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Decreased expression of acetaminophen-metabolizing enzymes and glutathione in asthmatic children after acetaminophen exposure

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Summary
Children with moderate-to-severe asthma have decreased expression of acetaminophen metabolizing genes and glutathione that may account for the previously-reported risk of acetaminophen in this vulnerable population.

Keywords
Asthma; children; acetaminophen; glutathione; glutathione-S-transferases; oxidative stress; drug metabolism; cytochrome p450

To the Editor:
We have read, with interest, the growing body of literature on the potential risk of acetaminophen in children with asthma summarized in the Journal1 and elsewhere.2 While the causal (or potentially confounded) nature of the association between acetaminophen and asthma remains controversial, we were intrigued by reports of increased disease severity in asthmatic children with frequent acetaminophen exposure.3,4 Others have hypothesized that these findings may be related to alterations in key antioxidant enzymes such as glutathione S-transferases that increase acetaminophen risk through decreased glutathione biavailability.5,6

In keeping with this hypothesis, we previously demonstrated significant depletion of glutathione in children with severe asthma associated with inhibition of the transcription factor, Nuclear factor (erythroid-derived 2)-like 2 (Nrf).7 Because Nrf2 also protects against acetaminophen toxicity through transcriptional regulation of genes important for Phase I and Phase II xenobiotic metabolism, we questioned whether acetaminophen-related gene expression was similarly impaired in these children. We report that children with moderate-to-severe symptomatic asthma have decreased mRNA expression of genes encoding the Aryl Hydrocarbon Receptor (AHR)/Aryl Hydrocarbon Receptor Nuclear Translocator

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(ARNT) heterodimer, a transcriptional regulator of toxin metabolism, after ex vivo acetaminophen exposure. These observations were also associated with decreased expression of several Phase II enzymes such as glutathione S-transferase pi 1 (GSTP1) and blunted extracellular glutathione release. These findings lend potential biologic plausibility to previously-reported acetaminophen-asthma associations.

Venipuncture for peripheral blood mononuclear cell isolation was performed in asthmatic children 6–17 years (n = 11, 64% male) and healthy young adults 18–30 years of age (n = 8, 75% male).7 CD14+ monocytes were isolated with magnetic beads (Miltenyi Biotec, Germany) and recovered for 3 hours at 37°C. Monocytes were treated with ±15 µg/mL acetaminophen (Sigma Aldrich, St. Louis, MO) for 1 hour in keeping with peak plasma concentrations observed in febrile children after a single 12.5 mg/kg dose.8 Cell viability was assessed by trypan blue staining and was at least 95% for all experiments. RNA was isolated7 and mRNA gene expression was determined by a real-time PCR array containing 84 genes (Human Drug Metabolism RT2 Profiler™ SABiosciences, Frederick, MD). Data were normalized to 5 housekeeping genes (B2M, GAPDH, ACTB, HPRT1, and RPL13A) which were used to calculate delta cycle threshold (ΔCT) values for each subject. Extracellular glutathione release was quantified in the cell culture media by HPLC.7 Group differences were assessed using t-tests with a significance level of 0.01. Pathway analysis was performed with GNCPro™ software (SABiosciences).

Asthmatic children (11 ± 3 years) had moderate-to-severe symptomatic asthma with symptoms at least twice weekly or a history of an emergency visit or hospitalization for asthma within the preceding 6 months. These children were treated with 455 ± 310 mcg inhaled fluticasone/day and at least one additional controller medication (montelukast, n = 7; long-acting beta-agonist, n = 5) and were further characterized by mild airflow limitation (FEV1/FVC ratio, 0.75 ± 0.06) and increased online exhaled nitric oxide concentrations (48 ± 34 ppb). Given age differences between the asthmatic and control groups, confirmatory experiments were also performed on banked RNA samples from six atopic, non-asthmatic children with allergic rhinitis (10 ± 4 years, 50% male). The atopic non-asthmatic children and the healthy young adults were not receiving medications.

mRNA expression of cytochrome p450 enzymes did not differ between children with moderate-to-severe symptomatic asthma and healthy controls either at baseline or after acetaminophen exposure. However, children with asthma were characterized by lower expression of several Phase II metabolizing enzymes, including dehydrogenases, hydrolases kinases and oxidoreductases both at baseline and after acetaminophen exposure (Table I). Although acetaminophen increased expression of glutathione peroxidases and paraoxonases in children with moderate-to-severe asthma, acetaminophen decreased expression of other Phase II metabolizing enzymes in these children, including GSTP1 (Table I). Furthermore, whereas extracellular glutathione release did not differ between groups at baseline (11 ± 6 vs. 13 ± 6 nM for control vs. asthma), glutathione release increased in controls after acetaminophen exposure (26 ± 4 nM, p = 0.018) but remained unchanged in children with asthma (17 ± 9 nM). This observation was accompanied by a significant decrease in expression of the transcription factor AHR, as well as its heterodimer, ARNT, and a downstream gene, ArsA arsenite transporter, ATP-binding, homolog 1 (bacterial) (ASNA1) (Figure 1, A–C). Pathway analysis further suggested that impaired AHR/ARNT signal transduction may underlie the alterations in Phase II metabolizing enzyme gene expression.

