

Drug Transporters

Transporter proteins are embedded in plasma membranes and actively transport their substrates (drugs, food components or endogenous compounds) into or out of the cell. Transporters play an important role in the absorption, distribution and excretion of drugs. Drug-transporter interactions can cause unwanted drug-drug or drug-food interactions resulting in either decreased efficacy or enhanced toxicity. In the broad range of DMPK services of TNO, several methods are available to study active drug transport. These specific models can be very helpful in predicting potential drug-drug interactions.

TNO's services

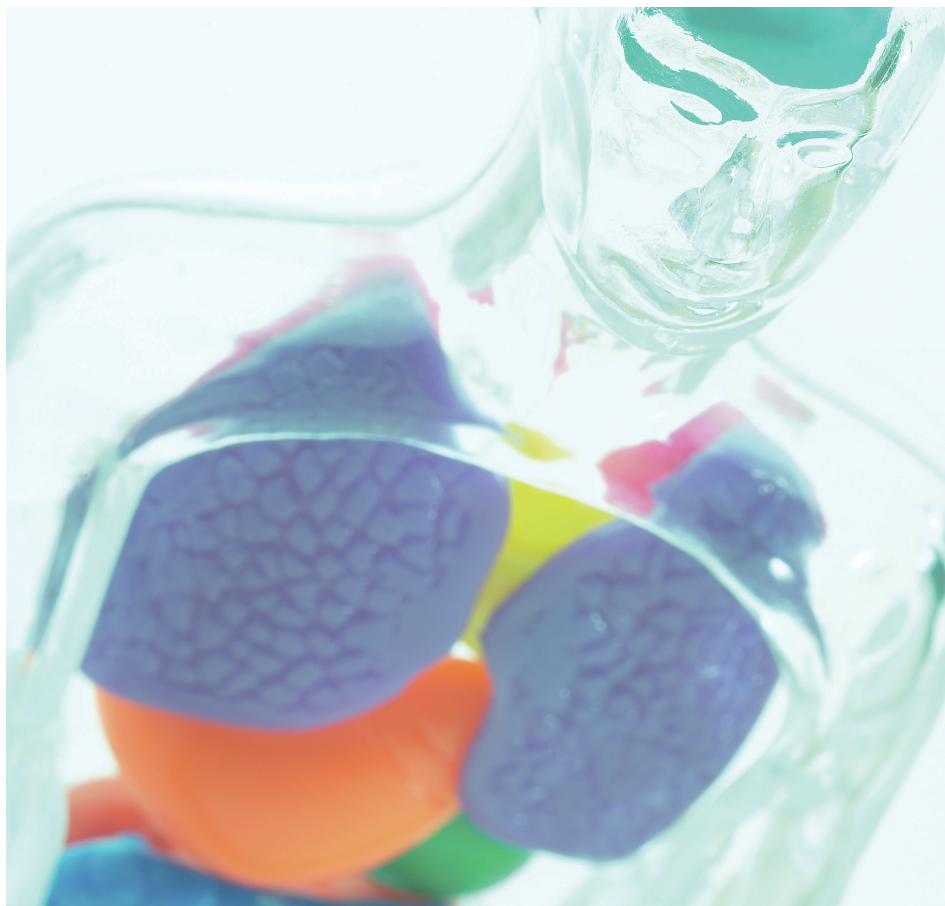
Transporters are expressed in various excretory organs, such as the liver, kidney and intestine, and play an important role in determining the bioavailability of drugs. Some transporters are also present in

so-called tissue sanctuaries, such as the brain, testes, or placenta, where they protect these tissues by reducing the uptake of potentially toxic compounds. Transporters often have broad and overlapping substrate specificities. Therefore, it is possible that

two or more co-administered drugs or food-components both interact with the same transporter(s), leading to unwanted transporter-mediated drug-drug or drug-food interactions. During drug development it is extremely important to investigate possible interactions of compounds with one or more transporters. TNO helps design optimal studies to assess whether transporter related processes are relevant for your compound of interest.

Your advantage

- Studies are adjusted to the properties of your compound and consequently provide useful answers.
- Fast turn-around times of well designed, executed and reported studies allow you to efficiently integrate studies in your pre-clinical development programme.
- GLP compliant studies provide you with a report on drug-drug interactions that is required for regulatory approval. It is written in your format.



