DRUG DEVELOPMENT COMMUNICATIONS, 2(6), 439-451 (1976)

THE ROLE OF MODEL SYSTEMS IN ASSESSING BIOAVAILABILITY OF ORALLY ADMINISTERED DRUGS

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INTRODUCTION

The therapeutic performance of a drug in man is the prime reason for drug development and utilization. However, in the development of formulations and the subsequent, or preferably concurrent, assessment of bioavailability, it is not always possible or desirable to undertake such studies in man. Analytical, ethical, and economic factors are all involved. Consequently, it is necessary to develop reliable screening methods or model systems that do not involve man but which will provide reasonable assurance of correlating with the profile in man.

Naturally, there are shortcomings whenever one organism or mechanical device is substituted for another organism, in this case, man. However provided these potential deficiencies are appreciated and taken into account wherever feasible, it is fair to say that studies involving model systems have a significant place in dosage form development. This is not a new situation for, in a very real sense, the basics of any

drug development program are based on model systems - namely, the use of animals as pharmacological screens to evaluate the spectrum of activity of new compounds.

The thrust of this article is with model systems that can be used to evaluate the effect of formulation upon the bioavailability of the drug under development. Naturally, the usefulness of any model system lies in how well and consistently it is subsequently shown to correlate with what happens in man. Greatest weight and reliance is then placed upon such systems. Test systems where such correlations have not been previously shown, or cannot be shown, must be regarded as of minor value in a biological sense, and serve simply as physical quality control procedures.

It is reasonable to suppose that the further a model system departs from the true *in vivo* situation, the less likely is the chance for demonstrating total and consistent correlations. However, even in the absence of drug correlation, *in vitro* test systems can be useful in indicating and delineating the mechanisms that control a particular process. In particular potentially interfering *in vivo* factors can be "built out" of the system.

IN VITRO SYSTEMS

The drug and/or metabolite concentration in plasma and urine as a function of time following the oral administration of a drug depends on the relative rates and extent of drug dissolution, absorption, distribution, metabolism and elimination. Drug absorption from the gastrointestinal tract may be rate-limited by either dissolution into the aqueous

gastrointestinal fluids or permeation through the lipoidal gastrointestinal wall. In many cases where bioavailability problems arise, it is the rate of drug dissolution which is controlling the rate and extent of absorption.

Solubility

Insofar as one of the factors upon which dissolution rate depends is solubility, this property may perhaps be regarded as the first, albeit crude, *in vitro* model. Of particular importance with a new compound is a knowledge of its pH-solubility profile. Experience suggests that at biological pH's, aqueous solubilities of less than 0.1 - 1.0% $^{w}/_{v}$ may result in bioavailability problems. Drugs having solubilities in excess of this range are less likely to be so affected. If the solubility decreases with pH, the chances of problems arising are increased.

A relationship between solubility and the initial dissolution rate has been shown with a wide range of compounds,[1] although exceptions have been reported.[2] Solubility can therefore be regarded as a qualitative, but not necessarily quantitative index of bioavailability.

Dissolution studies

Drug dissolution studies can be a relatively useful index of drug availability. While the current USP and NF include dissolution specifications for a number of drug products, the number of well documented cases where *in vitro* - *in vivo* correlations have been

established is quite small. Within the U.S. compedia, correlation has only really been demonstrated with digoxin tablets. Obviously, further work in this area needs to be undertaken. It must, however, be emphasized that dissolution rate studies on drugs and drug products are useful simply to confirm that under a given set of arbitrary conditions (i) the drug under study is able to dissolve from the dosage form, and (ii) the formulation has not compromised the natural dissolution rate of the drug.

Some of the more useful model systems for evaluating dissolution have been reviewed elsewhere.[3,4,5] While the list of drugs found, or suspected, to have bioavailability problems is quite significant,[6,7,8] examples of a correlation between *in vitro* dissolution and *in vivo* availability in man of commercially available products are comparatively few. Some examples are tetracyclines,[9,10] aspirin,[11-14] para-aminosalicyclic acid,[15] griseofulvin,[16,17] salicylamide[18], erthromycin[19], and digoxin.[20,21].

