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MEETING REPORT

NEW PERSPECTIVES ON TRANSPORTERS IN DRUG DISCOVERY AND DEVELOPMENT: ADME AND BEYOND

HIGHLIGHTS FROM THE SOCIETY FOR MEDICINES RESEARCH SYMPOSIUM, HELD ON JUNE 23, 2011 AT THE MILLENNIUM GLOUCESTER HOTEL & CONFERENCE CENTRE, LONDON, UK

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SUMMARY

The importance of transporters in drug discovery and development is increasingly being recognized and there has been significant progress towards identifying the individual membrane transporters and determining their function and substrate preferences. Accompanying this explosion of new information now available to scientists and regulators involved in the discovery of new therapies, there has been a clear need to provide guidance on the most important drug transporter interactions in both the preclinical research setting and in support of clinical studies. This symposium brought together a panel of international experts from the pharmaceutical industry and academia to present and discuss valuable insights into the key role of transporters in drug discovery and development. Topics included the importance of transporters in drug uptake, human transporters from regulatory perspectives, transporters as targets in modulating disease and drug case histories describing the challenges of drug transport for oral and inhaled administration.

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THE CENTRAL IMPORTANCE OF TRANSPORTERS IN DRUG UPTAKE

In the opening lecture, Dr. Paul Dobson (Department of Chemical and Biological Engineering, University of Sheffield, U.K.) presented the historical evidence that is shifting current scientific thinking around substance permeability across lipid membranes. In his opinion, and that of his fellow scientific collaborators, the early "lipid-dominant view" was supported primarily by the use of artificial membranes and the data generated therein. Substance movement from an aqueous environment to a lipid environment is very much dependent on the formation and cleavage of hydrogen bonds and lipophilicity, which are the same parameters used by Lipinski's "rule of 5" predictor of drug likeness and oral absorption. These correlations imply that no transporter-mediated processes are involved and that the supporting data generated using artificial membranes simply predict the potential of substances to interact with hydrophobic environments.

Drawing on an evidence-based approach published recently (1), Dr. Dobson suggested that, rather than being the exception, carrier (or transporter)-mediated and active uptake of drug substances are far more common than currently assumed. Transporters per se are generally regarded as substrate-specific, yet while there are tens of thousands of substrates there are only hundreds of transporters. In the "drug space", there are currently > 400 drug–transporter interactions cited in the literature, yet > 80% of these substrates obey Lipinski's "rule of 5". The "solute carrier" (SLC) group contains > 300 transporters, organized into 47 families. The solute carrier family 15 peptide transporter family contains the peptide transporter 1 (*PEPT1/SLC15A1*), which is reported to transport all naturally occurring di- and tripeptides, and some 8,400 substrates, including several drugs, such as amoxicillin, ampicillin and valaciclovir. The SLC transporter for organic ions such as bile salts and eicosanoids also interacts with a number of drugs, including atorvastatin, rosuvastatin, methotrexate and rifampicin. Similarly, the solute carrier family 22 transporter associated with organic anions, cations and zwitterions is also linked with a wide range of structurally diverse drugs, including aciclovir, cimetidine, propranolol and quinidine. Thus, Dr. Dobson argued that, based on literature evidence alone, certain transporters display considerable promiscuity with the potential to cover a wide area of chemical space. Direct experimental evidence was provided by a systems biology-based approach (2) using yeast transporter assays. Using a chemical genomics platform developed using fermenters and robotics to study how drugs get into yeast cells (wild-type vs. transporter knockout), of the nine drugs studied in detail to date, only one (amiodarone) showed no interactions with any transporters, one (L-canavanine) was a substrate for only one transporter, and yet the other seven interacted with at least one and up to four transporters.

The implications of a more transporter-dominated as opposed to passive permeability-based landscape are varied. A comparison of transporters by species and tissue would lead to a better understanding and interpretation of differential drug distribution. The impact of disease on transporter expression and function will provide greater insight into the development of physiologically based pharmacokinetic/pharmacodynamic models with improved clinical translation, and, coupled with individual patient genomic data, opens up real prospects for personalized medicine.

