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Exploring Intestinal Barrier Function And Drug Delivery

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ABSTRACT

In recent years, significant efforts have been made to understand oral drug absorption mechanisms and to develop new research models. These studies have been conducted using both in vitro and in vivo models and play a crucial role in determining the rate and extent of drug absorption in the intestines. Specifically, the permeability value (Peff value) is a common measure used to assess drug intestinal passage. Today, there is an increasing need for reliable gastrointestinal absorption models that can be used in preclinical studies to develop new drugs and appropriate dosages. However, collaboration and integration across different disciplines are important for further advancements in this field. Strengthening connections between pharmaceutical, biopharmaceutical, biochemical, and physiological research areas can contribute to a better understanding of drug absorption mechanisms and biopharmaceutical progress. Particularly, the use of animal models that mimic human intestinal drug permeability and the role of human cell culture models in investigating drug absorption in the intestine are highlighted as significant steps in this regard. Such research enables a more detailed examination of intestinal drug diffusion processes, which are crucial for the biopharmaceutical advancement of pharmaceutical compounds. Understanding factors such as the interactions between drug molecules and membrane transport molecules that affect the intestinal Peff value can further advance the assessment and improvement of drug absorption processes.

Keywords: Intestinal, Peff value, diffusion processes, Drug absorption.

INTRODUCTION

In every period of history, people have used various methods to treat diseases. Drogs obtained from various living things and sources have increased in number in the light of various studies and researches over the years and have begun to be understood in terms of their effects and properties.As a result of the studies and developments in the field of medicine, the pharmaceutical sector has become increasingly important. Drugs have become one of the indispensable objects of human life. Medicine, along with its production relations and techniques, has developed and changed its shape. In the production phase, it met with the industrial revolution and mass production. Today, the pharmaceutical industry has grown and diversified on a global scale. With this diversification, monopolization has occurred in drug production. The robot, artificial intelligence and especially nano technology brought by the industry have developed. These developments have influenced and developed the chemical and pharmaceutical industry in its context. (Özer, M.T., 2019)In general, it is accepted

that drugs create their effects on living structures by connecting to or affecting some points specific to them. These cellular macromolecular structures that drugs interact with in the body are generally called receptors; These are of vital importance for the survival of the cell, and therefore of the living thing.Since drugs are rarely given directly to the place of action, the drug must be moved from the place of administration to the place of effect in order to observe the therapeutic effect. Reaching the systemic circulation, the drug is distributed through the blood not only to the place of action, but also to all organs in the body. After acting on the target organ, it is removed from the body through the excretion organs. (Tutor, H., 2018)Although there are many parameters of the mechanism of absorption, distribution, excretion of the drug, intestinal permeability is one of these parameters. It is important to know the kinetics of these mechanisms. With various experiments, these parameters; It can be observed how it follows a path in changes such as different drug active ingredient, different disease, health status of the person.Pharmacokinetics describes the absorption, distribution, metabolism and excretion of drugs from the body.The passage of drugs into the systemic circulation by the route they are administered is called 'absorption'. 'Bioavailability' is defined as the ratio of the drug dose in the systemic circulation. Intravenous drugs have full bioavailability because they participate directly in the systemic circulation. Non-intravenous drugs pass through a series of biological membranes before participating in systemic circulation, so only some of them are involved in systemic circulation. After oral administration, some of the drugs undergo a first-pass effect in the liver, and this effect reduces absorption.Another important barrier for absorption is the gastointestinal (GI) mucosa. Factors such as intestinal and hepatic cytochrome (CY) P450 enzymes or presystemic metabolism associated with intestinal bacteria, transport activity such as the amount of serum albumin associated with the transport of the drug in the blood, and the balance between drug intake and excretion also affect bioavailability. (Sarı and Ersoy, 2016) The composition of intestinal fluids that affect absorption and intestinal permeability vary during childhood. The absorption of drugs administered orally is affected by these factors. (Work and other.,2017)

