Complex Multi-factorial pathophysiological Processes in sickle cell disease: Recent Therapeutic Advances

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Complex Pathophysiology in SCD

- Sickling
- CVD, VTE, Stroke, PAH,
Pathophysiology of SCD

1. Molecular
2. Cellular
3. Vascular
4. Biochemical
5. Clinical
Molecular pathology of SCD
Normal versus sickle beta globin

**Normal**

\[ \beta^A \]

1 2 3 4 5 6 7 8 9 10 146

**Sickle**

\[ \beta^S \]

1 2 3 4 5 6 7 8 9 10 146

glu

val
Pathophysiology of SCD

1. Molecular
2. Cellular
3. Vascular
4. Biochemical
5. Clinical
Pathophysiology of SCD: Cellular
Pathophysiology of SCD

In a red blood cell containing mostly Hb S...

When oxygenated...

...single Hb S molecules in solution; allows red cell to be soft, and round

When deoxygenated

- O₂

+ O₂

Hb S molecules polymerize into long fibers; mishapen, dehydrated and adherent sickle cells.
Pathophysiology of SCD

1. Molecular
2. Cellular
3. Vascular
4. Biochemical
5. Clinical
Pathophysiology of SCD: Cellular
SCD Pathogenesis
Cellular Adhesion to ECs

Pathophysiology of SCD

1. Molecular
2. Cellular
3. Vascular
4. Biochemical
5. Clinical
<table>
<thead>
<tr>
<th>Pathogenesis Factor</th>
<th>Effect on Biomarkers in SCD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal Adhesion To Vascular Endothelium</td>
<td>↑ VCAM</td>
</tr>
<tr>
<td></td>
<td>↑ ICAM</td>
</tr>
<tr>
<td></td>
<td>↑ L-selectin</td>
</tr>
<tr>
<td></td>
<td>↑ P-selectin</td>
</tr>
<tr>
<td>Inflammation</td>
<td>↑ TNF-α</td>
</tr>
<tr>
<td></td>
<td>↑ IL-1β</td>
</tr>
<tr>
<td></td>
<td>↑ IL-6,</td>
</tr>
<tr>
<td></td>
<td>↑ IL-8,</td>
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<tr>
<td></td>
<td>↑ MCP-1</td>
</tr>
<tr>
<td></td>
<td>↑ MIP-α</td>
</tr>
<tr>
<td></td>
<td>↑ IFN-γ</td>
</tr>
<tr>
<td>Hemolysis, Reperfusion Injury and Nitric Oxide</td>
<td>↑ Soluble L-selectin</td>
</tr>
<tr>
<td></td>
<td>↓ NO</td>
</tr>
<tr>
<td>Hypercoagulation</td>
<td>↑ D-dimer</td>
</tr>
<tr>
<td></td>
<td>↑ Soluble P-selectin</td>
</tr>
</tbody>
</table>
Pathophysiology of SCD

Consequences of Hb S polymerization RBC sickling

- Red cell injury and Hemolysis
- Adhesion of RBC to endothelium
- Formation of hetero-cellular aggregate
- Propagation of vaso-occlusion in adjacent vasculature
- Deficits in vasodilator mediators (NO)
- Increased inflammation
- Hyper-coagulation (VTE), complement activation, …
Pathophysiology of SCD

1. Molecular
2. Cellular
3. Vascular
3. Biochemical
5. Clinical
1. Anemia

2. Vaso-occlusion

3. Chronic organ damage
The goal is to relieve the pain; prevent infections, organ damage, strokes and control complications.

Pain medicine: acetaminophen, non-steroidal anti-inflammatory drugs (NSAIDs), and narcotics such as morphine, oxycodone, and etc.

- Heating pads

- Hydroxyurea, Folic Acid, L-Glutamic
## Pharmacotherapy of SCD
### Hb F Induction

<table>
<thead>
<tr>
<th>Agent</th>
<th>Mode of action</th>
<th>Human trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyurea</td>
<td>Stress erythropoiesis; selection of F-cells</td>
<td>Phase 3, successful</td>
</tr>
<tr>
<td>Short chain fatty acids</td>
<td>Histone deacetylase inhibitor; reactivation of $\gamma$-gene expression</td>
<td>Promising in small studies; high doses required; variable responses</td>
</tr>
<tr>
<td>(Butyric Acid)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decytabine</td>
<td>Cytosine analog; DNA methyltransferase inhibitor</td>
<td>Promising in small pilots</td>
</tr>
</tbody>
</table>
## Pharmacotherapy of SCD
### Nitric oxide donors/regulators

<table>
<thead>
<tr>
<th>Agent</th>
<th>Mode of action</th>
<th>Human trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO</td>
<td>Improve micro-vascular blood flow</td>
<td>Shortens duration of pain</td>
</tr>
<tr>
<td>Arginine</td>
<td>NO synthase substrate</td>
<td>Reduce PA systolic pressure</td>
</tr>
<tr>
<td>Sildenafil</td>
<td>Phosphodiesterase-5 inhibitor</td>
<td>May help in pulmonary hypertension and priapism</td>
</tr>
</tbody>
</table>

**Oral L-Glutamine**

**Oral Voxelotor (previously called GBT440) Phase 2/3**

Hematopoietic stem cell transplantation and Gene Therapy
### Pharmacotherapy of SCD
#### Anti-adhesion agents