In concluding, Dr. Dobson offered the following personal observations:

- It is not a question of whether transporters are involved with drug distribution, but which transporters are involved
- The science of ADME needs to think of this as a systems biology challenge as opposed to a biophysics-based problem
- In order to unravel and understand the problem, the pharmaceutical industry (which is better at compound screening than academia) needs to collaborate more with academia (which is better at informatics than the pharmaceutical industry)

WHAT'S ALL THE FLUX ABOUT? INTERPRETATION AND APPLICATION OF THE INTERNATIONAL TRANSPORTER CONSORTIUM WHITE PAPER AND EMA DRAFT GUIDANCE

Dr. Joseph Polli (Drug Metabolism and Pharmacokinetics, GlaxoSmithKline, Research Triangle Park, North Carolina, U.S.) is a member of the International Transporter Consortium (ITC) and coauthor of the drug transporter white paper "Membrane Transporters in Drug Development" (3).

The ITC is comprised of experts in the field of drug transporters drawn from both academia and industry in the U.S., Europe and Japan. Their role was to: 1) provide an overview of the key transporters known to be involved in drug absorption and disposition; 2) provide examples of the approaches currently used to understand, elucidate and ultimately predict potential drug–drug interactions; and 3) provide criteria that could be used to enable the clinical investigation of such interactions. Highlighting the challenges that the group faced, Dr. Polli discussed the rapid and ever-increasing scientific knowledge base with data emerging from a number of in vitro, preclinical and clinical sources and publications. In contrast to the established science around the cytochrome P450 (CYP) family of enzymes, the number of reagents, tools and validated clinical probes is limited. Simply measuring systemic drug exposure in blood or plasma may be of limited value, since it is the tissue concentration at the site of action, be it safety or efficacy, that will be the key determinant in understanding the risk to the patient, an observation acknowledged by the current European Medicines Agency (EMA) draft guidance document published on April 22, 2010 (4). Consequently and not unsurprisingly, there are a number of conflicting messages to physicians, prescribers, patients and regulatory bodies that reflect the complexity of the problem.

In order to promote flexible and open discussion around the evolving and developing nature of the science, Dr. Polli highlighted a decision tree-based approach to clinical interaction studies. Such an approach must be clinically focused and consider the therapeutic area, clinical population and likely comedications. Timings should be driven by the clinical plan and the level of risk associated with the disposition of the drug, but with the objective of characterizing the key transporters in question by phase III. This strategy will inevitably be a cautious one, with only limited data on potentially unvalidated clinical probes available at the outset. However, with constant monitoring, updating and revision, the science can evolve and develop, thus avoiding the pitfalls of creating a process that would be regarded as too prescriptive and rigid when it came to the interpretation of the findings. Detailed examples of the decision tree-based approach were presented around potential P-glycoprotein (P-gp)/breast cancer resistance protein (*BCRP*, *ABCG2*) interactions using lapatinib and digoxin and a wide variety of potential organic cation transporter (OCT)/organic anion transporter (OAT) substrate and inhibitor interactions. In each case, Dr. Polli highlighted the complex interplay between drug transporters and drug-metabolizing enzymes, underpinning the need to integrate both preclinical in vitro data and clinical data in order to understand and contextualize any effect on drug safety or efficacy.

HUMAN TRANSPORTERS AND RELEVANCE OF PRECLINICAL MODELS FROM A REGULATORY PERSPECTIVE

Dr. Glynis Nicholls (AstraZeneca, Alderley Park, U.K.) described the importance of key transporters in drug disposition, the available preclinical models and their relevance to the clinical setting.

Key to successful drug therapy is achieving the right dose/schedule for patients, and thus there is a need to understand changes in drug

concentration versus response with time that may be due to transporter interactions. This may influence drug absorption, clearance and organ exclusion, as well as lead to potential toxicological effects, for example, drug–drug interactions (leading to a lack of efficacy or toxicity), inhibition of physiological functions, drug resistance (induction or inhibition) and accumulation.

