In vitro prediction of gastrointestinal absorption and bioavailability: an experts’ meeting report

Received: 27 April 2001 / Accepted in revised form: 3 August 2001 / Published online: 2 October 2001
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Abstract The most convenient route of drug administration is peroral. To reach their target, drug molecules must be absorbed from the gastrointestinal tract and enter 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 contrast to drugs, which are administered intentionally for therapeutic reasons, chemical residues in food and other matrices enter the body unintentionally. Hence, in this case, a low systemic availability would be advantageous. For many reasons, but particularly because of financial and ethical (reduced use of animals) considerations, in vitro and ex vivo approaches to this problem have been pursued 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. The purpose of this report is to review the current status of the field and to identify major gaps in our knowledge. 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. Some strengths and weaknesses of these approaches are presented, together with a discussion of genetic, environmental, physiological and pathological factors responsible for interspecies and inter-individual 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 priorities 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 compound 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 of bioavailability is still very debatable and will require their full validation. It is clear, however, that the results from such in vitro tests are important in shaping drug discovery and the early preclinical drug development process. For other environmental, industrial and household chemicals to which humans are exposed, in particular new chemicals, results from in vitro studies might be the only source of information concerning systemic availability.

Keywords Absorption · Systemic availability · Bioavailability
Introduction

The peroral route is the most convenient and widely used means of drug administration. Hence, during drug discovery it is of crucial importance to identify compounds with characteristics that make them suitable for this route of delivery. In addition, in most instances therapeutic response appears to be proportional to the amount of drug delivered to the site of action (pharmacokinetic/pharmacodynamic relationship), a surrogate measure of which might be the area under the plasma concentration–time curve (AUC). In other cases, the response is dependent on peak (maximum) concentration or is another, often complex, function of concentration. Indeed, for some therapeutic targets it is necessary to reach the peak concentration within a short time to achieve the therapeutic goals (e.g. headache, sleep induction). Hence, a major objective of pharmacokinetic studies in drug development is the prediction of the plasma concentration–time curve for a new chemical entity. Prediction of the AUC may also serve as a surrogate for the internal dose of non-therapeutic xenobiotics such as biocides, pesticides and chemicals in general. Here, the focus of interest is on adverse effects.

In modern pharmaceutical research the need for early estimates of absorption, distribution, metabolism and excretion properties is increasingly being driven by the implementation of combinatorial chemistry and high-throughput screening. Estimates of effective clinical dose are based on a combination of estimates of oral absorption, bioavailability (as discussed below, bioavailability relates to more than just absorption), clearance and volume of distribution.

Bioavailability is determined by the extent of absorption and presystemic metabolism. These factors also determine the early phases of the plasma concentration–time curve, together with the rate of absorption. However, it is not always the chemical substance itself that determines its absorption characteristics; pharmaceutical formulation may also be of importance. Then, in essence, release of a substance from the formulation determines its rate and extent of absorption and possibly also the extent of presystemic metabolism. In addition, conditions in the gut can have a crucial effect on the absorption characteristics of a pharmaceutical or other chemical (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 value to be able to predict intra- and inter-individual variability in the bioavailability and in the AUC caused by exogenous and endogenous factors, such as diet, gut environment, disease and genetics. In early drug development, and for many environmental chemicals, it is not possible to study variability directly, but it might be predictable by identifying the essential components involved (enzymes, transporters, etc.) in 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, knowing the extent of bioavailability may allow for route extrapolation and thus help to avoid unnecessary testing. In addition, further testing may be derogated in cases where no absorption takes place. Finally, prediction of variability in bioavailability and kinetics might allow substitution of default uncertainty factors by compound-specific adjustment factors leading to a more appropriate risk assessment.

Absorption and presystemic metabolism

It must be recognised that in the absorption process there are a time component (rate of absorption) and a quantitative component (extent of absorption) that are not, at least not invariably, interrelated. Bioavailability is the result of several competing processes, some of which favour the entry into the systemic circulation and others which impede it. The most important processes are the following:

- Extent of absorption (e.g. passive diffusion, active and facilitated transport, paracellular transport, endocytosis, gut flora metabolism)
- Efflux (e.g. P-glycoprotein, other efflux pumps)
- Presystemic metabolism [cytochrome P450 (CYP)3A4, other CYPs and phase-II enzymes in the gut, contribution of the liver]

The key question is how to predict these processes. 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 processes should be constructed.

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 greater than 200, which includes the vast majority of drugs and environmental chemicals, 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 transcellular passive diffusion is concerned.

However, drugs are administered as pharmaceutical preparations, in which the formulation may profoundly affect the rate of release of the active ingredient. In vitro