

Interaction of Pesticides with human Efflux Transporters

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Introduction

Human exposure to pesticides is continuously being studied and monitored. Due to its presence in food and the environment (see table 1) pesticides are easily taken up in the human body. Little is known, however, about the fate of pesticides after entering the body. ABC transporters could play an important role in excretion of pesticides as xenotoxins like food toxins (aflatoxin B1, PhIP), several pesticides, drugs and heavy metals have been shown to interact with efflux transporters like MDR1 (ABCB1), MRP1 (ABCC1) and MXR (BCRP, ABCG2) ^{1,2}.

Drugs and xenotoxins interacting with efflux transporters are known to accumulate to a lesser extent in the body than compounds that don't. This makes drugs and pesticides safer, but also less efficacious and thus ABC transporter interaction an important parameter in pharmacological and toxicological research and development. Moreover, MXR has been shown to concentrate many of its substrates into the milk of lactating organisms, increasing the exposure of suckling infants to xenotoxins. Also pesticide residues have been found in human breast milk, and in pasteurized cow milk².

Measuring interaction of pesticides with ABC transporters might thus provide valuable information on the absorption or excretion potential of pesticides in the human body.

Table 1: presence of pesticides in environmental and food samples

Name	Found in	Max cc (uM)
Acetochlor	Carpet dust	13,73 ⁽³⁾
Malathion	- Domestic and import feeds - Pasteurized milk	89 ^(4,5)
Chlorpyrifos	- Domestic and import feeds - Pasteurized milk	1,33 ^(4,5)
Fluazifop-p-butyl	Carpet dust	1,01 ⁽³⁾

Results

Over 50% of the measured compounds interacted with MDR1 and/or MXR, indicating an important role for these proteins in excretion or absorption of pesticides. Activation of MDR1 ATPase activity and inhibition of calcein AM efflux by pesticides malathion, chlorpyrifos, fluazifop-p-butyl, acetochlor and dimetachlor is shown in figure 3.

Large differences in interaction with both MXR and MDR1 were observed between pesticides showing only minor structural differences. Some results are summarized in table 2.

MRP1 was shown to interact with Chloroacetylcholine (CAIN) and its metabolite 7-Me-CAIN, but only in the presence of Glutathione (figure 2). Interestingly the glutathione conjugate of 7-Me-CAIN also interacted with MRP1 in the absence of glutathione.

Methods

More than 50 pesticides were screened 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).

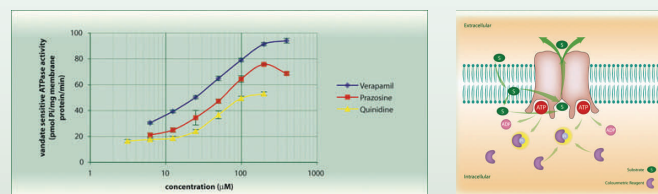


Figure 1: Principle (right) and typical output (left) of an ATPase activity assay

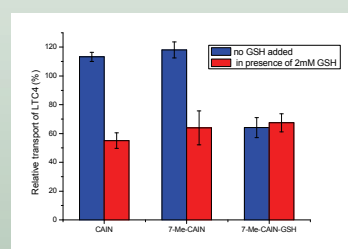


Figure 2: Inhibition of MRP1-LTC4 transport by CAIN and its metabolites at a concentration of 67 uM in the presence and absence of 2 mM glutathione.

Table 2: Structural differences of pesticides

Pesticide	Inhibition of E3S transport	Inhibition of Hoechst efflux	Activation of ATPase activity	Inhibition of CalceinAM efflux
K-113	+	-	-	-
K-1341	-	+	-	-
Dimetachlor	-	-	-	-
Acetochlor	+	+	+	+

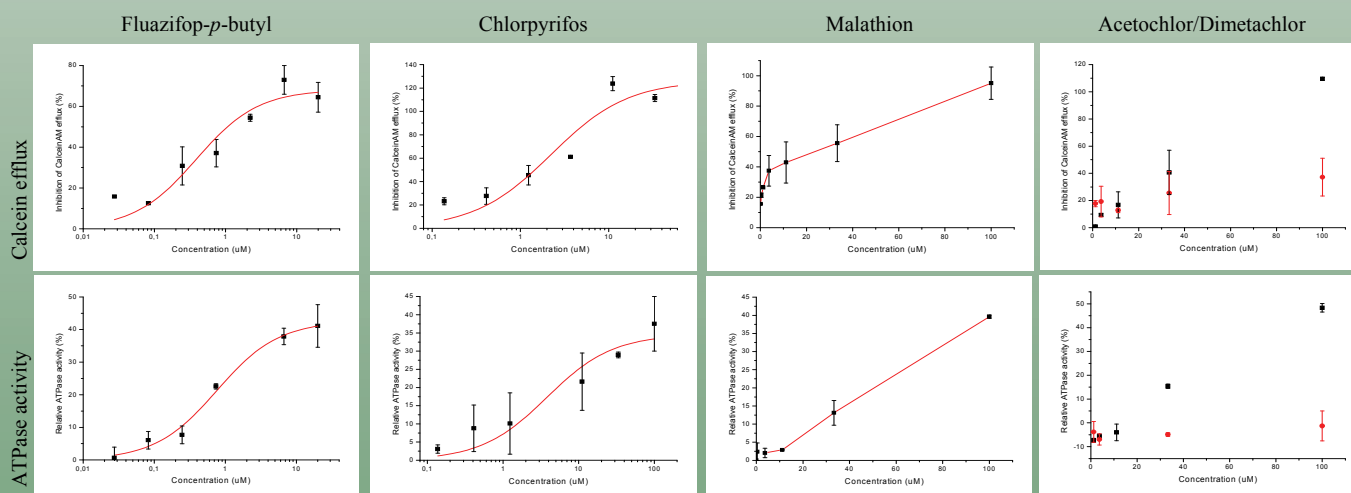


Figure 3: Inhibition of calcein AM efflux and activation of MDR1 ATPase activity by several pesticides. In the diagrams on the left acetochlor (■) and dimetachlor (●) are shown. As can be seen, dimetachlor does not activate MDR1 ATPase activity nor inhibit efflux of calcein AM, whereas acetochlor does. The structural difference between the two molecules, however, is minor (see table 2).

Conclusions

- Interaction of pesticides can easily be measured using whole cell or membrane based high throughput techniques.
- Many, but not all pesticides interact with efflux transporters, mainly MDR1 and MXR. Small changes in structure can have large effects on interaction patterns, indicating that ABC transporter substrate specificity is hard to predict.
- Measuring interaction of pesticides with ABC transporters could be a useful tool in risk assessment of pesticides.

References

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