential Therapeutic Actions of Heparin Mediated Through the Non Anticoagulant Components (Continued)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Potential Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammatory Bone disease</td>
<td>- Generalized inhibition of cell migration, decreased production of inflammatory mediators. (Controlled Clinical Trials)</td>
</tr>
<tr>
<td>migration,</td>
<td></td>
</tr>
<tr>
<td>Allergic Encephalomyelitis</td>
<td>- Anti-inflammatory effects, multiple mechanisms. (Experimental Observations)</td>
</tr>
<tr>
<td>Interstitial Cystitis</td>
<td>- Effective in both human and animal models of interstitial cystitis. (Clinical Reports)</td>
</tr>
<tr>
<td>models</td>
<td></td>
</tr>
<tr>
<td>Allergic Rhinitis</td>
<td>- Inhibition of cellular activation, anti-inflammatory effects. (Clinical Trials)</td>
</tr>
<tr>
<td>Miscarriage</td>
<td>- Multiple mechanisms. (Clinical Observations)</td>
</tr>
</tbody>
</table>
Anticancer Effects of Heparins and Related Glycosaminoglycans

- Thrombin Inhibition and Regulation
- Apoptosis
- Oncogene Expression
- Heparanase Inhibition
- Multiple Drug Resistance Proteins Glycosylation
- Angiogenesis
Molecular Heterogeneity of Heparin

Both functional and molecular heterogeneity is observed.
<table>
<thead>
<tr>
<th>Protease/Receptor</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>TF</td>
<td>Overexpression $\rightarrow$ ↑ histological grade of Cancer</td>
</tr>
<tr>
<td></td>
<td>↑ metastatic potential of tumours</td>
</tr>
<tr>
<td></td>
<td>↑ in vitro tumour cell invasion</td>
</tr>
<tr>
<td></td>
<td>↑ in vitro tumour growth</td>
</tr>
<tr>
<td>TF/FVIIa</td>
<td>Increased expression of uPAR in SW979 cells</td>
</tr>
<tr>
<td></td>
<td>Induced calcium flux in J82 cells</td>
</tr>
<tr>
<td></td>
<td>VEGF production in melanoma cells</td>
</tr>
<tr>
<td></td>
<td>Cytoskeletal reorganisation in J82 cells</td>
</tr>
</tbody>
</table>
Target Proteases in Cancer

Thrombin/PAR-1
- Induces TF expression in SW480 Ca colon cells
- Enhanced uPA expression in Ca prostate cells
- Enhanced invasiveness of Ca breast cells
- Expression of Cyr-61 in HeLa cells

FXa/PAR-1
- Expression of Cyr-61 in HeLa cells

Heparanase
- Overexpression, isoforms in various cancers

Endogenous GAGs play a role in the modulation of these proteases. Synthetic and hemi-synthetic analogues of GAGs represent potential therapeutic approaches.
Anti-angiogenic effects of heparins

Control
(5U/ml)

UFH
(5U/ml)

Dalteparin
(5U/ml)

Angiogenic score

Graph showing the angiogenic score for Control, UFH (5U/ml), and Dalteparin (5U/ml) treatments.
Lewis Lung Carcinoma (LLC) Tumor Model

Day 1
- LN7 Cell injection

Day 7- Day 10
- Taxol/Heparin administration

Day 23
- sacrifice

Tumor growth

Tumor volume monitoring:

\[ V = L \times l^2 \times 0.52 \]

- \( L \): largest tumor diameter
- \( l \): smallest tumor diameter

C57B1/6

Heparin Treatment: from D7 to D22, 1 inj daily, s.c.

