Impaired transsulfuration and oxidative stress in autistic children: Improvement with targeted nutritional intervention

S. Jill James, PhD, DAN! Conference, 2003

Oxidative Stress

The generation of reactive oxygen species (ROS) is an inevitable consequence of aerobic energy metabolism. A delicate oxidant-antioxidant balance within each cell maintains normal physiologic levels of ROS that serve as important signaling molecules for the activation of cGMP-dependent functions, for vasomotor tone, T cell activation, and normal gene expression\(^1,2\). Oxidative stress occurs when cellular antioxidant defense mechanisms fail to counterbalance and control ROS production. Unopposed ROS can result in damage to mitochondrial and nuclear DNA, alteration in protein structure, and membrane lipid composition\(^3\). Functionally, these aberrations translate into abnormal gene expression, membrane signal transduction, and altered rates of cell proliferation, differentiation, and apoptosis. A pro-oxidant microenvironment has been implicated in the etiology of numerous human diseases including cardiovascular disease, cancer, autoimmune disorders, and neurodegenerative conditions\(^4-8\). The potential role of oxidative stress in the etiology of autism has received less research attention.

Overview of the Methionine Transsulfuration Pathway

Abbreviations: THF: tetrahydrofolate; MS: methionine synthase; BHMT: betaine-
homocysteine methyltransferase; MAT: methionine adenosyltransferase; SAM: S-adenosylmethionine; SAH S-adenosylhomocysteine; SAHH: SAH hydrolase; ADA: adenosine deaminase; AK: adenosine kinase; CBS: cystathionine beta synthase

Under normal physiologic conditions, SAH is hydrolyzed by SAH hydrolase (SAHH) to adenosine and homocysteine. It is important to note, however, that this reaction is readily reversible with equilibrium dynamics that strongly favor SAH synthesis, rather than hydrolysis. In fact, the only reason that this reaction proceeds in the hydrolytic direction is efficient product removal. Thus, metabolic perturbations that interfere with the efficient removal of homocysteine and/or adenosine will lead to an increase in SAH. Adenosine can be efficiently removed by either the adenosine deaminase (ADA) reaction or the adenosine kinase (AK) reaction. An increase in intracellular adenosine will inactivate SAHH activity by binding to the active site and will cause SAH to accumulate resulting in inhibition of essential methyltransferase reactions. A decrease in ADA activity and a genetic polymorphism in ADA have been reported in children with autism.

Approximately 50% of homocysteine generated from methionine is metabolized to cystathionine by cystathionine beta synthase (CBS). This is a one-way reaction that permanently removes homocysteine from the methionine cycle and initiates the transsulfuration pathway for the synthesis of cysteine and glutathione. It is important to note that cysteine is a conditionally essential amino acid that is dependent on adequate methionine status. A decrease in intracellular methionine effectively increases the requirement for cysteine and a decrease in methionine is often associated with an equivalent decrease in cysteine levels. Methionine transsulfuration to cysteine and glutathione occurs primarily in the liver, the predominant organ for methionine metabolism. Cysteine is the rate limiting amino acid for the synthesis of glutathione; thus, low methionine and cysteine levels reduce glutathione synthesis and are associated with decreased intracellular glutathione.

**Tissue-specific Glutathione Synthesis:**

The tripeptide, g-glutamylcysteinylglycine, or glutathione (GSH), is the major thiol antioxidant in mammalian cells. The antioxidant capacity of glutathione resides in the –SH (thiol) group of cysteine within the tripeptide. Because the intracellular levels of glutathione (1-8 mM) greatly exceed extracellular levels (5-7 mM), the concentration gradient across the cell membrane strongly favors cellular export over uptake of glutathione. As a consequence, most cells are not able to take up intact glutathione and rely on the uptake of its precursors, cysteine, cysteinylglycine, or g-glutamylcysteine for GSH synthesis inside the cell. Quantitatively, most de novo glutathione synthesis occurs in the liver where it is constantly exported and degraded by extracellular g-glutamyltranspepsidase (GGT) into precursor amino acids for transport to and uptake by extrahepatic tissues. In this way, glutathione synthesis (and indirectly antioxidant function) in peripheral tissues is directly dependent on GSH synthesis and export by the liver. A decrease in hepatic glutathione synthesis or cysteine availability due to nutritional or genetic deficiencies would be expected to negatively affect antioxidant
capacity in vulnerable tissues, (e.g., brain, intestine, and thymus) that are dependent on hepatic cysteine for glutathione synthesis\textsuperscript{18}. A decrease in glutathione synthesis would increase vulnerability of these tissues to pro-oxidant environmental stressors and may have relevance to neurologic, gastrointestinal, and immune dysfunction in autistic individuals\textsuperscript{8,17,19-21}.

