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Review

Ex vivo and in situ approaches used to study intestinal absorption

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ABSTRACT

Over the recent years, intestinal absorption has been recognized as a critical factor affecting the bioavailability of oral drugs. Intestinal absorption is affected by many factors including the physicochemical property of the drug, the absorption mechanisms, and the need for absorption enhancers. Ex vivo and in situ methods have been used extensively to evaluate the intestinal absorption of new drugs. Biological performance can be obtained rapidly and reliably using these techniques. However, these approaches have many inadequacies which need to be recognized so that appropriate adjustments can be made to the methodology. These shortcomings also need to be accounted for during the interpretation and application of the results in vivo situations. This review describes ex vivo and in situ models of drug absorption, and compares their relative advantages and drawbacks to assist researchers in selecting appropriate models for different drug and therapeutic situations.

Contents

1. Introduction

Bioavailability is commonly defined as the extent and rate at which a drug becomes available in the general circulation (Mannhold, Kubinyi, Folkers, Waterbeemd, & Testa, 2009). It is one of the most important aspects of the drug development process, especially for natural compounds or new molecular entities with significant biological activity (Smith & O'Donnell, 2006). Bioavailability following oral administration is affected by factors such as dissolution, transit time, enzymatic transformation in digestive juice, intestinal permeability, biotransformation by intestinal flora tract, and gastrointestinal and hepatic metabolism (Testa & Waterbeemd, 2008). Among these factors, intestinal permeability is a principal determinant of drug absorption following oral administration. Indeed, transport across the intestinal barrier is a prerequisite for the clinical effect of most drugs (Amidon, Hussain et al., 2002), and, intestinal permeability is used for classifying drugs in the Biopharmaceutics Classification System (BCS) (CDER, 2000).

Numerous methods have been used to evaluate intestinal absorption early in the process of drug development in order to exclude new

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compounds with poor absorption properties (Holmstock, Annaert, & Augustijns, 2012). The perfusion technique of Loc-I-Gut is regarded as the 'gold standard' for evaluating human intestinal absorption. However, experimental models in small animals are routinely used as they are easy to undertake. Ex vivo and in situ models in small animals are also widely used when there is a good agreement between intestinal permeability results in rats and human subjects. Transcellular in vivo absorption properties of new chemical entities are usually determined early during the drug discovery process, together with the evaluation of the new drug's physico-chemical properties. In vitro techniques include simple artificial or biological membrane systems, or assays based on biological cell layers (e.g., Caco-2 cells, IAM, and PAMPA) (Clere, Desangle-Gouty, Genty, González, & Legendre, 2001; Gubernator, Kansy, & Senner, 1998). These techniques enable rapid determination of artificial or biological membrane permeability of drugs, making them suitable for high-throughput drug screening (Holmstock et al., 2012). However, differences between predicted passive permeability and observed permeability emphasize the need to assess absorption in vivo in order to obtain more complete knowledge of the transport mechanism(s) involved in the absorption process (Amidon, Hussain et al., 2002).

This review describes and evaluates ex vivo and in situ methods which are used to replicate the in vivo situation and allow more accurate prediction of human intestinal drug absorption (Table 1). Our objective is to present scholars with an overview of methods available for estimating intestinal drug absorption, and to facilitate the selection of the optimal model for drug investigation in late discovery and early development stages.

2. Ex vivo methods

Ex vivo methods provide a theoretical means of estimating human intestine absorption. They include intestinal perfusion, everted gut sac (Fig. 1), and Ussing chambers. Ex vivo models have a number of distinctive features that separate them from the in vitro Caco-2 model. Adequate paracellular permeability is provided by the small intestinal epithelium, a mucus layer is present in the model, and there is also expression of transport proteins and drug metabolism. However, results from ex vivo sometimes inappropriately estimate the degree of oral absorption, due to the interruption of normal blood flow and the lack of a nervous system. For example, investigation of an ester prodrug using ex vivo methods, failed to provide evidence of significant transport enhancement (Annaert et al., 2000). Despite these shortfalls, ex vivo methods are simple, and widely used in the design and testing of potential new drugs.

2.1. Intestinal perfusion

Since the experiments of Salvioli, numerous procedures have been devised to study the intestinal absorption and metabolism using isolated perfused intestinal segments (Parsons & Prichard, 1966). The surviving intestine preparation is set up apart from the body and has a viable mucosa. Pig intestine was used in early experiments and carbohydrate absorption has been studied from viable specimens of human intestine obtained at operation (Mansford, 1965). Krebs's bicarbonate saline, Krebs's phosphate saline, Tyrode's solution or Ringer–Locke's solution was used as perfusates, and was sampled at predefined time intervals.

Table 1
Strengths and limitations of each method, along with the earliest date of use.

	Strengths	Limitations	Earliest date of use
Ex vivo intestinal perfusion	The model has a viable mucosa, and it is a quick, simple technique for estimating of intestinal drug transport.	Tissue viability and integrity of the intestinal respiratory system have a marked effect on the results. And the barrier imposed by the intestinal wall and serosa may result in slower absorption rates than those obtained in intact animals.	R. Fisher and Parsons (1949)
Ex vivo everted gut sac experiments	The model is simple, and it is very useful for predicting the extent of transfer and intestinal metabolism of drugs.	The everted intestinal sacs gradually lose structural integrity.	Wilson and Wiseman (1953)
Ex vivo Ussing chamber	The method is well validated and it can be used to study the permeability of drugs that are poorly absorbed, the absorption mechanisms of different compounds, the drug–drug interactions, and drug transport processes.	The Ussing chamber method appears to be unsuitable for evaluating ester prodrugs. And it is also not suitable for use with rabbit tissues as the duodenal and jejunal sections are too thick for the diffusion chambers and leaks are observed.	Ussing and Zerahn (1951)
In situ closed-loop method	It enables intestinal absorption to occur at body temperature for an appointed time. The model also allows absorption to be measured separately at different regions of rat intestine, jejunum, ileum and colon. And it avoids the uncertainty of gastric emptying time.	The procedure does not allow estimation of absorption at steady state. It is also necessary to undertake a large number of experiments before statistically significant results can be obtained. And the operative procedure is complex.	Gibson and Wiseman (1951)
In situ Thiry–Vella fistula	It enables intestinal absorption to be studied in conscious animals with an intestine maintained at near normal physiological conditions.	It requires sophisticated surgical procedures and instrumentations.	Clarke et al. (1951)
In situ intestinal single-pass perfusion	It significantly reduces the number of animals utilized and animals act as their own controls for analyzing segmental-dependent membrane permeability.	It requires sophisticated surgical procedures and instrumentations.	Brodie et al. (1958)
In situ intestinal recirculating perfusion	It can magnify the concentration changes, which is suitable for studying drugs which are absorbed slowly.	It requires sophisticated surgical procedures and instrumentations.	Brodie et al. (1958)
In situ intestinal perfusion with venous sampling	It is a useful method for obtaining realistic drug absorption rates, and it allows the determination of intestinal metabolism without interference by the confounding effects of hepatic first-pass metabolism.	It requires sophisticated surgical procedures and instrumentations.	Kavin et al. (1967)
In situ vascularly perfused intestine–liver	It allows the investigation of the hemodynamics and metabolism for each organ, as well as the inter-relationships between the small intestine and liver.	It requires sophisticated surgical procedures and instrumentations.	Cherry et al. (1985)
In situ Loc-I-Gut	It is an accurate method that provides direct estimates of local drug absorption in human subjects. And it is not influenced by other gastrointestinal factors such as transit time and regional pH-differences.	It requires sophisticated surgical procedures and instrumentations.	Knutson et al. (1989)