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Conference Report

Channels and Transporters

Mini-Symposium of the Division of Medicinal Chemistry (DMC) of the Swiss Chemical Society (SCS) at the Department of Chemistry, University of Basel, May 27, 2010

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Abstract: During a half-day symposium, the topic 'Channels and Transporters' was covered with five lectures, including a presentation on 'Introduction and Basics of Channels and Transporters' by Beat Ernst, lectures on structure, function and physiology of channels and transporters ('The Structural Basis for Ion Conduction and Gating in Pentameric Ligand-Gated Ion Channels' by Raimund Dutzler and 'Uptake and Efflux Transporters for Endogenous Substances and for Drugs' by Dietrich Keppler), and a case study lecture on 'Avosentan' by Werner Neidhart. The program was completed by Matthias Hediger who introduced to the audience the National Center of Competence in Research (NCCR)-TransCure in his lecture entitled 'From Transport Physiology to Identification of Therapeutic Targets'.

Keywords: Ion channels · Membrane transport · Transporters

Introduction

Ligand- and voltage-gated ion channels have been known for decades to be involved in ion transport, electrical conductance and maintenance or modulation of membrane potentials, and have thus always had some relevance as targets of marketed and experimental drugs. The in-depth structural and functional understanding of specific channel types, however, became available only in recent years. Transporters represent even more complex molecular machineries: a plethora of membrane transporters for endogenous substances and drugs exist. Based on their transport mechanisms they are classified as uniporters, symporters, antiporters or as unidirectional membrane ATPases (efflux pumps). Many of them have been identified and characterized over the recent years in the membranes of the intestine, liver, kidney, and at the blood–brain barrier. A steadily increasing amount of structural and functional information about transporters is becoming available. Many drugs interact with several of them during their path through the body, but it is still rather the exception than the rule that drugs directly target transporters or that interaction with specific transporters is actively pursued during a drug development program.

During the Mini-Symposium, the five speakers (Fig. 1) provided insights into basic understanding, structure and function of ion-channels and transporters, as well as on the National Transporter Research Programs; a drug development case study completed the event.

Fig. 1. Dietrich Keppler, Matthias Hediger, Raimund Dutzler, Werner Neidhart and Beat Ernst.

Introduction and Basics of Channels and Transporters

Beat Ernst

In the first lecture Beat Ernst, University of Basel, provided an overview about structure and function of membrane transport proteins. Energy coupling is an important aspect of these transport systems. Uniporters simply facilitate passive diffusion as transport follows the direction of a concentration gradient. Primary, secondary and tertiary active transporters, on the other hand, move ions or neutral molecules against a concentration gradient (Fig. 2) with the help of energy provided either by ATP hydrolysis or by the downhill movement of an ion coupled to the uphill movement of the respective solute. Glucose transporters $GLUT_{1\text{-}12}$ that function in numerous organs are examples of uniporters transporting glucose along a concentration gradient. Sodium-dependent glucose co-transporters (SGLT) that are responsible for glucose transport in the small intestine, kidney and liver and peptide transporters which facilitate the uptake of peptides in the small intestine (PEPT1) and

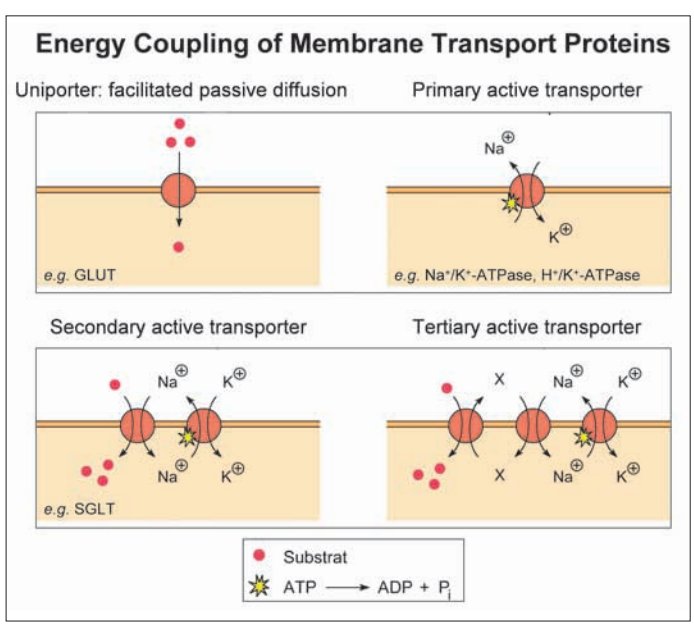

Fig. 2. Schematic representation of uniporters that facilitate passive diffusion and of primary, secondary and tertiary active transporters.

