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Intestinal Absorption Models

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Review Article

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ABSTRACT

The intestinal epithelium is the barrier which regulates the entry of xenobiotics and nutrients. Understanding of the absorption and metabolism of those materials at the intestinal mucosal level is very important because it regulates the bioavailability of those substances. Any xenobiotic entering the systemic circulation has to pass through the epithelial layer, section of the lamina propria, and the wall of the respective vessel. It is important to select suitable model for understanding the rate limiting step in the absorption process.

INTRODUCTION

Absorption models are broadly grouped into three i.e. *in vivo*, *in situ* and *in vitro* models. The choice of model depends totally on the properties of the xenobiotic being screened [1-12].

In vivo Models [13-15]

Advantages

In vivo models can integrate the dynamic components of the mucous layer, the mesenteric blood circulation and all other factors that can alter drug dissolution.

Cassette dosing studies can be performed to test large number of products (high throughput screening [HTS)],

Disadvantages

It is not possible to separate the variables involved in absorption process, i.e. identification individual rate-limiting factors is not possible.

In situ Models[16-20]

Advantages

Can integrate permeation and metabolism aspects

All physiological factors that influence absorption are present

Absorption at particular region of the intestine can be studied

Direct effects of the drug on intestinal absorption can be studied

Secretion of substances into intestinal lumen by P-gp etc. can be studied

Disadvantages

Involves surgical procedures with anaesthesia, and anaesthesia have effects on intestinal drug absorption. Hence precautions should be taken while selecting the anaesthesia.

The rise in luminal hydrostatic pressure during absorption studies at particular sites can influence intestinal permeability.

***In vitro* Models** [21-32]

Advantages

Availability of all intestinal cell lines (like caliciform cells, enterocytes, and lymphocytes) which can be used to study formulation effects, regional differences in permeability and intestinal metabolism/stability

Rapid and simple model

Can be used to study transport mechanisms

The test drug can be exposed to either apical or basolateral surfaces

Disadvantages

No physiological factors that influence permeation (bile salts, cholesterol, mucous)

Some of them have cancerous origin

Difficult to estimate the influence of P-gp

Hence each of these has their own advantages as well as disadvantages and is equally important in absorption studies. In this review we discuss about a few very commonly used models to study intestinal absorption [33-56].

COMMONLY USED INTESTINAL ABSORPTION MODELS

Rat Gut Sac

Rat gut sac model can be performed in two ways the everted gut sac model and the non-everted gut sac model, the former being more preferable. Small intestine is isolated from the anaesthetized rat and the intestine everted with the help of a glass rod, and placed in physiological solution containing the drug. Samples are collected from both sides of the intestine at regular time intervals and the drug diffusion rate is determined.

This model is used in determination of kinetic parameters with high reliability and reproducibility. Tissue can be maintained viable for up to 2 hours by following specific preparation techniques and using oxygenated tissue culture media. It can be used to study drug transport into the epithelial cells and across the intestine, by using sensitive detection techniques like use of radiolabelled compounds.

Earlier it is used to study the transport of macromolecules and liposomes but now it is being used to mostly to quantify the paracellular transport of hydrophilic molecules, and to estimate the effects of potent enhancers on their absorption. The apparent permeability (Papp) of mannitol a paracellular marker is 1.5×10^{-5} to 1.7×10^{-5} cm/s. This value is identical to the values reported with low-molecular weight hydrophilic drugs in human experiments. Much higher permeability value is found with molecules that cross the epithelial barrier by a transcellular route and can be accurately quantified using the everted sac system. Absorption at different regions in the small intestine and colon can be measured with ease. It can also be used for estimating the first-pass metabolism of drugs in intestinal epithelial cells.

Major drawback of this method is the presence of the muscularis mucosa, hence this model cannot reflect the actual intestinal barrier, because the drugs has to pass from the lumen into the lamina propria and through the muscularis mucosa **(Figure 1)** [57-59].

