

★ Through the progressive work of the MEMTRANS project an improved methodology to study the impact, mechanism and regulation of drug and xenobiotic transporters is close to realisation. We spoke to the project's coordinator **Dr Marival Bermejo** about her team's research

Exploring the role of membrane transporters

Currently, through a mixture of vague and differing methodologies as well as mass inter-laboratory variability, the study of drug and xenobiotic efflux transporters is heavily reliant on animal experimentation at a pre-clinical stage and is the cause of high failure rates – due to poor absorbability – in drug candidates. This fractured process is responsible for the slow development of new drugs for patients and the difficulty in accurately predicting and maintaining the cross over from in vitro to in vivo. To address these problematic issues, the MEMTRANS project (Membrane transporters: In vitro models for the study of their role in drug fate), led by Dr Marival Bermejo, was initiated.

“MEMTRANS is focused on the improvement of the current in vitro cell culture methods to predict human intestinal permeability,” explains Bermejo, as she begins to describe why the project was instigated. “Currently these models are quite reliable for drugs that are absorbed by passive diffusion but for those substances (drugs, toxicants, nutrients) using a transporter (either to be absorbed or to be secreted out of the body) the predictions are less accurate due to the different expression levels of the transporter in the in vitro system compared to the human intestine.”

Therefore there can be difficulty in maintaining the accuracy of predictions. To compound this, patients favour an oral route for drug administration due to convenience – a form of administration that relies heavily on absorbability – which leads to further difficulties in predicting with a high level of accuracy. This present inability to predict the behaviour of the transporter and the drug's absorbability

that it carries, inevitably leads to extensive animal and drug candidate testing, which is time consuming, expensive and ethically sketchy.

Ideally therefore, the more accurate in vitro testing can be – which is cheaper and results are able to be gained faster – the better. “There are several methods to predict oral absorption, but obviously the in vitro methods present the advantage of their high-throughput and, as a consequence, are the preferred ones from the point of view of the three R's policy on animal experimentation (replace, reduce,

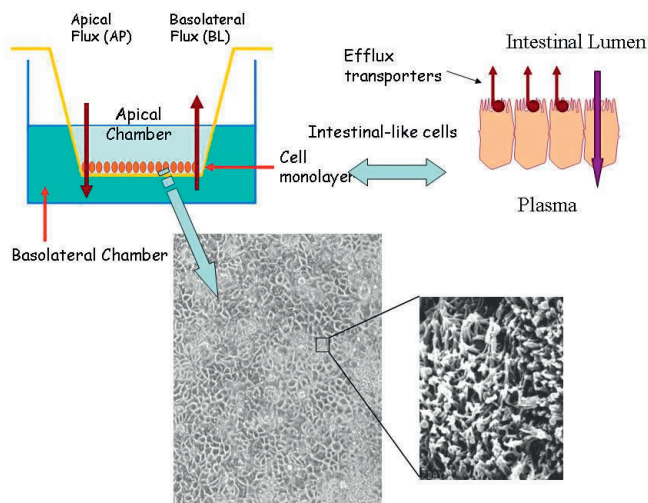
The challenge of in vitro to in vivo

Despite the obvious benefits of conducting experimentation in vitro, no matter how successful the prediction model, inevitably one has to transfer this to in vivo – something which raises numerous challenges. Firstly, in vitro methods are highly susceptible to inter-laboratory variability, with results – such as permeability – often being affected by pre-experimental, experimental and even and post-experimental variables. So, in essence, in vitro methods for determining drug models suffer from a

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refine,” notes Bermejo. “In vivo human or animal methods reproduce better the real situation as they include all the physiological variables, but are not adequate for screening a high number of compounds due either to ethical and economical reasons. So even if at the end the absorbability of a new drug is studied in vivo (in the preclinical phase in animals and in humans, once the candidate goes to the clinical phase) it is essential to have a good screening tool earlier to select the best ones among hundred of molecules. The project intends to improve the predictability of the current in vitro methods that are, so far, good but not at all perfect.”

lack of standardisation, with results between different laboratories being incomparable. “Standardisation has been central to the MEMTRANS project,” says Bermejo. “The first step was to agree about common Standard Operating Procedures (SOPs) for all the procedures and generating templates for data treatment. The SOPs used in intestinal cell culture models contain a big number of different variables to take into account and so the consequences are that each laboratory needs to run an internal validation before using the results for predictions and it is not possible to combine or to compare results from different groups.”



