As part of a team researching transmembrane transporter proteins for around two decades, Dr Gerhard Ecker has overseen major advances in the field and has high hopes for a future characterised by open access to data and a more personalised approach to treatment.

Can you begin by describing the many and varied functions of transmembrane transporters (TMTs) in the phospholipid membranes which surround cells?

Transporters are essential for moving compounds that cannot pass through the membrane by passive diffusion into the cell and also across cell membranes such as the blood-brain barrier. Efflux transporters have the opposite function, they transport toxic compounds out of cells, thus protecting them from xenotoxic molecules. These transporters also protect organs such as the brain from compounds that have passed the lipid membrane by pumping them immediately back into the extracellular space.

What is the significance of the ATP binding cassette (ABC) transporter family? Can you outline some of the crucial functions that proteins from this family play in the human body?

ABC transporters play a vital role in protecting cells and whole organs from toxic compounds. They are expressed at many barriers, including the intestine, blood-brain barrier and blood-cerebrospinal fluid (CSF) barrier, and due to their polyspecificity prevent the entry of a broad variety of structurally and functionally diverse compounds into the body or respective organ. Furthermore, as they also transport numerous anticancer agents, their (over)expression in tumour cells results in anticancer drug resistance.

Permeability glycoprotein (Pgp) is paradigmatic amongst the ATP binding cassette (ABC) transporter family; responsible for carrying a broad range of small molecules across the cell membrane, the permeation of structurally and functionally diverse xenotoxins allowed by Pgp can lead to multidrug resistance (MDR). Gaining a better understanding of Pgp’s polyspecificity with regards to its substrates and inhibitors makes them crucial defenders against harmful toxic agents. It also makes them vital players in allowing drugs to successfully reach their targets unhindered. Already, the benefits of a more in-depth understanding of the mechanisms underlying transporter biology have been felt for issues such as drug-resistant epilepsy, selective antidepressant drugs and chemotherapy resistance.
How have ABC transporters been linked with multiple drug resistance and other drug-drug interactions?

In 1978 P-glycoprotein (Pgp), a permeability protein, was described by Dr Victor Ling’s group as being responsible for reducing the accumulation of colchicine in multidrug resistant (MDR) Chinese hamster ovary cells. In 1981 verapamil, the first inhibitor of Pgp, was described as being able to restore drug sensitivity to MDR tumour cells.

Later on the role of Pgp in the blood-brain barrier was discovered and proven by MDR1 knockout mice.

The Pharmacoinformatics Group at the University of Vienna has been researching TMTs and particularly the polyspecificity of ABC transporters since the early 1990s; how has research in this field developed since then?

When we started there was almost no knowledge regarding the (quantitative) structure-activity relationships (QSAR) of Pgp inhibitors. Pharmacophore hypotheses were limited to at least two aromatic rings, high lipophilicity and a positive ionisable nitrogen atom. Since then, numerous SAR and QSAR have been performed, which have allowed the identification of the molecular features important for ligand-transporter interaction. With the publication of the mouse Pgp structure, structure-based modelling became possible and allowed us to derive binding hypotheses of selected paradigm inhibitors.

Why is there a particular focus on TMTs expressed in the liver?

With the increasing knowledge of the role and function of TMTs it became apparent that they are involved in numerous transport processes in the liver. If these transport processes are modulated by drugs, this may result in liver-toxic effects, such as cholestasis. Examples would be the protein ABCB11, which transports bile acids into the bile duct, and ABCB4, which transports the phospholipids necessary to form micelles with the bile acids. If this system gets out of balance by, for example, drugs blocking one of the transporters, it can result in toxic concentrations of bile acids either in the hepatocytes or in the bile duct.

Is there much variation between individuals in terms of TMTs present in their cell membranes? Is there any potential for the development of personalised medication based on this variation?

With the increasing knowledge of the importance of transporters it has also become evident that genetic variations might play a substantial role in individual drug response. However, little information is available at present. This will of course change in the future and pave the way for more individualised drug regimens.

How are open access projects contributing toward the future of medical research? Do you envisage any problems arising from them?

Open access projects will become indispensable. Most of the research in academia is conducted using public money and the results should therefore be provided to the public. Furthermore, only full commitment of the open access and open source community will ensure the sustainability of these projects as they are driven and maintained by the community itself. Major challenges are data quality and the use of proper standards.
INTELLIGENCE

TRANSMEMBRANE TRANSPORTERS IN HEALTH AND DISEASE

OBJECTIVES

The Pharmacoinformatics Research Group seeks to further understanding of transporter proteins and their interactions with drugs, with a particular focus on multidrug resistance in cancer. The development of the eTOX and Open PHACTS databases should encourage greater integration of pharmacoinformatics datasets so that more efficient in silico models can be created to aid the development of new drugs.

