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Spiral progression in the development of absorption enhancers based on the biology of tight junctions ☆

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ABSTRACT

Epithelium covers the body and, therefore, separates the inner body from the outside environment. Passage across the epithelium is the first step in drug absorption. Tight junctions (TJs) seal the space between adjacent epithelial cells and prevent the free movement of solutes through the paracellular space. Modulation of the epithelial barrier is the most important strategy for enhancing drug absorption. Development of the strategy has accelerated with progress in understanding of the biology of the TJ seal. The first-generation absorption enhancers were screened on the basis of their absorption-enhancing activity in vivo. However, TJs were not well understood initially. The identification of TJ components, including those based on occludin and claudins, has led to the development of new strategies for drug absorption. Accumulation of knowledge of claudins has provided new insights into the paracellular transport of drugs. This review examines the relationship between advances in understanding of TJ biology and paracellular transport of drugs and discusses progress in the development of mucosal absorption enhancers.

Contents

1. Introduction

The philosopher Hegel proposed that "change moves in spirals, not circles." In other words, things of the past will reemerge with progress.

Drugs are administered by routes that include oral, nasal, pulmonary, and epidermal routes or injection. Noninvasive routes are better than invasive ones, but drug administration via the nasal, pulmonary, and epidermal routes requires specialized equipment.

Abbreviations: TJ, tight junction; EDTA, ethylenediaminetetraacetic acid; AJ, adherens junction; DS, desmosome; GP, gap junction; CPE, *Clostridium perfringens* enterotoxin.

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These noninvasively administered drugs are expensive. Drugs administered orally without using specific equipment are the least expensive. Patients are often compliant in taking oral medication because this method of drug delivery is easy and pain-free. Therefore, orally administered drugs are ideal for drug development. However, the intestinal mucosa functions as a biological barrier, separating the outside environment from the inner body and preventing the free movement of solutes. Modulation of the epithelial barrier must be considered in the development of orally administered drugs.

The first report of mucosal absorption of drugs was published approximately 50 years ago [1]. Ethylenediaminetetraacetic acid (EDTA) was shown to enhance intestinal absorption of heparin and synthetic heparinoids, but the mode of action was unclear. Chelation of magnesium and calcium by EDTA was later shown to increase intestinal permeability to solutes, but the mechanism was not clarified [2]. EDTA was found to widen the intercellular junctional seal, enhancing intestinal permeability to solutes [3]. This was the first evidence that modulation of the intercellular seal in the mucosal epithelium may be a potent strategy for mucosal absorption of drugs.

The first breakthrough in the biology of the epithelial barrier was the identification of tight junctions (TJs) between adjacent epithelial cells, in 1963 [4]. This finding promoted the development of novel strategies for mucosal absorption of drugs by modulating the TJ seal. Development has progressed further with our understanding of the biology of the epithelial barrier.

Strategies for mucosal absorption of drugs are developing, accompanied by advancements in understanding of the biology of epithelial cells. This review describes the development of strategies for mucosal absorption of drugs as a result of the accumulation of knowledge of the epithelial barrier. We also describe the future directions in research on mucosal absorption of drugs.

2. First-generation mucosal absorption enhancers

The first advancement in the biology of the epithelial barrier was the discovery of the TJ (Table 1) [4]. High-resolution replica analysis revealed that TJs form a continuous band-like meshwork. Globular components bridge the width of the adjoining membranes linked together in the plane of the intercellular space [5]. However, it was unclear whether the TJ components are proteins or lipids. Membrane lipids, rather than membrane proteins, were thought to be the structural elements of TJ seals [6,7]. Therefore, the initial stage of the development of novel strategies for mucosal absorption of drugs was based on the knowledge that the intestinal mucosa is a barrier for drug absorption and that TJ components are responsible for intercellular sealing, but no molecular level information about TJ components was available.

