Chloroacetanilide herbicides and their metabolites interact with human Efflux Transporters and modulate drug absorption

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INTRODUCTION

Herbicides are widely used in agriculture. In 2000 herbicide usage accounted for 44% of overall pesticide usage, being over 500 million pounds of active ingredient. Among the most frequently used herbicides in the world are **Chloroacetanilide herbicides**. Compounds like Metolachlor, Acetochlor and Alachlor are used to control annual grasses and broadleaf weeds in corn, among others.

Due to their extensive usage, residues of chloroacetanilide are often found in environmental and food samples. Moreover, most compounds have been shown to have carcinogenic effects.

ABC transporter proteins are transmembrane proteins that actively transport their substrate across the cell membrane. Through their expression in important barrier organs, like intestine, liver, kidney and the blood brain barrier (BBB), transporters are the **first barrier of defense** against xenotoxins, as has been convincingly demonstrated in knock-out mice [1,2]. Using in vitro transporter assays, compounds can rapidly be screened for interaction with transporters. In this study five, structurally similar chloroacetanilide herbicides were tested for interaction with human efflux transporters **MDR1 (P-gp), MRP1, MRP2 and BCRP**. Both parental compounds as well as metabolites were screened using at least two different types of assay for each transporter.

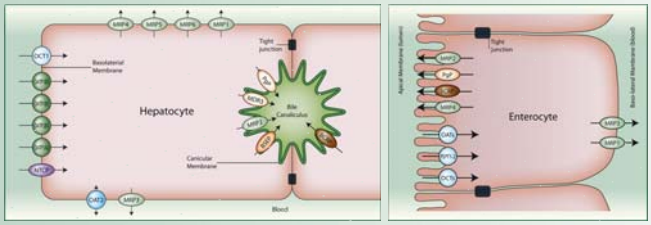
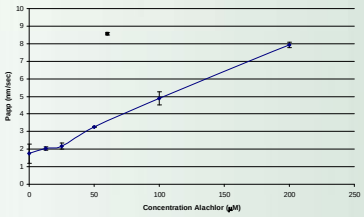


Figure 1: Transporter expression in hepatocytes (left) and enterocytes (right)



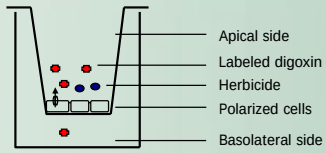


Figure 3: Inhibition of Digoxin transport on Caco-2 monolayers. Test compounds were incubated for 2 hours in the presence of 20nM radioactively labeled Digoxin. Digoxin is a substrate of MDR1, which prevents it from intestinal absorption. Chloroacetanilide herbicides act as competitive inhibitors and increase the permeability of Digoxin on Caco-2 monolayers.

RESULTS

Four out of five chloroacetanilide herbicides interacted with MDR1, whereas no interactions were observed with other transporters. Alachlor, Acetochlor, Metolachlor and Metazachlor all stimulate MDR1 ATPase activity and inhibit MDR1 mediated calcein-AM efflux. Results for Alachlor, Acetochlor and Metazachlor are shown in figure 2. Metolachlor weakly activates MDR1 ATPase activity, but strongly inhibits calcein-AM efflux. Dimetachlor does not activate ATPase activity and only at high concentrations (>200μM) weakly inhibits calcein-AM efflux. Interestingly, the structural difference between acetochlor and dimetachlor is minimal. Using Caco-2 monolayers, **alachlor, acetochlor and metazachlor were shown to modulate apical-to-basolateral transport of digoxin**, an MDR1 substrate. Results for Alachlor are shown in figure 3. The glutathione conjugate of the acetochlor metabolite deethoxymethyl acetochlor stimulated ATPase activity of MRP1. Using inside out membrane vesicles **ATP dependent transport of this compound could be demonstrated** (figure 5).

