Genetic Damage to Sperm Following Treatment with Azathioprine/6-Mercaptopurine in Men with IBD

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Introduction

• Little is known about the effects of AZA/6MP on fertility & pregnancy outcomes among fathers with IBD

• AZA/6MP, though FDA pregnancy category D, are often continued in pregnant women as the available data suggests low risk

• Data among fathers on AZA/6MP are mixed
  – Since AZA/6MP interferes with nucleic acid synthesis, treatment with these agents can theoretically produce germ cell mutations as well as teratogenic effects.
Animal Studies

• Mice treated with 6mp:
  – Chromosomal aberrations and SCE in germinal cells
  – Dominant lethal mutations (chromosomal aberrations that may not affect ability of sperm to fertilize ovum, but result in early embryonic death around the time of implantation)
    • Major congenital malformation rate 2% cases/controls
    • % females impregnated decreased in direct relation to escalating doses 6mp (2,5,8 mg/kg IP)
    • 6MP did not impair sperm production and morphology, however, significantly higher rates of nonviable pregnancies in the females indicated occult sperm damage

• Suggests that genetic evaluation of sperm is needed to detect damage from 6mp

Mosesso Mutation Research, 1993:296: 279
Ligumsky Scan J Gastro 2005:40:444-449
Human Studies

• Retrospective Case Series:
  – 37 men, similar outcomes regardless of 6mp use
  – WAGR (Wilms, aniridia, genital anom, retardation)
  – 4/13 pregnancies 6mp w/i 3 months of conception:
    • Risk vs. 6MP>3 mos (p<0.013) no exposure (p<0.002)

• Danish population cohort study
  – 54 exposed pregnancies vs. 57,195 control
    • Pts: glomerulonephritis, severe skin disease
  – 7.4% (4/54) vs. 4.1% (2334/57195) CA
  – OR= 1.8 (95% CI 0.7-5.0)
  – All male children
  – No CA if conception w/i 3 months of prescription

Rajapakse AJG 2000 (95) 684;Norgard APT 2004:19:679-685
Semen Analysis

• Semen quality
  – 23 IBD pts on AZA vs. WHO standards
  – AZA/SAS: markedly reduced semen morphology
  – AZA 1.5-2 mg/kg (n=18):
    • No difference before and after therapy with AZA.
  – However, some decrease in motility and morphology compared to normal suggesting effect of disease itself

Dejaco Gastro 2001 (121):1048-1053
Hypothesis

• Basic semen analysis is inadequate to determine damage to sperm from AZA/6MP

• AZA/6MP may lead to genetic damage that results in infertility rather than congenital malformations
  – Dominant lethal, nonheritable mutations

• Study Aim:
  – Compare rates of genetic damage to sperm among men with IBD exposed and unexposed to AZA/6MP using sperm chromatin structure assay (SCSA)
SCSA

• Established technique to study male infertility
• Rapid flow cytometry measurements of 5,000 individual sperm per sample
  – SCSA utilizes metachromatic properties of acridine orange to distinguish between native DNA (green fluorescence = double-stranded) and low pH denatured DNA (red fluorescence = single-stranded) at sites of DNA strand breaks.
  – Data are presented as DNA fragmentation index \( \text{%DFI} = \frac{\text{Red}}{\text{red} + \text{green}} \) fluorescence
  – \( \text{%HDS} = \% \) cells with high green fluorescence
    – Immature sperm with intact DNA
Methods

- All men in the UCSF IBD Database were contacted by phone or mail, as well as during routine office visits.
- Populations:
  - *Exposed* population: men with IBD on 6MP/AZA for at least 3 months, with a stable dose for at least one month.
  - *Unexposed*: men w/ IBD not on 6MP/AZA for at least 3 mos
  - Biologics, other immunosuppressives, corticosteroids, sulfasalazine, abx not allowed for either group. Mesalamine, budesonide allowed
  - Patients must be in remission. CDAI, MTWSI measured.
- Two semen samples were procured 2 weeks apart
  - Semen analysis w/ strict morphology on both samples
  - SCSA on 200-ul aliquot of raw, liquefied semen from 2nd
- A serum sample was drawn for AZA/6MP metabolites
- Subjects were identified by exposure status and then the prevalence of DNA damage between these populations was contrasted
# Results: Patient Demographics