Mayo, 1972; Young et al., 2001
Heparin Conversion to Semuloparin

Heparin
MW = 16.7 KDa

Phosphazene depolymerization

Semuloparin
MW = 2.9 KDa
Effect of High Affinity and Low Affinity Semuloparin on Tumor Volume in the Lewis Lung Mouse Model

All agents were administered at 1.0 mg/kg SC for 2 weeks (n=10/group).
All animals (N=10/group) were administered 1.0 mg/kg SC of each agent. Blood samples were drawn upon sacrifice and VEGF was measured.
* $p < 0.01$ vs. control,
One-way ANOVA followed by a Tukey post hoc test.
Comparative Studies on Heparin and LMWHs on Primary Tumor Growth, Angiogenesis and Apoptosis in a Heparin Deficient Transgenic Mouse Model
Animal Model/Rationale

Animal Model
Transgenic mice deficient in N-Deacetylase/N sulphotransferase (NDST-2) unable to synthesize endogenous heparins.

Rationale
These mice are not able to synthesize endogenous heparin. Thus, when challenged with pathogenic triggers respond more adversely to NDST-2 animals. Therefore it provides a novel model to investigate the regulatory roles of heparins.

Treatment
All animals were administered once a day for 2 weeks.
**Study Parameters**

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor volume</td>
<td>Daily</td>
<td>15 days</td>
</tr>
<tr>
<td>Apoptosis (Tunel)</td>
<td>At the end</td>
<td>15-18 days</td>
</tr>
<tr>
<td>Angiogenesis (Anti-CD31)</td>
<td>At the end</td>
<td>15-18 days</td>
</tr>
</tbody>
</table>

Statistical analysis included one-way ANOVA and individual comparisons against the control group (Dunet test). Significance p<0.001
Comparative Effect of UFH and LMWHs in an Experimental Tumor Model
Dosage: 250 ug/kg SC

* P vs. control
Comparative Effect of UFH and LMWHs in an Experimental Tumor Model

* P vs. control
Comparative Effect of UFH and LMWHs in an Experimental Tumor Model

* P vs. control
Studies on Walker 256 Carcinosarcoma
### Tumor Burden and Thrombogenesis in a Rat Walker 256 Carcinosarcoma Model

<table>
<thead>
<tr>
<th>Duration</th>
<th>Stasis Clot Score</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>FEIBA (2.5 U/kg)</td>
</tr>
<tr>
<td>Baseline (0 time)</td>
<td>0</td>
<td>1.6±0.7</td>
</tr>
<tr>
<td>1 week</td>
<td>0.7±0.3</td>
<td>2.3±0.9</td>
</tr>
<tr>
<td>2 weeks</td>
<td>1.2±0.5</td>
<td>3.1±0.7</td>
</tr>
<tr>
<td>4 weeks</td>
<td>1.9±0.8</td>
<td>3.8±1.6</td>
</tr>
</tbody>
</table>

Individual groups of rats were injected with filtered cellular homogenates of Walker 256 CS intraperitoneally and thrombogenesis was assessed at different times.
**Anticancer Effects of Various Low Molecular Weight Heparins**

<table>
<thead>
<tr>
<th>Agent</th>
<th>% Decrease of Tumor Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>0</td>
</tr>
<tr>
<td>Unfractionated heparin</td>
<td>14±5.1</td>
</tr>
<tr>
<td>Enoxaparin</td>
<td>18±6</td>
</tr>
<tr>
<td>Semuloparin</td>
<td>20±7</td>
</tr>
<tr>
<td>HAF-Semuloparin</td>
<td>18±4</td>
</tr>
<tr>
<td>LAF-Semuloparin</td>
<td>22±6</td>
</tr>
</tbody>
</table>

All agents were dosed at 1.5 mg/kg BID for 2 weeks. Saline was injected at a comparable volume of 0.25 ml.
Anti-Metastatic Effects of Heparins in a B16 Melanoma Model of Metastasis
Beside Anticoagulation, Heparins Have a Major Role in Cancer Management

- NO
- PGI₂
- TFPI
- TNF-α
- CRP
- Heparinases
- TAFI

Xa, IIa, IXa, plus other AT-dependent Coagulation factors

Protein Matrix
Inflammation
Non-Anticoagulant Heparins and Related Agents

1. AT fractionated heparin components
   a. High affinity
   b. Low affinity

2. Molecularly modified heparins
   a. De-sulfated heparins (Regioselective)
   b. Glycol split heparin

3. GAG analogues and homologues
The comparative effects of three different fractions of semuloparin on the inhibition of FXa and thrombin generation following supplementation in NHP

Anti-Xa

Mean IC$_{50}$ value ± the standard deviation of three individual experiments.