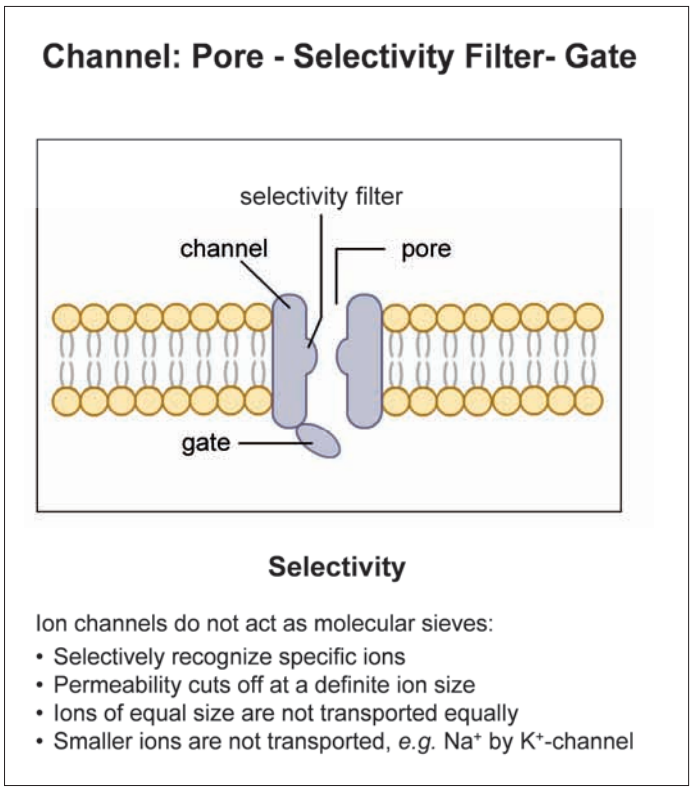

Fig. 3. Channel structure with pore, selectivity filter and gate at the inner end of the pore.

promote re-absorption of peptides in the proximal tubuli of the kidney (PEPT2) represent examples of secondary active transporters.

Ion channels catalyze the selective and rapid diffusion of ions down their electrochemical potential. These proteins consist of a transmembrane pore with a narrow selectivity filter and a gate that opens and closes the ion permeation path (Fig. 3). Channels are classified according to their mode of gating: Voltage-gated Na^+, K^+, Cl^-, Ca^{++} channels are gated in response to changes in the transmembrane electric field, but there exist also examples of non-gated members within the respective families. Ligand-gated nicotinic ACh-receptors, NMDA-receptors, and $GABA_A$-receptors are gated by their respective ligands acetylcholine, glutamate and GABA. The M2-protein (Influenza A) represents an example of a proton-gated channel whereas mechanical stimuli gate certain bacterial channels. In other cases ion channels can be regulated in response to signals received from non ionotropic neurotransmitter receptors of the GPCR family such as muscarinic ACh-receptors, serotonin-receptors and $GABA_B$ receptors.

Ion channels have been widely used as drug targets: potassium channel blockers like Glibenclamide and Tolbutamide are well-established antidiabetics. Sodium channel blockers Procaine, Lidocaine and Sotalole act as local anesthetics. Calcium channel blockers Nifedipine, Verapamil and Diltiazeme are highly effective antihypertensives, and Amantadine and Rimantadine are potent blockers of viral M2-channels. 'Moderne Pharmakokinetik – Transport durch Membranen' by Beat Ernst and Alexander Vögtli[1] is recommended for further reading.