**Abnormal methionine transsulfuration metabolites in autistic children:**

Baseline levels of methionine transsulfuration metabolites were measured in plasma from twenty autistic children using HPLC with electrochemical detections\textsuperscript{22,23}. Relative to levels measured in age-matched control children, the plasma thiol profile of autistic children was severely abnormal.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Control Children</th>
<th>Autistic Children</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methionine (µmol/L)</td>
<td>30.6 ± 6.5</td>
<td>19.3 ± 9.7</td>
<td>0.001</td>
</tr>
<tr>
<td>SAM (nmol/L)</td>
<td>90.0 ± 16.2</td>
<td>75.8 ± 16.2</td>
<td>0.01</td>
</tr>
<tr>
<td>SAH (nmol/L)</td>
<td>20.1 ± 4.3</td>
<td>26.1 ± 5.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Homocysteine (µmol/L)</td>
<td>6.3 ± 1.2</td>
<td>5.4 ± 0.9</td>
<td>0.01</td>
</tr>
<tr>
<td>Adenosine (µmol/L)</td>
<td>0.28 ± 0.16</td>
<td>0.39 ± 0.19</td>
<td>0.05</td>
</tr>
<tr>
<td>Cysteine (µmol/L)</td>
<td>210 ± 18.5</td>
<td>163 ± 14.6</td>
<td>0.001</td>
</tr>
<tr>
<td>Total glutathione (µmol/L)</td>
<td>7.9 ± 1.8</td>
<td>4.1 ± 0.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Oxidized Glutathione (nmol/L)</td>
<td>0.3 ± 0.1</td>
<td>0.55 ± 0.2</td>
<td>0.001</td>
</tr>
<tr>
<td>GSH/GSSG Ratio</td>
<td>25.5 ± 8.9</td>
<td>8.6 ± 3.5</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Because methionine is the precursor for cysteine, the rate-limiting amino acid for glutathione synthesis, it is not surprising that low methionine levels are associated with the low cysteine and glutathione levels in these children. The significant increase in adenosine is consistent with the increase in SAH since adenosine binds to the active site of SAH hydrolase and inhibits its activity. The increase in SAH is of concern in autistic children because of its ability to inhibit SAM-dependent methyltransferase activity and cellular methylation reactions. The reduction in total glutathione (GSH) and increase in oxidized glutathione resulted in a 3-fold reduction in the ratio of reduced (active) GSH to oxidized glutathione (GSSG). This is of major concern because it reflects a significant decrease in antioxidant capacity associated with an increase in oxidative stress in the autistic children.

Cellular consequences of decreased glutathione antioxidant potential: possible implications for symptoms of autism
1. Reduced ability to detoxify environmental toxicants and heavy metals\(^2\): neurotoxicity
2. Oxidation of cysteine thiol (SH) groups in proteins\(^2\): altered structure/function
3. Decreased liver GSH synthesis\(^2\): Reduced export/transport of cysteine to brain
4. Degeneration of gut epithelium\(^1\): Increased gut permeability and autoimmunity
5. Increased Th2, altered thymic T cell subsets\(^5\): Autoimmunity
6. Reduced S-adenosylmethionine synthesis and increased SAH accumulation\(^2\): Methyltransferase inhibition and reduced cellular methylation capacity
7. Reduced total antioxidant capacity (active Vitamin C and E depend on GSH)\(^2\): Exacerbates all of above

Cellular consequences of reduced methylation capacity: possible implications for symptoms of autism

1. Reduced DNA methylation: Reduced methylation and silencing of integrated viral sequences \(^2\)
2. Reduced protein methylation: altered activity/function \(^1\)
3. Decreased catecholamine-O-methyltransferase activity: altered neurotransmitter synthesis
4. Reduced membrane phosphatidylcholine synthesis \(^3\): altered membrane fluidity and signaling
5. Reduced methylation and detoxification of arsenic: increased oxidative damage

**Results of a targeted nutritional intervention trial with folinic acid and betaine (trimethylglycine) in children with autism**

In an attempt to improve baseline plasma methionine/cysteine/glutathione levels and increase antioxidant and methylation capacity, the 20 autistic children were given supplements of 800 mg of folinic acid b.i.d. and 1000 mg betaine (trimethylglycine) b.i.d. for a period of 3 weeks. Folinic acid (5-formylTHF) enters the folate pathway in a reduced form which is more easily assimilated into folate metabolism than the synthetic vitamin form, folic acid. Folinic acid is converted to 5, 10-methyleneTHF which will support purine and thymidylate synthesis and also methionine synthesis. Betaine provides a folate-independent pathway for methionine regeneration via the betaine-homocysteine methyltransferase (BHMT in figure 1) that occurs primarily in the liver.

