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**IN VITRO PREDICTION OF GASTROINTESTINAL ABSORPTION AND
BIOAVAILABILITY**

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on behalf of other members of the Working Group 1 of the Action COST B15 and for the invited COST B15 Experts*

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ABSTRACT

The most convenient route of drug administration is peroral. To reach their target, drug molecules have to be absorbed from the gastrointestinal tract and enter to the systemic circulation in sufficient quantities. For this reason, understanding and anticipating the mechanisms and factors affecting gastrointestinal absorption and metabolism are of the utmost importance in developing new drugs. In contrary to drugs, which are intended for the treatment, chemical residues in food and other matrices are entering the body unintended. Hence, a low systemic availability would be an advantageous property. For many reasons, but particularly because of financial and ethical (reduced used of animals) considerations, *in vitro* and *ex vivo* approaches have been pursued in this area over the last few years. The use of *in vitro* methods, however, inherently creates questions about the validity of extrapolation to the *in vivo* situation. To review the current status of the field and to identify major gaps in our knowledge, an expert meeting was organised by the COST Action B15 in Berlin, May 5-6, 2000. This paper summarises some highlights of that meeting.

Currently, there are a number of *in silico*, *in vitro*, cultured cell-based and *ex vivo* approaches available to predict the cell permeation, absorption and gastrointestinal metabolism of molecules under development. Some strengths and weaknesses of these approaches are presented, together with a discussion of genetic, environmental, physiological and pathological factors responsible for interspecies and interindividual variability in these processes. Recent advances in our understanding of active processes such as gut epithelial transporters, involved in absorption, and drug-metabolising enzymes, responsible for intestinal presystemic metabolism, are highlighted. Some major research needs are identified, including the need for high-quality, information-rich databases, against which testing methods being developed can be prevalidated and validated. Preclinical drug development is changing rapidly and the role of *in vitro* and *ex vivo* approaches in this process is becoming increasingly more important. Methods available now are very useful in the drug discovery and development process, including lead selection and optimisation and in the design of very early clinical studies, but whether any of them will eventually obviate the need for clinical trials is still very debatable and will depend on their full validation. It is clear, however, that the results from such *in vitro* tests are important in shaping the drug discovery and the early preclinical drug development process. . For other, environmental,

industrial or household, chemicals exposing humans, in particular new chemicals, results from *in vitro* information might be the only source of information concerning systemic availability .

Keywords: absorption, systemic availability, bioavailability, *in vitro* methods, drug discovery, drug development, pharmacokinetic modelling, *in silico*

(Footnote: in this paper the term bioavailability is used to describe both bioavailability which means the systemic availability from a specific dosage form, and systemic availability which means the availability of a drug substance or a xenobiotic in the systemic circulation.)

***In vitro* prediction of gastrointestinal absorption and metabolism**

1. Introduction

The primary objective of pharmacokinetic studies in drug development is to predict plasma concentration-time curve for a NCE. This is of primary importance, because it is assumed that in most instances therapeutic response is proportional to the amount delivered to the site of action (PK/PD relationship), a surrogate measure of which might be the area under the concentration time curve (AUC). There are also cases where the response is dependent on peak (maximum) concentration or is another, often complex function of concentration. In other cases the therapeutic response is sometimes complex function of concentration. Actually, for some therapeutic targets it is needed to reach the peak concentration within a short time in order to fulfill the therapeutic goals (headache; sleep induction). Prediction of plasma concentration time curve may also serve as a surrogate for the effect of non-therapeutic xenobiotics such as biocides, pesticides and chemicals in general. Here, the interest focuses on adverse effects.

In modern pharmaceutical research the need to early estimates of ADME properties is driven by the event of combinatorial chemistry and high-throughput screening. Estimates of effective clinical dose are based on a combination of estimates of oral absorption, bioavailability (as mentioned next page bioavailability relates to more than just absorption), clearance, volume of distribution.

The most important factors affecting the early phases of plasma concentration time curve are rate and extent of absorption and the extent of any presystemic metabolism. However, it is not always the chemical substance itself that determines its absorption characteristics, but often pharmaceutical formulation is of importance. Then, in essence, release of a substance from the formulation determines its rate and extent of absorption. In addition, conditions in the gut can have a crucial effect on the absorption characteristics of a pharmaceutical (food effect).