KEY COLLABORATORS

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GERHARD ECKER is Professor of Pharmacoinformatics at the University of Vienna. He has a doctorate in natural sciences from the University of Vienna and underwent his postdoctoral training at the group of J Seydel (Germany). In addition, he is the coordinator of the Open PHACTS project, Editor of Molecular Informatics and held the position of President of the European Federation for Medicinal Chemistry from 2009-11.

THE PROPafenONE Promise

Ecker’s work aims to characterise Pgp’s interactions in order to achieve inhibition and restore drug sensitivity in cancer therapy following MDR. In addition, Pgp’s tendency to influence the absorption, distribution, metabolism, excretion and toxicity (ADMET) of many compounds has led to the suggestion that new drug candidates should ideally be screened for Pgp interaction. If, for example, a Pgp inhibitor and substrate are administered together, side effects related to the central nervous system may arise as a result of increased substrate levels crossing the blood-brain barrier. This type of scenario can seriously hamper the drug discovery process, and reliable in silico methods to characterise Pgp interactions would undoubtedly improve the situation. This is no easy task, however, as the protein’s polyspecificity makes in silico characterisation difficult to determine.

Until recently the structure of human Pgp has remained an elusive goal due to the lack of high-resolution crystal ABC-exporter structures necessary for running homology models. Having previously relied on less than adequate bacterial homologues, the recent characterisation of Pgp’s crystal structure in mice has been of great benefit, as an 88 per cent match in sequence identity with human Pgp has allowed Ecker to make great progress in homology modelling, with a combined homology and docking approach.

To counteract the negative effects of scoring-function approximations in homology modelling, Ecker employs docking methods as a means of identifying ligand-protein interactions. Due to their clear structure-activity relationship (SAR) pattern, propafenone derivatives provide Ecker with the tools to judge the reliability of the docking poses. Using this combined strategy, a strong correlation between Pgp inhibition and lipophilicity has been observed but, interestingly, of the five propafenone derivatives used, those with hydroxyl in their piperidine moieties were more active by a factor of 10 than those without, despite an equivalent lipophilicity. As well as identifying promising levels of inhibition in this group, Ecker’s research also indicates an important role played by the amino acid residue Y307 in binding propafenone to Pgp transmembrane helices. In addition to this, Y307 may lead to permanent ATPase activation as suggested by its close vicinity to amino acid residue I306 which, when mutated into cysteine and covalently linked with verapamil, possesses this activating quality.

INTEGRATING INFORMATION

Establishing the role transporters such as Pgp have in mediating toxicity is of fundamental importance to the development of in silico prediction tools, an area in which Ecker’s research provides an invaluable contribution. “We try to establish in silico models for prediction of ligand-transporter interactions and to link these profiles to in vivo toxicities,” he explains. EFPIA and EC’s collaborative eTOX project aims to liberate the vast quantity of high quality toxicology data effectively buried in the archives of pharmaceutical companies. Currently, safety testing in drug development consists of time-consuming, expensive trials on rats, dogs and monkeys. Reliable in silico methods could greatly reduce instances of time, money and resources being wasted following the discovery of negative toxicological side effects in the later stages of drug development by recognising them earlier in the process. In merging public and private EFPIA datasets, the unprecedented eTOX database and the predictive in silico models derived from it should reduce the need for animal studies and the duration of preclinical testing phases; as well as improving the success rate of new substances becoming drugs. A knock-on effect of reductions in the costs of drug development is that more money will be freed up to support a broader range of disease areas.

Key to the project’s success is the ease with which researchers can access public and private data. The Open PHACTS Discovery Platform has been developed by the Open PHACTS Consortium, another project running under the framework of the Innovative Medicines Initiative, to provide the information gathered through integration of public data sources, allowing users to posit complex research questions and analyse the results in real time, a mode that greatly benefits the in silico approach. It is possible, for example, that soon one could request all the compounds associated with liver toxicity and their interaction profile with all the transporters expressed in the liver. As eTOX and Open PHACTS progresses, Ecker is confident that his research will eventually see transporters gain wider recognition as important agents when it comes to the ADMET properties of candidate drugs. Bolstered considerably by data gleaned from the literature and combined ligand- and structure-based studies, it is all the more likely that significant advances in in silico modelling will be made – leading to safer, cheaper drugs.