



Evaluation of Herb-Drug Interactions with MetMax™ Pooled Donor Human Enterocytes with Commonly used Herbal Supplements

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Scientific Rationale

- Herbal supplements are used extensively world-wide for the putative health benefits, with a U. S. revenue of >6 billion USD
- Herbal adverse effects include both toxicity (mainly hepatotoxicity) and drug interactions
- As orally administered substances, herbal supplements may have effects on enteric drug metabolism but not on hepatic metabolism due to the relatively low concentrations of the inhibitors in the systemic circulation after absorption
- Effects of herbal supplements on enteric drug metabolism have not yet been reported
- We report here the potential drug interaction potential of 28 frequently used herbal dietary supplements plus grapefruit juice on enteric drug metabolism using a novel experimental system, **MetMax™ cryopreserved human enterocytes**

MetMax™ Efficiency

MetMax™ Enterocytes

Freezer to Incubation:
<5 minutes

- Retrieve from -80 C freezer
- Thaw in a 37 C water bath
- Add equal volume to 2X test article
- Incubate

Intact Enterocytes

Freezer to Incubation:
>30 minutes

- Retrieve from LN2 Freezer
- Thaw in a 37 C water bath
- Add to recovery medium
- Centrifuge
- Microscopically quantify viability and cell number
- Adjust to 2X final cell density
- Add at equal volume to 2X test article
- Incubate

Materials & Methods

- Enterocyte isolation and cryopreservation:** Enterocytes were isolated and purified from human small intestine segments obtained from the International Institute for the Advancement of Medicine (IIAM, Exton, PA) using a proprietary method. The cryopreserved enterocytes were stored in liquid nitrogen storage containers.
- Preparation of MetMax™ Enterocytes:** Enterocytes from multiple donors were recovered from cryopreservation, pooled, permeabilized using a proprietary procedure, and supplemented with cofactors, and recryopreserved and stored in a -80 degree C freezer at a cell density of 2 million cells/mL
- Quantification of enteric CYP3A4 activity:** Effects of herbal supplements on enterocyte CYP3A4 activity were evaluated using luciferin IPA (LIPA ; Promega, Madison, WI) with luminescence quantified on a Perkin Elmer Wallac 1420 Victor microplate reader.
- Herbal drug interaction studies:** The herbal supplements were obtained commercially. The daily recommended dose was dissolved in 50 mL (4X of 100% concentration) of HQM (IVAL), pH adjusted to 7.0 to 7.2, and sterilized by filtration. For the drug interaction studies, aliquots of 50 uL of MetMax™ enterocytes suspension per well were added to 96-well plates, followed by an addition of 25 uL of the 4X herbal supplements and 25 uL of 4X LIPA. CYP3A4 activity was quantified upon an incubation period of 60 minutes.
- Data Analysis:** Results are expressed as relative normalized CYP3A4 activities using the following equations:
 $Relative\ CYP3A4\ activity = \frac{CYP3A4(treatment)}{N-CYP3A4\ (negative\ control)} \times 100\%$

MetMax™ Enterocytes: Cofactor-Supplemented Permeabilized Enterocytes

MetMax™ Advantage: Complete Drug Metabolizing Pathways (as with Intact Enterocytes) and Simplified Use Procedures (as with S9/microsomes)

Complete

Organelles	MetMax™	Intact Enterocytes	Microsomes	S9
Endoplasmic Reticulum	☺	☺	☺	☺
Cytosol	☺	☺		☺
Mitochondria	☺	☺		
Lysosomes	☺	☺		
Golgi	☺	☺		
Plasma Membranes	☺	☺		
Nucleus	☺	☺		

Efficient

Organelles	MetMax™	Intact Enterocytes	Microsomes	S9
Storage	-80 C	LN2	-80 C	-80 C
Centrifugation	No	Yes	No	No
Microscopic Examination	No	Yes	No	No
Cell Counting	No	Yes	No	No
Cofactor Addition	No	No	Yes	Yes
Thaw and Use	Yes	No	No	No

Results

Enhanced DME Activities of MetMax™ Pooled Human Enterocytes

Metabolic Pathway	Substrate	Marker Metabolite	Metabolite Activity (pmole/10 ⁶ /min)		
			Pooled Enterocytes	MetMax Pooled Enterocytes	MetMax™/Intact Enterocytes Ratio
CYP2C9	Diclofenac	4-OH Diclofenac	4.05 ± 0.16	5.78 ± 1.13	142%
CYP2C19	S-Mephenytoin	4-OH S-Mephenytoin	0.55 ± 0.03	3.36 ± 0.32	610%
CYP3A4-1	Midazolam	1-OH-midazolam	1.21 ± 0.03	4.23 ± 1.22	349%
CYP3A4-2	Testosterone	6OH-testosterone	10.6 ± 3.3	147 ± 14.5	1386%
UGT	7-OH-Coumarin	7-Hydroxycoumarin Glucuronide	16.05 ± 0.32	275 ± 79.5	1713%
SULT	7-OH-Coumarin	7-Hydroxycoumarin Sulfate	7.24 ± 0.34	13 ± 0.69	179%
2J2	Astemizole	O-Demethyl Astemizole	0.92 ± 0.43	5.14 ± 1.53	558%
CES2	Irinotecan	SN38	0.37 ± 0.14	0.38 ± 0.27	102%

Dose-dependent Inhibition of CYP3A4 Activity* of MetMax™ Enterocytes by Herbal Supplements (*Relative Activity of Control)

Concentration	0%	1.56%	3.12%	6.25%	12.5%	25%	50%	100%
Green Tea	100.0	102.3	101.5	101.5	47.9	32.5	14.1	2.4
Grapefruit Juice	100.0	62.8	49.1	49.1	24.0	20.0	12.9	6.9
St. John's Wort	100.0	116.1	100.6	100.6	49.8	34.3	21.2	8.1
Echinacea	100.0	116.5	88.8	88.8	85.4	82.1	52.2	19.9
Ginger	100.0	114.8	100.8	100.8	70.9	73.5	45.0	25.3
Horehound	100.0	117.3	94.6	94.6	57.4	50.7	47.9	36.7
Spirulina	100.0	102.2	86.0	86.0	72.8	58.0	54.0	38.5
Milk Thistle	100.0	104.3	93.3	93.3	66.9	54.3	57.2	41.3
Black Elderberry	100.0	141.4	118.8	118.8	92.3	82.0	88.0	42.9
Ginkgo	100.0	114.1	98.8	98.8	88.8	93.5	60.6	49.2
Cinnamon	100.0	110.9	111.9	111.9	104.5	99.0	87.4	51.6
Ginseng	100.0	124.6	95.5	95.5	87.6	75.3	66.5	58.9
Garlic	100.0	118.6	119.4	119.4	121.0	112.8	99.8	66.6
Black Cohosh	100.0	124.0	108.9	108.9	90.6	86.2	76.9	67.0

Conclusions

- MetMax™ pooled donor human enterocytes represent a convenient and metabolically active in vitro experimental system for the evaluation of enteric drug metabolism and food-drug interactions**
- 24 commonly used herbal supplements were evaluated for their inhibitory effects on enteric CYP3A4**
 - Dose dependent inhibition was observed for all 24 supplements
 - Green tea extract, grapefruit juice, St. John's wort, echinacea, and ginger herbal supplements caused ≥75% inhibition of CYP3A4 activity at the recommended dose, suggesting that they may have clinically significant inhibitory effects on orally-administered drugs that are CYP3A4 substrates**