Processes determining the pharmacokinetic behaviour of a substance exhibit considerable variability amongst individuals and time-dependent variation within an individual. Thus, it

would also be of use to be able to predict intra- and interindividual variability in the plasma concentration-time curve caused by exogenous and endogenous factors, such as diet, gut environment, disease, and genetics. In early drug development, it is not possible to study variability directly, but it can be predicted by identifying the essential components involved (enzymes, transporters, etc) affecting the behaviour of the compound and extrapolating the results on the basis of what is known about variation in these processes to the *in vivo* situation. For non-therapeutic xenobiotics, prediction of variability in kinetics may allow for a substitution of default safety factors by substance specific safety factors leading to a more appropriate risk assessment.

2. Rate of absorption and presystemic metabolism

It has to be recognized that in the absorption process there is a time component (rate of absorption) and a quantity component (extent of absorption), which are not, at least not invariably, interrelated. The general equation describing bioavailability is the following:

$$\text{Bioavailability (F)} = A - E - M$$

A = extent of absorption [passive diffusion vs active transport, pumps]

E = efflux [P-glycoprotein, other efflux pumps]

M = metabolism [CYP3A4, other CYPs and phase II enzymes in the gut; contribution of the liver]

The key question is how to predict these terms? It is useful to look at each separately and determine whether there are *in vitro* tests available. Then, if possible, a model encompassing all of these different steps should be constructed.

2.1. *In vitro* dissolution and absorption

In general, passive diffusion is the most important transfer process for drugs in the gut. Diffusion can occur either transcellularly or paracellularly. However, for compounds with a molecular weight >200, which includes the vast majority of drugs, the paracellular route is

negligible. Diffusion is determined primarily by the molecular and physicochemical properties of a substance (pKa, lipophilicity, molecular size, hydrogen bonding, etc) and by the properties of the intestinal membranes, through which the drug passes. There is evidence that the membranes in different parts of the gastrointestinal tract (and indeed, elsewhere in the body) are comparable in terms of passive transport. However, there is also some evidence that an endothelial barrier (blood-brain barrier) behaves differently from an epithelial barrier (Caco-2), even when pure trans-cellular passive diffusion is concerned.

However, drugs are administered as pharmaceutical preparations, in which various components may profoundly affect the rate of release of the active ingredient. *In vitro* tests for rate and extent of dissolution are available and are in routine use in drug development. Several *in vitro* methods are also available for measuring permeation across membranes (using either artificial or natural membranes). . A general consensus is that these *in vitro* methods can reliably differentiate between extremes, i.e. drugs with poor membrane permeation vs those that permeate well. There are, however, two important caveats: active transport processes and molecules in the "grey area" of physicochemical characteristics with respect to membrane permeation. To overcome these limitations, *in vitro* systems of a higher level of complexity are required (see later discussion on cellular models). There are also some, perhaps more subtle, problems related to the conditions prevailing in such systems. One of these is the static nature of most *in vitro* systems in the sense that they lack dynamic *in vivo* behaviour such as constant transit of drug-containing matrix along the length of the gut, the action of the microvilli in stirring the intervening aqueous layer between the gut contents and the epithelium and the sink phenomenon of permeability for dissolution (mesenteric blood flow). To the extent to which these phenomena are of importance in model building for prediction using *in vitro* systems has yet to be determined.

2.2. Contribution of intestine to transport and metabolism

Absorptive transport

A number drugs are synthetic analogues of endogenous ligands for receptors ligands or enzymes and hence it is not surprising that they can also serve as ligands for various membrane pumps. Recently, it has become apparent that analogous to the role of certain

drug metabolising enzymes in detoxifying a wide range of non-nutrient compounds, originally from the diet but now including many synthetic chemicals, there are several transporter systems that appear to exist to protect the organism from non-nutrient compounds. The best characterised of these is the multi-drug resistance protein family (MRPs). The role of the various pumps in the gastrointestinal absorption of pharmaceuticals is not yet very well established. Further, many novel drugs are structurally quite complex, amphipathic molecules, which require carrier mediated transport for absorption. Transporters and transport processes in the liver, both at the sinusoidal and canalicular membrane, have been more extensively characterised than those in the intestine (Meier). A number of drugs *per se*, but many more conjugated metabolites, are ligands for various transporters belonging to the families of polyspecific organic anion transporting polypeptides (Oatps), organic cation transporters (OCTs) and organic anion transporters (OATs).