There are now many examples of the clinical relevance of transporter-mediated drug–drug interactions and drug labels increasingly include information on transport-mediated interactions. Noteworthy examples of transporter inhibition resulting in toxicological effects are the inhibition of solute carrier organic anion transporter family member 1B1 (OATP-C; *SLCO1B1, OATP1B1*) by rifampin, resulting in an increase in atorvastatin exposure, and *BCRP* inhibition by GF-120918, leading to an increase in topotecan exposure.

Regulatory authorities and others have published a number of articles on recommended transporter studies. There is now an expectation that pharmaceutical companies will evalute the impact of the key "clinically relevant" transporters on both drug safety and efficacy prior to regulatory submission. As a result, transporter information is now a core part of the drug development process and better "tools" are being developed to meet this need. Dr. Nicholls highlighted the transporter "toolbox" that is now generally being used, including in vitro preclinical assays (ATPase, calcein AM, membrane vesicles, cell lines, primary cells), in situ tissue perfusion, knockout animals and in vivo/pharmacokinetic studies, as well as clinical drug–drug interaction studies. The use of decision trees to help in the decision-making process was highlighted.

Two case examples of using preclinical data to predict/explain clinical drug–drug interactions were presented, one of which was rosuvastatin (Crestor®) and its interaction with gemfibrozil. In vitro preclinical data indicated that the drug is a substrate for organic anion-transporting polypeptide (OATP) uptake transporters (mainly *OATP1B1*), with its uptake being inhibited by gemfibrozil and ciclosporin in oocytes (IC_{50} = 4.0 and 2.2 µM, respectively) and by gemfibrozil in rat hepatocytes (IC_{50} = 80.2 µM). Rosuvastatin is also a substrate for ileal sodium/bile acid cotransporter (ISBT), organic anion transporter 3 (OAT3) and organic anion transporter 4 (OAT4), but not organic anion transporter 1 (OAT1). In vivo studies suggested that biliary secretion was reduced in both C-C motif chemokine 9 (macrophage inflammatory protein-related protein 2, MRP-2)-deficient rats and breast cancer resistance protein 1 homolog (*Bcrp1*) knockout mice compared to controls, indicating that MRP-2 and *Bcrp1* may play a role in the elimination of rosuvastatin (5). In the clinic, the systemic exposure of rosuvastatin (80 mg) increased ~2-fold when given in combination with gemfibrozil, without a significant change in the half-life of rosuvastatin. In addition, the systemic exposure of the *N*-desmethyl metabolite of rosuvastatin (formed by CYP2C9) was reduced by ~50%. Although gemfibrozil inhibits CYP2C8, 2C9 and glucuronide formation, the effect on metabolism did not explain the increase in rosuvastatin plasma concentrations, since there was no clinically relevant change in exposure when coadministered with fluconazole. These data therefore suggest that the inhibition of *OATP1B1*-mediated rosuvastatin hepatic uptake is a likely major contributor to the mechanism of drug–drug interactions in the clinic (6).

There was also a brief discussion on the impact of pharmacogenetics, suggesting that the functionally important *BCRP* 41C>A mutant allele, which has a high prevalence in the Chinese/Japanese population, may be a possible explanation for the ethnic differences observed in rosuvastatin exposure (7).

In conclusion, the use of preclinical transporter tools helps to provide a mechanistic approach to explaining variability in pharmacokinetics, pharmacodynamics and safety in the clinic. However, we need to better understand the limitations of our current models and approaches, with a view to improving their predictive ability. If transporter interactions are studied early, it may be possible to identify patients at risk of developing adverse reactions and manage such interactions by exclusion or dose adjustment, as well as identifying at-risk drug combinations.

MITOCHONDRIAL DYSFUNCTION IN MOTOR NEURON DISEASE – A POTENTIAL PROBLEM?

The presentation from Dr. Scott Allen (Sheffield Institute for Translational Neuroscience [SITraN], Sheffield University) was on amyotrophic lateral sclerosis (ALS), the likely causes and current therapy.

ALS, or motor neuron disease (MND), is a progressive and severely disabling fatal neurological disease characterized by initial muscle weakness followed by muscle atrophy, spasticity and eventual paralysis and death from respiratory failure, typically within 3-5 years after onset of symptoms. The cause of the spasticity, paralysis and death is progressive degeneration and elimination of upper motor neurons (MNs) in the cerebral cortex, and lower MNs in the brainstem and spinal cord (8).

