AMS for measuring absolute bioavailability of new drugs

In the past it has been difficult to calculate absolute bioavailability of drugs based on extravascular and intravenous drug dosing studies because of issues associated with concentration-dependent kinetics. Accelerator Mass Spectrometry is a new isotope ratio-based technique designed to overcome these problems and thus lead to improved drug discovery and development.

Obtaining information on the metabolic fate of a drug in humans is a key requirement in drug development. Of the various metabolic (pharmacokinetic) parameters such as half-life and clearance, absolute bioavailability is one parameter that is not routinely measured. Although some data on the bioavailability of new drugs has to be submitted as part of the drug approval process, pharmaceutical companies may not necessarily perform a full absolute bioavailability study in humans in every case. Although there are scientific benefits to such studies, the pharmaceutical industry does not perceive the cost benefit to be sufficiently favourable to warrant the performance of an absolute bioavailability study unless there are very good reasons; such as when the regulatory agency demands it.

There are many reasons for low bioavailability of a drug, including incomplete dissolution when administered as a solid, inability to permeate the membranes separating the absorption site from the systemic circulation, and metabolic instability as the drug moves from the absorption site to within the systemic circulation (first pass metabolism). Understanding the contribution of each of these sources of loss is important in drug development. For example, if the major problem is poor dissolution, often this can be overcome by appropriate reformulation of the product, whereas poor permeability generally cannot be overcome.

Absolute bioavailability studies are unpopular

Absolute bioavailability studies in humans are unpopular in the pharmaceutical industry, primarily because such studies require the inclusion of an intravenous reference dose in the clinical study design. An intravenous dose guarantees that all the administered drug reaches the systemic circulation. Such intravenous studies come at considerable cost, not least of which is the necessity to conduct intravenous preclinical toxicity tests in two animal species, one of which is a non-rodent to ensure adequate safety. In addition, there are potential problems due to solubility limitations. Indeed, for drugs where the intended clinical route of administration is purely extravascular, then an absolute bioavailability study in humans may be the only occasion when the drug is administered intravenously.

There are, nevertheless, certain situations where ignoring the need for an absolute bioavailability study might be unwise. For example, an orally administered anti-infective that is poorly absorbed may have to be given at doses that have adverse effects on gut microflora leading to diarrhoea with the accompanying side-effects. More generally, bioavailability determined from animal models is not always predictive of that in humans and unexpected low bioavailability in humans is perhaps not that uncommon.

MEET GRAHAM LAPPIN AND COLIN GARNER OF XCELERON

With over 25 years in the business, Dr Graham Lappin started by researching into the metabolism of terpenoids in plants. His Bachelors degree and PhD were obtained from the University of Westminster, London, UK, followed by post-doctorial research at the University of Glasgow in Scotland. After this he spent a number of years specialising in mammalian metabolism and today he is dedicated to the study of drug metabolism in humans. He is a fellow of the UK's Institute of Biology and Royal Society of Chemistry and is currently the Head of Research and Development at Xceleron Ltd.

Professor Colin Garner is a graduate in pharmacy from London University where he also conducted his PhD studies in biochemical toxicology. After a two year post-doctoral fellowship at the University of Wisconsin he returned to the UK where he set up a cancer research laboratory at the University of York. His research focus has been on gene/environment interactions especially relating to cancer cause and prevention. In 1997 he created the University of York spin-out company, Xceleron, where he is currently the CEO. Professor Garner became the UK's first Professor of Molecular Epidemiology in 1995. In the same year London University awarded him a Doctor of Science for outstanding contributions to cancer research.

A traditional protocol for an absolute bioavailability study in humans comprises a two-way crossover design, with an intravenous arm and an extravascular (eg oral) arm. Following dosing, the plasma drug concentrations are measured over time, typically by liquid-chromatography mass-spectrometry (LC-MS).

The plasma concentrations are used to determine the systemic exposure to the drug (the area under the plasma concentration-time curve, or AUC). Since the intravenous route equates to 100 per cent absorption, then the absolute bioavailability is calculated by dividing the dose-normalised AUCs for the extravascular dose with that of the intravenous dose.

Scientifically, the crossover type of bioavailability study is open to criticism in that, if the plasma concentrations attained for the intravenous and extravascular doses are significantly different, then concentration-dependent pharmacokinetics might occur.

An alternative approach using isotopes

An alternative approach was pioneered in the 1970s, where the intravenous dose, labelled with a stable isotope (eg ^{13}C or ^{15}N), was administered simultaneously with the extravascular dose. Plasma concentrations were determined by LC-MS and the isotopically-labelled drug was distinguished from the non-labelled drug by virtue of their different molecular weights. AUCs were then compared, as above, to determine the absolute bioavailability. Because of the simultaneous dosing, only one plasma sample is taken at each time point yielding data for both the oral and intravenous doses. In effect, the systemic intravenous dose is isotopically diluted with absorbed drug originating from the extravascular dose. By simply measuring the amounts of each isotope, the concentration arising from each administration can be determined (Figure 1). This study design is not only pharmacokinetically elegant, it also has the advantage that only one dose occasion is required and therefore the clinical times and costs are reduced.

