



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.

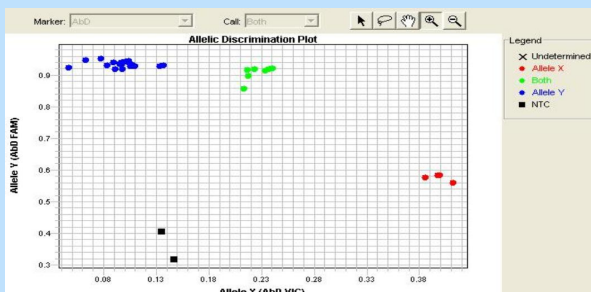
Introduction

- ITCs are natural nonnutritive components in the diet that have putative antioxidant and chemopreventive properties.
 - Sulforaphane (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 Pregnane-X-Receptor (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 of 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.
- 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



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).

ID #	Genotype	Average uninduced MDZ AUC ng*ml/min
2269	Wt (G/G)	451
2214	Wt (G/G)	630
2233	Wt (G/G)	421
2297	Wt (G/G)	870
2313	Wt (G/G)	619
2326	Wt (G/G)	1125
2335	Wt (G/G)	409
2341	Wt (G/G)	471
2378	Wt (G/G)	435
2394	Wt (G/G)	739
2415	Wt (G/G)	474
2434	Wt (G/G)	571
2476	Wt (G/G)	413
2482	Wt (G/G)	325
2493	Wt (G/G)	729
2499	Wt (G/G)	570
2202	Het (G/A)	568
2268	Het (G/A)	401
2280	Het (G/A)	569
2310	Het (G/A)	822
2369	Het (G/A)	497
2371	Mut (A/A)	431
2439	Mut (A/A)	476

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

	average AUC	Regression
Wt	546	Stata was used with
CYP3a5+	530	DT's compared to a binary
P val =	0.64	variable 0=WT 1=Homo or Heterozygous for A allele as shown to the left.

Results (cont'd)

Above is gel electrophoresis of GSTT1 positive (highest), B-globulin control (middle), and GSTM1 positive (lowest).

ID #	GST			average uMol ITC
	T	GSTM	low/high	
2214	0	0	0	199
2202	1	1	0	206
2280	1	0	0	161
2313	1	0	0	165
2335	1	0	0	184
2342	1	0	0	99
2394	1	0	0	123
2415	1	0	0	123
2434	1	0	0	110
2438	1	1	0	166
2371	1	0	0	43
2310	0	0	1	224
2326	0	0	1	267
2378	0	1	1	247
2233	1	1	1	292
2268	1	1	1	254
2476	1	0	1	327
2482	1	0	1	314
2493	1	1	1	261
2499	1	0	1	238
2369	1	0	1	328

Logistic regression was used regressing ITC (Low ITC=0, High ITC=1) to GSTT (GSTT1+ =1, GSTT1-=0) and GSTM (GSTM1+=1, GSTM1-=0) P = .21 for GSTT1 and P=.24 for GSTM1

# ptnts	GSTT1 +	GSTT1 -	GSTM1 +	GSTM1 -
low ITC	10	1	2	9
high ITC	7	3	4	6

Conclusion

- 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.
- Future studies will consider a larger sample size.

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