Augmenting Nitric Oxide Signaling in SCD

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Disclosures

• Research support from Bayer AG

• Consultant for Mast Pharmaceuticals
Augmenting NO Signaling

- Background
- Inhaled NO
- Sildenafil
- L-arginine
- Riociguat
- Topical sodium nitrite
- Hydroxyurea
- PDE9 inhibitors
• 46 year old African American woman with sickle cell anemia
• Leg and foot ulcers for many years
• Pain crisis twice a year
• Diagnosed PAH, treated with sildenafil with clinical improvement
• Died during pain crisis complicated by sepsis
Sickle Cell Leg Ulcer

Minniti et al., Am J Hematol 2014
Low Bioavailability of Nitric Oxide

Nolan et al. BJH 2006
Kato, Steinberg & Gladwin, Blood Rev 2007
Connes et al., 2014
Overlapping spectrum

- Serum LDH
- Reticulocyte count
- Plasma Hb and arginase
- Pulmonary HTN, Priapism, Leg ulcers
- Stroke?

- Hemolysis-Endothelial Dysfunction
  - Proliferative Vasculopathy

- Viscosity-Vaso-occlusion
  - Erythrocyte Sickling

- Hemoglobin level
  - Vaso-occlusive pain crisis
  - Acute chest syndrome
  - Osteonecrosis

- $\alpha$-thalassemia shifts subphenotype

Kato, Steinberg & Gladwin, Blood Rev 2007
# SCD Endophenotypes

<table>
<thead>
<tr>
<th>Clinical Features</th>
<th>Effect of Hyperhemolysis</th>
<th>Effects of α thalassemia</th>
<th>Protection by HbF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain or dactylitis</td>
<td>Reduced risk</td>
<td>Increases risk</td>
<td>Protective</td>
</tr>
<tr>
<td>Acute chest syndrome</td>
<td>Neutral</td>
<td>Increases risk</td>
<td>Protective</td>
</tr>
<tr>
<td>Leg ulcers</td>
<td>Increases risk</td>
<td>Reduces risk</td>
<td>Equivocal</td>
</tr>
<tr>
<td>Osteonecrosis</td>
<td>Reduced risk</td>
<td>Increases risk</td>
<td>Equivocal</td>
</tr>
<tr>
<td>Priapism</td>
<td>Increases risk</td>
<td>Reduces risk</td>
<td>Not protective</td>
</tr>
<tr>
<td>Nephropathy</td>
<td>Increases risk</td>
<td>Reduces risk</td>
<td>Not protective</td>
</tr>
</tbody>
</table>
| Cerebrovascular         | Increases risk           | Reduces risk             | Infants: Not protective  
                        |                          |               | Adults: Possibly protective |
| Cholelithiasis          | Increases risk           | Reduces risk             | Protective       |
| Retinopathy             | Neutral                  | Equivocal                | Possibly protective |
| ↑TRV or ↑SBP            | Increases risk           | Equivocal                | Not protective   |
| Mortality               | Increases risk           |                         | Protective       |
Nitric oxide resistance

SMOOTH MUSCLE

ENDOTHELIELUM

BLOOD VESSEL LUMEN

eNOS

NO

sGC

GTP → cGMP → Relaxation → INCREASED BLOOD FLOW
Nitric oxide resistance

Atorvastatin Reduces Serum Cholesterol And Triglycerides With Limited Improvement In Vascular Function In Adults With Sickle Cell Anemia

Candice Bereal-Williams, Roberto F. Machado, Vicki McGowan, Amy Chi, Christian J. Hunter, Gregory J. Kato
Haematologica November 2012 97: 1768-1770; Doi:10.3324/haematol.2011.054957

Atorvastatin in SCD

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iNO

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NO

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GTP → cGMP → Relaxation → INCREASED BLOOD FLOW

Hb

Hb
Inhaled nitric oxide

- DeNOVO trial
- RCT 150 adolescents and adults in VOC
- Inhaled NO by Inovent followed by Inopulse
- No benefit
- What went wrong?