Permeation cells

Various *in vitro* permeation cells have been designed and utilized to study drug transport across biological membranes.[22,23] The basic design of these units is of a "lipid" phase (comprising either a liquid oil, and oil-impregnated filter, or a polymeric membrane) separating two aqueous phases. The pH's of the aqueous phases are adjusted so as to mimic, on the one side, the stomach, and, on the other, plasma. While correlations between transport rate constants have been achieved,[24,25] their significance in regard to formulations is limited.

The main use of these devices is in studying the effects of substituents on the relative absorbability of a series of homologs or otherwise closely related compounds. However, to the extent that some of the better correlations are observed between the forward transport rate constants and partition coefficients, it is probably as meaningful - and certainly less time consuming - to simply determine this latter parameter.

Isolated gut techniques

Model systems that are a step closer to the situation in man can be developed based on the use of animal gastrointestinal tracts. Systems using isolated membranes in this way include the Wilson-Wiseman and the Crane-Wilson everted intestinal sac.[26]

In this latter procedure, the section of small intestine is washed, everted, tied at one end, and attached to a cannula at the other. The whole is placed in a container and filled with a Ringer's solution at 37°C. Oxygen is bubbled through the solution in contact with the mucosal surface and drug is added also to this solution. Samples of the serosol fluid, inside the everted sac, are easily taken at known time intervals and assayed for drug concentration. A drawback is uncertainty as to the viability of functionality of the everted sac with time. However, the technique is quite widely used, again mostly as a screen.[27] In conjunction with separate dissolution studies, this technique can be used to indicate whether a particular compound is likely to show impaired absorption *in vivo* because of dissolution and/or permeation problems.[28,29]

IN SITU METHODS

These methods, lying between *in vitro* and *in vivo* techniques, may be defined as techniques in which the animal's blood supply remains intact. This is a major advantage over the isolated gut techniques, since it provides a physiologic situation closer to that found *in vivo*.

Bates and Gibaldi[27] have reviewed the several techniques used. These differ primarily in their handling of the drug and perfusing fluid. While these techniques are mostly used for studies of intestinal absorption, they have also been readily modified to follow gastric absorption. The rat is the animal of choice.

The perfusion tec nique developed by Schanker et al.[30] depends on passing a solution containing the drug under study through the gut lumen at a constant known rate. By measuring the drop in concentration in the perfused fluid, it is possible to calculate the amount absorbed. The small intestine is cannulated at the duodenal and ileal ends, with the rat under anaesthesia throughout the entire experiment. A perfusion pump is then connected to the duodenal cannula, the intestinal contents are washed out, whereupon the drug solution is perfused. The technique can be either single or multiple pass. Using seventeen different sulfonamides, Koizumi et al.[31] were able to correlate rates found with this model to chloroform/water partition coefficients.

The technique developed by Doluisio et al.[32] involves cannulating the duodenal and ileal ends of the small intestine with L-shaped cannula. As before, the rats are anaesthetized. Stopcocks are added such that drug solution can be introduced into the intestine and left there for known time intervals. Samples of solution can be readily obtained and a drug absorption *versus* time profile can thus be built up.

As in all *in situ* work, it is necessary to correct for any intestinal absorption of water from the drug solution, since this can lead to errors in calculating the amount absorbed.

The method devised by Levine and Pelikan[33] is similar to that described by Doluisio et al. except that the gut is simply ligated at both ends of the desired segment. Drug solution (circa 0.5 ml.) is introduced directly into the gut lumen via a hypodermic syringe, the intestinal loop is replaced in the abdominal cavity, and the incision is closed. The animal is then allowed to recover from the anaesthesia. After the desired time interval, the animal is sacrificed, the intestinal loop excised and homogenized, and then the amount of unabsorbed drug determined. That this is a "one point" procedure, thereby requiring the use of several animals for each time point, is an obvious disadvantage. Offsetting this is the fact that the animals are non-anaesthetized and are ambulatory during the experiment.

