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

<table>
<thead>
<tr>
<th>MetMax™ Enterocytes</th>
<th>Intact Enterocytes</th>
<th>Freezer to Incubation:</th>
<th>&lt;5 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1. Retrieve from LN2 freezer</td>
<td>2. Thaw in a 37°C water bath</td>
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</tbody>
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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, supplemented with cofactors, re-cryopreserved, 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 luminometry 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 H2O (IVL), 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:

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 wart, 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