The causes of sporadic ALS are unknown, although there is evidence that diet and metabolism play a key role, since ALS patients are hypermetabolic and hyperlipidemic, with high LDL/HDL ratios, low fat storage, typically have a lean body mass and are frequently thermogenic. Athleticism and lean body mass may be important and there is a great deal of evidence for an elevated incidence of ALS in Italian football players for example (9, 10). However, factors such as environmental toxins and pesticides have been implicated in the disease and cannot be ruled out. Patients with ALS and type 2 diabetes have delayed disease onset and feeding ALS mice a high-fat diet delays disease onset.

In the last 10 years, approximately 20 drugs have been evaluated in clinical trials for ALS, but only riluzole (Rilutek®) has emerged as a viable product, although it has limited efficacy. The drug preferentially blocks tetrodotoxin (TTX)-sensitive sodium channels, which are associated with damaged neurons, reducing the influx of calcium ions and indirectly preventing the stimulation of glutamate receptors. However, the action of riluzole on glutamate receptors has been controversial, as no binding of the molecule has been shown for any known receptor. Novel candidate drugs (NCDs) are being developed based on the pathogenesis of ALS and findings from genetic, pathological and biochemical post mortem evaluations. The NCDs have been chosen for their potential to reduce excitotoxicity, apoptosis, MN degeneration, bioenergetics or oxidative stress. Oxidative stress has been implicated in the pathogenesis of ALS, and therapies have been developed to evaluate antioxidants as a

means of controlling this process. However, results from clinical trials based on this approach have so far yielded unimpressive results.

Despite the identification of specific genes and proteins that are invariably associated with neurodegenerative disorders, there are currently no validated molecular targets and no effective therapies to slow or cure these devastating diseases. To avoid the pitfalls of target-based drug discovery, phenotypic screening using primary neurons has been used to select compounds in a relevant pathophysiological context, with a view to identifying and validating new druggable targets. Using primary neurons that express relevant target(s) under conditions that lead to their dysfunction or death, such as excitotoxicity or trophic factor withdrawal, a simple assay endpoint has been designed based on cell survival. Many cellular processes are affected in MND and oxidative stress, and mitochondrial dysfunction is a hallmark of neurological disorders and is thought to sit on the early phase of disease progression. Oxidative stress can appear in different forms, such as DNA-damaged patients with MND, lipid peroxidation (staining in a mouse spinal cord model) and reactive oxygen species in the cytosol of a neuronal cell model. All three can be measured in models of MND and are also detected in ALS patients with the disease.

Familial forms of ALS have autosomal dominant or autosomal recessive inheritance patterns and make up approximately 10% or less of all cases of ALS. Mutations linked to ALS occur in the genes encoding superoxide dismutase 1 (*SOD1*), alsin (*ALS2*), senataxin (*SETX*, *ALS4*), vesicle-associated membrane protein-associated protein B/C (*VAPB*), dynactin (*DCTN*), TAR DNA-binding protein 43 (*TARDBP*) and RNA-binding protein FUS (*FUS*, *TLS*). The most recent genes implicated in familial MND are *TARDBP* and *FUS*, which are RNA splicing regulators, Current knowledge is based on superoxide dismutase 1 (*SOD1*) mutations in ALS leading to a toxic gain of function. Superoxide dismutase 1 normally functions as a homodimer with Cu and Zn required for action, where it converts potentially harmful superoxides to H_2O_2.

In mutated *SOD1*, a whole host of toxic gain of functions occur, including aggregation in the mitochondria. Mutant *SOD1* is preferentially imported into or deposited onto mitochondria in affected tissues, which may interfere with the elements of the electron transport chain, thereby disrupting ATP-generating oxidative phosphorylation. Mutant *SOD1* may also disrupt mechanisms by which mitochondria buffer cytosolic calcium levels. Mutant *SOD1* aggregates can interfere with components of mitochondrial-dependent apoptotic machinery, such as apoptosis regulator Bcl-2, thereby triggering premature activation of an apoptotic cascade, including cytochrome c release into the cytosol. In addition, mutant *SOD1* may indirectly affect similar pathways linked to mitochondria by physically blocking the protein import machines, TOM and TIM. Oxidative damage incurred by various mitochondrial proteins may also contribute to overall mitochondrial dysfunction. Collectively, these mechanisms (or a combination thereof) are predicted to disturb cellular homeostasis (within glial and/or motor neurons), ultimately triggering motor neuron death.