<table>
<thead>
<tr>
<th>Agent</th>
<th>Mode of action</th>
<th>Human trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>RheothRx</td>
<td>Improve micro-vascular blood flow</td>
<td>Phase 3, reduced duration of pain episode, analgesic use</td>
</tr>
<tr>
<td>Anti-Selectins</td>
<td>Improve micro-vascular blood flow</td>
<td>Preclinical, Phase 1/2</td>
</tr>
<tr>
<td>LMWH</td>
<td>Multiple Mode of actions</td>
<td>Double blind Randomized Multicenter (Qari et al: Thrombosis Hemostasis 2007)</td>
</tr>
</tbody>
</table>

Several other Strategies are under exploration in preclinical and Clinical investigations include the followings: r-Thrombomodulin, L-Glutamine, Thiol containing compounds, 5-hydroxymethyl 2 furfural (5-HMF), a breakdown product from glucose, Endothelin antagonists, weaker LMWH, Sulfated Non-Anticoagulant LMWH (S-NACH)
LMWH - Sevuparin – Weaker Anticoagulant

Sevuparin

IV Infusion

Intact pentasaccharide sequence
Role of Ultra-Heparin Derivatives in the Management of Sickle Cell Disease

S-NACH
A. Unfractionated heparin:

\[ n = 20 - 35, \, R_1 = H \text{ or } SO_3^-, \, R_2 = H \text{ or } Ac \text{ or } SO_3^-, \, R_3 = H \text{ or } Na^+ \]

B. Tinzaparin:

\[ n = 4 - 10, \, R_1 = H \text{ or } SO_3^-, \, R_2 = H \text{ or } Ac \text{ or } SO_3^-, \, R_3 = H \text{ or } Na^+ \]

C. NAC heparin:

\[ R_1 = H \text{ or } SO_3^-, \, R_2 = H \text{ or } Ac \text{ or } SO_3^-, \, R_3 = H \text{ or } Na^+ \]

Figure 1. Chemical structure. Molecular structure of: (A), unfractionated heparin; (B), Tinzaparin; and (C), NAC heparin.
## Structure Differences Between S-NACH and Enoxaparin

<table>
<thead>
<tr>
<th>Differences</th>
<th>S-NACH</th>
<th>Enoxaparin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average M. Wt.</td>
<td>4,000</td>
<td></td>
</tr>
<tr>
<td>4,500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fractions &lt; 2000 Da</td>
<td>≤ 20%</td>
<td>≤ 20%</td>
</tr>
<tr>
<td>2,000-6,000 Da</td>
<td>60-70%</td>
<td>≤ 60%</td>
</tr>
<tr>
<td>&gt; 6,000-8,000 Da</td>
<td>≤ 10%</td>
<td>≤ 15%</td>
</tr>
<tr>
<td>Sulfate/Carboxylate</td>
<td>2.5-3.5/1</td>
<td>1.5-1.8/1</td>
</tr>
</tbody>
</table>
Multiple Pathways Targeted by UFH/LMWH and S-NACH

UFH/LMWH
Plasmatic + vascular activity

S-NACH
Vascular affinity
Complex Pathophysiology in SCD

- Sickling
- CVD, VTE, Stroke, PAH,
SNACH Restores Normal Round Shape to sickled RBCs from Sickle Cell Patient under hypoxia

Human blood drawn from Sickle Cell patients, incubated with and w/o S-NACH

- S-NACH 24 hr. incubation at 37 C, 1-10 µg/ml
  *Essentially 100% of cells returned to normal round shape*

- Control 24 Hour incubation at 37 C
Anti-Sickling Efficacy of SNACH in Blood from Sickle Cell Subject

Percentage of sickle cell (80.3 %)

Percentage of sickle cell (1.5 %)
Anti-Sickling Efficacy of SNACH in Blood from Sickle Cell Subject

**P < 0.001**
Anti-Sickling Efficacy of SNACH in Blood from Sickle Cell Subject

Patient 5
Incubation at 25 C

**PBS**

- Red arrow = Sickled cell
- Green arrow = Normal cell
- Percentage of sickle cell (80.3 %)

**SNACH**

- Red arrow = Sickled cell
- Green arrow = Normal cell
- Percentage of sickle cell (1.5 %)
Average of sickle cells in the all 12 patients

Sickling decreased by 80%
Could Nanotechnology Enable us to convert Injectable LMWH / S-NACH into an Oral Dosage form?

\[
m = 1 \text{ to } 25, \quad R = H \text{ or } \text{SO}_3\text{Na}, \quad R1 = H, \text{SO}_3\text{Na} \text{ or } \text{COCH}_3, \quad R2 = H \text{ and } R3 = \text{COONa} \text{ or } R2 = \text{COONa} \text{ and } R3 = H, \quad \text{and } \text{N-}(\text{3Dimethylaminopropyl})' -\text{N'-ethylcarbodiimide hydrochloride} = \text{EDC}
\]
Oral Bioavailability of Nano-LMWH versus LMWH
SNACH: Mechanism – Sickle Cell

• Anti-Sickling
• Nanoformulated with Maximal Oral Bioavailability
• Prevents cellular adhesions
  • Inhibits P, L, S selections - prevents deformed blood cells from attaching to blood vessel walls and causing VOC and painful crisis
• Mitigates vascular occlusions
  • Induction of endothelium-dependent relaxation mediated by nitric oxide
  • Local Anti-thrombotic effect via inhibition of TF
• Vascular specificity
  • Sulfation Increased affinity for vascular endothelium
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