Effects of GSTT1, GSTM1, and CYP 3A5 Polymorphisms on Levels of Isothiocyanate Metabolites and Midazolam

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Objectives
- Investigate how GSTT1 and GSTM1 affect isothiocyanate (ITC) excretion rates in vivo.
- Investigate how CYP3A5 affects the rate of metabolism of Midazolam (MDZ) in vivo.

Introduction
- ITCs are natural nonnutritive components in the diet that have putative antioxidant and chemopreventive properties.
  - Sulfurophane (SFN) is an ITC highly concentrated in cruciferous vegetables (e.g. broccoli and broccoli sprouts).
- CYP3A4 is a gene involved in the biotransformation of more than 50% of pharmaceuticals and has important pharmacological and toxicological implications including drug clearance and efficacy.
  - CYP3A4 activity is dramatically decreased by SFN in primary cultures of human hepatocytes via a PXR-mediated mechanism.
- Rifampicin, a first line treatment for tuberculosis (TB), increases CYP3A4 levels to the extent that it metabolizes antiretroviral drugs for HIV/AIDS so quickly that they are contraindicated and rendered ineffective. SFN could prevent the rifampicin mediated CYP3A4 induction in TB/AIDS patients.
- The CYP3A5 gene affects metabolism of MDZ.
- CYP3A5 is related to CYP3A4 in that it has similar substrate specificity but is polymorphic in the human population.
- GST is a family of genes involved in carcinogen detoxification including GSTT1 and GSTM1.
  - GSTs may affect the extent to which SFN is available in the body because they conjugate SFN/ITC with glutathione and facilitate clearance.
- Hypothesis: GSTT1 and/or GSTM1 positive individuals will clear ITCs more rapidly than GSTT1 and/or GSTM1 null subjects as reflected in urinary ITC levels, and persons expressing CYP3A5 will metabolize MDZ at a faster rate than those who lack it.

Methods and Materials
- 23 healthy adults received (400umoles/day) of SFN daily for one week.
- Participants were dosed with 1 mg MDZ, and blood was drawn at defined time-points.
- MDZ area under the curve (AUC) was used as a measure of CYP3A4/CYP3A5 activity. MDZ ng/ml in blood plasma was calculated using HPLC MSMS. AUC was calculated using a non-compartmental method in the program WinNonLin. Stata 11 was used to perform regression. P values < .05 were significant.

GSTs may affect the extent to which SFN is available in the body because they conjugate SFN/ITC with glutathione and facilitate clearance.

The graph above shows the CYP3A5 genotypes separated. Blue represents Wild Type (G/G), red represents Mut (A/A), and green represent Het (G/A).

The table above shows the participant’s ID #, the corresponding genotype, and the average concentration of MDZ.

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GST is a family of genes involved in carcinogen detoxification including GSTT1 and GSTM1.

Methods and Materials
- Genotyping of GSTT1/GSTM1 was completed by PCR and gel electrophoresis while CYP3A5 was performed on Applied Biosystem’s 7900 with ABI’s assay-by-design.

Results

<table>
<thead>
<tr>
<th>ID #</th>
<th>Genotype</th>
<th>Average uninduced MDZ AUC</th>
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<tbody>
<tr>
<td>2268</td>
<td>WT (G/G)</td>
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</tr>
<tr>
<td>2271</td>
<td>WT (G/G)</td>
<td>430</td>
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<td>2297</td>
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</tr>
<tr>
<td>2499</td>
<td>WT (G/G)</td>
<td>312</td>
</tr>
</tbody>
</table>

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Future studies will consider a larger sample size.

Conclusions
- 19% (4/21) of participants were GSTT1 null
- 57% (15/21) were GSTM1 null
- 70% (16/23) of participants were found to be WT (G/G)
- 22% (5/23) were found to be Het (G/A)
- 8% (2/23) were found to be Mut (A/A)
- No correlation was found between GSTT1 and/or GSTM1 genotypes and urinary ITC levels or CYP3A5 genotypes and MDZ levels.

Acknowledgments

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