The Use of Hydroxyurea in Adults with Sickle Cell Disease

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Standard Therapy for SCD-1990

- **Infection prophylaxis**
  a. Oral penicillin
  b. Vaccination (HIB, pneumovax)

- **Aggressive Treatment of Infections**

- **Management of Pain Crisis**
  a. Hydration
  b. Analgesics

- **Transfusions**
Beneficial Effects of Increased Hb F

**In Sickle Cell Disease:**
- Exclusion from deoxy Hb S polymer
- Inhibition of sickling

**In β-Thalassemia:**
- Compensation for decreased/absent β-chain synthesis
- Amelioration of globin chain imbalance
New Therapeutic Approaches to SCD

- **Augmentation of Hb synthesis**
  - a. Hydroxyurea
  - b. Butyrate derivatives
  - c. Erythropoietin
  - d. Epigenetic therapies (HDACi/ DMT1i)
  - d. Combined therapies

- **Prevention of K+ loss and RBC dehydration**

- **Prevention of cell adhesion**

- **Bone Marrow Transplantation**

- **Gene Therapy**
Hb F Induction

- Anti “switching” therapy beneficial for both beta thalassemia and SCD
- Agents that induce Hb F in adults
  - Hydroxyurea (HU)
  - Butyrate and short-chain fatty acids
  - 5’Azacytidine
  - Decitabine
  - Thalidomide derivatives
BASELINE Hb F LEVELS IN SCD

~50% variation:
- FCP on Xp22
- -158 C→T at Gγ (Xmn 1 polymorphism)

~20-30% variation (12 SNPs in 6q22.2-23.2)
- Phosphodiesterase 7 (PDE7)
- Microtubule associated protein 7 (MAP7)
- Peroxisomal biogenesis factor 7 (PEX7)
- Mitogen activated protein kinase 5 (MAP3K5)
Hydroxyurea in SCD: Milestones

- PLATT et al (1984): Increase in Hb F in two SS patients treated with intermittent (5 day) courses of Hydroxyurea
- DOVER et al (1986): Analysis of Hb F and F-cell production in response to Hydroxyurea in SS patients
- RODGERS et al (1990): Hematologic response to HU in 10 SS patients treated with an intermittent dosing schedule
CHARACHE et al (1992): Multi-center study of open label HU in 32 SS patients using a daily dose with escalation to MTD


CHARACHE et al (1995): MSH; Efficacy of HU in decreasing crisis frequency in SS patients
Hydroxyurea in SCD: Milestones

Hydroxyurea in SCD: Milestones

- De MONTALAMBERT ET AL (2006): Efficacy of HU in 225 children with SCD treated for a median of 3.8 years. No severe toxicity observed

- WANG et al (2002): BABY HUG Study
- WARE et al (2005): SWiTCH Trial
Phase III, multicenter, randomized study of HU in adult SCD patients with >3 crises per year (21 centers, MCG enrolled 15 patients)

- 299 patients enrolled (152 HU; 147 placebo)
- Study stopped early because of significant HU effect
  - 44% reduction in crisis
  - 50% reduction in transfusion
  - 50% reduction in acute chest syndrome

HU approved by FDA for SCD in 1997

14 year follow-up
The Relationship Between Hydroxyurea Usage and Mortality

The following hydroxyurea usage categories (which were defined a priori) were considered in our analysis:

**HU Continuous** was defined as a patient using hydroxyurea more than 80% of the time he/she was followed (from MSH through MSH PFU Ext.1, AV08)

**HU Episodic** was defined as a patient using hydroxyurea anywhere from 20% to 80% of the time he/she was followed (from MSH through MSH PFU Ext.1, AV08)

**HU Never** was defined as a patient using hydroxyurea less than 20% of the time he/she was followed (from MSH through MSH PFU Ext.1, AV08)
The Relationship Between Hydroxyurea Usage and Mortality

Figure 1

MSH: Cumulative Mortality

According to HU Continuous, Episodic and Never

HU Continuous(Solid)  Episodic(Dot)  Never(Dashed)
The Relationship Between Hydroxyurea Usage and Mortality

Patients who used hydroxyurea greater than 80% of the time they were followed (from MSH through MSH PFU Ext.1, AV08), had significantly better survival outcome than those patients who used hydroxyurea less than or equal to 80% of the time they were followed when the early to mid-portion of the survival curves were considered.