The Structural Basis for Ion Conduction and Gating in Pentameric Ligand-Gated Ion Channels

Raimund Dutzler

Raimund Dutzler from the University of Zurich discussed structural and functional properties obtained from X-ray structures of two prokaryotic pentameric ligand-gated ion channels, which share the overall architecture of their eukaryotic counterparts with conservation in functionally important residues. Although both structures are overall similar they show different conformations of the ion conduction pore. The structure of the ion channel from *Erwinia chrysanthemi* (ELIC) depicts a non-conducting state of the channel with a narrow transmembrane pore that is interrupted by conserved hydrophobic residues.[2] A second structure from a homologous protein from the cyanobacterium *Gloebacter violaceus* (GLIC) has revealed a conducting conformation where the hydrophobic constriction is removed.[3] The two structures thus suggest a novel gating mechanism for the family where pore opening proceeds by structural changes in the transmembrane region (Fig. 4). Fig. 5 shows the α2 helices defining the pore region in ELIC and GLIC. Assuming that the two structures depict the closed and open conformations of the ion conducting pore, opening occurs as consequence of ligand binding to a site in the extracellular domain. This event causes a conformational change in the ligand-binding domain that is transmitted to the transmembrane part and triggers a change in the tilt of helices α2 and α3 of the pore domain. These helices move as a rigid body around an axis that is located about halfway across the membrane and that runs parallel to its plane. As result of this movement the ion permeation path in GLIC has opened to a funnel-like aqueous pore, which represents a conducting conformation. This pore is wide at its hydrophobic extracellular half, it narrows at its hydrophilic intracellular half and it contains negatively charged residues at the cytoplasmic entry that play an important role for ion-protein interactions (for a systematic representation see Fig. 6). This open conformation is reversibly inhibited by channel blockers such as the local anaesthetic Lidocaine. The Lidocaine binding site is located at an extended site in the center of the pore and is likely conserved within the cation selective part of the family. The two prokaryotic ion channels thus provide first structural insight into the ion permeation and gating mechanisms of pentameric ligand gated ion channels, but we should be aware that our understanding of channel function is still at an early stage.

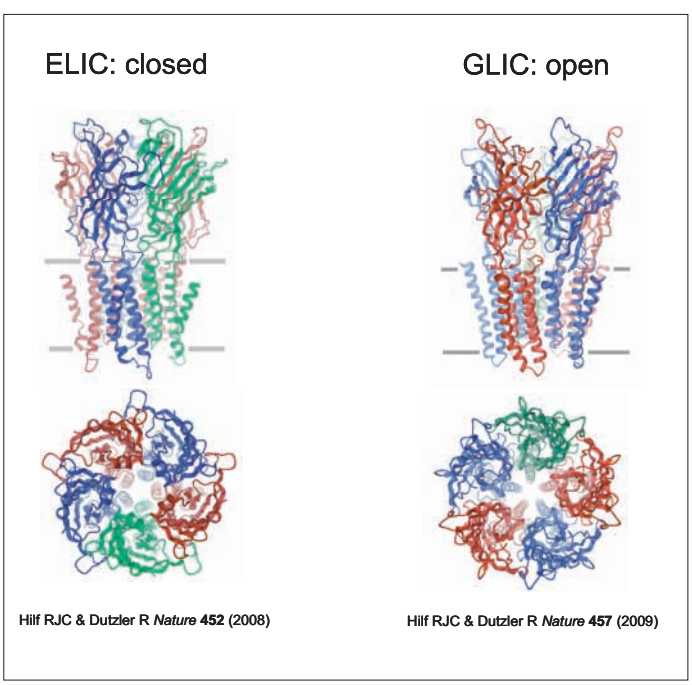

Fig. 4. Ribbon representation of ELIC (closed channel conformation) and GLIC (open channel conformation) viewed from within the membrane with the extracellular solution above and from the extracellular side along the pore axis.[2,3]

