Physiological Considerations in Dosage Form Design

Ping-Yang Yeh*, Philip L. Smith

Pharmaceutical Development, GlaxoSmithKline Pharmaceuticals,
1250 South Collegeville Road, PO Box 5089, Collegeville, PA 19426-0989, USA

SUMMARY: Absorption across intestinal mucosal membranes is recognized as a rate-limiting step for systemic delivery of therapeutic agents following oral administration. Our understanding of the factors that limit transport across mucosal membranes has developed in part from *in vitro* methodologies. Such understandings are being further expanded with advances made in pharmacogenomics. Cloning and expression of drug transporters provide tools to drug discovery scientists for optimizing candidate selection. Our increased awareness of these physiological factors should provide the impetus for more efficient candidate selection and physiology-based dosage form design in the future.

Introduction

The unprecedented advances in drug discovery in the past decade have substantially enhanced our understanding of the biology underlying various diseases and the rationalization in the design of better therapeutics. The advent of molecular biology and, in particular, of genomic sciences is having a deep impact on pharmaceutical industry in terms of the way new medicines are discovered[1,2]. This change has been propelled by a variety of factors including: (i) advances in molecular biological approaches to isolate and evaluate gene sequences, which have allowed more rapid identification of potential new targets for human diseases[3-5]; (ii) development of combinatorial chemistry approaches to increase the number of chemicals which can be synthesized and evaluated[6,7]; and (iii) application of high throughput screening techniques to more rapidly identify lead compounds for progression into development[8]. Although these approaches promise to provide an increased number of novel medicines for evaluation in the treatment of a greater number of diverse diseases, realization of the potential benefit of these new therapeutics will only be achieved by successful formulation development of safe, convenient and physiological-relevant delivery system for these compounds[9,10].

The aforementioned advances in the drug discovery process have significantly increased the number of "drug-like" molecules. However, since these molecules are being selected through combinatorial chemistry and pharmacological screens, their physicochemical (e.g., solubility,

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lipophilicity, polymorphism) and pharmacokinetic (e.g., intestinal absorption, liver metabolism) properties are often less optimized. The consequence of moving compounds into development prior to evaluating their physiochemical and pharmacokinetic properties is often an increase in the complexity of the clinical trials. These issues are motivating the pharmaceutical industry to change the paradigm by which compounds are identified for development (Fig. 1).

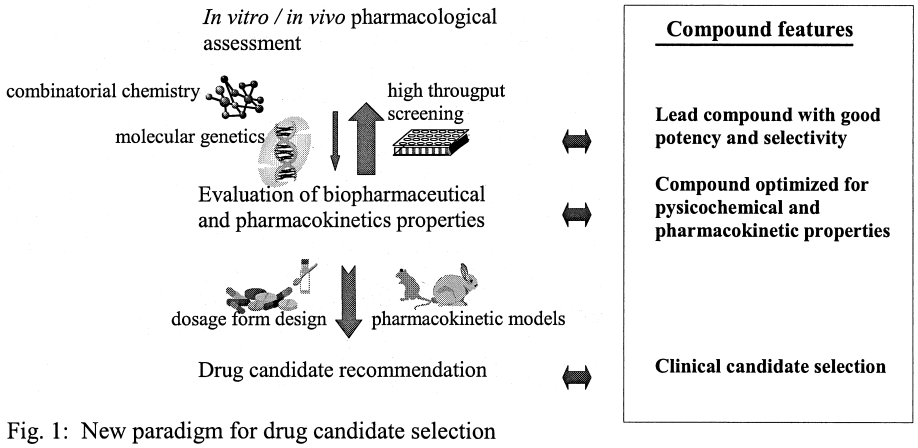


Fig. 1: New paradigm for drug candidate selection

Thus in addition to identifying molecules which are potent and selective at the putative pharmacological targets, compounds should also be evaluated for their developability (e.g., pharmaceutics, pharmacokinetics, safety) prior to being put into clinical trials. This often results in selection of a molecule which is not optimized for one attribute (e.g., potency, selectivity, absorption, metabolism, etc.) but is a compromise of all these properties which is selected to enhance its chances of success in clinical trials. In this presentation, attention is being paid to oral delivery since this remains the most preferred route of administration, particularly for chronic therapy. It is, however, fully recognized that alternative routes of administration (e.g., parental, pulmonary, transdermal, nasal, buccal) are being continuously developed for specific therapeutic applications.