Marival Bermejo©

In vitro models for intestinal permeation: Cells (Caco-2, MDCK, MDCK-Mdr1) are grown on porous filters to form a confluent monolayer. Fluxes and Permeabilities (P_{ef}) of drugs can be measured in both directions (apical to basal and basal to apical). The picture shows a confluent monolayer of caco-2 cells and in detail the microvilli

Secondly, and even more problematic than inter-lab standardisation, is the differing behaviour of transporters. “In vitro systems are quite good for obtaining estimations about absorbability of compounds when the absorption mechanism is passive diffusion, but this is not the case with regard to carrier-mediated processes,” explains Bermejo, as she elaborates on the problems faced by the team. “As the expression level of P-Glycoprotein (Pgp – a transporter under analysis by MEMTRANS) is different from the in vitro to the in vivo scenario, the transference of the results from one system to the other is not easy. Further, drugs that are identified as P-gp substrates in vitro sometimes show no limitation in their oral absorption (and could be potentially rejected as good drug candidates) while others having a light affinity for this transporter present bioavailability problems in vivo.”

Transporting the message

Predicting the behaviour of the drug transporter is therefore key to MEMTRANS, and the project has made great strides in both creating accurate mathematical models in vitro and then mitigating the problems of their transference through control of system parameters to in vivo. By controlling these parameters (paracellular, transcellular permeability and expression

level), MEMTRANS can use them as scaling factors to transfer the results from one cell line to another, or from one laboratory to another.

As well as allowing a greater level of transference from one transporter to another, both in and out of the laboratory, the MEMTRANS project is transferring its research knowledge to other projects, institutions and the scientific community in general through extensive dissemination activities. Already this year the project has presented its results at the 34th International Symposium on High-Performance Liquid Phase Separations and Related Techniques in Dresden, and the PharmSciFair held in Nice. In addition, the project is in the process of organising its next major workshop, which is to be held in Saarbrücken from the 17-18 of September and is where preliminary results shall be presented.

With this wide-reaching remit, and dedicated research and development team, the MEMTRANS project is currently in a strong position to drive research forward in this field, something that Bermejo is keen to see realised. “The next stage for us will be to use the same approach used on the MEMTRANS project but with more cell lines and other cell cultures used for other barriers in the organism – for instance the blood-brain barrier, the skin and the pulmonary route.” ★

At a glance

Full Project Title

Membrane transporters: In vitro models for the study of their role in drug fate (MEMTRANS) - FP6 LSHB-CT-2006-518246 funded by European Commission

Project Objective

The general goal of the project is to optimise and prevalidate existing in vitro models for the study of the impact, mechanism and regulation of drug and xenobiotic efflux transporters. Specifically, we wish to identify their influence on concentration-time profiles of xenobiotics in the circulation (absorption, distribution, metabolism, and excretion – ADME).

Project Partners

University of Valencia (UVEG), Spain
 • Across Barriers GmbH (ACB), Germany
 • Solvo (SOLV), Hungary
 • Aukstieji Algoritmai (AA), Lithuania
 • Hungarian Academy of Sciences (HAC) / Institute of Enzymology, Biological Research Centre, Hungary

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Marival Bermejo is Associate Professor of Biopharmaceutics at the University of Valencia and member of the Board of Directors of the Drug Delivery Foundation (www.ddfint.org). She is Area Head in the 'BIOSIM' Network of Excellence and coordinator of the STREP 'MEMTRANS', devoted to the improvement of in vitro methods and the use of modelling and simulation in drug development.

