UNDERSTANDING THE IMPACT OF METABOLISM BY AO AND XO ON METABOLITE SAFETY AND PK PREDICTIONS

Michelle Gleave, Angus Nedderman, Mark Savage and Julian Haynes
Unilabs York Bioanalytical Solutions, Discovery Park, Ramsgate Road, Sandwich, Kent, CT13 9ND

INTRODUCTION

As our understanding of cytochrome P450-mediated metabolism grows and chemical design is able to effectively modulate P450 clearance, our attention turns to other metabolising enzymes and their potential impact on clinical efficacy and safety. Notably, aldehyde oxidase (AO) and xanthine oxidase (XO) may be potentially important contributors to drug clearance, particularly in certain areas of chemical space. This work describes an investigation of the metabolism of precedented AO and XO substrates in a range of matrices.

EXPERIMENTAL

Zoniporide, carbazeran, caffeine and xanthine were incubated in human, Sprague Dawley rat, Wistar Han rat and mouse liver cytosol (1µM; 250µM hydralazine or 100µM allopurinol). Aliquots (25µl) of the incubations were sampled and precipitated with acetonitrile (75µl) at various time points (0, 10, 20, 30, 45 and 60 minutes). Following centrifugation, an aliquot (25µl) of the supernatant was diluted with H_2O (75µl); samples containing carbazeran were further diluted 1 in 5 prior to analysis by LC-MS/MS. Quantitative clearance data were obtained using MRM on an API5000.

Additional aliquots (100µl) were sampled at 60 minutes and precipitated with acetonitrile (300µl); following centrifugation, the supernatant was reduced to dryness under nitrogen and the extract reconstituted in 5% acetonitrile prior to analysis by LC-HRMS. Zoniporide and carbazeran were also incubated in human hepatocytes (10µM, 5h), and the samples prepared as described above. Qualitative information was obtained on a LTQ Orbitrap by acquiring full scan HRMS data +/- data-dependent MS^n.

RESULTS

Quantitative analysis:

LC-MS analysis yielded a range of intrinsic clearance values for zoniporide and carbazeran in incubations with cytosol from human, rat (SD and WH) and mouse (Figures 1 and 2; Table 1). Zoniporide clearance was highest in Wistar Han rat and mouse liver cytosol, but was dramatically reduced in the presence on the AO inhibitor hydralazine. Zoniporide clearance was low in human and Sprague Dawley rat liver cytosol.

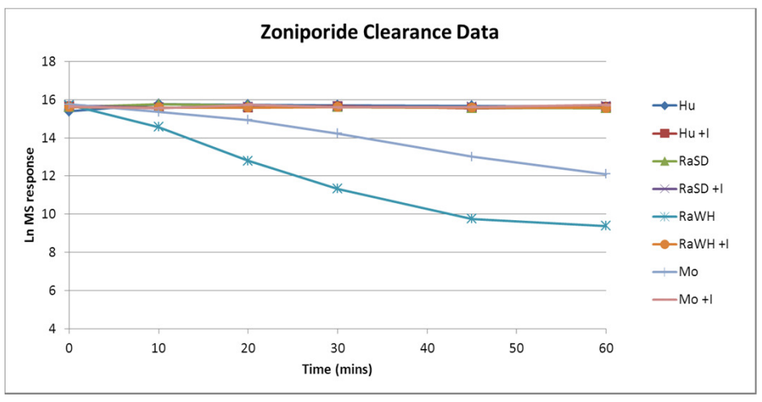

Figure 1: Comparison of zoniporide clearance in cytosol of various species (+I = incubated with AO inhibitor hydralazine).

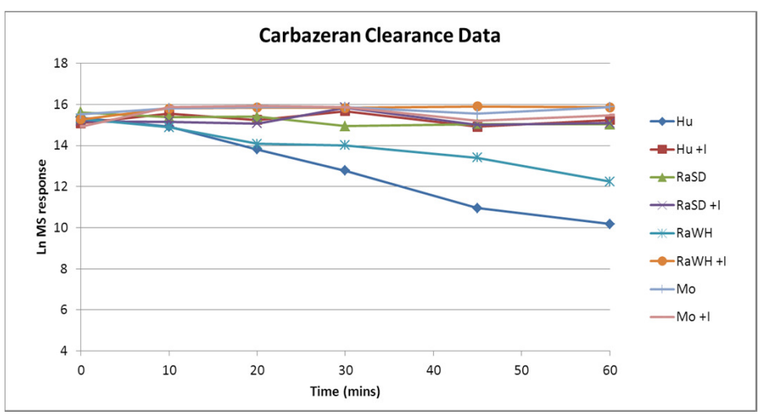

Figure 2: Comparison of carbazeran clearance in cytosol of various species (+I = incubated with AO inhibitor hydralazine).