Efflux pumps

The properties of P-glycoprotein, the product of the MDR1 gene, have been extensively characterised. P-glycoprotein is the so-called efflux pump, which can extrude a wide range of structurally diverse substances from the cell. It is expressed along the entire length of the gut and also in the liver (canalicular membrane), kidney, blood-brain barrier and placenta.

The substrate and inhibitor selectivity of P-glycoprotein has been quite extensively, but not yet exhaustively, investigated. There is some degree of overlap with CYP3A4 substrates and inhibitors, although this is not absolute. Interestingly, the expression of P-glycoprotein can be induced by a number of compounds, some of which are also inducers of CYP3A4, e.g. rifampicin, dexamethasone, cisplatin.

As indicated above, some cellular models such as Caco-2 and MDCK cells, express P-glycoprotein. As Caco-2 cells are human in origin, it might be expected that the kinetic characteristics of P-glycoprotein in such cells are similar to those of the pump expressed *in vivo*. However, this has not yet been established directly. MDCK cells originate from dog and consequently, there may be significant differences in their substrate and inhibitor specificities and in their kinetic properties from those of the human transporter. It will be of interest to determine whether the kinetic constants for a substance measured using the expressed protein would predict its efflux in a model system, e.g. Caco-2 cells.

Verapamil (and its metabolites) has been extensively employed as a model compound for P-glycoprotein, both as a substrate and as an inhibitor. Talinolol has also been a useful compound with which to explore the role of intestinal absorptive and secretory activities (Gramatte and Oertel 1999).

The role in the intestine of other efflux transporters, for example multidrug resistance related proteins (MRP-2 and others) has been explored with respect to the vectorial transfer of organic anionic drug metabolites (e.g. glucuronide and sulphate conjugates), but very little information has been obtained on pharmacologically active drugs (Suzuki and Sugiyama 2000).

Metabolizing enzymes

Most of the principal drug-metabolising enzymes are expressed in the gut, but there are large variations in their localization, both cellular and regional, with respect to both pattern of expression and factors affecting metabolism. P450 enzymes belonging to families 1 to 3 are the most important in xenobiotic metabolism, but the current evidence suggests that most CYP-associated activities are very low in the gut. CYP3A4 is the most abundant of the CYP enzyme present, representing approximately 70 % of the total CYP population, but CYP2C enzyme(s) (specific form(s) present yet to be identified) is also expressed to some extent. Both the pattern and extent of expression are dependent on location: in proximal parts of the small intestine (i.e. duodenum) CYP3A4 is the predominant enzyme, whereas in the large intestine CYP3A5 is more abundant. In distal parts of the small intestine (i.e. ileum) CYP3A4 expression is much lower than in more proximal parts. Characteristics of hepatic CYP3A4 have been extensively studied and the intestinal form seems to behave similarly (as far as it has been studied), but more work is clearly needed [possible basis of any difference?]. Pharmacokinetic studies have established that intestinal CYP3A4 activity could be an important limiting determinant of the bioavailability of several CYP3A4-metabolised drugs, but further studies are needed to elucidate the role of other CYPs, particularly CYP2C, in the intestine.

Among phase II or conjugation enzymes, UGT, GST, SULT and others are variably expressed in the different parts of the human gastrointestinal tract. There is no consistent

pattern of expression, some being higher in one part of the intestine and others being evenly expressed. The activities of these enzymes are often similar to, and sometimes even higher than, those of the corresponding hepatic activities (Pacifci et al). Very little is known about the role of intestinal phase II enzymes in determining the bioavailability of pharmaceuticals. However, in principal, given the localization of enzymes in the gut, both regional and cellular, one should anticipate an effect on intestinal metabolism and bioavailability.