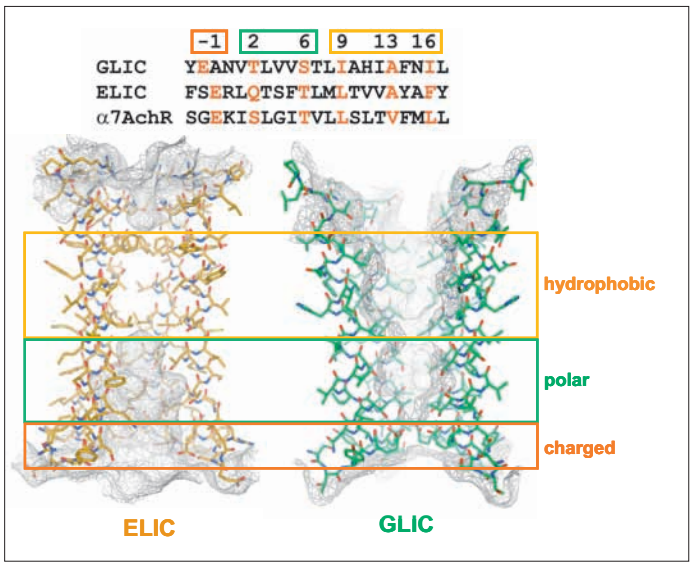

Fig. 5. View of the α2 helices defining the pore regions in ELIC (left) and GLIC (right). The front subunit has been removed for clarity. The molecular surface is shown as white mesh.

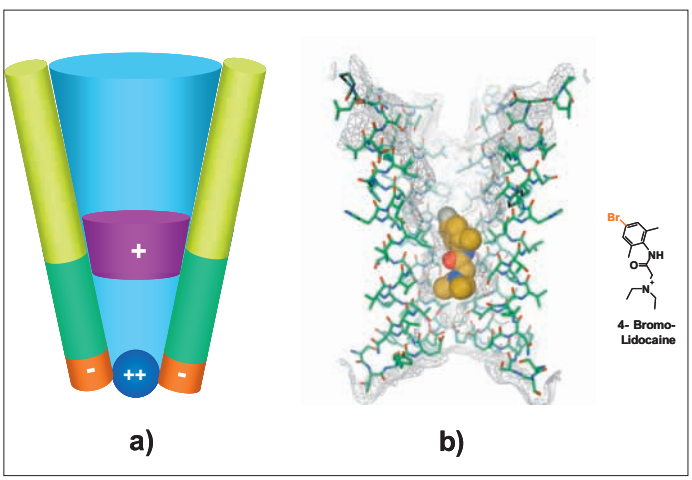

Fig. 6. Schematic representation of the pore in the open GLIC channel with a divalent cation (blue) bound to the narrow intracellular part, the selectivity filter of the channel and a positively charged pore blocker bound in the center of the membrane (a). View of the α2 helices defining the pore region in the open GLIC channel with a 4-bromo-lidocaine molecule bound to an extended site in the center of the membrane (b).

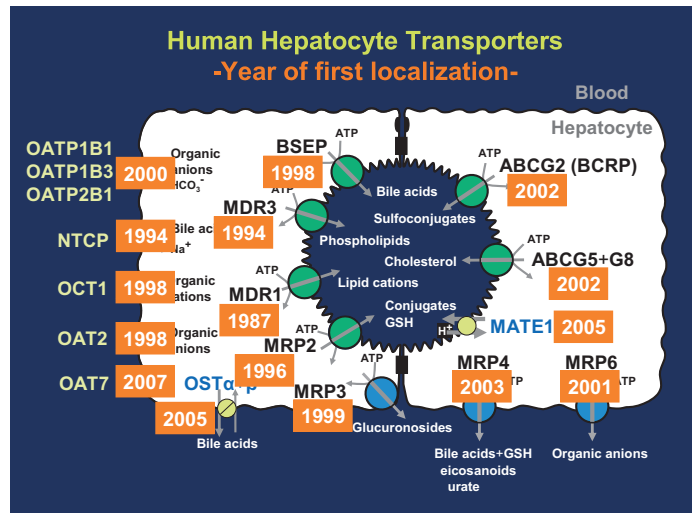

Fig. 7. Human Hepatocyte Transporters: overview and year of first localization.