One-way ANOVA followed by a Bonferroni multiple comparison test.

*$p < 0.001$ vs. non-AT AF semuloparin; $^\wedge$ $p < 0.01$ vs. semuloparin and non-AT AF semuloparin.
TFPI Release by NON AT Affinity Heparins

1. Low affinity (AT) Heparins
2. Astenose
3. 2-0 desulfated heparins
TFPI release in Non-human Primates Treated with semuloparin and its Oligosaccharide Fractions
Dosage: 1.0 mg/kg IV
TFPI measured 15 minutes post administration

All results represent a mean±SD of 4-6 primates.
TFPI Release by Semuloparan and it's Fractions
Modulation of Nitric Oxide by Heparin and Its Fractions

![Bar chart showing NO (uM) levels for Heparin, HAF, LAF, and Saline]
Bianchini – Pioneering Contributions in GAG Development
Non-anticoagulant activities of GAGs

- Sulfomucopolysaccharide
- (mixture of Heparin, Heparan sulf., dermatan, condroitin sulf)

- Sulodexide
- (mixture of Heparan sulf, dermatan, condroitin sulf)

- Senile dementia
- Emotional dysfunctions
- Cellulitis (vials mesotherapy)

- Atherosclerosis
- Peripheral vascular disease
Non-anticoagulant activities of GAGs

• Genes involved in heparin and Heparan sulfate metabolism are also involved in the regulation of lipids metabolism (probable single and multiple polymorphisms are involved)
• This may have impact on both lipid metabolism and angiogenesis
  [D’ippolito t al. Plos One 2012]
that are commonly dysregulated in cerebral and peripheral diseases related to microcirculation
Non-anticoagulant activities of GAGs

- Neuroparin [C3] a ULMWH [4-14 saccharides] made by γ irradiation
- Senile dementia
- Emotional dysfunction
- Cisplatin nerve damage (peripheral and central)

[three international patents]
Non-anticoagulant activities of GAGs

• Hepcidin inhibition
• [hepcidin is a ploypetide (25AA) produced in the liver only that binds and induces the degradation of ferroportin, the only cellular iron exporter]
  [Poli et al Blood 2011]

This gives us an indication for the treatment / prevention of parkinson’s disease
GAG Symposia 1991-1995

- Structure activity relationship in GAGs
- Antithrombotic and bleeding effects of GAGs
- **Synthetic and biosynthetic analogues of GAGs**
- Recognition of GAG science as a moving target in drug discovery
- Analytical methods for characterization (NMR, MS, HPLC, CE, GCMS and other methods)
- Protein interactions with GAGs
- **Anti-inflammatory actions of GAGs**
- TFPI release
- PK/PD of heparins
- **Chemically modified heparins**
- Cellular interactions of GAGs and their relevance to physiologic and pathologic responses
GAG Symposia 1991-1995 (continued)

- Role of GAGs in cancer
- PK/PD of GAGs and related agents
- Role of heparan sulfate as a signaling molecule
- Growth factor interactions with GAGs
- Heparin and angiogenesis
- Neuroprotective effects of GAGs
- Original discussions on the development of recombinant hirudin
- Surface coating of GAGs to develop non-thrombogenic material
- Species specificity in GAGs structure
- Differentiation of porcine and bovine heparins
GAG Symposia 1996-2001

- Structural diversity workshop
- Novel clinical applications of GAGs
- Antithrombin drugs
- Clinical validation of newer anticoagulants
- Synthetic bifunctional oligosaccharides
- Clinical development of newer antithrombins and antiplatelet drugs
- Newer approaches in DVT and PE management
- Cellular biology of GAGs
- Unresolved issues in thrombosis
- Heparin-induced thrombocytopenia
- Standardization of hirudins
GAG Symposia
1996-2001 (continued)