Gastrointestinal physiology

The gastrointestinal tract presents a complex and heterogeneous environment with different segmental functions serving various physiological requirements (see Table 1). On the other hand, these regional differences also provide various unique opportunities for the pharmaceutical scientists to tailor mechanisms into dosage forms through which the drug release is controlled (e.g., when, where, at what rate).

Table 1. Characteristics of human gastrointestinal tract[11)]

Section	Length (m)	Surface area (m^2)	pH	Transit time (h)
Stomach	0.2	0.1	1-2	0.5 - variable[a]
Small intestine	7	120[b]		4-5
duodenum	0.3	0.1	6.6	-
jejunum	3	60	7.4	-
ileum	4	60	7.5	-
Large intestine	1.5	0.3	6.7	12-24

[a] Depending upon fasted or fed state. [b] This number would be even larger (4500 m^2) when the microvilli covering the mucosal cells are taken into account.

For a dosage form designed for drug absorption within the gastrointestinal tract, there are a variety of morphological and physiological characteristics which can influence the performance of a dosage form. The first to be considered is regional pH. This can affect the lumenal ionization state (or lipophilicity) of the drug and hence membrane permeability and solubility. The second is the transit time through the stomach, small intestine and large intestine. Transit time can affect the systemic drug level if there exists an "absorption window" for drug absorption. The third is regional differences in lumenal digestive factors, such as bile salts, which can affect the solubility and/or dissolution of the drug and stability of the formulation. The last to be exemplified is the presence of epithelial transporters which can be involved in the active uptake or secretion of a drug. All these various factors are important when considering dosage form design. Oral dosage form design has traditionally been focused on the physicochemical aspects of the formulation, such as salt form, excipients, polymorphism, particle size, stability, dissolution rate, etc. These aspects, while very important to consider in formulation development, only account for the means to "present" drug in a soluble form to the surface of mucosal membranes. It has long been recognized that absorption across intestinal mucosal membranes is often the rate-limiting step for systemic delivery of therapeutic agents, particularly for oral drug delivery.

Mechanisms of transepithelial transport

The intestinal epithelium is the first barrier encountered following oral administration. A drug molecule must traverse this barrier before it can reach the systemic circulation. Transport of drug across the intestinal epithelium can occur by a variety of pathways including both the paracellular and transcellular routes[12)]. These are presented in Fig. 2. The passive paracellular pathway (Fig. 2) is the junctional aqueous pores between adjacent cells. Transport of drug molecules via the paracellular pathways takes place in these aqueous channels. It has been reported that in man, the equivalent pore diameter is between 6-8 Å.

However, pore diameter varies with disease and along the length of the gastrointestinal tract[13,14]. The paracellular pathway is not considered a high-capacity pathway since it accounts for less than 1% of the total surface area of the intestine[13]. A variety of approaches for enhancing absorption through this pathway for hydrophilic or large molecules, such as peptides and proteins, have focused on opening or regulating these junctional pores[15-17]. These approaches, however, have met with limited success. In general, delivery of a compound through the paracellular route will be successful only if the compound is low-dose (i.e., with high potency) and has an appropriate molecular size and hydrophilicity to allow passage through the aqueous pores.

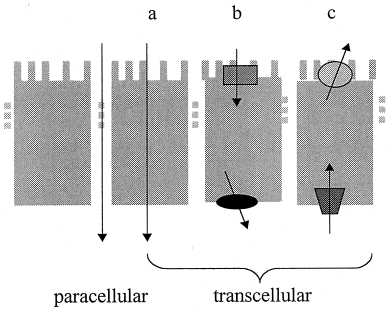


Fig. 2: Various drug transport pathways across intestinal epithelia

Another transport pathway is the passive transcellular route (Fig. 2a)[12]. For a compound to traverse this route, it must have sufficient hydrophilicity to interact with the lipid bilayers of the intestinal cell membrane. Passive transcellular transport is probably the most significant (high-capacity) pathway for transport of drug molecule across the intestinal epithelium because the surface area of cell membrane accounts for more than 99 % of the total surface area of the intestine. In addition, the passive transcellular route allows absorption of a compound along the entire intestinal length, unlike some carrier-mediated transport mechanisms that are localized within specific regions of the intestine (e.g., bile acid transporter in the ileum).