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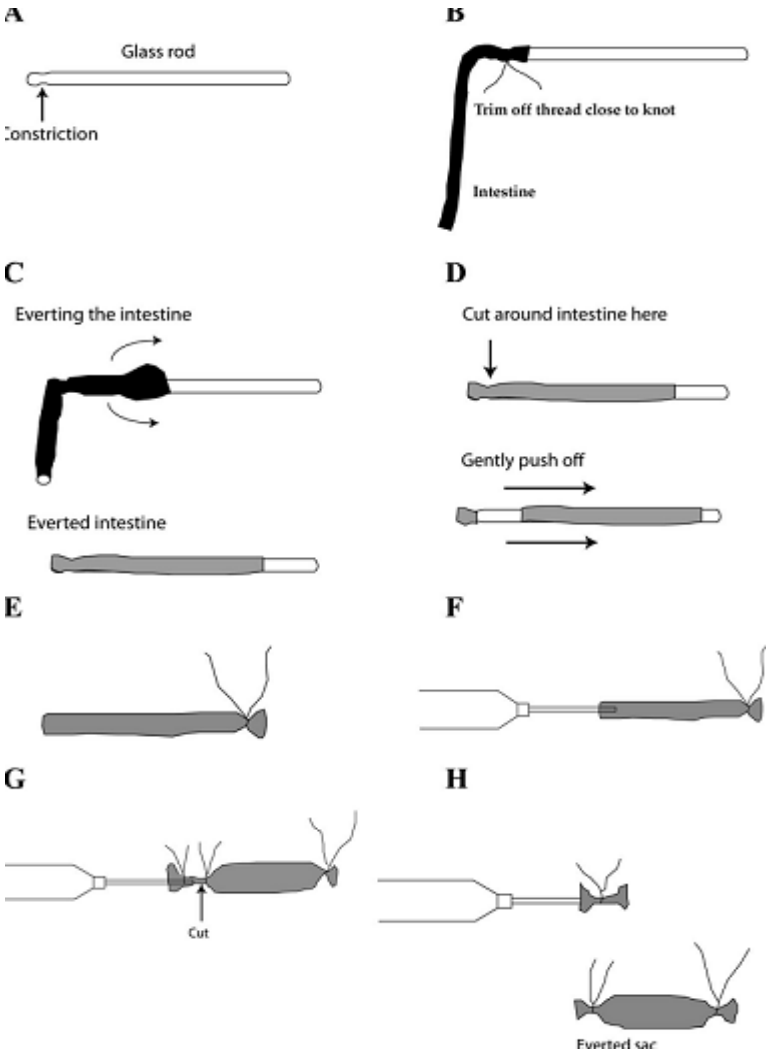

Figure 1: Schematic representation of gut sac eversion.

Intestinal Perfused Segments

Curran et al. proposed this model to study ion and water fluxes in the ileum of rats. In this method the perfusion tube and drainage tube are inserted into the proximal and distal intestinal segments, respectively by laparotomy. The drug solution is perfused through the intestinal cavity with the help of a peristaltic pump at a specific rate. The difference in drug concentration at the influx and the efflux is measured and is used to calculate the drug absorption rate and Peff, respectively (**Figure 2**).

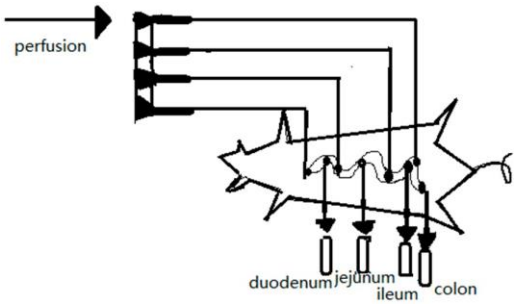

Figure 2: Schematic representation of intestinal perfused segments.

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Based on the patterns of perfusion, intestinal perfusion is divided into

Circular Perfusion Single-pass Perfusion

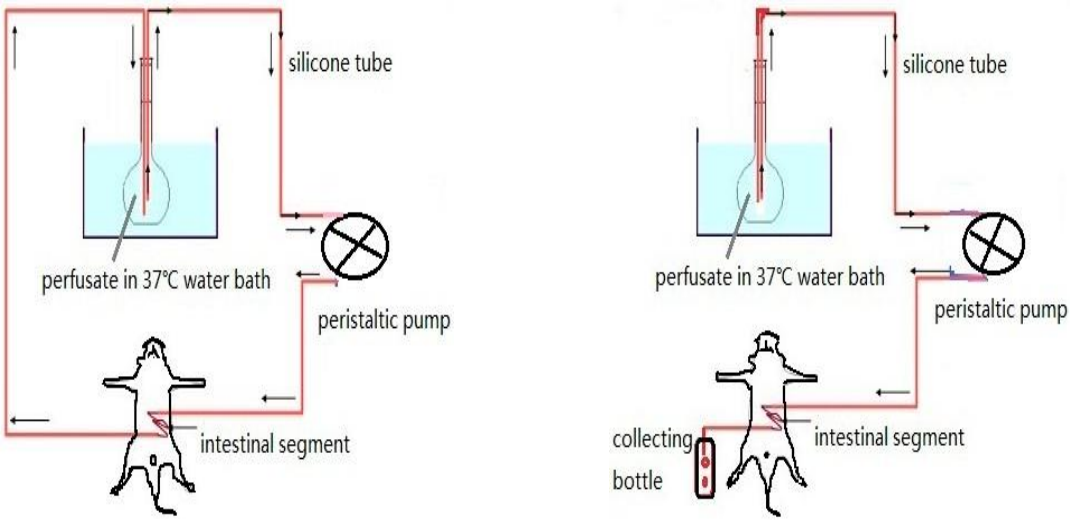

Figure 3: Schematic representation of circular perfusion and single pass perfusion respectively.