The epithelial lining of the gastrointestinal tract forms a regulated, selectively permeable barrier between the contents of the lumen and the underlying tissue compartments. Permeability along the epithelium is determined, in part, by the velocity-limiting barrier of the paracellular pathway, the most apical intercellular linkage, called the tight coupling (TJ). TJ consists of a multiprotein complex attached to the underlying apical actomyosin ring. TJ structure-function, and therefore epithelial permeability, is influenced by a variety of physiological and pathological stimuli (Nusrat et al., 2000).Intestinal permeability is the property that dissolves between the lumen and tissues and allows the exchange of fluids; intestinal barrier, on the other hand, refers to extracellular barrier components, such as mucus. Furthermore, the TJ barrier function and modification of paracellular permeability are dynamically regulated by a variety of extracellular stimuli (Lee, 2015).The intestinal barrier is a complex structure that separates the internal environment from the lumen environment. Physically, the barrier includes cellular and stromal components from the vascular endothelium to the epithelial cell lining, and the mucus layer, which consists of a gel with the interaction of various mucosal secretions, namely mucins, alfalfa peptides and surfactant lipids. In addition to physical disability; There is a chemical barrier consisting of digestive secretions, antimicrobial peptides and other cell products (cytokines, inflammatory mediators, etc.). The gut microbiota can also be considered a barrier. Finally, immune function and mobility contribute to the barrier (Cummings et al., 2004).

Factors Affecting Intestinal Permeability

The dynamic structure of tight junctions allows the passage of nutrients, water and ions into the blood. At the same time, it does not allow harmful external factors to enter the body and the permeability of this tight junction can be modified by the action of hormones, cytokines, bacterial toxins and drugs (Ateş, 2013).In cases of hunger-satiety; in hormonal or neurological signals; in the presence of mast cell secretions, inflammatory mediators, bacterial or viral pathogen-induced stimuli; physiologically or pathologically, tight ligaments open and close and change intestinal permeability (Kocakaya, 2009).Pathogenic bacteria and viruses can cause intestinal infection and altered intestinal permeability. In the presence of intestinal pathogenic Escherichia coli, Occludin, Claudin-1 and Zonula Occludens-1 proteins, which are located among intestinal epithelial cells, are progressively reduced, resulting in barrier dysfunction.To summarize, intestinal permeability is affected by bacterial toxins, cytokines, growth factors and various foods. (Paradise, 2018)

Intestinal Permeability Assessment Methods: Many methods are used for the detection of intestinal permeability, the common features of the substances used in these methods are; non-toxic, soluble in water, has a small molecular structure, does not carry an electrical charge, is not metabolized in the intestine and does not collapse into the tissue where it is located when absorbed, is collected in the kidneys and excreted in the urine, and is easily separated from similar endogenous structures and the amount is reliably determined (Kocakaya, 2009).

Paracellular Material Transition Kitsm: The permeability of a specific area in the gastrointestinal tract can be evaluated with probes specific to the investigated area; Examples include sucrose, lactulose, sucralose, crEDTA (Cennet, 2018).

FITC-dextran and FITC-inulin: From fluorescently labeled compounds; fluorescent isothiocyanate (FITC)-dextran and fluorescent isothiocyanate (FITC)-inulin are used as markers in paracellular permeability (Jimison et al., 2012).

51Cr-EDTA Clearance: Chromium-Ethylenediaminetetraacetic acid (51Cr-EDTA) spends the longest time in the gastrointestinal tract in the colon in proportion to the highest water absorption and is therefore indicated to indicate colon permeability, and its detection is achieved by giving 3.7 mBq 51Cr-EDTA orally and monitoring the rate of passage into the blood (Jenkins et al., 1992).

Sucrose : Sucrose, which acts distally in the gastroduodenal region and is broken down by the Sucrase-Isomalase enzyme, is used to determine the permeability of that region (Meddings et al., 1993). Sucrose natural sugar is the probe used for the evaluation of the gastroduodenal area (Arrieta et al., 2006).

Sucralose: Artificially created sucralose is used to assess colonic permeability (Arrieta et al., 2006).

Ramnose: Rhamnose, which has a monosaccharide structure, shows the permeability of the small intestine since it is destroyed by the bacteria in the colon, but this method, which evaluates the amount of sugar in the urine with HPLC, decreases in reliability due to the breakdown of sugars in the lumen in case of overgrowth of intestinal bacteria (Kocakaya, 2009).

Lactulose and Mannitol: Oligosaccharides Lactulose and Mannitol are used in the determination of small intestine permeability due to their fermentation in the large intestine (Kocakaya, 2009). Lactulose is the probe used for the evaluation of the gastroduodenal space (Arrieta et al., 2006). Mannitol is absorbed by paracellular transport (Bijlsma et al., 1995).