Compound	Half-life (mins)			
	Human	Rat SD	Rat WH	Mouse
Zoniporide	87	120	6	11
Carbazeran	7	60	15	>120

Table 1: Comparison of metabolic half-life data for zoniporide and carbazeran in cytosolic incubations.

Carbazeran clearance was highest in human and Wistar Han rat liver cytosol. The presence of the AO inhibitor hydralazine significantly reduced clearance in both species. Carbazeran clearance was lower in mouse and Sprague Dawley rat liver cytosol. Caffeine clearance was low in all species and strains studied (data not shown).

Qualitative analysis:

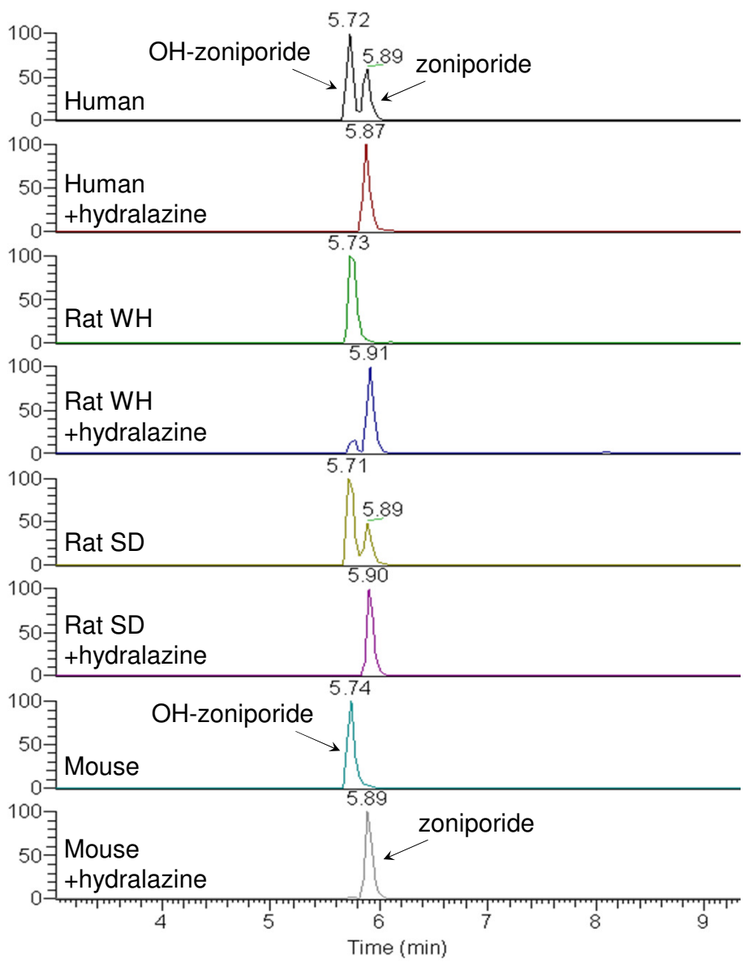

Figure 3: Illustration of the metabolism of zoniporide in cytosolic incubations (extracted ion chromatograms for zoniporide and 2-oxozoniporide).

The metabolic profiles obtained via HRMS analysis (Figure 3) correlated well with the clearance data obtained via MRM; in Wistar Han rat and mouse liver cytosol zoniporide was observed to have been almost completely turned over to 2-oxozoniporide.

Human hepatocyte incubations with carbazeran and zoniporide also demonstrated the formation of the AO-mediated oxidised metabolites (Figure 6). In both cases, relative levels of the AO-mediated oxidised metabolites were lower in hepatocyte incubations than in cytosol.

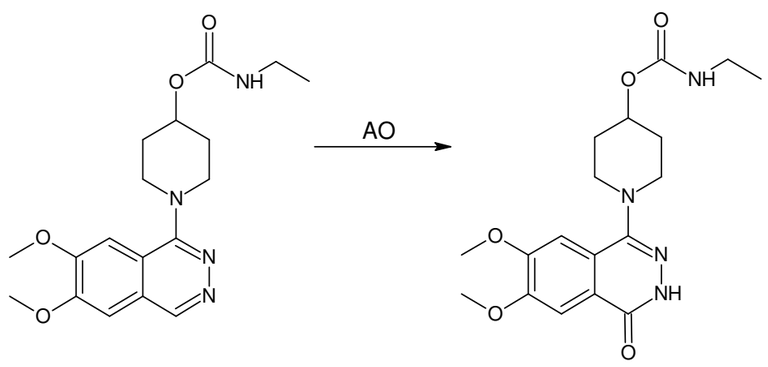

Figure 4: Oxidation of carbazeran to the phthalazinone by aldehyde oxidase.