Cellular models

Absorption of compounds by passive diffusion can be reasonably well predicted on the basis of physicochemical properties, such log D (distribution), delta log D (octanol/water – alkane/water), solubility, or using simple hydrophobic membrane systems (immobilised artificial membranes: PAMPA (Roche), HDM (Novartis), liposomes). However, to improve prediction., account must be taken of active processes of metabolism and transport.

Several cellular models are available to study the absorption characteristics of pharmaceuticals. Two of the most widely used are Caco-2 cells in monolayer – or their sub-clone TC-7 - and MDCK cells in monolayer. These models both have their advantages and disadvantages. A potential advantage of Caco-2 cells, for example, is that that they are enterocyte-derived cells with a microvillous surface, although in fact they are derived from human colon. Also, considerable experience and a fairly large data set have been accumulated with their use over the years. Disadvantages of this system include the fact that they need long culturing times - two to three weeks - to express fully differentiated functions (and even then expression is not at the *in vivo* level), they form very tight junctions in monolayer and they exhibit a high transepithelial electrical resistance relative to that *in vivo*. Whilst p-glycoprotein is expressed in Caco-2 cells, significant CYP3A4 expression required culture for some time in the presence of vitamin D.

All single cell *in vitro* models, exhibit considerable differences from the situation *in vivo*: these include lack of flow of gut contents ("perivillous layer") and lack of basolateral blood flow ("sink effect"). When a large number of compounds with a range of physicochemical properties were studied with this model, it was found that the permeation curve is sigmoidal, with very steep slope, i.e. there is a sharp threshold and absorption *in vivo* of compounds at

the threshold permeability varies considerably. Furthermore, the threshold value varies from laboratory to laboratory. A Caco-2 cell line expressing transfected CYP3A4 is also available, but there is little published information about its usefulness and it seems that expression levels are currently not adequately stable or very high (Crespi et al 2000).

Recently, an MDCK cell model has been investigated as a possible screening tool for absorption studies. There are some advantages when compared with Caco-2 cells: MDCK cells have a short culturing time and their electrical resistance is lower than in Caco-2 cells, closer to that *in vivo*. However, like Caco-2 cells, although they express P-glycoprotein, expression of CYP3A4 is very low or absent. Another (very basic) potential disadvantage is that these cells are derived from dog kidney. The performance of these cells has not been thoroughly studied. Recombinant CYP3A4 has also been expressed in MDCK cells and expression is stable and relatively high.

2.3. Is it possible to predict T_{max} and/or C_{max}?

In some therapeutic situations, such as the use of analgesics and antipyretics, there is need for a rapid and high peak concentration of the drug and consequently absorption should be fast and extensive. In these particular situations, but also more generally from a pharmacokinetic point of view, it would be very useful to be able to predict the shape of the initial plasma concentration-time curve, i.e. how rapidly is the maximum concentration achieved (T_{max}) and how high is the maximum concentration (C_{max}). There is little experimental data available to help make such predictions, but extensive simulations show that T_{max} and C_{max} are more related to clearance than to absorption (Tom Tozer). Consequently, for the prediction of these parameters, information about the clearance of a compound might be more useful.

3. Intra- and interindividual variability and interactions

Genetic variability

Whilst oral bioavailability is often consistent within an individual, many drugs exhibit considerable interindividual variability in their bioavailability. The reasons for this are not

known, but may include genetic factors. Transporters and enzymes being gene products, could, and some do, display genetic polymorphisms. Two more extensively studied examples, which might be of importance in drug absorption and bioavailability, are polymorphisms of P-glycoprotein and CYP3A4. Hoffmeyer et al (2000) demonstrated that the exon 26 single nucleotide polymorphism of MDR1 correlates with intestinal expression and function. They showed that variant heterozygotes and homozygotes expressed less MDR-1 in the duodenum, which most likely explains why these individuals have higher AUCs and maximal plasma concentrations with digoxin (a P-glycoprotein ligand) than non-variant homozygotes. Several variant alleles for CYP3A4 have been identified, but their metabolic and pharmacokinetic consequences remain unclear. It is quite intriguing to note that no deletion alleles have been found in humans either for P-glycoprotein or for CYP3A4, although deletion mutants are quite common for other drug-metabolising enzymes. Even less is known about genetic variability of other transporters and enzymes in the gastrointestinal tract.