Inhaled nitric oxide

- NO gas has a half life about 1 second
- NO not expected to circulate from lungs to tissues
- But prior phase 2 data had been positive
- Lesson: phase 2 data limited to assess efficacy
SMOOTH MUSCLE

ENDOTHELIUM

BLOOD VESSEL LUMEN

eNOS

sGC

NO

GTP → cGMP → Relaxation

INCREASED BLOOD FLOW

Hb

PDE5

GMP

Sildenafil
Sildenafil

• Walk-PHaSST trial
• RCT designed for 132 patients
• Age ≥12 yr, TRV ≥2.7 m/s, 6MWD < 500m
• 16 weeks of sildenafil vs placebo

Hospitalization for pain in patients with sickle cell disease treated with sildenafil for elevated TRV and low exercise capacity

Sildenafil

• Stopped at 74 pts due to high SAE rate
• VOC 35% sildenafil vs 14% placebo, P=0.03
• Nonsignificant improvement in 6MWD
• What went wrong?
No NO, no pain? The role of nitric oxide and cGMP in spinal pain processing

Achim Schmidtko, Irmgard Tegeder and Gerd Geisslinger

Guanylate cyclase-C/cGMP: an emerging pathway in the regulation of visceral pain

Gerhard Hannig, Boris Tchemychev, Caroline B. Kurtz, Alexander P. Bryant, Mark G. Currie and Inmaculada Silos-Santiago*

Sildenafil induces hyperalgesia via activation of the NO-cGMP pathway in the rat neuropathic pain model

C. S. Patil¹, S. V. Padi, V. P. Singh, and S. K. Kulkarni*

0925-4692/06/020022-6
DOI 10.1007/s10787-006-1511-y
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Sildenafil

- Sildenafil may have increased pain sensitivity in the dorsal root ganglia
- Only 56% of target 132 enrolled due to early closure
  - Only 29 completed study (22% of target)
  - Low statistical power for efficacy
- Anecdotal cases of clinical improvement
  - May not increase pain in all patients
Current clinical trials

• L-arginine
• Riociguat
• Topical sodium nitrite
• PDE9 inhibitors
SMOOTH MUSCLE

ENDOTHELIUM

BLOOD VESSEL LUMEN

eNOS

Arg

Hb

INCREASED BLOOD FLOW

BLOOD VESSEL LUMEN

ENDOTHELIUM

SMOOTH MUSCLE

Arg → eNOS → NO → sGC → GTP → cGMP → Relaxation → INCREASED BLOOD FLOW

GMP
L-arginine: VOC

- Precursor to NO synthesis
- Claudia Morris, MD
- Phase 2 RCT, 38 children, 56 episodes
- L-arginine 100 mg/kg q8hr
- 56% reduction in opioid use (p=0.02)
- Trend to lower LOS (0.7 days, p=0.34)
- Now in phase 3 trial

L-arginine: VOC opioid use

L-arginine: Pain scores

SMOOTH MUSCLE

ENDOTHELium

BLOOD VESSEL LUMEN

eNOS

NO

sGC

GTP → cGMP → Relaxation → INCREASED BLOOD FLOW
Riociguat (Adempas®)

• Soluble guanylate cyclase stimulator
• Approved in 2014 for:
  • Pulmonary arterial hypertension
  • Chronic thromboembolic pulmonary hypertension
• Bayer HealthCare Pharmaceuticals
Adempas sensitizes soluble guanylate cyclase (sGC) to endogenous NO by stabilizing sGC-NO binding.

Adempas directly stimulates sGC independently of NO via a different binding site.

INDEPENDENTLY OF

IN THE PRESENCE OF

http://www.adempas-us.com/hcp/how-adempas-works/
Riociguat

- Well-tolerated oral vasodilator
- Efficacious in PAH/CTEPH
- Good solution for defects in NO bioavailability
Hypotheses

- Riociguat is safe and well tolerated in adults with SCD
- Riociguat will provide preliminary efficacy in reversing:
  - Systemic hypertension
  - Pulmonary vasculopathy
  - Proteinuria
Target complications

Systemic HTN ⇒ Proteinuria ⇒ High TRV

Proteinuria ⇒ STROKE, CKD, PH, DEATH
STERIO-SCD

- Safety, Tolerability and Efficacy of Riociguat in Adults with Sickle Cell Disease
- Investigator-initiated, industry-funded
- Sponsor is University of Pittsburgh
- Funded by Bayer Pharmaceuticals
- PI’s Gregory Kato and Mark Gladwin
12 week, ascending dose RCT

