Selection of Human Liver S9 and Cytosol Fractions for Evaluating Clearance by Aldehyde Oxidase (AO): The Impact of Low Versus High AO Activity Lots.

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Introduction

Aldehyde oxidase (AO) is a cytosolic enzyme present in the liver of humans and other mammals that catalyzes various oxidation and reduction reactions. Biotransformation by AO is an important clearance mechanism for many drugs and drug candidates, with increasing importance in certain chemical spaces, and in some cases, such as zaleplon, AO metabolism leads to rapid in vivo clearance. Several publications have demonstrated the under-prediction of in vivo human clearance from in vitro clearance data, which are typically conducted with human liver subcellular fractions, such as S9 or cytosol. Zientek and colleagues (2010)[1] described a rank order approach, or 'yard-stick' approach, to categorize known AO substrates into low, medium or high clearance categories based on in vitro CL_{int} data. With this approach, new drugs candidates can be evaluated in vitro in S9 or cytosol and the predicted in vivo clearance can be qualitatively evaluated. These subcellular fractions, S9 and cytosol, are commercially available from multiple sources and in many formats (individuals and pools of various sizes), which leads to variation in AO activity.

Because of the necessity to scale AO clearance with a rank-order approach, the present study set forth to determine which human liver S9 and cytosol lots (individual or pooled) can be utilized to predict AO clearance once threshold values are determined with appropriate probe drugs. Therefore, this study evaluates the impact of low versus high AO activity in human liver S9 and cytosol preparations on the prediction of scaled clearance for AO substrates with the 'yard-stick' approach.

Methods

Individual and pooled human liver cytosol (n =1 or n = 50) and S9 (n = 1 or n = 200) fractions were prepared internally. Phthalazine, zoniporide, O^6-benzylquanine, zaleplon and methotrexate were purchased from Sigma-Aldrich (St. Louis, MO). Carbazeran and deoxypenciclovir were purchased from Toronto Research Chemicals (Toronto, Ontario).

Metabolic clearance was evaluated in human liver S9 and cytosol fractions selected from five individuals that spanned >10-fold range in AO activity based on previous internal characterization. Each S9 and cytosol sample was previously characterized by measurement of phthalazine (20 µM, 10 min) oxidation (incubation conditions similar to those described below). Metabolic stability was evaluated by monitoring the disappearance (parent loss) of six drugs (at 1 µM) with varied AO clearance, namely carbazeran, deoxypenciclovir, zoniporide, benzylquanine, zaleplon and methotrexate. Briefly, incubations (200 µL, 37°C) were conducted in duplicate with human liver S9 (2.5 mg/mL) or cytosol (1 mg/mL) in phosphate buffer (50 mM, pH 7.4) for up to 4 hours in the presence of one of the selected drugs. Time points were adjusted to account for expected half-life (final time points ranged from 15 to 240 min). Reactions were terminated by the addition of organic solvent (50:50 ACN/water) with an appropriate internal standard. Parent drug was monitored by LC-MS/MS and remaining drug was quantified with a standard curve prepared for each compound in the appropriate matrix. Disappearance of drug at each time point was determined by comparison to the zero time control (% remaining). Half-life ($t_{1/2}$) was determined by log transformation of the parent loss data with Excel (Microsoft, 2007).

References

1. Zientek et al., *Drug Metab Dispos* **38**:1322-1327, 2010

Tables and Figures

Figure 1. Metabolic stability (clearance) of the AO substrates carbazeran, zoniporide and zaleplon in individual (five) and pooled (n = 50) human liver cytosol fractions