Knowledge of the extent of interindividual variability is an important information for the risk assessment of non-therapeutic xenobiotics as it allows to properly define safety factors.

Host factors

It is known on the basis of research on gastrointestinal physiology and pathology that a myriad of factors can affect the structure and function of those parts of the gastrointestinal tract most important for the absorption of drugs. For example, chronic diseases of the small and large intestine, such as Crohn's disease or colon irritable, cause structural changes in the epithelium, with consequent changes in the expression and activity of enzymes and transporters. However, research on how these changes affect drug absorption and bioavailability is scanty. It is also not clear how, and to what extent, such changes should be taken into consideration when drugs are tested preclinically. From the perspective of the risk assessment of chemicals in general, more knowledge would be welcome as it would allow to define subpopulations at risk which should be taken into consideration.

Drug-drug interactions

In theory, potential drug-drug interactions should become entirely predictable as soon as the roles of P-gp and CYP3A4 in the absorption and metabolism of a specific substance have been established. Already, some important, previously unexplained, interactions, such as those between digoxin and quinidine and between digoxin and rifampicin, are now explicable on the basis of P-glycoprotein. Quinidine is a potent inhibitor of P-glycoprotein and digoxin serves as a substrate. In contrast rifampicin induces P-glycoprotein and the increase in expression leads to a decrease in the systemic absorption of digoxin. It has been difficult to study the contribution of intestinal CYP3A4 in the presystemic metabolism and interactions of its substrates, because of the predominance of hepatic CYP3A4, but there is now clear evidence that intestinal CYP3A4 contributes significantly to the presystemic metabolism and drug interactions of at least some drugs, such as midazolam and cyclosporin A. Some other CYP3A4 substrates are not affected. Because of the overlap of substrates and inhibitors for P-glycoprotein and CYP3A4, it has been suggested that they have a synergistic role in the intestinal elimination and consequently, potential interactions, of “bisubstrates”.

Effect of food (drug-diet interactions)

This is a topic that has not been systematically explored, despite the fact that a plethora of dietary factors, at least in theory, could affect the absorption characteristics of drugs. Perhaps it is because of the complexity of the diet that exploration of this has been sporadic. Among dietary factors that, theoretically, could affect drug absorption are the overall characteristics of food: volume, composition, pH, caloric density, viscosity; individual components: carbohydrates, protein, fat, fibers; and specific components: alcohol, caffeine, isoflavones. This array of dietary factors are involved in a complex interplay with functional status, motility, acidity, etc, of the gastrointestinal tract, together with the physicochemical properties, formulation and dissolution characteristics of the drugs involved (“solubilisation by food”) and also with specific components of the enterocytes, such as transport and metabolising proteins (grapefruit juice!). Furthermore, children (especially young) and the elderly may be special groups in terms of drug absorption because their diets may differ, often considerably, from the “standard adult diet” and because they will differ in their physiological characteristics, although little specific information is available.

Biopharmaceutics classification system

Recently, a biopharmaceutical classification system has been presented, in which drugs are classified on the basis of their solubility (S) and permeability (P) (either high (H) or low (L) for both criteria) into four classes:

- * Class I: HS:HP - controlled by gastric emptying (paracetamol., valproate, NSAIDs, verapamil)
- * Class II: LS:HP - controlled by dissolution rate, gastric emptying, fat content (griseofulvin, digoxin, phenytoin, cyclosporin, spironolactone)
- * Class III: HS:LP - controlled by transit time, diffusion barriers (frusemide-but absorption variable, bidisomide)
- * Class IV: LS:LP - absorption decreased by food (amphotericin)

An important issue is whether, and to what extent, standardisation of diet and food effects is necessary in clinical studies and how these potential interactions could and/or should be studied in the preclinical phase. Even the need for such studies is questioned, probably because the true incidence and clinical significance of drug-food interactions is largely unknown (Gauthier and Malone 1998).