The two other transcellular transport pathways, depicted in Fig. 2b and 2c, are carrier-mediated transport pathways. Drugs transported via these pathways are mediated by membrane proteins. Membrane proteins which mediate absorption of drug from the intestinal lumen (Fig. 2b) include the proton-dependent peptide transporter[18], sodium-dependent sugar or amino acid transporters and bile acid transporters[13]. Several classes of drugs including antibiotics and ACE inhibitors are transported by the proton-dependent peptide transporters[18]. There are also amino acid carriers at the basolateral membrane[13]. In Fig. 2c, there are

membrane proteins, whose functions have been identified in the past few years, which mediate transport of drug from the intracellular or basolateral space into the intestinal lumen. These include P-glycolprotein on the apical membrance[19] and organic anions or cations on the basolateral membrane[20]. P-glycolprotein has been identified as participating in an important mechanism for limiting absorption of a variety of therapeutic agents[19]. It has been proposed to work in conjunction with cytochrome P-450 in limiting toxic agents from accessing the systemic circulation[21].

In vitro models for intestinal transport evaluation

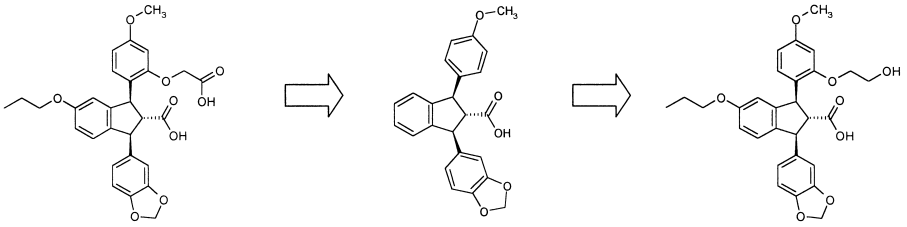
A number of physicochemical methods[22,23] and *in vitro* models[24] have been developed over the past decade as predictive tools to assess intestinal absorption of drug molecules in man. Among these, isolated intestinal tissues and intestinal cells[24] grown as confluent monolayers have been routinely employed by the pharmaceutical industry for the study of drug transport. These studies involve placing the tissue or cell culture monolayers in a diffusion chamber, and determining the transport of drug by introducing the drug into the solution on one side of the tissue and measuring its appearance on the opposite side. Viability and integrity of the tissue or cell monolayers can be evaluated by electric properties, biochemical markers, or transport markers[24,25]. Advantages of using tissues or cell monolayers include (i) ease of use, (ii) ability to study mechanism[26], (iii) positive correlation with human absorption[27], and (iv) the finding that they are amenable to constructing high-throughput screens[28].

Case Studies

Case studies to demonstrate the increased integration of pharmaceutical discovery and development have been recently presented[9]. In the following discussion, examples of the role of intestinal transport studies in preclinical evaluation and compound selection will be presented.

Case I: Passive transport

The first example is the identification of an orally active endothelin receptor antagonist. Endothelin-1 (ET-1) is a very potent vasoconstrictor. It has been reported that ET-1 plays a role in the pathophysiology of disease[29]. Development of nonpeptide endothelin receptor antagonists has been undertaken to alleviate the pathophysiological effects associated with endothelin interaction at its receptors. Potent and selective nonpeptide endothelin receptor antagonists have been developed (Fig. 3).



SB 209670 (0.003 cm/h) SB 202994 (0.172 cm/h) SB 217242 (0.095 cm/h)

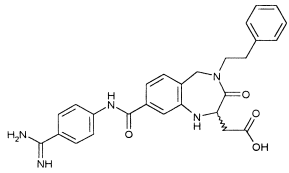
Fig. 3: Structure and *in vitro* permeability in rabbit distal colon of three endothelin receptor antagonists

However, when administered orally in animal models, the oral bioavailability was low[30] (3-5 %). To better understand the reasons for the low bioavailability seen with SB 209670, *in vitro* studies were conducted to evaluate intestinal permeability of this compound. These studies showed that the intestinal permeabilities of SB 209670 was very low in both rabbit intestinal tissues and caco-2 cells[30]. Additionally, the permeabilities of SB 209670 were similar in mucosal-to-serosal (m-to-s) and reverse (s-to-m) directions, suggesting that SB 209670 is transported across the intestinal epithelium by passive diffusion. An examination of the structure of SB 209670 reveals that it contains two carboxylic acid groups with pK_a of 3.9 and 5.6. It was, therefore, hypothesized that ionization of these groups could contribute to the limited permeability and oral bioavailability of SB 209670. Comparison of the permeability of SB 209670 with that of an inactive, monocarboxylic acid analogue, SB 202994 demonstrated that permeability in the presence of a single charged group was approximately 0.2 cm/h in rabbit intestine. Further support for the hypothesis that the presence of two charged groups impeded passage across the intestinal epithelium was provided by the finding that SB 217242, a highly potent monocarboxylic acid analogue of SB 209670, also has high intestinal permeability (about 0.1 cm/h). From a variety of studies performed with SB 217242, it was demonstrated that this compound traverses the intestinal epithelium via a passive transcellular pathway and has an oral bioavailability of 60-70 % in animals[30]. Thus, from these *in vitro* studies and from pharmacological evaluation, it was possible to identify a highly potent, orally bioavailable endothelin receptor antagonist, of which the characteristics for development, in terms of delivery and pharmaceutics, have been enhanced.