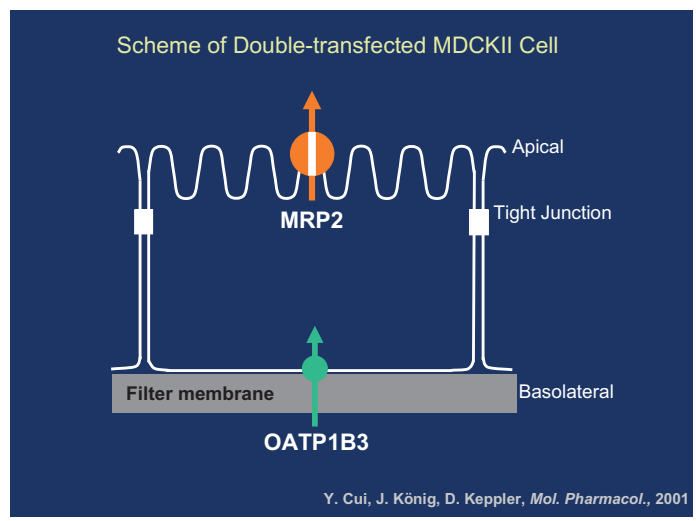

Fig. 8. Double-transfected MDCKII Cells: double-transfected Madin-Darby canine kidney (MDCK) cell line permanently expressing a recombinant uptake transporter for organic anions in the basolateral membrane and an ATP-dependent export pump for anionic conjugates in the apical membrane.

Uptake and Efflux Transporters for Endogenous Substances and for Drugs

Dietrich Keppler

The relevance of membrane transporters in humans was the focus of the lecture by Dietrich Keppler, German Cancer Research Center, Heidelberg. Precise knowledge about localization of specific transporters and their substrate specificity allows understanding and prediction of uptake and elimination routes in the body. Transporters exert their functions at any relevant epithelium all over the body, as in the intestine, in hepatocytes, in cholangiocytes, in the gallbladder, in kidney proximal tubules, at the blood–brain barrier, in the choroid plexus, in the airways, in the placenta, and in fetal capillaries. Hepatocyte transporters have been the focus of intense research activities for at least two decades. Fig. 7 summarizes relevant transporters in human hepatocytes together with their year of first localization.

Human genetic variants as well as knockout animal models have proven to be very helpful in elucidating the role of transporters in drug disposition, *e.g.* the hereditary deficiency of the human bile salt export pump (ABCB11) in familial intrahepatic cholestasis (PFIC2), or Mdr1 p-glycoprotein deficiency in Mdr1a knockout mice and Collie dogs resulting in an about 100-fold increased sensitivity to the centrally neurotoxic pesticide Ivermectin (due to the absence of the Mdr1 p-glycoprotein which prevents in control animals Ivermectin from crossing the blood brain barrier).

Monolayers of double-transfected polarized cells provide useful systems to study the transport of endogenous substances, drugs, and toxins (Fig. 8). In such systems, vectorial transport of substrates of both transporters (uptake and efflux transporters) can be observed at a much higher rate than in non-transfected or single-transfected cells.

Frequently, xenobiotics and drugs are substrates for several different transport proteins: a list of the most relevant transport proteins includes OATP1B1, OATP1B3, OATP2B1, OAT1, OAT3, OCT2, OCT1 for cellular uptake; and MDR1 P-gp (ABCB1), BCRP (ABCG2), BSEP (ABCB11), MRP2 (ABCC2), MRP4 (ABCC4)

for cellular efflux. Detailed knowledge of the interactions with all of them is indispensable for in-depth understanding of uptake and elimination routes in the body. For a recent review see Giacomini *et al.*, 'Membrane transporters in drug development'.[4]

From Transport Physiology to Identification of Therapeutic Targets

Matthias Hediger

Matthias Hediger, University of Bern, gave a brief overview about the National Center of Competence in Research (NCCR)-TransCure, a joint research project that was recently funded by the Swiss National Science Foundation (SNSF). Matthias Hediger has identified and characterized novel transporters of various classes. These include the Na^+glucose cotransporter SGLT1 and the intestinal oligopeptide transporter PEPT1. Subsequently, he has elucidated the physiological, pathological and pharmaceutical aspects of the transporters of interest using a diverse array of approaches. The vision of the NCCR-TransCure is to exploit excellence in membrane transporter research for the treatment of human diseases. Key to success will be a unique research collaboration involving three major disciplines: i) physiology/pathology/medicine, ii) structural biology and ii) organic chemistry/ligand design (Fig. 9). The NCCR-TransCure is composed of 18 scientific laboratories from Basel, Bellinzona, Bern, Lausanne and Zurich.

