MEMTRANS

Membrane transporters: *in vitro* models for the study of their role in drug fate

Contract No	LSHB-CT-2006-518246
Project type	Specific Targeted Research Project
EC contribution	€ 1 900 000
Starting date	1 April 2006
Duration	36 months

Background and objectives:

MEMTRANS seeks to optimise and prevalidate *in vitro* cultured cell models so as to predict oral absorption and pharmacokinetics of efflux systems substrates (e.g. P-gp, MRP2, BCRX), as well as to establish an *in vitro* cut-off for the risk of secretion-associated problems and to study the structure-affinity-transport relationships. These actions would allow the following:

- a reduction in the number of animal experiments to study efflux processes;
- the *in vitro* study of drug-drug (including phytopharmaca) and drug-food interactions related with secretion transporters;
- the gathering of useful information for modelling transporter interactions in other normal and transformed tissues (blood brain barrier, lung mucosa, tumours).

The objectives of the project will be achieved by using a systems biology approach that involves modelling and simulating the complex dynamic interactions between proteins (transporters), metabolites (i.e. substrates) and cells (lipoidal barriers). Computational and mathematical predictive models will be generated from the data, based on the system parameters and on the drug characteristics. The experimental data generated in the project will be analysed from a mechanistic point of view in order to split all the individual steps involved in transport. The mathematical models, in combination with the right physiological, physicochemical and chemical information, could be applied to predict drug transport in a wide range of membranes in the organism (blood brain barrier, liver, kidney, tumours, etc.), but especially in the gastrointestinal tract.

Approach and methodology:

The MEMTRANS partners target the following items.

- Prevalidation of different methods to predict gastrointestinal absorption of secretion transporters substrates.
- Standard operation procedures for maintaining and working with the developed models in order to reduce inter-laboratory variability and to allow exchange of data obtained in different laboratories. This might lead to a reduction of animal and human studies, as shown by the validation of cell culture models for gastrointestinal absorption, which is accepted by the FDA in its guidance about biowaivers based on the Biopharmaceutical classification system. These concepts are also included in the EMEA bioequivalence guidelines.
- Mathematical models to predict from *in vitro* data the *in vivo* intestinal efflux characteristics and its impact in drug ADME.

Expected outcome:

With this improved validated *in vitro* methodology, avoiding extensive animal experimentation

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in preclinical phases to study efflux processes and to prevent failures of drug candidates due to poor absorbability would be possible. At the same time, an appropriate *in vitro* model with a level expression of P-glycoprotein — similar to that found in experimentation animals and with less interlaboratory variability — would allow the combination of data obtained in different laboratories. In this way, it will improve the statistical signification of the established relationships. All these facts will be reflected in the faster development of new drugs for patients.

The oral route for drug administration is the most convenient and preferred by patients, and the prediction of drug absorption from the GI tract is a key issue for a potential new medication in the process of drug design and development. The early prediction of favourable pharmacokinetic properties is the new paradigm in the screening of drug candidates. Current *in vitro* models for the predictions of drug transport across biological membranes include cell cultures that reproduce physiological characteristics of different barriers, such as the intestine, the blood-brain barrier or the kidney and liver. These models have shown good performance for compounds that are transported by passive diffusion. However, for drugs with a carrier-mediated mechanism, the predictions are less accurate, mainly due to the differences in expression levels and affinity for the carriers *in vitro* versus the *in vivo* situation.
It is of paramount importance to know in the early stages of drug discovery, whether a new compound is or is not a substrate and/or an inhibitor of an efflux pump, in order to prevent absorption problems, drug-drug, and drug-food interactions, and to minimise pharmacological resistance.

The *in vitro* cell cultures currently used (as Caco-2 cells) for high-throughput screening of absorbability-distribution of new drugs show good performance with passively absorbed drugs, but in the case of compounds absorbed by active mechanisms the predictability is still poor. The expression level of the transporters differs between clones and laboratories, and it is highly dependent on the culture conditions. Standardised procedures and the use of quality controls to verify the functionality of the transporters are essential for achieving good predictive performance. On the other hand, stable cell lines with good expression of the transporter of interest are scarcely available.
The MEMTRANS team will collaborate with the BIOSIM Network of Excellence in the dissemination tasks. Therefore, the MEMTRANS results will be disseminated within the BIOSIM as a complementary channel.

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