In order to better understand and exploit the circumstantial evidence linking mitochondrial dysfunction with neuronal dysfunction culminating in neurodegenerative disease, a project called Mito-Target has been formed, which involves 17 partners across Europe. A major aim is to test whether drugs targeted to specific targets or mechanisms involved in mitochondrial dysfunction can affect the diseases in question. One such drug is **olesoxime** (TRO-19622; Fig. 1), which has been granted orphan drug status for ALS in the U.S. and the E.U. and for spinal muscular atrophy (SMA) in the E.U. (11).

The drug was initially identified based on its survival-promoting activity in purified cultured rat motor neurons deprived of neurotrophic factors. Treatment of $SOD1^{G93A}$ mice with olesoxime delayed the onset of many disease signs, including the decline in body weight, and significantly increased life span of mice. Because the delay in onset was similar in magnitude to the prolongation of life span, it was concluded that the major beneficial effect of the drug was on disease onset rather than progression. Preclinical studies have shown that the drug is likely to act via a number of mechanisms to promote the function and survival of neurons under disease-relevant stress conditions. This includes having a protective function in the $SOD1^{G93A}$ mouse model of ALS, interaction with the mitochondrial permeability transition pore (mPTP), prevention of mitochondrial depolarization, reduction of caspase activation/apoptosis and inhibition of cytochrome c release. Phase I/Ib studies have been completed in ALS patients and the drug has been shown to be well tolerated, with an excellent safety profile. Further work includes elucidating the mechanism of action/efficacy.

THE ROLE OF HEPATOBILIARY TRANSPORTERS IN DRUG-INDUCED LIVER INJURY

The presentation from Dr. Simone Stahl (AstraZeneca, Alderley Park, U.K.) described the role of hepatobiliary transporters in drug-induced liver injury. The formation of bile and the role of bile flow in the excretion of endogenous and exogenous molecules are an essential function of the liver. Hepatobiliary transport systems, such as the bile salt export pump (*ABCB11*) and multidrug resistance-associated protein 2 (*ABCC2*, *MRPC*), play an important part in the maintenance of this role (12, 13). Perturbation of these physiological processes can result in hepatobiliary disease.

Drug-induced liver injury is a major concern to regulatory agencies and pharmaceutical companies. Drug-induced liver injury caused by an individual drug is a consequence of multiple drug-related and patient-related hazard and risk factors (14, 15). Transporter-mediated safety issues were then presented and included cellular accumulation of drug/metabolites; impaired efflux of endogenous bioactive molecules (e.g., bile acids), drugs and metabolites; alteration of nutrient transport (uptake or elimination); altered drug

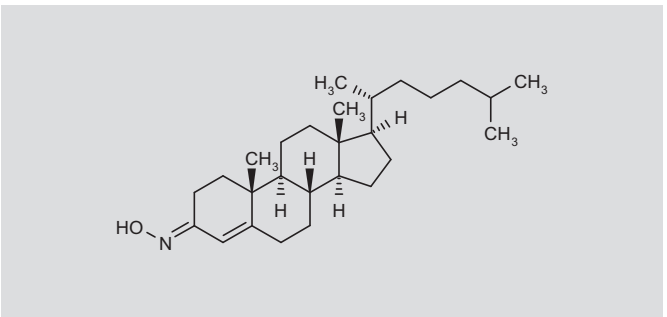

Figure 1. Structure of olesoxime.

disposition, i.e., concomitant medications (drug–drug interactions), saturation of transporters leading to nonlinear pharmacokinetics, inhibition or altered expression (e.g., induction, change in pharmacokinetics upon multiple dosing) and variable pharmacokinetics due to genetic polymorphism.