5. *In vivo* studies

Whatever the system developed for use during drug discovery and development to predict absorption, a reliable and sufficiently large *in vivo* data base on human and possibly other species absorption as well as bioavailability data is needed for even small-scale validation. As rather detailed information on the absorption characteristics of a drug is needed for registration purposes, there is a considerable amount of data available in public literature. This is usually in the form of the *in vivo* pharmacokinetic behaviour of the drug, from which its absorption characteristics can be derived.

From the mechanistic point of view, the most important question is: What really happens to the drug in the gut? Recently, an *in vivo* experimental system, the human jejunal regional single pass system, has provided detailed information to answer this question. In this system it is possible to change various factors affecting absorption. At the present moment information is available for more than 20 substances (Lennernäs). There is a very good

correlation with *in vivo* absorption determination from clinical studies. The only exception was enalapril, which is a prodrug. However, much further work is needed to obtain sufficient high-calibre *in vivo* data to be able to validate *in vitro* models.

6. Species differences

Although many comparative data on interspecies differences and similarities in gastrointestinal anatomy and physiology are available, much less is known how this translates into explanations for interspecies differences and similarities in drug absorption and bioavailability. As far as passive transport phenomena are concerned, it is commonly assumed that the gastrointestinal tract and membrane barriers from different species behave in roughly similar ways in terms of drug permeation. However, there are also differing views: rough similarities might only be true for trans-cellular passive diffusion, but when considering para-cellular passive diffusion (via the tight junctions), it is a well known fact that dog has slightly wider pores than rat or man and therefore exhibits a higher permeability. Broadly speaking, rat is believed to be closer to man but dog is known to be quite different depending on the compound. Furthermore, when active processes, catalysed by enzymes and transporters have to be taken into consideration, species differences become inevitable. Furthermore, differences in anatomy and physiology, such as gut length and motility, amount and composition of gut microflora, coprophagy, enterohepatic recycling, intestinal transit time, may profoundly affect drug absorption in different species. In drug development, one of the basic questions is whether the dose of a drug used in test animals can be scaled, and on what basis, to the human situation. To answer this question would require extensive comparative knowledge of anatomical, physiological and pharmacological factors affecting drug absorption in a test species and in humans (which is currently not available, at least to the extent required).

7. *In silico* predictions

In silico predictions involve approaches from relatively simple quantitative structure-activity relationships (QSAR) to the use of complex physiologically based pharmacokinetic and/or

pharmacodynamic (PBPK, PKPD) models. Whatever the actual model, any predictions need good-quality and value-rich databases of appropriate descriptors. A side issue, though of some importance, is the fact that database availability, i.e. issues of ownership and confidentiality, may sometimes become a problem. Computational methods exist to enable calculation of permeation from single or multiple descriptors of molecular and physicochemical properties of drug molecules and these are generally regarded to provide useful information. Techniques are available to calculate physicochemical constants from molecular structure that are important in determining absorption, although there are difficulties in the *de novo* calculation of log D. Parameters of use include lipophilicity, hydrogen bonding and molecular size/shape. There are several different parameters reflecting these properties, and sometimes it is difficult to decide which is most appropriate for use in such calculations. One of the most critical issues for the successful prediction of absorption is how active processes should be modelled and whether there is sufficient information available to develop quantitative structure activity relationships.

In the risk assessment of chemicals *in silico* predictions rate high. For new chemicals, for example, *in vivo* kinetic data have only be submitted when the production volume of a specific chemical is higher than 1000 t/year; however, such a case has never occurred in the last 20 years. Thus, no other than QSAR derived information is available.

8. Validation of models

Whether the methods used to predict the behaviour of drugs *in vivo* in humans are *in vitro*, *ex vivo*, or in experimental animals, some validation is necessary. Validation is a process by which the reliability and relevance of a procedure are established for a particular purpose. It has to be stressed that none of the many *in vitro* approaches described above have been properly validated, in the formal sense. However, this is not to say that these methods are not useful or that they do not perform reasonably well in the process of drug development. . With respect to the importance of *in vitro* methods in the assessment of chemicals there is an urgent need to perform this exercise.