Through research in the field of membrane biology, the NCCR-TransCure aims to understand the mechanisms of common diseases like diabetes, hypertension, cancer and neurodegenerative disorders, and to develop, based on this knowledge, innovative therapeutic strategies and novel treatment drugs.

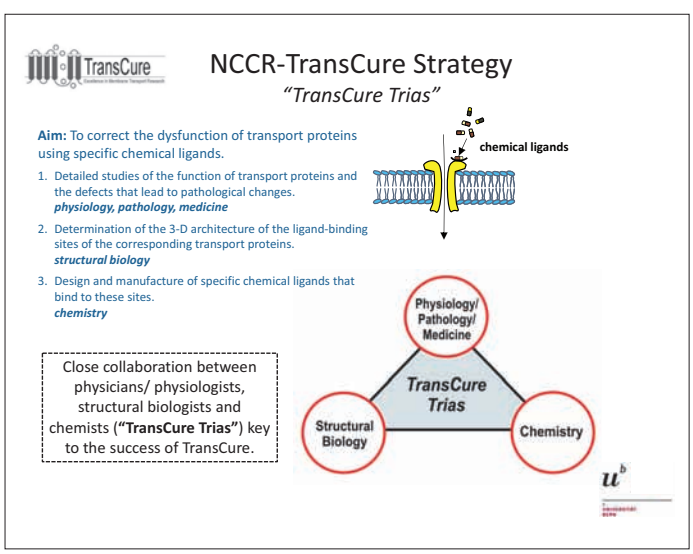

Fig. 9. NCCR-TransCure Strategy: TransCure Trias.

Avosentan

Werner Neidhart

In his case study lecture, Werner Neidhart, F. Hoffmann-La Roche Ltd, Basel, talked about the identification of Avosentan, an endothelin A receptor (ETA-R) selective follow-up compound of RO0485695 (Fig. 10). RO0485695 had to be withdrawn from clinical trials after an eight-day clinical phase I study. Liver enzyme increase had been observed and blood chemistry data indicated an induction of cholestasis through interference with the excretion pathway of bile acids as main underlying cause. Impairment of bile formation, by blockage of the canalicular bile salt export pump

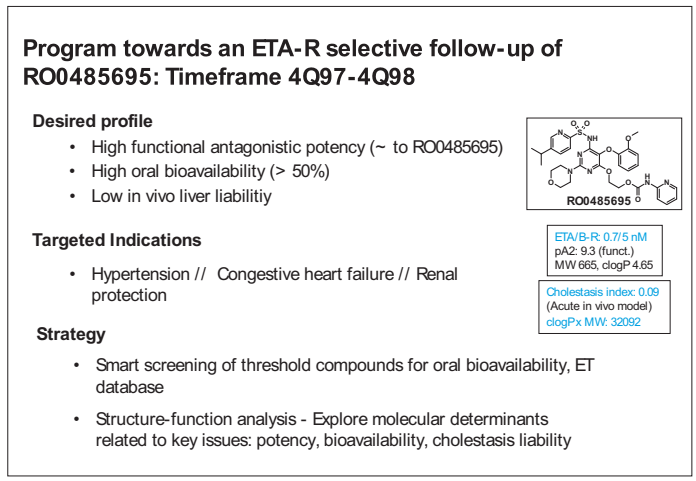

Fig. 10. Concept for the identification of an ETA-R selective follow-up compound of RO0485695.