Gehan-Breslow Generalized Wilcoxon Test  
Tarone-Ware Test  

p-value = 0.0361  
p-value = 0.0415
Hydroxyurea: Indications for Therapy at MCG

- Frequent VOCs (>3/year): 88
- Anemia/Hyperhemolysis: 39
- History of CVA: 9
- Priapism: 6
- Leg Ulcers: 4
- Acute Chest Syndrome: 3

TOTAL 149
Hydroxyurea: Demographic Information

- **AGE:**
  - Mean: 30.7
  - Median: 29
  - Range: 15-54

- **GENDER:**
  - Male: 69
  - Female: 80

- **TREATMENT:**
  - Mean: 42.1 mo.
  - Median: 39 mo.
  - Range: 7-127

- **DOSE:**
  - Mean: 18.6 mg/Kg
  - Range: 5.6-33.3 mg/Kg
Hydroxyurea: Treatment Results

- COMPLIANCE: 71.1% (106/149)
- VOC’s: Pre: 8.9/year, Post: 2.2/year (p=0.0001)
- WEIGHT: Pre: 60.0 Kg, Post: 63.4 Kg
- LEG ULCERS: No effect
# Hydroxyurea: Effect on Laboratory Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre</th>
<th>Post</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl)</td>
<td>8.2</td>
<td>9.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hb F (%)</td>
<td>5.7</td>
<td>18.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hb F (g/L)</td>
<td>4.7</td>
<td>18.4</td>
<td>&lt;0.01</td>
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<tr>
<td>Hct (%)</td>
<td>24.0</td>
<td>30.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>92.2</td>
<td>111.6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>31.6</td>
<td>37.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Retics (X10^3)</td>
<td>297.2</td>
<td>111.6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>WBC (X10^9)</td>
<td>12.4</td>
<td>9.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ANC (X10^9)</td>
<td>7.0</td>
<td>4.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Platelets (X10^9)</td>
<td>413.2</td>
<td>352.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>T. Bili (mg/dl)</td>
<td>3.6</td>
<td>2.7</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
Hb F Response for Pts on HU > 4 Yrs
Hydroxyurea: Hematologic Toxicity

- ANC: $\leq 2000$
- PLATELETS: $\leq 80,000$
- RETICS: $\leq 80,000$
- Hb: $\leq 4.5$ G/dl
Hydroxyurea: Hematologic Toxicity

- **TOXICITY:**
  - 26/149 Patients (17.4%)
  - 62 Episodes (1-7)

- **HU DOSE:**
  - 20.6 mg/Kg (mean)
  - 11.3-33.3 (range)

- **AGE:**
  - 35.9 (mean)
  - 24-55 (range)
Hydroxyurea: Hematologic Toxicity

- ↓ ANC: 41.8%
- ↓ HB AND RETICS: 46.3%
- ↓ PLATELETS: 11.9%
Hydroxyurea: Hb F Increase and Haplotypes
Hb F Response to Hydroxyurea (HU)

- Collaborative study between Boston U. and MCG
- 214 Hb SS patients on HU: poor, moderate or good response
- 226 SNPs in 46 candidate genes associated with drug metabolism, Hb F regulation and hematopoiesis
- SNPs in two genes associated with good response:
  - CYP2C9 (cytochrome p450 family)
  - Aquaporin 9 (AQP9: membrane channel involved in urea transport/transport of uncharged solutes)
Hb F Response to Hydroxyurea (HU)

- 137 patients enrolled in MSH
- 320 tagging SNPs in 29 candidate genes analyzed
- Association considered significant when $p \leq 0.01$
- 17 SNPs were associated with the change in % Hb F
- 20 SNPs associated with change in absolute Hb F

Ma et al, Pharmacogenomics Journal, 2007, ePUB
Hb F Response to Hydroxyurea: MSH

- Association with Hb F %
  - 5 in TOX
  - 2 in MAP3K5
  - 3 in FLT1
  - 2 in NOS1
  - 2 in ARG2
  - 2 in NOS1
  - 1 in HAO2