It is advantageous as work is carried out on an intact organ, with physiological cell-cell contacts and normal intracellular matrixes preserved. The major drawbacks are the short viability, use of anesthesia and amenability to changing physiological conditions **(Figure 3)** [60-65].

Ussing Chambers

Ussing and Zehran in 1951 first proposed this model in isolated frog skin to stud the active transport of sodium as a source of electric current in short-circuited skin. Later on, they were extensively used to study ion transport across different membranes.

Using chamber contains a receiving pool and a diffusion pool with test drug separated by human or animal intestines or mucous membranes. After the incubation period samples are collected from the receiving pool at fixed intervals and are replaced with fresh media maintained at 37°C, the collected samples are analyzed to determine the rate of drug absorption through the membrane.

This method is not only used to study intestinal transport but also used for intestinal metabolism studies. This method can be used to expose the drugs to apical or the basolateral surface of the enterocytes **(Figure 4)** [66-71].

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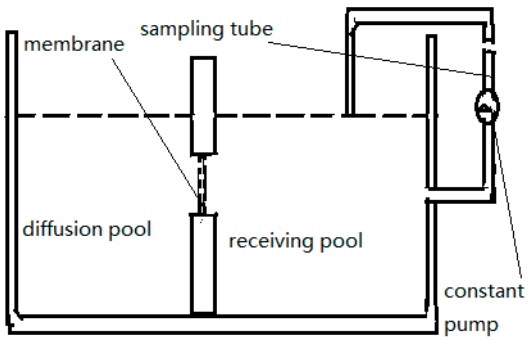

Figure 4: Schematic representation of Ussing Chamber.

Cell Models

Absorption mechanisms can be best studied in models containing only absorptive cells, without the interference of mucus, the muscularis mucosa and/or the lamina propria. Hence epithelial cell cultures are handy in drug transport mechanism studies. However, difficulty to culture and limited viability are the hurdles for the use of isolated intestinal epithelial cells.

Human cell culture systems were found to show loss of crucial *in vivo* anatomical and biochemical features hence attention has shifted over to human adenocarcinoma cell lines, like Caco-2 and HT-29, which reproducibly retained many characteristic of differentiated intestinal cells. Moreover sensitive and automated measurement techniques were parallel developed along with these cell lines.

These models are relatively simple, and are readily suitable for HTS and automated procedures. However as they lack *in vivo* physiological correlation of the data to the *in vivo* situation renders difficult [72-85].

Non-intestinal cell systems

Madin and Darby isolated Madin Darby canine kidney (MDCK) cells from a dog kidney. They are currently used to study drug metabolism, transport at the distal renal tubule epithelial level, toxicity and the regulation of cell growth [86-89].

Caco-2 cells

Caco-2 cells are popular cellular models used in permeability and transport studies. They are derived from human colorectal adenocarcinoma. During culture they differentiate themselves into polarised intestinal cells with tight junctions between adjacent cells and apical brush border, typical microvillar transporters and express hydrolases. This cell line is initially used to study intestinal epithelial differentiation, and later being used to estimate the relative contributions of transcellular passage and paracellular in drug absorption.

Though they are colonic in origin, they express most of the morphological and functional characteristics of absorptive cells of small intestinal, including phase I and phase II enzymes **(Figure 5)** [90-100].

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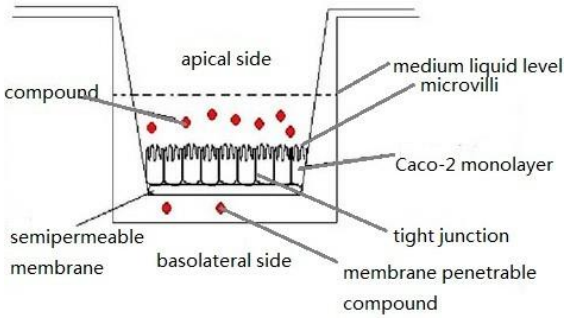

Figure 5: Schematic representation of Caco-2 cell monolayer

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