

Phenacetin as a Marker in Evaluation of CYP1A2 Induction in Human Hepatocytes

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Introduction

Human CYP1A2 has been previously demonstrated to catalyze phenacetin O-deethylation (1), 4-aminobiphenyl N-oxidation (2), caffeine 3-demethylation (2), and ethoxyresorufin O-deethylation (3). Ethoxyresorufin is widely used as an isoform selective substrate for CYP1A2. Our laboratories have routinely used ethoxyresorufin to evaluate induction of CYP1A2 in human hepatocytes. However, ethoxyresorufin O-deethylation can be mediated by both CYP1A1 and CYP1A2. Phenacetin has been widely used as the preferred marker for detecting CYP1A2-based inhibition potential *in vitro* (4). The purpose of this study was to evaluate the use of phenacetin as a selective marker and compare it with ethoxyresorufin as a probe for the evaluation of CYP1A2 induction in human hepatocytes. The assay used to monitor the O-deethylation of phenacetin uses HPLC or LC/MS/MS and has been validated based on bioanalytical method validation guidelines.

Methods

Human hepatocytes cultures

- Hepatocytes were isolated and cultured based on a previously published method (5).
- Cells were plated after viability was measured using Trypan blue exclusion, then cultured for 48 hours prior to initiation of treatment.
- Hepatocytes were treated with vehicle (1% acetonitrile), omeprazole, or rifampin for 48 hours, then treated with ethoxyresorufin for 1 hour or with phenacetin for 0.5-2 hours.

Analysis

- Phenacetin O-deethylase: The activity of phenacetin O-deethylase was determined by measuring the formation of acetaminophen with HPLC or LC/MS/MS.
- Ethoxyresorufin O-deethylase: The activity of ethoxyresorufin O-deethylase was determined by measuring the formation of resorufin using fluorescence.

Results

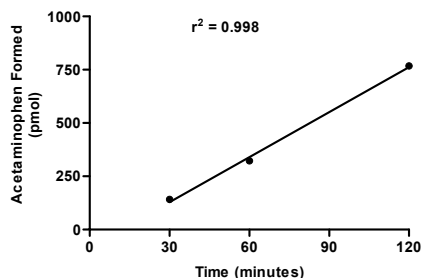


Figure 1. Acetaminophen formed in relation to the incubation time. Hepatocytes were incubated with phenacetin (100 μ M) for 30, 60, and 120 minutes, following incubation with omeprazole (50 μ M) for 48 hours

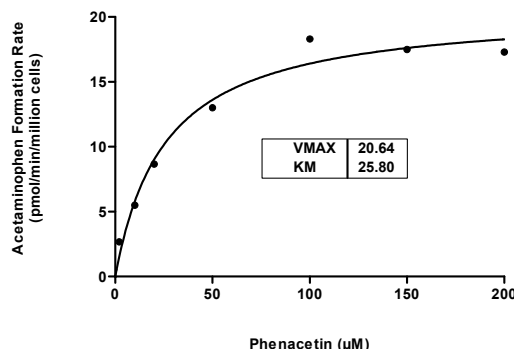


Figure 2. Acetaminophen formation rate in relation to phenacetin concentration. Hepatocytes were incubated with phenacetin (2-200 μ M) for 2 hours following treatment with 50 μ M omeprazole for 48 hours.

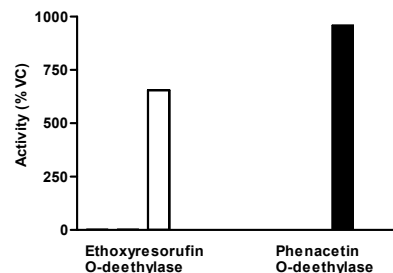


Figure 3. Comparison of ethoxyresorufin O-deethylase and phenacetin O-deethylase activity in fresh human hepatocytes following treatment with omeprazole

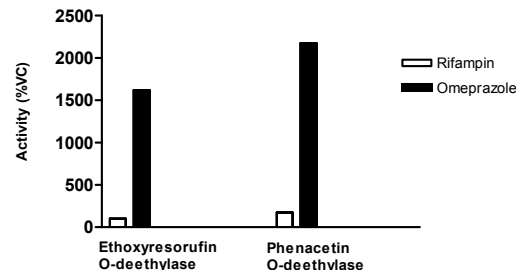


Figure 4. Comparison of ethoxyresorufin O-deethylase and phenacetin O-deethylase activity in cryopreserved human hepatocytes following treatment with omeprazole or rifampin

Table 1: Induction of phenacetin O-deethylase activity in fresh human hepatocytes by omeprazole at multiple concentrations

Omeprazole Concentration	Acetaminophen Formation Rate	Percent of Vehicle Control
12.5 μ M	5.85 \pm 1.03	306
25 μ M	6.58 \pm 0.466	344
50 μ M	5.11 \pm 0.682	267

Enzyme activity is expressed as pmol/minute/million cells. Data are expressed as mean \pm standard deviation.

Table 2: Induction of phenacetin O-deethylase activity in human hepatocytes by omeprazole

Lot / Donor Number	Hepatocyte Type	Gender	Age	Percent of Vehicle Control
RUJ	Cryopreserved	Female	74	1183
NOG	Cryopreserved	Male	46	1479
BDF	Cryopreserved	Male	50	1499
1	Freshly Isolated	Male	36	792
2	Freshly Isolated	Male	40	2221
3	Freshly Isolated	Male	70	763

Conclusions

- Phenacetin provides a more selective and sensitive alternative marker for the quantitation of CYP1A2 induction in human hepatocytes.
- The optimal assay conditions are 100 μ M phenacetin and 2-hour incubation period.
- Induction of phenacetin O-deethylase activity by omeprazole was similar in both cryopreserved and fresh human hepatocytes.
- The assay for phenacetin O-deethylase using LC/MS/MS provides significantly increased ability to detect the low levels of CYP1A2 present in the vehicle control when compared to the fluorimetric-based ethoxyresorufin O-deethylase assay.

References

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