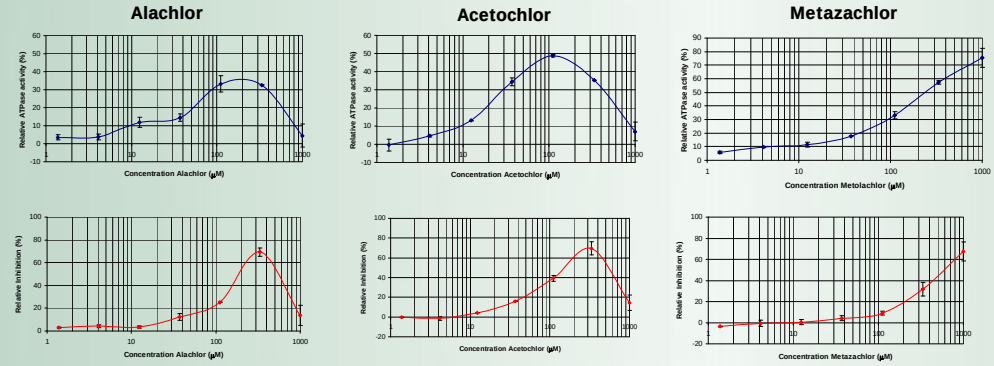


Figure 2: ATPase activity (upper) and Calcein-AM efflux inhibition (lower) results for Alachlor, Acetochlor and Metazachlor

CONCLUSIONS

- Using SOLVO's *in vitro* assay systems interactions of transporters with agrochemicals can easily be measured
- Chloroacetanilide herbicides are likely substrates of MDR1 and might cause unwanted modulation of the intestinal absorption of drugs.
- Small structural differences can strongly influence interaction with MDR1
- A major glutathione conjugate of acetochlor is a transported substrate of MRP1
- Transporter assays could become a useful tool in risk assessment of newly developed herbicides

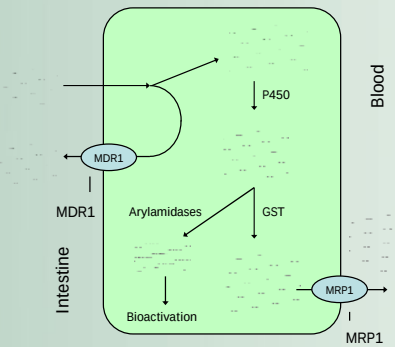


Figure 4: Metabolic pathway for the bioactivation of Acetochlor, including transporter data. Based on Coleman et al. (2000) [3].

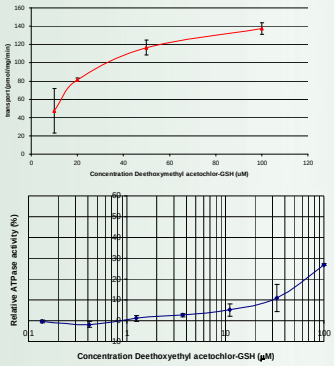


Figure 5: Upper: active uptake of deethoxymethyl acetochlor-GSH in MRP1 expressing membrane vesicles. Lower: MRP1 ATPase activation.

Methods

Compounds were tested for interaction with MDR1, MXR, MRP1 and MRP2, using both cellular and membrane based methods. ATPase activity and inhibition of vesicular transport were measured on transporter expressing Sf9 cell membranes, expressing the transporter of interest, as described by Sarkadi *et al.* (1992), and Bodo *et al* (2003). Efflux of calceinAM (substrate of MDR1) and Hoechst 33342 (substrate of MXR) was measured on cell lines overexpressing MDR1 or MXR (Holló *et al.*, 1994 , Özvegy *et al.*, 2002).

Digoxin permeability was measured on Caco2 monolayers. Samples were incubated for 2 hours in the presence of 50nM radioactively labeled Digoxin.

Direct transport of Deethoxymethyl acetochlor-GSH was measured in MRP1 expressing vesicles. Samples were incubated for 20 min. at 37oC. Membranes were disrupted using methanol. After drying samples were dissolved in eluent (85% water, 15% acetonitril). Samples were measured using LC/MS.

References:

1: Schinkel AH, Smit JJ, van Tellingen O, Beijnen JH, Wagenaar E, van Deemter L, Mol CA, van der Valk MA, Robanus-Maandag EC, te Riele HP, et al.(1994); Disruption of the mouse mdr1a P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs; Cell, vol 77(4), pp. 491-502

2: Van Heerwaarden AE, Schinkel AH (2006); The function of breast cancer resistance protein in epithelial barriesrs, stem cells and milk secretion of drugs and xenotoxins; TRENDS in Pharm. Sci.; vol 27, pp 10-16

3: Coleman S, Linderman R, Hodgson E, Rose RL (2000); Comparative MEtabolism of Chloroacetamide Herbicides and selected metabolites in human and reat liver microsomes. EHP, vol 108 (12), pp 1151-7