The only significant drawback of using stable isotopes in absolute bioavailability studies is that analytical methods that distinguish stable isotopes are inherently insensitive. This insensitively arises from the relatively high background levels of stable isotope in the environment (eg the natural abundance of ^{13}C is 1.1 per cent). On the other hand, radioisotopes have much lower natural abundances (eg the natural abundance of ^{14}C is around 10^{-11}per cent). Radioisotopes are however, by definition radioactive and there are severe restrictions on how much radioactivity can be administered to human volunteers.

AMS - a new technology

In the 1990s a technology new to the pharmaceutical industry emerged, known as Accelerator Mass Spectrometry (AMS). Invented in the 1970s for archaeological carbon dating, AMS accelerates isotopic ions to very high energies, thereby facilitating their separation and measurement to an extremely high level of precision and accuracy. The advent of AMS strategically changed the way in which absolute bioavailability studies could be conducted.

Designing an absolute bioavailability study around the use of AMS, it is possible to administer minute amounts of radioactivity-labelled drug intravenously. This has four principal advantages:

First, the amounts of radioactivity are so low that they are no longer classified as radioactive studies and the regulatory burden of conducting these studies is removed. Providing the bioavailability study is conducted appropriately, then dosimetry studies involving animal models to calculate radioactive exposure are not required.

Second, the dose levels are so low (typically 100 micrograms or less) that there is no need to conduct a preclinical safety package for the intravenous administration. Providing the systemic levels arising from the intravenous route (using the very small dose) are significantly lower than those arising from the oral administration (using the therapeutic dose) then the toxicity data from the oral route can be used to justify the intravenous dose. As a consequence, in situations where there is no intravenous toxicology data to support the intravenous route of administration in humans, the cost of the absolute bioavailable study falls considerably.

Third, administration of an intravenous dose to humans in the bioavailability study also enables some fundamental pharmacokinetic parameters to be calculated (eg clearance and volume of distribution). Without the intravenous dose, then these parameters can only be estimated - and not always accurately.

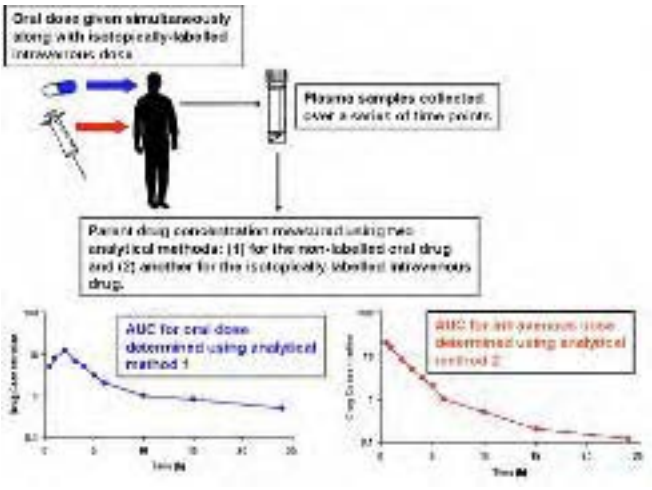

Fig 1. The principle of determining absolute bioavailability from the simultaneous administration of labelled intravenous and non-labelled oral doses of drug to human volunteers.

Finally, problems that can arise in the solubility and formulation of an intravenous dose do not apply to such very small doses. If there are solubility problems using a 100 microgram dose, then the chances are this will present a much greater problem than any bioavailability issues.

Cost benefits

Since the major barriers to conducting absolute bioavailability studies in humans have been lifted with the use of AMS, the cost-benefit equation shifts significantly towards benefit. Some pharmaceutical companies are now even factoring such studies into their routine development package. For example, if the oral bioavailability of a drug is low when a simple solution is administered, then there is little chance of increasing this value no matter how much effort the formulation chemists might expend.

Furthermore, it is possible to combine a traditional Phase I study and the bioavailability study by including the simultaneous very small intravenous dose alongside one of the oral doses. This approach reduces the clinical costs even further and allows the absolute bioavailability of the drug to be obtained in humans at the earliest possible time.

Absolute bioavailability studies, which in general terms can currently be considered nothing more than an expensive irritation in drug development, may well be about to be promoted to not only desirable studies in their own right but studies providing a multitude of useful data all combined within the one experiment. sp²

FURTHER INFORMATION

Graham Lappin and Colin Garner
Xceleron Ltd
The Biocentre
Innovation Way
Heslington
York Y010 5NY
United Kingdom
Tel: +44 1904 561561
Fax: +44 1904 561560

Internet Links: **Email: graham.lappin@xceleron.com or**
Email: colin.garner@xceleron.com
Web: www.xceleron,com