The first advancements evoked the idea that disruption of the mucosal epithelial barrier or TJ seal would lead to the development of novel strategies for mucosal absorption of drugs (Table 2). Nonionic, anionic, and cationic surfactants are also intestinal absorption enhancers [8,9]. These surfactants were believed to disturb the integrity of the plasma membrane [9]. Surfactant–surfactant interactions occur at high surfactant concentrations in the plasma membrane and can result in dissolution of the plasma membrane into surfactant–membrane mixed micelles. Surfactants can also extract proteins from the plasma membrane. Surfactant-enhanced membrane permeability is generally assumed to be nonspecific and cytotoxic [9]. Some surfactants, such as polyoxyethylene esters and dodecylmaltoside, exhibit absorption-enhancing effects and toxic effects in the intestine [10–13].

EDTA, a calcium chelator, enhances mucosal absorption of drugs [1]. EDTA modulates TJ barrier integrity by opening intracellular TJ seals [14]. Some surfactants also enhance intestinal absorption by sequestering calcium ions [10].

Various fatty acids, including caprate, caprylate, and laurate, enhance membrane permeability [15]. Mucosal absorption of insulin and cefmetazole is increased with 1% caprate treatment, but only absorption of insulin is enhanced by treatment with 0.25% caprate. One possible explanation for the differential effects on mucosal absorption of drugs was that the electrically repulsive effects of the paracellular route might affect paracellular absorption of a neutral molecule, insulin, and an acidic molecule, such as cefmetazole [15]. This finding indirectly suggested that modulation of the paracellular route can lead to mucosal absorption of solutes in a solute-specific manner. A series of analyses aimed at determining the mode of action of sodium caprate indicated that the compound activates phospholipase C, elevates intercellular calcium levels, and subsequently stimulates contraction of calmodulin-dependent actin–myosin filaments, thereby opening TJ seals [16,17].

Cationic chitosan increases epithelial paracellular permeability [18]. Chitosans bind to the epithelial cell membrane through a charge-dependent interaction, resulting in F-actin depolymerization and separation of TJ components. This event triggers enhanced epithelial permeability. Polylysine also enhances epithelial paracellular permeability by opening TJ seals [19].

There are two issues in the development of absorption enhancers: the toxicity of these substances and the risk of opening TJ seals. The first-generation absorption enhancers disrupted the cell membrane and modulated the TJ seal. Transient modulation of TJ seals by EDTA, fatty acids, and polycations would be less toxic than disruption of the cell membrane by surfactants. Opening the intercellular TJ seal might lead to the influx of solutes other than drugs, including undigested food, metabolites of intestinal microorganisms, and bile salts. Mucosal

Table 1
Progressive elucidation of TJ biology.

Year	Event
1963	Identification of TJ [4]
1973	Identification of TJ strands [5]
1982	Membrane lipid hypothesis [6,7]
1986	Identification of ZO-1 [96]
1993	Identification of occludin[24]
1998	Identification of claudin [33]
1999 onward	Clarification of TJ barrier function of claudins [47] Identification of paracellular ion transport via claudins [94]
2011	Identification of transcellular transport coupled to claudin-based TJ strands [95]

TJ: tight junction.

Table 2
Progress in the development of absorption enhancers.

Category	Enhancer	Possible mode of action
First-generation absorption enhancers	EDTA	Sequestration of Ca^{2+}[14][a]
	Surfactants	Perturbation of the plasma membrane [8,9]
	Sodium caprate	Phospholipase C [16,17][b]
	Chitosan	Depolymerization of actin [19]
Second-generation absorption enhancers	Occludin peptide	Perturbation of occludin [26–29][c]
	C-CPE	Binding to claudin-4 [47,50]
	FSH-fused occludin peptide	Perturbation of occludin in BTB [30]
	Claudin peptide	Binding to claudins and occludin [77]

EDTA: ethylenediaminetetraacetic acid; FSH: follicle-stimulating hormone; BTB: blood–testis barrier.

[a] Activation of protein kinase C is partly involved in modulation of the TJ barrier by chelation of Ca^{2+}[97,98].

[b] Activation of phospholipase C increases intracellular calcium levels, followed by contraction of calmodulin-dependent actin–myosin filaments and subsequent opening of the TJ-seal [16,17].

[c] Occludin peptides cause a decrease in the cellular content of occludin or perturbation of localization of occludin [26–28]. An occludin peptide interacts with occludin and claudin-1 [99].