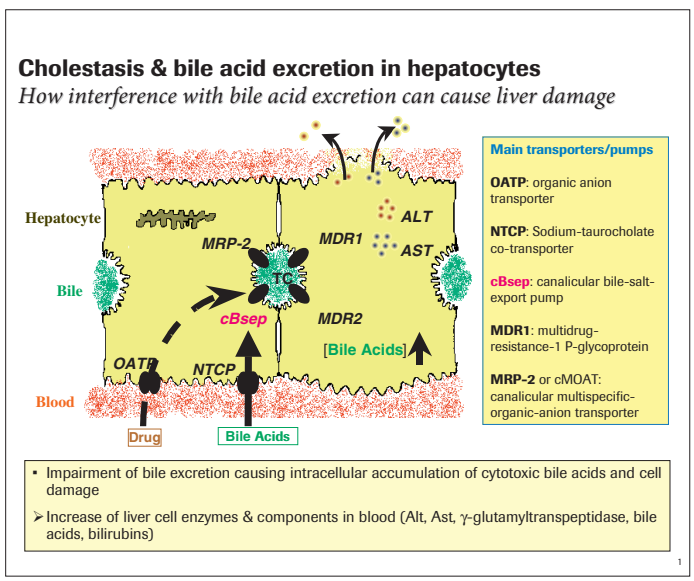

Fig. 11. Cholestasis & bile acid excretion in hepatocytes demonstrating how interference with bile acid excretion can cause liver damage.

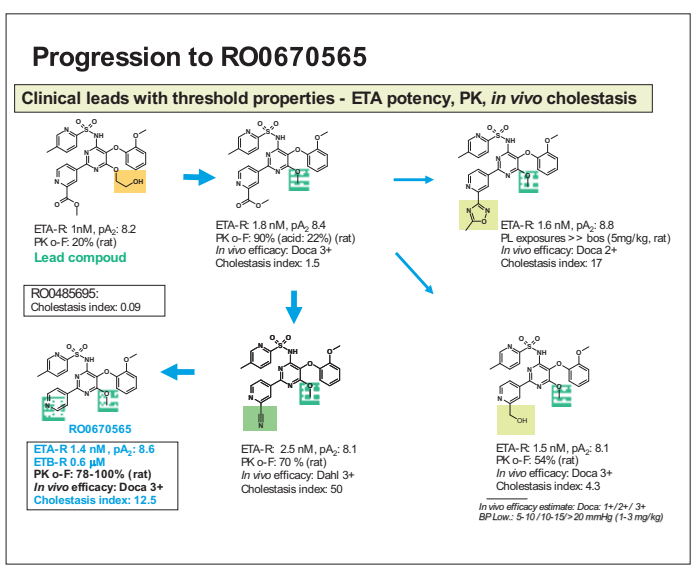

Fig. 12. The compound optimization path leading to RO0670565, Avosentan.

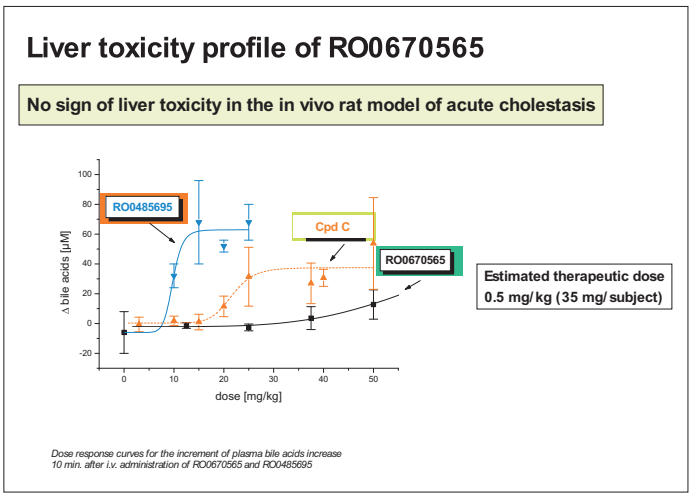

Fig. 13. Liver toxicity profile of RO0670565, Avosentan, showing no sign of liver toxicity in the *in vivo* rat model of acute cholestasis.

(cBsep), was assumed to be the main culprit; in other words, the intracellular accumulation of cytotoxic bile acids and the subsequent cell damage was assumed to be responsible for the observed liver enzyme increase (Fig. 11). Compounds with favorable Cholestasis indices [Cholestasis index = ED 50/(δ10 + δ30) (efficacy vs time curve of plasma bile acid increase)] were favored during the compound optimization process (Fig. 12) which finally resulted in the identification of RO0670565, Avosentan, as the optimized compound (Fig. 12, 13).

All preclinical parameters, efficacy, pharmacokinetics, cholestasis prediction, and overall toxicity profile translated very well into the clinical behavior of the compound, and no liver liability has been observed thus far in humans.

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