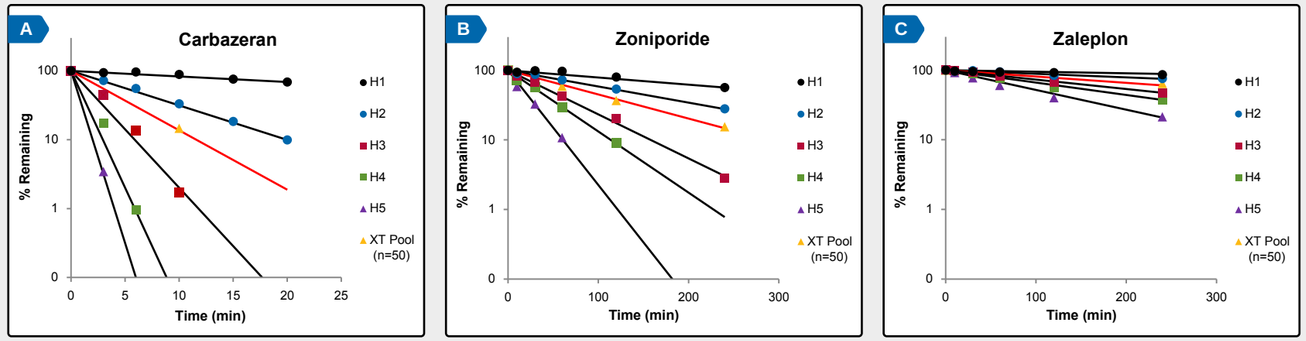

Figure 2. Metabolic stability (clearance) of the AO substrates carbazeran, zoniporide and zaleplon in individual (five) and pooled (n = 200) human liver S9 fractions

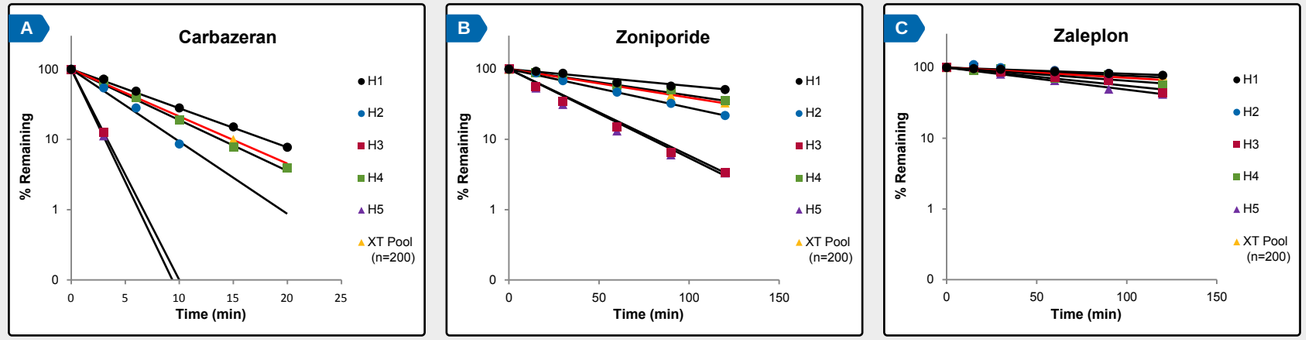

Table 1. Rank order of AO activity in various individual and pooled human liver cytosol fractions as determined by in vitro half-life

		Half-life (minutes)					
Substrate (1 µM)[a]	$CL_{int, AO}$ (in vitro)[b]	H1	H2	H3	H4	H5	XT Pool (n=50)
Carbazeran	High	36	6	1.7	0.9	0.6	4
Deoxypenciclovir	Med	>240	96	25	22	12	71
Zoniporide	Med	>240	133	47	35	19	89
Benzylguanine	Med	>240	144	56	38	21	113
Zaleplon	Low	>240	>240	218	163	108	>240
Methotrexate	Low	>240	>240	>240	>240	>240	>240
Phthalazine[c]		382	711	1524	2417	3148	1286

a. Sorted based on rank order of XT Pool results
b. Based on 'Yardstick Approach' described by Zeintek et al., 2010[1]
c. Enzymatic rate for phthalazine oxidation (pmol/mg protein/min)

Table 2. Rank order of AO activity in various individual and pooled human liver S9 fractions as determined by in vitro half-life