Case II: Carrier-mediated efflux

The second example of the role of intestinal transport studies in preclinical evaluation is the development of orally active vitronectin receptor ($\alpha_v\beta_3$) antagonists. The $\alpha_v\beta_3$ receptor is a

member of the integrin family of the transmembrane heterodimeric glycoprotein complex, which function in cell-cell and cell-substrate adhesion and communication[31]. The integrin $\alpha_v\beta_3$ is expressed on the surface of a variety of cell types, including osteoclasts, vascular smooth muscle cells, endothelial cells, and tumor cells, and has been shown to mediate several biologically relevant processes, including adhesion of osteoclasts to the bone matrix[32], vascular smooth muscle cell migration[33], and angiogenesis[34]. Consequently, $\alpha_v\beta_3$ antagonists are expected to have utility in the treatment of osteoporosis, restenosis, and diseases involving neovascularization, such as cancer[35]. Initial studies focused on the development of nonpeptide mimetic antagonists and resulted in the identification of compounds such as SB 207448 (Fig. 4).



SB 207448 (< 0.001 cm/h)

Fig. 4: Structure and *in vitro* permeability in rabbit intestinal tissues of a vitronectin receptor antagonist

This compound showed very poor intestinal transport (e.g., permeability < 0.001 cm/h). In further studies, it was found[26] that at physiological pH, the positively charged amidine group is the major contributing factor to the limited permeability of SB 207448 and modification of this functional group should therefore have a positive influence on the permeability of this class of compounds. In subsequent studies, highly potent compounds of similar structure and with less basic terminal amino groups were synthesized[36] (Fig. 5).

Despite the reduction in basicity of the terminal amino groups, SB 223245 also has low permeability in rabbit intestinal tissues. Because SB 223245 is still a zwitterionic compound, the carboxyl group was methylated to produce an ester, SB 223243. SB 223243 has high permeability in the s-to-m direction but, surprisingly, poor permeability in the m-to-s direction.

These results suggest that by modifying the structure to enhance permeability, the compound has become a substrate of the carrier-mediated efflux system. The presence of a carrier-mediated efflux process for SB 223243 could limit intestinal absorption and also contribute to other development issues, such as drug-drug interactions, variability in oral bioavailability,

and food effects[37]. This result highlights the importance of understanding the mechanisms underlying intestinal transport and the use of such information for drug candidate selection.

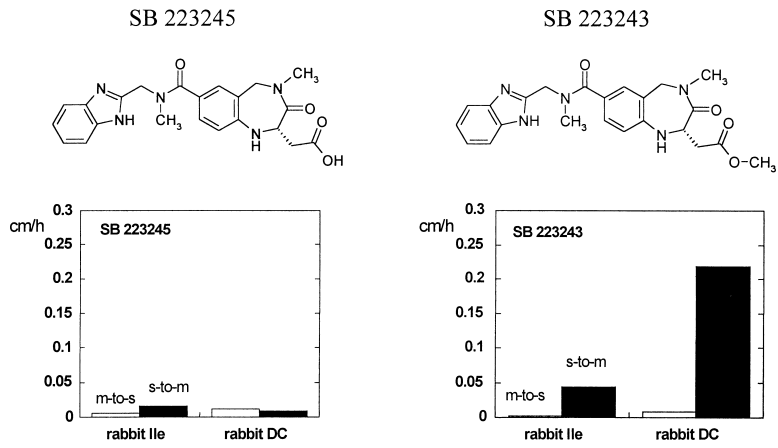


Fig. 5: Structure and *in vitro* permeability in rabbit intestinal tissues of two vitronectin receptor antagonists

Conclusions

As can be seen from the above discussions, integration of intestinal permeability screens early in the discovery process has provided an approach to identify and eliminate issues that previously would not have been observed until late in the process of development. *In vitro* methods could also aid in assessment the influence of drug excipients on basic intestinal physiological functions to provide an understanding of the development of safe formulations. Further advances in analytical methods, molecular biological approaches, and computational modeling should provide the increase in capacity required to meet the needs in the future.

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