Defective hepatic transporter activity has been shown to result in cholestatic drug-induced liver injury in humans. In particular, functional impairment of bile flow arising from genetic deficiencies in the bile salt export pump, such as progressive familial intrahepatic cholestasis and benign recurrent intrahepatic cholestasis, or multidrug resistance-associated protein 2 leads to different forms of cholestasis or hyperbilirubinemia (12). A number of drugs reported to cause cholestatic liver injury in humans and rodents inhibit the bile salt export pump in vitro, such as troglitazone, bosentan, ketoconazole, nefazodone, chlorpromazine, erythromycin and ciclosporin. This has led to the suggestion that inhibition of the bile salt export pump by such drugs could play a role in the development of drug-induced liver injury.

At AstraZeneca, in vitro models such as sandwich cultures of hepatocytes or recombinant vesicles, prepared from transfected Sf21 insect cells, are used to identify candidate drugs which have a high propensity to cause drug-induced liver injury in humans. In vivo rat models are also used to study functional effects alongside conventional toxicology parameters. Indicators of in vivo functional perturbance of transporter function are, e.g., changes in plasma bile acid levels and hepatic transporter expression, as well as excretory function measured via in vivo imaging. In vitro assays were evaluated by profiling marketed drugs (19-31 per group) and their predictive value to aid in drug-induced liver injury hazard identification (cholestatic, hepatocellular and control) was presented. A significant number of compounds causing cholestatic drug-induced liver injury in humans were identified as inhibitors of transporter activity in vitro. A good correlation was also observed between rat hepatocyte and vesicle models using [^{3}H]-taurocholate as the substrate.

In vitro models allow the study of compound interactions with transporter proteins and can be used early in discovery to influence chemical design and aid in the selection of compounds with reduced potential to cause drug-induced liver injury. However, the in vitro inhibitory potency did not predict the in vivo drug-induced liver injury frequency or severity. The challenge for the future is to improve risk assessment strategies and develop models to measure functional in vivo effects in preclinical species, using techniques which are translatable to humans. In vivo rat data were presented using glibenclamide and ciclosporin, which are potent bile salt export pump and multidrug resistance-associated protein 2 inhibitors in vitro. Administration of these compounds resulted in elevations of plasma bile acids, which is an indication of functional impairment of bile flow through inhibition of the bile salt export pump. Bile acids provide a possible translational biomarker which can also be measured in humans.

OATP TRANSPORTERS IN HEPATIC CLEARANCE: A CASE HISTORY

Dr. Hannah Jones (Department of Pharmacokinetics, Dynamics and Metabolism, Pfizer Global Research and Development, Sandwich, U.K.) described the development of a model for the prediction of human pharmacokinetics for OATP substrates.

Success in the effort to reduce CYP450-mediated clearance during the early phases of drug discovery has resulted in a shift towards transporter-mediated pharmacokinetics becoming more prevalent with drug candidates. This has driven the development of a number of in vitro assays with varying complexity that allow assessment of these uptake processes (16-19). The prediction of human pharmacokinetics and plasma concentration–time profiles is reasonably well established using physiologically based pharmacokinetic techniques for compounds with passive mediated pharmacokinetics. However, the prediction of transporter-mediated human pharmacokinetics is less well established, with only a few reports of success in the literature.

At Pfizer, using a sandwich culture human hepatocyte system together with a passive mediated pharmacokinetics model, they assessed the predictability of human pharmacokinetics for seven known OATP substrates where clinical intravenous (i.v.) pharmacokinetic data were available (16-19), and subsequently, a passive mediated pharmacokinetics prediction strategy was developed. This model was then assessed further by applying this prediction strategy to another four OATP substrates.

Passive mediated pharmacokinetics models have been developed that adequately describe the pharmacokinetics of the initial seven compounds tested. However, for each of these compounds the active transport clearance into the liver was underpredicted and an empirical scaling factor was required to more accurately capture the plasma concentration–time profile (Fig. 2). The application of this empirical scaling factor to a further four test compounds resulted in accurate predictions of human pharmacokinetics, especially when compared to other more empirical approaches. This work represents a step forward in the prediction of human pharmacokinetics for OATP substrates. A particular advantage is the ability to capture the multiphasic plasma concentration–time profiles for such compounds using in vitro data. Additional work is ongoing to understand further the requirements for empirical scaling factors.