A number of guidelines describing the process of prevalidation of a test method are available (some examples: <http://altweb.jnhsp.edu/>, ECVAM (ECVAM and DG XI) (1995) Concept of a prevalidation phase; OECD (1996) Acceptance of principles for validation). The full process is a stepwise approach, which could well take at least 7-9 years to reach regulatory acceptance.

Because the full validation process is very cumbersome and long, industry, at least, has taken the view that preclinical *in vitro* tests serve more in the preparation for the clinical phases of testing than for preclinical regulatory purposes. On the other hand, regulatory authorities have recently prepared some guidelines on the judicious use of specific *in vitro* tests, to help drug developers to use such tests in a meaningful way so that they avoid unnecessary clinical studies. Furthermore, such advice should be very useful also for other developers of other chemicals (e.g. agrochemicals etc) in order reduce the number of animal studies in the test programme of chemicals. For those types of models discussed here, internal validation is satisfactory as far as it has been achieved using recognized internal references.

9. Future trends

The principal goal of the expert meeting was to identify important topics for future research through the identification of gaps in our current knowledge. Some of the most important issues are listed in table 1 and a number of others can be found in the text. It is apparent that the current trend is towards the integration of information obtained using a number of different approaches during various phases of drug development which, with the help of model building, will lead to the *in silico* prediction of absorption and presystemic metabolism. This is precisely the goal of COST Action B15 and there is ample evidence of a similar approach in the EUFEPS initiative “New Safe Medicines Faster”. Furthermore, this meeting served well the intentions of Action COST B 15 to transfer knowledge gained in the field of drug development to the field of risk assessment of chemicals.

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Table 1. Important topics for future research in the area of drug permeation and bioavailability in drug development.

High-quality human *in vivo* data on absorption and bioavailability

- currently some data are available from about 250-300 compounds
- bioavailability data is available for ca 600 compounds
- need of data from further *in situ* human absorption studies (now about 25 compounds?)

Good P-glycoprotein efflux model with SAR

Prediction of role of intestinal CYP3A4 (and other XMEs)

Knowledge of how the roles of P-glycoprotein and CYP3A4 inter-relate

High-throughput screens for membrane permeability

Characterisation and prevalidation of cellular models for permeation and bioavailability

- competing interests: *in vivo*-like, maximum expression of active processes, need for high throughput

Characterisation of genetic and non-genetic factors affecting active processes

- MDR1, other absorptive and efflux transporters, CYP3A4 and other XMEs

Interindividual variability in various passive and active processes

- is there a need to model variability?

Drug-drug and food-drug interactions

- usefulness of biopharmaceutical classification system based on permeability and solubility

Creating appropriate databases for interspecies comparisons of drug absorption and bioavailability

- importance of anatomical and physiological differences for drug absorption

In silico prediction

- finding the “best” molecular parameters for successful predictions
 - intimately linked to the development of high-quality *in vitro* and *in vivo* databases
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Table 2. Common absorption and bioavailability models currently in use.

System	Model	Pros	Cons
<i>In silico</i>	Various computational approaches using SAR/molecular properties	HT, easy	lacks active processes (at present) depends heavily on quality and quantity of available data
<i>In vitro</i>	Artificial membrane systems (PAMPA, IAM, etc)	HT (only some), easy to use, analytically easy	measures only transcellular permeation lacks active processes
	Cell-based systems (Caco-2, MDCK, etc)	moderate to high throughput active and passive transport human intestinal epithelium for Caco-2 cells	labour intensive analytically more difficult intra- and interlab variability many phenomena model-dependent
	Brush Border Membrane Vesicles (BBMV)	moderate throughput active and passive transport	labour intensive analytically difficult variability
<i>In situ</i>	Rat intestinal perfusion	closer to <i>in vivo</i> situation transporters, enzymes relevant tight junctions	labour intensive species differences possible effects of manipulations
<i>In vivo</i>	Rat portal vein studies	closer to <i>in vivo</i> transporters, enzymes relevant tight junctions presystemic metabolism	labour intensive species differences possible effects of manipulations