These preliminary observations suggest that acetaminophen detoxification by AHR/ARNT-mediated signal transduction may be impaired in symptomatic children with moderate-to-severe asthma as a function of altered glutathione homeostasis. Given that AHR also regulates Nrf2, which upregulates genes responsible for glutathione synthesis and

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antioxidant defense, these findings are in keeping with our previous observations of impaired Nrf2 activation and signaling and decreased glutathione availability in this population. Indeed, the mechanisms of the AHR and Nrf2 transcription factors are similar, in that both undergo nuclear translocation upon activation and bind to specific response elements in the DNA to promote gene transcription. Whether there is a common mechanism underlying AHR and Nrf2 dysfunction in children with moderate-to-severe symptomatic asthma is not clear. Furthermore, because the children enrolled in this study were treated with a number of asthma controller medications, the effect of current asthma treatment as well as other key asthma-related phenotypic variables on AHR/ARNT signaling and Phase II enzyme-mediated acetaminophen metabolism is not understood. While we were unable to obtain liver samples from participating children due to ethical limitations, the monocytes used here do express cytochrome p450 2E1 and other Phase I metabolizing enzymes similar to other extrahepatic tissues and therefore may be useful for future mechanistic studies of drug metabolism in this population. Although additional in vivo pharmacokinetic studies are also clearly warranted, these preliminary findings are intriguing and beg the question whether there is indeed a vulnerable group of children with moderate-to-severe symptomatic asthma for whom acetaminophen may pose an increased risk. However, we caution against extrapolation of these findings to the larger population of children with asthma given limited mechanistic data to either support or refute previously-reported acetaminophen-asthma associations.

Acknowledgments

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REFERENCES

Figure 1.
mRNA expression of (A) AHR, (B) ARNT, and (C) ASNA1 in healthy adult controls (AC) and children with moderate-to-severe asthma after 15 µg/mL acetaminophen (APAP) exposure. Baseline expression data from age-matched, atopic pediatric controls (PC) are also shown for comparison.
### Table I

mRNA gene expression (2^−ΔΔCT values relative to controls) of Phase II metabolizing enzymes and other related genes in healthy adult controls and children with moderate-to-severe asthma before and after acetaminophen exposure.

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th><strong>Baseline</strong></th>
<th><strong>Acetaminophen (15 µg/mL)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy Controls</td>
<td>Moderate-to-Severe Asthma</td>
</tr>
<tr>
<td><strong>Decarboxylases</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>GAD1</em></td>
<td>1.30 ± 0.79</td>
<td>3.00 ± 3.41</td>
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<tr>
<td><strong>Dehydrogenases</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>ALDH1A1</em></td>
<td>1.06 ± 0.34</td>
<td>0.28 ± 0.32**</td>
</tr>
<tr>
<td><strong>Glutathione peroxidases</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>GSTM2</em></td>
<td>1.31 ± 1.09</td>
<td>3.66 ± 3.30</td>
</tr>
<tr>
<td><em>LPO</em></td>
<td>1.30 ± 1.95</td>
<td>4.27 ± 3.54</td>
</tr>
<tr>
<td><strong>Glutathione S-Transferases</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>GSTP1</em></td>
<td>1.01 ± 0.17</td>
<td>0.87 ± 0.35</td>
</tr>
<tr>
<td><strong>Hydrolases</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>EPHX1</em></td>
<td>1.16 ± 0.64</td>
<td>0.50 ± 0.30*</td>
</tr>
<tr>
<td><em>FBP1</em></td>
<td>1.24 ± 0.84</td>
<td>0.82 ± 0.39</td>
</tr>
<tr>
<td><strong>Kinases</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>HK2</em></td>
<td>1.11 ± 0.44</td>
<td>0.30 ± 0.34**</td>
</tr>
<tr>
<td><em>PKM2</em></td>
<td>1.02 ± 0.26</td>
<td>0.89 ± 0.51</td>
</tr>
<tr>
<td><strong>Oxidoreductases</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>BLVRA</em></td>
<td>1.03 ± 0.28</td>
<td>0.58 ± 0.24**</td>
</tr>
<tr>
<td><em>BLVRB</em></td>
<td>1.02 ± 0.22</td>
<td>0.58 ± 0.34**</td>
</tr>
<tr>
<td><em>MTHFR</em></td>
<td>1.09 ± 0.44</td>
<td>0.59 ± 0.37*</td>
</tr>
<tr>
<td><strong>Paraoxonases</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>PON1</em></td>
<td>1.61 ± 1.83</td>
<td>6.35 ± 0.39</td>
</tr>
<tr>
<td><strong>Transferases</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>COMT</em></td>
<td>1.04 ± 0.30</td>
<td>0.85 ± 0.47</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>MARCKS</em></td>
<td>1.13 ± 0.51</td>
<td>0.59 ± 0.53*</td>
</tr>
<tr>
<td><em>SNN</em></td>
<td>1.05 ± 0.35</td>
<td>0.80 ± 0.39</td>
</tr>
<tr>
<td><em>ABCB1</em></td>
<td>1.06 ± 0.38</td>
<td>3.33 ± 2.79*</td>
</tr>
</tbody>
</table>

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*  
\[ p \leq 0.01 \text{ vs. controls,} \]

**  
\[ p \leq 0.001 \text{ vs. controls.} \]