After nutritional intervention for only 3 weeks, a highly significant increase in plasma methionine, cysteine, and glutathione levels were associated with almost 2-fold increase in the ratio of reduced to oxidized glutathione (GSH/GSSG). These results would suggest that supplementation with folinic acid and betaine had a strong positive impact on antioxidant capacity in the autistic children. Although SAM levels were significantly increased, the decrease in SAH and adenosine levels did not reach statistical significance due to high inter-individual variability. Eight of the 20 children continued the intervention with betaine and folinic acid for an additional 3-4 months. After the extended intervention period, SAM levels increased to 112 nmol/L, SAH levels
decreased to 17 nmol/L, and adenosine levels decreased to 0.18 µmol/L; all well within normal ranges.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Baseline</th>
<th>After Intervention</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methionine (µmol/L)</td>
<td>19.3 ± 9.7</td>
<td>28.0 ± 7.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SAM (nmol/L)</td>
<td>75.8 ± 16.2</td>
<td>81.4 ± 10</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>SAH (nmol/L)</td>
<td>26.1 ± 5.4</td>
<td>25.1 ± 8.4</td>
<td>NS</td>
</tr>
<tr>
<td>Homocysteine (µmol/L)</td>
<td>5.4 ± 0.9</td>
<td>7.0 ± 1.0</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>Adenosine (µmol/L)</td>
<td>0.39 ± 0.2</td>
<td>0.33 ± 0.1</td>
<td>NS</td>
</tr>
<tr>
<td>Cysteine (µmol/L)</td>
<td>163 ± 14.6</td>
<td>225 ± 37</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>Total glutathione (µmol/L)</td>
<td>4.1 ± 0.5</td>
<td>5.7 ± 1.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Oxidized Glutathione (nmol/L)</td>
<td>0.55 ± 0.2</td>
<td>0.48 ± 0.2</td>
<td>NS</td>
</tr>
<tr>
<td>GSH/GSSG Ratio</td>
<td>8.6 ± 3.5</td>
<td>13.8 ± 4.8</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Implications for intervention strategies in autistic children:**

Targeted nutritional intervention with folinic acid and betaine successfully increased both methylation capacity and glutathione antioxidant capacity in the 20 participating autistic children, especially after 3-4 months of intervention. A list of supportive nutrients or synthetic precursors is presented below.

**Micronutrients supportive of methionine synthesis:**

1. Zinc: MS, BHMT, and methyltransferases are zinc-dependent enzymes involved in methionine cycle.
2. Folinic Acid: Supports nucleotide synthesis by increasing 5,10-methylene THF; supports methionine recycling by increasing 5-CH3THF.
4. Methyl-B12: Replaces need for methyl group transfer from 5methylTHF; potential for trapping folate as 5-methyl-THF and reducing synthesis of metabolically active THF.
5. Choline: Spares phosphatidylcholine breakdown to betaine for BHMT-mediated methionine synthesis.

**Micronutrients and precursors to support glutathione synthesis**

1. Glutathione methyl ester: Oral or injectable glutathione is not readily taken up by most cells due to large concentration gradient favoring export (plasma levels increase but not intracellular levels). The methyl or ethyl esters of glutathione
cross the cell membrane and have been shown to increase intracellular glutathione levels 31.

2. N-acetylcysteine: Stable form of cysteine that readily crosses cell membrane and increases intracellular glutathione levels; toxic only at extremely high doses (1.5 gm)

3. 2-oxothiozolidine -4- carboxylate (OTC): Stable derivative of cysteine that is readily converted to cysteine and increases glutathione synthesis 32

4. Vitamin B6: CBS and cystathionine lyase are both B6 dependent enzymes that are involved in the transsulfuration of homocysteine to glutathione

5. Selenium: Glutathione peroxidase is a selenium-dependent enzyme; selenium deficiency is common in malabsorption and gastrointestinal disease 33

6. Glutamine: Glutamine enhances gut glutathione production 34.

7. Antioxidants: Vitamin E, vitamin C, lipoic acid

Increased turnover of the transsulfuration pathway and increased glutathione antioxidant capacity in females: Implication for sex ratio in autism:

It is well-established that females have lower homocysteine levels than males. After menopause, however, the difference in homocysteine levels between sexes narrows, suggesting that estrogen regulates the rate and activity of methionine turnover and transsulfuration 35. Consistent with this possibility, females have been shown to have higher MAT activity than males in humans 36. Estrogen has been shown to increase plasma glutathione levels in a dose-dependent manner in experimental animals 37. By enhancing the activity of glucose-6 phosphate dehydrogenase, estrogen supports the regeneration of reduced glutathione from NADPH and increases antioxidant potential 38. Mitochondrial glutathione levels and antioxidant capacity have been reported to be higher in females than in males 39. Taken together, the evidence suggests that both cellular methylation capacity and antioxidant activity are higher in females than males. The increased rate of methionine transsulfuration and glutathione antioxidant activity in females may have a protective effect against the development of autism.