- Association with Hb F (g/dl)
  - 5 in PDE7B
  - 5 in TOX
  - 5 in FLT1
  - 2 in NOS1
  - 2 in ARG2
  - 1 in HAO2

Ma et al, Pharmacogenomics Journal, 2007, ePUB
<table>
<thead>
<tr>
<th>Gene</th>
<th>Location</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOX (Thymus high mobility group binding protein)</td>
<td>8q11-q12</td>
<td>DNA binding</td>
</tr>
<tr>
<td>PDE7B (Phosphodiesterase 7B)</td>
<td>6q22-q23</td>
<td>Cyclic nucleotide metabolism</td>
</tr>
<tr>
<td>ASS1 (Argininosuccinate synthase)</td>
<td>9q34.1</td>
<td>Penultimate step in arginine synthesis</td>
</tr>
<tr>
<td>MAP3K5 (Mitogen activated protein kinase 3)</td>
<td>6q22-q23</td>
<td>Signal transduction</td>
</tr>
<tr>
<td>HAO2 (Hydroxyacid oxidase)</td>
<td>1p13.3-p13.1</td>
<td>Fatty acid oxidation</td>
</tr>
<tr>
<td>NOS1 (Nitric oxide synthase in NOS)</td>
<td>12q24.2-q24.31</td>
<td>No synthesis</td>
</tr>
<tr>
<td>NOS2A (iNOS) (Nitric oxide synthase, )</td>
<td>17q11.2-q12</td>
<td>No synthesis</td>
</tr>
<tr>
<td>ARG2 (Arginase 2)</td>
<td>14q24.1-q24.3</td>
<td>Hydrolysis of arginine</td>
</tr>
<tr>
<td>FLT1 (VEGFR1)</td>
<td>13q12</td>
<td>Cell proliferation and differentiation</td>
</tr>
</tbody>
</table>
Hydroxyurea: Early effects on RBC

- Decreased adhesion to HUVEC in 2 weeks
- Improved RBC hydration by proton NMR by 4 weeks
- Increase in K⁺ content and fall in K⁺/Cl⁻ co-transport in 4 weeks
- No change in F-cells and F-retics in 2 and 4 weeks
- Increase in delay time of Hb S polymerization (10 fold by 24 weeks)

(Bridges et al., 1994)
Expression of CD36 and VLA4 subunits on reticulocytes were significantly lower in HU treated SCD patients, similar to those of normal controls.

Hydroxyurea: Effect on Endothelial Cells

- Increase in Endothelial Cell size and formation of multinucleated giant cells (0.3 mmol/L)
- Decreased protein content (50% at 0.3 mmol; 80% at 3.0 mmol)
- Increase in cell Na$^+$ and K$^+$ content
- Decreased adherence of AA and SS RBC to endothelial cells

(Adragna et al., Blood, 83:553, 1994)
Hydroxyurea: Potential Beneficial Effects

- Improved RBC Rheology
  \((\uparrow \text{MCV}; \uparrow \text{RBC hydration})\)

- \(\downarrow\) WBC/Neutrophils

- \(\downarrow\) Platelets

- Effects on endothelial cells
  \((\downarrow\) expression of adhesion molecules; decreased protein synthesis\)
Hydroxyurea: Established Benefits in Sickle Cell Disease

- Decrease in painful episodes
- Decrease in frequency of ACS
- Improvement in priapism
- Attenuation of hemolysis and improvement in anemia (most cases)
- Decrease in transfusion requirements
- Decrease in mortality
Leg Ulcers and HU Therapy

- Reports suggestive of leg ulcers with HU therapy (myeloproliferative disorders)
- Is HU therapy causative of leg ulcers in SCD?
Leg Ulcers and HU Therapy: MCG Data

- 421 adult SS patients – median age 30 (range 15-61)
- 152 on HU: 269 not exposed to HU
- Overall incidence: 25 patients (5.9%)
- 17/25 on HU: 8/25 not on HU
- 16/17 HU patients had previous Hx of leg ulcers (prior to HU exposure)
  - 8/269 (3%) incidence of de novo ulcers in non-HU patients
  - 1/135 (0.7%) incidence of de novo ulcers in HU patients
- Conclusion: HU therapy does not appear to be causative of de novo leg ulcers in SCD
HU Mortality

- **Causes of death in 34 patients who died from sickle cell-related causes:**
  - 12 acute chest syndrome (ACS)
  - 5 multi-organ failure
  - 4 stroke
  - 2 ESRD
  - 1 each from cardiac arrest, sepsis, respiratory distress, and PE
  - In 7 patients, the cause of death could not be determined
Characteristics of Deceased Patients

- 39 patients (17 male, 21 female)
- Mean age at death - 35.8 ±11.4 vs 32.1 ± 10.0 years for the surviving patients
- HU instituted at a later age in deceased pts.