In addition to solutions, this technique has been used successfully for capsule charges and suspensions.[34] A similar technique could be developed for emulsion and tablet granulations, thus widening significantly the application of this method.

Perrier and Gibaldi[35] have recently compared the in situ rat method with the everted rat gut technique mentioned earlier. The results led these workers to conclude that the best correlation with the absorption of antibiotics in man was exhibited by the in situ intestinal loop preparation. Using a similar type of preparation, Pelzmann[36] has been able to follow the time course of drugs in the bile of rats.

DRAWBACKS OF IN VITRO AND IN SITU METHODS

There are several disadvantages to all the previously discussed methods. With the isolated gut techniques, tissue is removed from the body and separated from its blood supply. The gut is not therefore anatomically or physiologically "true"; worse, it deteriorates with time, often to an unknown extent during the course of an experiment. In situ methods equate drug loss from the gut lumen with absorption - an assumption that may be in error if significant amounts of drug are either metabolized or accumulated in the gut wall. However, from the point of view of using these models to investigate relative effects due to modification of formulations, these deficiencies are not too serious. However, the types of dosage form one can administer are limited. Many of these drawbacks may be overcome by the use of in vivo animal models.

IN VIVO MODEL SYSTEMS

The choice of suitable in vivo models is not as large as one might think. For example, the following criteria should apply in the selection of a suitable animal.

a) the gastrointestinal system should be similar to man
b) it should be possible to take multiple point plasma levels and/or urine samples over a prolonged period of time
c) the animal must be capable of repeated use, necessary for "crossover" studies
d) it should be possible to administer orally formulated drug products to the animal, as well as i.v. solutions. The opportunity to also administer drug directly into the intestine is an advantage
e) the size of the animal, the dose administered and the volume of sample taken should be compatible with available analytical methodology.

While there are well demonstrated differences between various animal species in terms of duration of drug response, these are very probably due to variations in the rate of excretion and/or metabolism.[35] Such differences are not too significant when using an animal model to study the bioavailability of various formulations. Of greater significance are such factors as the volume and surface area of the gut, rate and extent of peristalsis, pH conditions throughout the gut, acid capacity of the stomach, and gastric emptying time.

The relative advantages and disadvantages of the most commonly used animals are summarized below:

rats and mice - inexpensive; small, difficult to administer solid dosage forms, limited number of plasma and/or urine samples possible, low "cross-over" potential.

rabbits - gastrointestinal tract different from man, very slow emptying time, stomach not emptied by fasting.

monkeys - good models; difficult to handle unless properly restrained.

dogs - good models; potential to vomit.

mini-pigs - good models, expensive, large storage facilities required.

Based on the above considerations, the dog is probably the best routine choice. A useful modification is the Thiry-Vella dog, in which a U-shaped loop of intestine is isolated, both ends being made accessible from the exterior by passage through the abdominal wall. The *in situ* loop can be used repeatedly and provides good control capability and reproducibility. Using such a preparation, Sample *et al.*[38] showed that semi-quantitative predictions could be made of the absorption of acetaminophen in man.

Recently, Nayak and Benet[39] have described the use of unanaesthetized rhesus monkeys to follow the gastrointestinal absorption of drugs. By surgically implanting venous and arterial catheters, plus stomach and duodenal cannulae in the monkey, dosage forms can be administered and plasma levels monitored

under a wide range of experimental conditions. Undoubtedly, this represents the most sophisticated and comprehensive model system reported on to date with which to assess the bioavailability in man of orally administered drugs.

NOTES

Presented, in part, at a symposium on Formulation and Biopharmaceutics, Leura, NSW, Australia, August 1975.

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