<table>
<thead>
<tr>
<th></th>
<th>Unexposed</th>
<th>Exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=9</td>
<td>N=16</td>
</tr>
<tr>
<td>Mean Age (yrs)</td>
<td>34.2 (28-44)</td>
<td>32.9 (23-45)</td>
</tr>
<tr>
<td>Current Tobacco/ETOH</td>
<td>2 tobacco/8 etoh</td>
<td>1 tobacco/10 etoh</td>
</tr>
<tr>
<td>Married</td>
<td>3 (33%)</td>
<td>4 (25%)</td>
</tr>
<tr>
<td>Prior Pregnancies</td>
<td>6: 1 preg/ 5 term</td>
<td>1: miscarriage</td>
</tr>
<tr>
<td>Venereal Disease</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>IBD Type: UC CD</td>
<td>6 (3 IPAA)</td>
<td>9</td>
</tr>
<tr>
<td>Mean IBD Duration (yr)</td>
<td>8.7 (6-13)</td>
<td>7.1 (1-22)</td>
</tr>
<tr>
<td>Mean CDAI MTWSI</td>
<td>32.3 (7-61)</td>
<td>93 (57-165)</td>
</tr>
<tr>
<td>Metabolite Lvls: 6TG 6MMP</td>
<td></td>
<td>211 (75-400)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3048 (245-5011)</td>
</tr>
</tbody>
</table>
## Results

<table>
<thead>
<tr>
<th>Mean values [WHO normal]</th>
<th>Unexposed N= 9 (range)</th>
<th>Exposed N=16 (range)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days Abstinent</td>
<td>3.3 (2, 4.5)</td>
<td>3.4 (2.0, 6.5)</td>
<td>0.75</td>
</tr>
<tr>
<td>Volume [1.5-5 ml]</td>
<td>2.9 (1.4, 4.8)</td>
<td>3.4 (1.1, 6.3)</td>
<td>0.38</td>
</tr>
<tr>
<td>Concentration [&gt;20 mln/ml]</td>
<td>58.9 (25.5,136)</td>
<td>66.5 (5.5,140)</td>
<td>0.65</td>
</tr>
<tr>
<td>Motility [&gt;50%]</td>
<td>65% (52,80)</td>
<td>52% (41,66)</td>
<td>0.0007</td>
</tr>
<tr>
<td>Progression [1-4]</td>
<td>2.88 (2.38, 3.5)</td>
<td>2.62 (1.8, 3.3)</td>
<td>0.18</td>
</tr>
<tr>
<td>Total Motile Ct [&gt;40million]</td>
<td>101.4 (50,141)</td>
<td>106.2 (8,231)</td>
<td>0.83</td>
</tr>
<tr>
<td>Normal oval forms [&gt;14%]</td>
<td>7% (1.5,12.5)</td>
<td>6.4 % (0,14)</td>
<td>0.73</td>
</tr>
</tbody>
</table>
## SCSA Results

<table>
<thead>
<tr>
<th>Sample #2 Mean values</th>
<th>Unexposed N= 9 (range)</th>
<th>Exposed N=16 (range)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFI (%)</td>
<td>9.1% (3.9,14.7)</td>
<td>13.9% (3.7,31.6)</td>
<td>0.071</td>
</tr>
<tr>
<td>&lt;15% excellent</td>
<td>9 excellent</td>
<td>11 excellent</td>
<td>0.123</td>
</tr>
<tr>
<td>&gt;15 to &lt;30% good</td>
<td></td>
<td>4 good</td>
<td></td>
</tr>
<tr>
<td>&gt;30% fair to poor</td>
<td></td>
<td>3 fair-poor</td>
<td></td>
</tr>
<tr>
<td>HDS [&lt;15% = normal]</td>
<td>5.6% (4-8.7)</td>
<td>7.71% (3.1-22.9)</td>
<td>0.17</td>
</tr>
</tbody>
</table>
Summary

• Confirmed that men with IBD have sperm morphology below normal levels

• Men with IBD in remission exposed to AZA/6MP compared to those not exposed had
  – Significantly lower sperm motility rates
  – A trend towards higher % DNA Fragmentation

• Men with IBD on AZA/6MP having difficulty conceiving should undergo semen evaluation and consider stopping AZA/6MP during the conception period