		Half-life (minutes)					
Substrate (1 µM)[a]	$CL_{int, AO}$ (in vitro)[b]	H1	H2	H3	H4	H5	XT Pool (n=200)
Carbazeran	High	6	3	1	4	1	5
Deoxypenciclovir	Med	72	38	15	63	12	57
Zoniporide	Med	104	54	25	84	21	74
Benzylguanine	Med	101	58	25	132	22	86
Zaleplon	Low	>120	>120	105	>120	86	>120
Methotrexate	Low	>240	>240	>240	>240	>240	>240
Phthalazine[c]		256	554	1080	1266	1946	609

a. Sorted based on rank order of XT Pool results
b. Based on 'Yardstick Approach' described by Zeintek et al., 2010[1]
c. Enzymatic rate for phthalazine oxidation (pmol/mg protein/min)

Results

- **Figure 1** illustrates the metabolic clearance of carbazeran, zoniporide and zaleplon in individual (five) and pooled (n =50) human liver cytosol fractions. The in vitro clearance of carbazeran (**Figure 1A**), a high clearance AO substrate[1], increased in accordance to the rank order previously determined for phthalazine oxidation (**Table 1**). Across the range of individuals, the half-life of carbazeran ranged from 0.6 to 36 minutes. In pooled cytosol, the observed half-life of carbazeran was four minutes which was similar to the median individual sample.
- In a similar manner, the in vitro clearance of zoniporide (**Figure 1B**), a moderate clearance AO substrate[1], increased in accordance to the rank order previously determined for phthalazine oxidation (**Table 1**). Across the range of individuals, the half-life of zoniporide ranged from 19 to >240 minutes. In pooled cytosol, the observed half-life of zoniporide was 89 minutes which was similar to the median individual sample.
- The in vitro clearance of zaleplon (**Figure 1C**), a low clearance AO substrate[1], increased in accordance to the rank order previously determined for phthalazine oxidation (**Table 1**). Across the range of individuals, the half-life of zaleplon ranged from 108 to >240 minutes. In pooled cytosol, the observed half-life of zaleplon was >240 minutes which was similar to the median individual sample.
- High, moderate and low clearance drugs (**Table 1**) could be segregated based on the observed half-life in all cytosol samples (individual and pooled) with the exception of the lowest activity individual (H1).
- **Figure 2** illustrates the metabolic clearance of carbazeran, zoniporide and zaleplon in individual (five) and pooled (n =200) human liver S9 fractions. The in vitro clearance of carbazeran (**Figure 2A**), a high clearance AO substrate[1], increased in accordance to the rank order previously determined for phthalazine oxidation (**Table 2**). Across the range of individuals, the half-life of carbazeran ranged from 1 to 6 minutes. In pooled S9, the observed half-life of carbazeran was 5 minutes which was similar to the H2 individual sample.
- In a similar manner, the in vitro clearance of zoniporide (**Figure 2B**), a moderate clearance AO substrate[1], increased in accordance to the rank order previously determined for phthalazine oxidation (**Table 2**). Across the range of individuals, the half-life of zoniporide ranged from 21 to 104 minutes. In pooled S9, the observed half-life of zoniporide was 74 minutes which was similar to the H2 individual sample.
- The in vitro clearance of zaleplon (**Figure 2C**), a low clearance AO substrate[1], increased in accordance to the rank order previously determined for phthalazine oxidation (**Table 2**). Across the range of individuals, the half-life of zaleplon ranged from 86 to >120 minutes. In pooled S9, the observed half-life of zaleplon was >120 minutes which was similar to the median individual sample.
- High, moderate and low clearance drugs (**Table 2**) could be segregated based on the observed half-life in all S9 samples (individual and pooled).

Conclusions

- In human liver S9, half-lives and scaled clearance could be calculated from all five individuals, including the lowest activity sample. In each case the 'yard-stick' approach could be applied to all samples tested with reasonable thresholds, including the lowest and highest activity donors representing over a 10-fold range in AO activity.
- In human liver cytosol, half-lives and scaled clearance could be calculated from four of five individuals, the exception being the lowest AO activity sample. Similarly, the 'yard-stick' approach could be applied with reasonable thresholds for all samples tested, except for the lowest AO activity sample.
- These data indicate that, because of the necessity to scale AO clearance with a rank-order approach, nearly all human liver S9 and cytosol lots (individual or pooled) can be utilized to predict AO CL_{int} once threshold values are determined with appropriate probe drugs.