Screening

Randomization

Week 0

Riociguat

1.0 mg TID

1.5 mg TID

2.0 mg TID

2.5 mg TID

Placebo

Sham titration

Titration Phase

Maintenance Phase

End of treatment safety follow-up

Week 2

Week 4

Week 6

Week 8

Week 10

Week 12
Eligibility Criteria

• Age 18 years or older; any form of SCD
• At least one of the following findings:
  • Systolic blood pressure >130 mm Hg
  • Urine albumin to creatinine ratio > 300 mg/g
  • TRV > 2.9 m/sec
• Willingness to provide a specimen for DNA
• If on hydroxyurea or vasodilator therapy:
  • Stable therapy for three months
Primary endpoints measure

- Overall incidence of treatment emergent SAE in the riociguat arm compared to the placebo arm
Secondary endpoints

- VOC
- Overall adverse events
- Brief Pain Inventory
  - And electronic daily pain diary at some sites
Main efficacy outcomes

• 6 minute walk distance
• blood pressure
• Tricuspid regurgitant velocity
  • echo markers of LV diastolic dysfunction
• NT-proBNP
• Modified Borg Dyspnoea Scale
• urine albumin, creatinine, eGFR
Main efficacy outcomes

- Hemoglobin, LDH, reticulocyte count, white blood cell count, fetal hemoglobin
- Sickle Cell related clinical complications
  - acute chest syndrome
  - Priapism
  - new leg ulcer
  - new stroke
  - other new non-CNS thromboembolic event
SMOOTH MUSCLE

ENDOTHELIUM

BLOOD VESSEL LUMEN

Nitrite

eNOS

Hb

sGC

NO

INCREASED BLOOD FLOW

PDE5

GTP

cGMP

Relaxation

GMP

BLOOD

Hb

FLOW

Nitrite

eNOS

Hb

Nitrite is an NO prodrug

$\text{NO}_2^- \xrightarrow{\text{Low } O_2} \text{NO} \cdot$

- Hemoglobin
- Myoglobin
- Neuroglobin
- Cytochromes
- Xanthine Oxidase
Nitrite Increases Regional Blood Flow

Increased peri-wound skin temperature by infrared imaging

Before Sodium Nitrite application

mean ± std for 30-min imaging

After Sodium Nitrite application

Alex Gorbach, NIBIB
Topical sodium nitrite

- Well tolerated
- Increases regional blood flow
- Decreases pain
- Reduces wound size

Topical sodium nitrite for chronic leg ulcers in patients with sickle cell anaemia: a phase 1 dose-finding safety and tolerability trial

Caterina P Minniti, Alexander M Gorbach, Dihua Xu, Yuen Yi Hon, Kara-Marie Delaney, Miles Seidel, Nitin Malik, Marlene Peters-Lawrence, Carly Cantilena, James S Nichols, Laurel Mendelsohn, Anna Conrey, George Grimes, Gregory J Kato

www.thelancet.com/haematology
http://dx.doi.org/10.1016/S2352-3026(14)00019-2
Hydroxyurea is an NO donor

King SB
A role for nitric oxide in hydroxyurea-mediated fetal hemoglobin induction.
Hydroxyurea is an NO donor

Cokic VP, et al.  
*J Clin Invest 2003; 111:231-9.*

Gladwin MT, *et al.*  

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**Academy for Sickle Cell and Thalassaemia (ASCAT): London**
Cell adhesion in vaso-occlusion
PDE9 inhibitors

Decreased neutrophil adhesiveness

Decreased endothelial adhesiveness

PDE9 Inhibitor

- IMR-687
- Sickle cell mouse
- increases HbF levels
- reduces red cell sickling
- reduces leukocytosis
- reduces microvascular stasis
- ASH abstract
PDE9 inhibitors

- Bayer BAY73-6691
- Pfizer PF-04447943
  - Phase 1 study ongoing in adults with SCD
- Imara IMR-687
  - Preclinical studies
Augmenting NO Signaling

- Background
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- L-arginine
- Riociguat
- Topical sodium nitrite
- Hydroxyurea
- PDE9 inhibitors
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