DRUG TRANSPORTERS IN THE LUNG

Dr. Cynthia Bosquillon (School of Pharmacy, University of Nottingham, U.K.) described the role of drug transporters in the lung (20). Many transporters present in the intestine, liver, kidney or brain are also found in the pulmonary tissue, although their pattern of expression appears to be specific to the lung. In addition, common inhaled drugs, such as glucocorticoids and cationic bronchodilators, have been reported to interact with these transporters (21). Active transport systems in the lung have received very little attention and their impact on the disposition of inhaled drugs remains poorly understood due to a combination of factors, including: 1) the relatively small number of new drug entities developed for the inhalation route; 2) the complexity of lung physiology, which can lead to difficulty in interpreting pharmacokinetic data after pulmonary drug administration; 3) the lack of validated and largely accepted simplified models to study drug transporters in the lung; and 4) the therapeutic administration of inhaled drugs to inflamed or infected pulmonary tissue, which may have altered expression levels of transporters when compared to healthy tissue.

The presentation started with an overview of the current published knowledge on the expression and localization of transporters in the lungs (20, 21), with the recommendation that a select group of transporters would be important for pulmonary drug delivery (multidrug

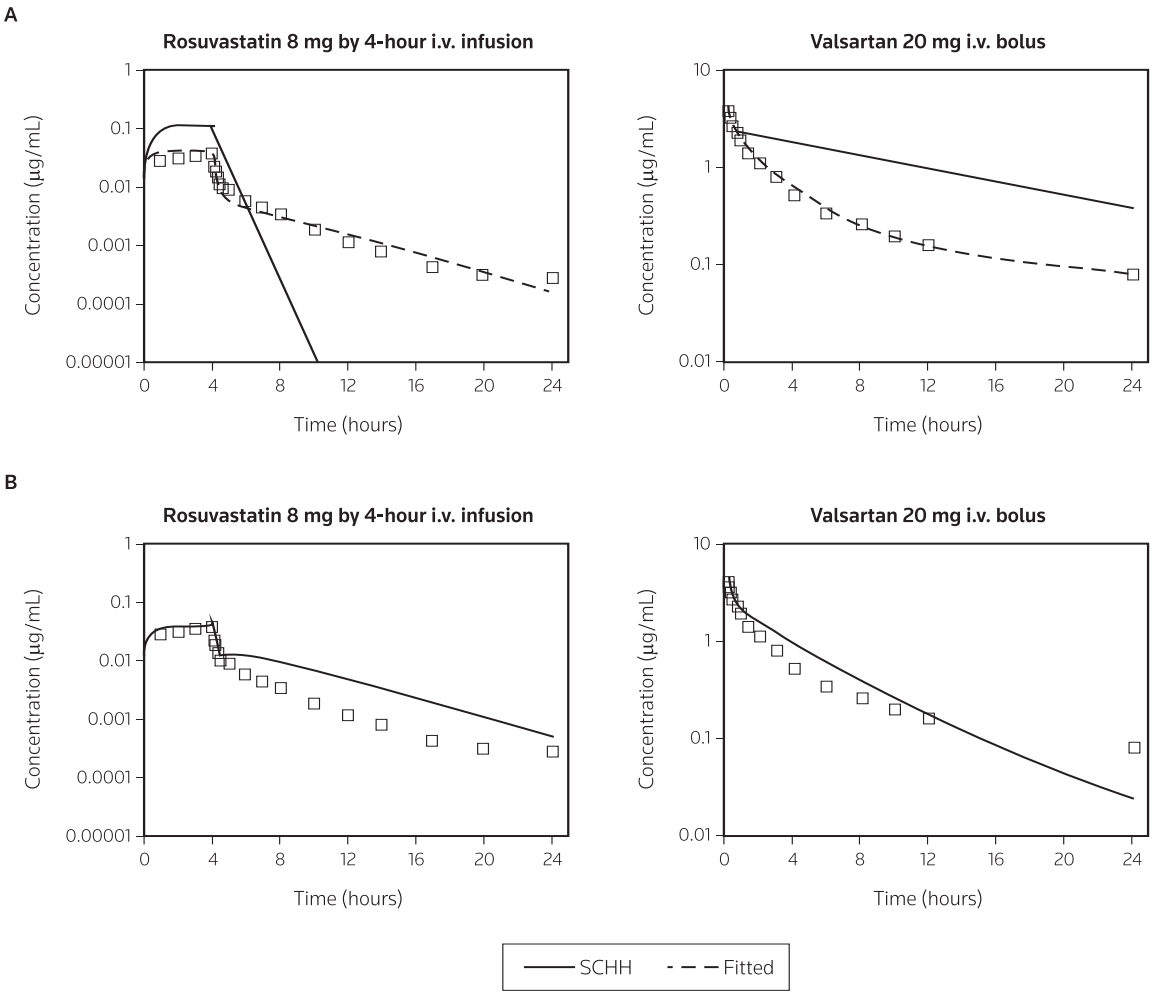

Figure 2. Two representative examples of sandwich culture human hepatocyte (SCHH) predictions of human i.v. pharmacokinetic profiles using a passive mediated pharmacokinetics modeling approach. (**A**) Without empirical scaling factors; (**B**) with empirical scaling factors. ☐ Observed data.

resistance-associated protein 1 [*MRP1*], multidrug resistance-associated proteins 3, 4, 5, 6 and 7 [*MRP3, MRP4, MRP5, MRP6* and *MRP7*], breast cancer resistance protein [*BCRP*], organic cation/carnitine transporter 1 [*OCTN1*], organic cation/carnitine transporter 2 [*OCTN2*], peptide transporter 1 [*PEPT1*], peptide transporter 2 [*PEPT2*], OATPs). The suitability of human lung adenocarcinoma Calu-3 and normal human bronchial epithelial (NHBE) cells as cell culture models of the respiratory epithelial