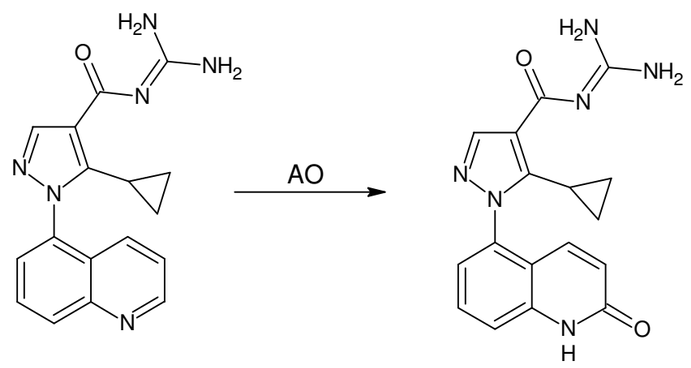

Figure 5: Oxidation of zoniporide to 2-oxozoniporide by aldehyde oxidase.

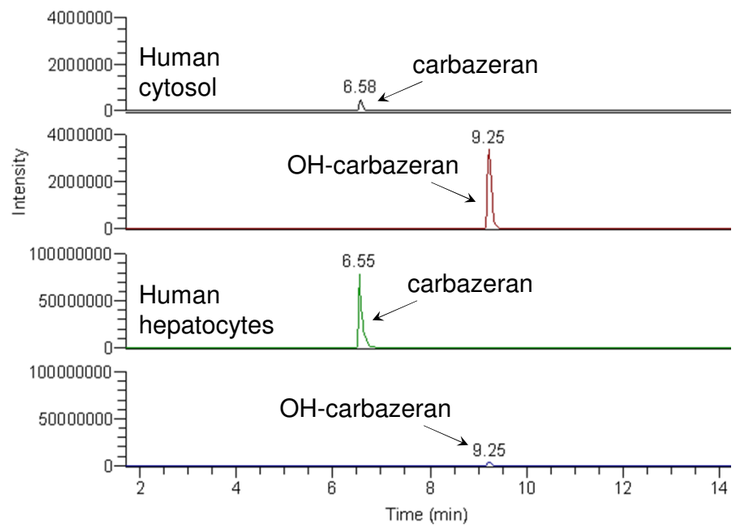

Figure 6: Illustration of the metabolism of carbazeran in human cytosol and hepatocyte incubations (XICs).

DISCUSSION

The experimental data showed that a wide range of metabolic clearance values for carbazeran and zoniporide were obtained from liver cytosol incubations in different species and strains. As expected, cytosol was an effective matrix for studying AO-mediated metabolism, and use of the AO inhibitor hydralazine was a simple method to confirm AO as the primary enzyme responsible for the clearance, or for the formation of a specific metabolite. The high carbazeran clearance observed in human cytosol correlates with in vivo data and with published cytosolic clearance values[1]; progression of carbazeran was terminated due to very low bioavailability and a lack of efficacy in humans. Comparing the metabolism of zoniporide and carbazeran between human liver cytosol and hepatocyte incubations, showed that the AO-mediated metabolite was formed in both matrices, although the relative levels were significantly lower in hepatocytes. The variation in clearance values between the species and strains studied and between compounds demonstrates some of the complexity in predicting a compounds susceptibility to AO metabolism in a particular species. It is well known that there are notable species differences in AO metabolism, and strain and sub-population differences have also been reported[2]. The general view is often that AO activity is high in humans and monkeys, low in rats and almost absent in dogs; a pattern that appears to be followed by carbazeran, but not zoniporide in this study. The variability in AO activity means that species (and strain) selections for PK, toxicology and efficacy studies, as well as for in vitro studies, are particularly important for potential AO substrates.

CONCLUSION

This work demonstrates the utility of liver cytosol for investigating AO vulnerability, and demonstrates some of the difficulty in interpreting in vitro AO-mediated clearance. Careful choices need to be made about the appropriate matrices, species and strains used when investigating AO clearance to ensure the data can impact decision making and compound progression appropriately.

REFERENCES

1. Zientek M, Jiang Y, Youdim K, Obach RS. *Drug Metab Dispos.* 38, 1322-1327 (2010).
2. Pryde DC, Dalvie D, Hu Q, Jones P, Obach RS, Tran TD. *J Med Chem.* 53, 8441-8460 (2010).

Unilabs
Bioanalytical Solutions · York · Sandwich · Copenhagen