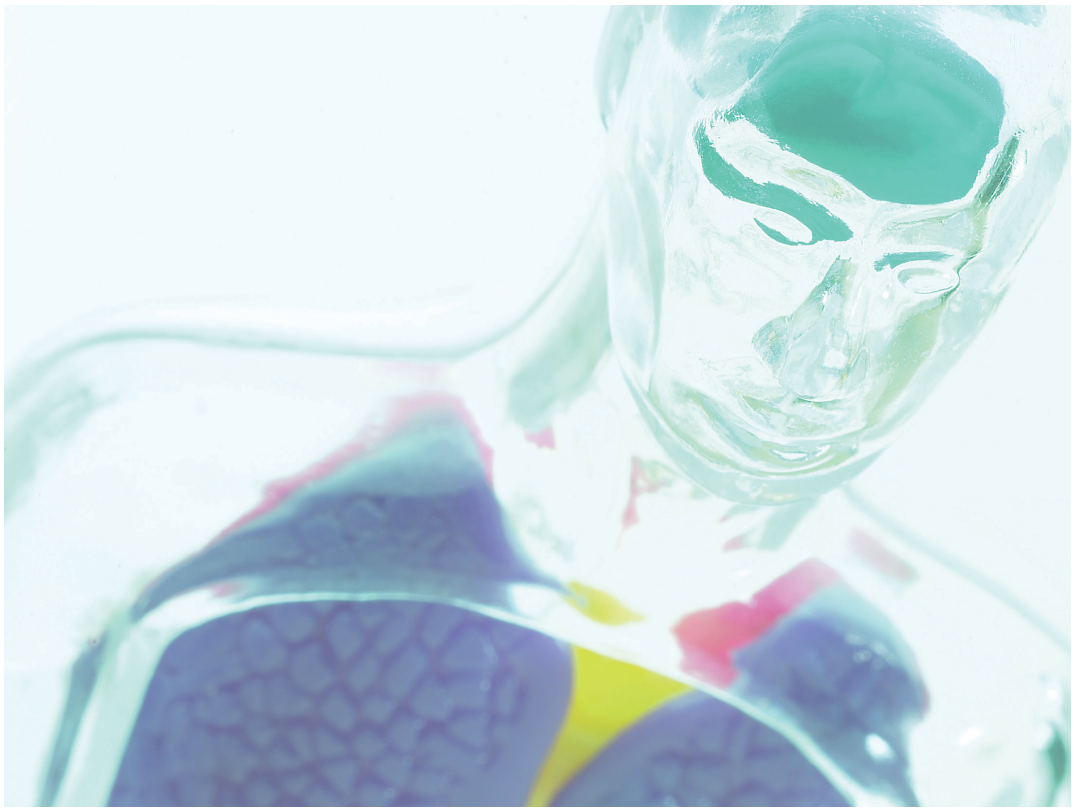
DRUG TRANSPORTERS



TNO innovation for life

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 a significant 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 research 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 therefore important to investigate possible interactions of compounds with one or more transporters.

TNO helps to 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. The report can be 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 (stable or transient) transfected cell-lines over-expressing a single uptake transporter. This method is ideally based on cell lines from human origin (e.g. HEK293 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.

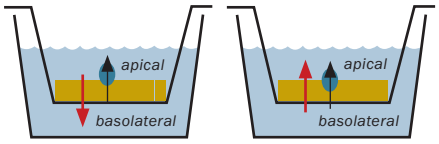


Figure 1. Bi-directional transport assay. When an efflux protein at the apical membrane is expressed (depicted in blue), transport of its substrates towards the apical direction will be higher than towards the basolateral side.

INSIDE-OUT VESICLE ASSAYS

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

CUSTOM MADE IN VITRO ASSAYS

As knowledge about 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.

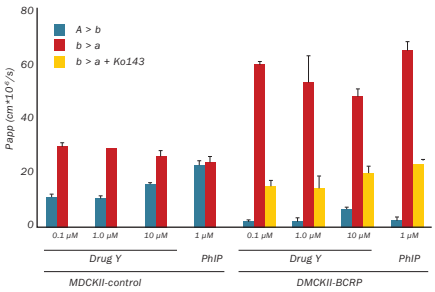


Figure 2. Results of a bi-directional transport assay using MDCKII-BCRP cells in comparison with the MDCKII-control cells. Drug Y is a clear BCRP substrate which transport was inhibited by the known BCRP inhibitor Ko143.

	Human transporters		Rodent transporters
MDCKII (control)	HEK293 (control)	HEK-OCT1	MDCKII-mBcrp1
MDCKII-MDR1	HEK-OATP1B1	HEK-OCT2	MDCKII-mMrp2
MDCKII-BCRP	HEK-OATP1B1*15	HEK-MRP4	LLC-PK1-mMdr1a
MDCKII-MRP1	HEK-OATP1B3	HEK-MATE1	HEK-rBcrp
MDCKII-MRP2	HEK-OATP2B1	HEK-URAT1	HEK-rOatp1b2
MDCKII-MRP3	HEK-OAT1	Caco-2	HEK-rOatp1a4
MDCKII-MRP5	HEK-OAT2	Hepatocytes	Hepatocytes
LLC-PK1 (control)	HEK-OAT3		
LLC-PK1-MDR1			
Other cellular / tissue systems (under development, and upon request)			

Table 1. Cellular assays for transporter studies at TNO.

RELATED RESEARCH

- Combination of cellular/tissue studies and TIM (TNO Intestinal Model)
- Abundance measurements of transporter proteins using UPLC-MS-MS
- In vivo KO or transgenic animal models
- In vivo imaging
- In silico modeling of transporter-related processes

Selected references

1. Vlaming M, Verwei M, de Groot J, Wortelboer H (2009) Drug-drug interactions: tools for drug transporter protein studies. *Eur Pharm Rev 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. *Environ Toxicol and Pharmacol 26: 102-108.*
3. Van Zanden JJ, van der Woude H, Vaessen J, Usta M, Wortelboer H, Cnubben NHP, Rietjens IMCM (2007) The effect of quercetin phase II metabolism on its MRP1 and MRP2 inhibiting potential. *Biochem Pharmacol 74: 345-351.*

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TNO HEALTHY LIVING

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