Cell-based transporter assays

In bi-directional transport assays the influence of a transporter on the actual flux of a drug from the apical to the basolateral side of a cell monolayer and vice versa can be studied in detail (Figure 1). This assay can be performed using (MDCKII) cells that overexpress the relevant transporter (compared to mock-transfected cells), or with Caco-2 cells in combination with specific inhibitors of the transporters. TNO studies interactions of a drug with uptake transporters using (transient) transfected cell-lines overexpressing a single uptake transporter. This method is ideally based on cell lines from human origin (e.g. HEK 293 cells) as plasma membrane lipid composition can affect transporter

function. In transfected cells, the time and concentration dependent uptake of the drug or the effects of the drug on the uptake of a model substrate are monitored.

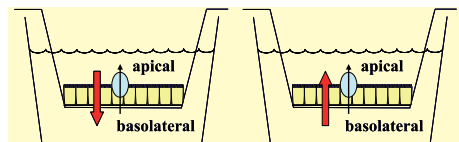


Figure 1. Bi-directional transport assay. When an efflux protein at the apical membrane is expressed (depicted in blue), transport of its substrates towards the apical direction will be higher than towards the basolateral side.

Inside-out vesicle assays

TNO offers inside-out vesicles, derived from the plasma membranes of cell lines (HEK293 or Sf-9) and overexpressing the efflux transporter of interest, to study the transport of compounds that do not easily enter cells. Alternatively, we examine the interaction of a drug with the transport of a model substrate of the transporter to investigate potential drug-drug interactions.

Table 1. Cellular assays for transporter studies at TNO.

	Available cell lines	Transporter studied
Bi-directional transport (Efflux transporters)	MDCKII-MII	Control cells
	MDCKII-MDR1	MDR1 (P-gp, ABCB1)
	MDCKII-BCRP	BCRP (ABCG2)
	MDCKII-MRP1	MRP1 (ABCC1)
	MDCKII-MRP2	MRP2 (ABCC2)
	MDCKII-MRP3	MRP3 (ABCC3)
	MDCKII-MRP5	MRP5 (ABCC5)
	Caco-2	MDR1, BCRP, MRP2 in combination with specific inhibitors
Cellular uptake assay (Uptake transporters)	HEK293	Control cells
	HEK293-OATP1B1	OATP1B1 (OATP-C, SLC01B1)
	HEK293-URAT	URAT1 (SLC22A12)
	HEK-OCT1	OCT1 (SLC22A1)
	HEK293-OCT2	OCT2 (SLC22A2)
Other transporters (under development, and upon request)		

Custom made in vitro assays

As knowledge about drug transporters is still growing, it is possible that there are

no existing *in vitro* models for a specific transporter. Therefore, TNO offers a service to develop cell-based models on request. Transporters can be transfected into HEK- or MDCKII cell lines, or vesicles can be prepared, depending on the sponsor's area of interest.

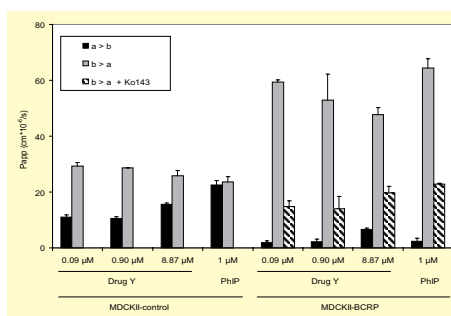


Figure 2. Results of a bi-directional transport assay using MDCKII-BCRP cells in comparison with the MDCKII-control cells. Drug Y is a clear BCRP substrate which transport was inhibited by the known BCRP inhibitor Ko143.

In vivo KO or transgenic mice studies

A wide range of (knock-out and transgenic) mouse models has been developed to study the *in vivo* function of transporters. These models are very valuable during research into the effect of specific transporters on the fate of new drugs in the body. These models help investigate how new drugs interact with specific transporters in great detail, especially in combination with specialized techniques such as microdialysis, gall/urine bladder cannulations or metabolic cage experiments (all available at TNO).

Selected references

1. Vlaming M, Verwei M, DeGroot J, Wortelboer H, (2009) Drug-drug interactions: tools for drug transporter protein studies. *European Pharmaceutical Review* 4: 47-52.
2. Wortelboer HM, Balvers MGJ, Usta M, van Bladeren PJ, Cnubben NHP, (2008) Glutathione-dependent interaction of heavy metal compounds with multidrug resistance proteins MRP1 and MRP2. *Environmental Toxicology and Pharmacology* 26 (1), p.102-108.
3. Van Zanden JJ, van der Woude H, Vaessen J, Usta M, Wortelboer H, Cnubben NHP, Rietjens IMCM, The effect of quercetin phase II metabolism on its MRP1 and MRP2 inhibiting potential. *Biochemical Pharmacology* (2007) 74, 345-351.

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