absorption barrier were evaluated by comparison of gene and protein expression transporter levels when compared to human lung tissue. There proved to be a similar correlation for these systems, but with the exception that the Calu-3 cell line expressed the multidrug resistance protein 1 (*ABCB1*, *MDR1*) transporter absent in both NHBE cells and lung tissue, and neither Calu-3 nor NHBE expressed multidrug resistance-associated protein 8 (*ABCC11*, *MRP8*) and 9 (*ABCC12*, *MRP9*), which are present in lung tissue.

Recent findings on the expression, function and regulation of the P-gp and the OCT family in layers of Calu-3 and NHBE cells showed some interesting results leading to the following conclusions:

- mRNA transporter levels do not reflect protein levels for P-gp
- Functionality studies must be interpreted with caution as no transporter substrate/inhibitor is truly specific, and digoxin is not a suitable probe for assessing P-gp activity in bronchial epithelial cell culture models
- Some unidentified transporters are responsible for active drug transport across bronchial epithelial cells in vitro (possibly multidrug resistance-associated protein or OATP).

Proposed areas of future high-value research will be to determine if transporter expression is altered in diseased lung tissue and whether

transporters present in the lungs are involved in pulmonary drug toxicity. Ultimately, if lung transporters are proven to play a key role in the pathophysiology of respiratory disease, will it be feasible to identify new chemical/molecular entities that selectively modulate transporter function for the treatment of respiratory diseases?

DISCLOSURES

Dr. Jack Allen is a DMPK project leader at AstraZeneca R & D, U.K. Dr. Paul Fish is the exploratory chemistry leader for the Regenerative Medicine unit at Pfizer, U.K. Dr. Phil Jeffrey is the Head of Translational Discovery in the Epinova DPU, GlaxoSmithKline, U.K. The SMR Committee organizes conferences on behalf of the Society for Medicines Research four times a year. These one-day conferences are multidisciplinary in nature and focus on various aspects of medicines research. Details of forthcoming meetings can be found at http://www.smr.org.uk or by e-mail to secretariat@smr.org.uk.

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