Of the 4 common haplotypes (BEN, BAN, SEN, CAM): homozygous BAN or heterozygous CAM haplotypes were significantly more likely to be in the deceased group (=6.16, p=0.013 for BAN -/- and +/- vs +/+ ; =5.71, p=0.017 for CAM -/- vs +/-)
<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre-HU</th>
<th>Post-HU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb F%</td>
<td>5.8±4.3</td>
<td>18.5±9.3</td>
</tr>
<tr>
<td>Hb F (g/dl)</td>
<td>0.5±0.4</td>
<td>1.8±1.0</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>8.4±1.3**</td>
<td>9.3±1.6</td>
</tr>
<tr>
<td>RBC</td>
<td>2.8±0.5*</td>
<td>2.5±0.5*</td>
</tr>
<tr>
<td>RDW %</td>
<td>21.8±3.8</td>
<td>18.9±3.9</td>
</tr>
<tr>
<td>PCV</td>
<td>0.3±0.1</td>
<td>0.3±0.1</td>
</tr>
<tr>
<td>Retics</td>
<td>287±130</td>
<td>167±110</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>89.7±9.8</td>
<td>109±15.3**</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>30.9±3.8</td>
<td>37.1±5.4*</td>
</tr>
<tr>
<td>WBC</td>
<td>12.7±4.1</td>
<td>9.5±4.1*</td>
</tr>
<tr>
<td>Polys</td>
<td>7.2±3.5</td>
<td>5.1±3.1*</td>
</tr>
<tr>
<td>Platelets</td>
<td>415±164</td>
<td>356±140</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>3.7±2.3</td>
<td>2.7±2.7</td>
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## HU Response: Deceased

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<tr>
<th>Variable</th>
<th>Pre-HU</th>
<th>Post-HU</th>
</tr>
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<tbody>
<tr>
<td>Hb F%</td>
<td>4.4±2.2</td>
<td>21.4±8.7</td>
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<tr>
<td>Hb F (g/dl)</td>
<td>0.4±0.2</td>
<td>2.0±1.0</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>7.7±1.3</td>
<td>8.7±1.9</td>
</tr>
<tr>
<td>RBC</td>
<td>2.5±0.7</td>
<td>2.3±0.7</td>
</tr>
<tr>
<td>RDW</td>
<td>23.4±3.9</td>
<td>20.1±4.4</td>
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<tr>
<td>PCV</td>
<td>0.2±0.04</td>
<td>0.3±0.1</td>
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<tr>
<td>Retics</td>
<td>254±110</td>
<td>144±88.7</td>
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<td>MCV (fl)</td>
<td>92.0±10.6</td>
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<td>MCH (pg)</td>
<td>31.7±4.4</td>
<td>39.8±6.2</td>
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<tr>
<td>WBC</td>
<td>12.5±4.6</td>
<td>7.8±2.7</td>
</tr>
<tr>
<td>Polys</td>
<td>7.0±3.9</td>
<td>3.9±1.8</td>
</tr>
<tr>
<td>Platelets</td>
<td>447±147</td>
<td>379±177</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>3.1±1.9</td>
<td>2.1±2.4</td>
</tr>
</tbody>
</table>
Hb F%

Hb g/dl

Surviving Patients: $r = 0.35$, $r^2 = 0.12$, $p = <0.0001$
Deceased Patients: $r = 0.14$, $r^2 = 0.02$, $p = <0.39973$
Hydroxyurea: Unanswered Questions

- **PREVENTION OF ORGAN DAMAGE:**
  - CNS disease
  - Splenic function
  - Renal disease
  - Avascular necrosis

- **LONG TERM SAFETY:**
  - Leukemogenicity/carcinogenicity
  - Teratogenicity