- Biotechnology derived heparin oligosaccharides
- Structural components of GAGs and their role in protein and cellular binding
- The discovery and early development of melagatran
- GAGs in diabetic nephropathy
- Cellular proliferation and growth modulation by GAGs
- Sequencing of specific protein binding GAGs
- Endothelial protection of GAGs
- Synthesis and biologic properties of nitro heparins
- Regioselective structural analysis of GAGs and their derivatives
- Heparinase-1 as a neutralizing agent for heparin
- Recombinant Heparanase as a tool to develop anticancer drugs
GAG Symposia 2002-2007

Broadened scope to include other areas related to GAGs

• Novel tools to isolate and analyze GAGs
• Heparin oligosaccharides in senial dementia
• TFPI in the control of thrombogenesis
• Glycosaminoglycan protein interactions in the control and regulation of metastasis
• Argatroban as an anticoagulant for heparin compromised patients
• GAG mediated modulation by TAFI
• Ultra low molecular weight heparins
• Generic versions of LMWHs
• GAGs in renal disease
• Heparinase biology and pharmacology including development of inhibitors
GAG Symposia 2002-2007 (continued)

• Role of heparins in cancer
• **Engineered heparins**
• Ultra LMWHs, octaparin and related agents
• **Modulation of osteoporosis and calcification**
• Physical methods to prepare LMWHs
• Alternate route to administer heparin and related GAGs
• **Heparin in Alzheimer’s disease**
• Identification of novel structural sites interacting with AT and other proteins
• Novel application of NMR and mass spect to analyze LMWH
• GAGs in the nervous system
GAG Symposia 2008-2011

- Additional biologic effects of heparin
- Contaminants in heparin
- Biophysical aspects of GAGs
- In depth characterization of heparins and LMWHs
- Anticoagulants beyond heparin
- Focused discussions on heparan sulfate including chemistry, biochemistry and molecular biology
- Inflammation and cancer are targets for GAGs
- Conversion approaches to analyze heparin
- Pharmacological aspects of heparin anticoagulation
- Finding a needle in a haystack! The wonders of new technologies
- Heparinase biology and pathology
GAG Symposia 2008-2011 (continued)

- Adhesion molecules and GAGs
- Molecular biology of GAGs with reference to gene transcription
- US FDA approval of the generic version of enoxaparin
- Oral anti-Xa and anti-IIa drugs
- Chemoenzymatic design of heparins
- Proteoglycans and GAGs in health and disease
- In depth topics on GAGs as anticancer and anti-inflammatory agents
- Advances approaches in the analysis of GAGs
- Heparins including LMWH, ULMWH, bioheparins and unique oligosaccharides
- Newer anticoagulants
GAG Symposia 2012
Topics with Potential Implications of Non-Anticoagulant Applications of GAGS

- Heparan Sulfate Derived Oligosaccharides as Inhibitor of β-Secretase as potential therapeutic agents in AD - Turnbull
- GAG Signals Involvement in Pulmonary Metastasis of Tumor Cells - Sughara
- Inhibition of Melanoma Metastasis in Mice - Schneider
- Heparin Interference with Adhesion Receptor Signaling - Bendas
- Heparin as an Inhibitor of Metastatic Colonization - Borsig
- Cell Surface Heparan Surface in Breast Cancer Mstastasis - Goette
- Rational Design of Heparanase Inhibitors _ Vlodavsky
- Clinical Trials Design on SST0001 - Barbieri
- Iron Oxide Nanoparticles – Heparin Hybrid Systems – Vismara
- Non-Anticoagulant Activities of GAGS - Fareed
THANKS TO PROFESSORS CASU and HARENBERG
AND ALL THOSE WHO HAVE CONTRIBUTED TO THIS OUTSTANDING
SERIES OF SYMPOSIA DURING THE PERIOD 1991-2012