Polyethylene Glycol Polymers (PEG)

PEG's; There are many polymers such as PEG400, PEG600, PEG900, PEG1000 and PEG4000, and P400 is used to determine intestinal permeability by giving 5-10 g after a night fasting and looking at the amount excreted in the urine. However, it is not preferred due to the fact that the taste is unpleasant and unreliable results have been detected in intravenous use (Kocakaya, 2009).

Electron microscopy: Intestinal epithelial integrity is determined by monitoring the transition from the lumen to the basement membrane in electron microscopy by adding 1 mM of the element called lanthanium, which can seep under the epithelium and reach under the epithelium depending on the degree of tightness of the bonds between the cells (Kocakaya, 2009).

Caco-2' Cell Model: Cell lines formed from intestinal epithelial cells of humans and animals are used to evaluate permeability, and the Caco-2 (Colon Adeno Carcinoma Cell) cell line is formed from human colon adenocarcinoma as a reference model and the functional status of the intestinal barrier is evaluated (Artursson et al., 2001).

Measurement of Transepithelial Electrical Resistance (TEER)

TEER measurement is performed for experimental control of the integrity of intestinal epithelial cells and this measurement is specified as a system that evaluates ion mobility with voltmeter with electrode-like rods (Abbasi et al., 2009). The tight junction protein network is dynamic, changes occur in its structure in physiological and pathophysiological conditions, and these adaptations also affect the electrical resistance of the intestinal mucosa (Fasano, 2001).

Plasma Citrulline: Citrulline, which is produced by enterocytes differentiated from the amino acid glutamine in the intestine, has been shown in studies to be a biomarker in the quantitative evaluation of the integrity of the epithelical barrier; it does not reflect a reliable outcome in individuals with kidney disease (Cennet, 2018).

Intestinal Explant Model : In order to evaluate intestinal integrity and permeability, a device called Ussing Chamber is used, which provides culture media to the placed intestinal segments; however, the culture duration is short and does not allow the evaluation of long-term effects (Cennet, 2018).

Diamine Oxidase: Diamine oxidase (DAO), a type of endocellular enzyme, is found only in the cytoplasm of the intestinal stratum supravasculare villi in mammals. When intestinal epithelial cells are injured, endocellular DAO is released into the intestinal intercellular space, enters the lymphatic vessel and blood, and eventually results in stable high levels of DAO in the blood plasma. Therefore, the activity of DAO in the blood indicates the maturity and integrity of the intestinal mucosa (Nieto et al., 2000).

Claudin in urine-3: Non-invasive, the major obstructive Claudin-3 as measured in urine demonstrates protein loss in intestinal tight junction (Grootjans, 2010).

Evaluation of Tight Junction Proteins: Tight junction proteins involved in the differentiation and proliferation of epithelial cells act as a major barrier in the intestine by forming an anastomosis network close to the intestinal luminal section and inhibiting the paracellular transport of antigens located in the intestinal

cavity. Different techniques such as ELISA, Western Blot, Immunohistochemistry can be used in the evaluation of Tight Junction proteins. Among these methods, immunohistochemistry is more advantageous because it shows the dispersion in the tissue.

Local Inflammation Markers: The detection of proteins produced by neutrophils such as lactoferrin, calprotectin, elastase, which are markers of inflammation, in the stool is associated with intestinal permeability (Langhorst et al., 2005).

Zonulin : One of the toxins secreted by the Gram-negative bacterium Vibrio cholerae is zonula occludens (ZOT, Zonula Occludens Toxin), which binds to the receptor located on the apical membrane of the epithelial cell of the intestine and increases paracellular permeability. The receptor called Zonulin, to which the toxin is bound, has been indicated in studies to be located on the membrane due to its main physiological effects as well as causing such pathological results (Wang et al., 2000). Zonulin, which reflects the physiological activity of the receptor to which ZOT binds and binds to this receptor, is in the form of a single chain and acts on the separation of tight junction proteins from each other (Duerksen et al., 2010). In the study carried out on recombinantly produced pre-haptoglobulin 2 (Zonulin), it is stated that pre-HP2 has a function on intestinal permeability as well as hemoglobin stabilization (Fasano, 2012).

In bacterial infections and Celiac Disease, the increase in the amount of Zonulin produced in the lamina propria and released into the intestinal lumen affects the pathophysiology of intestinal permeability (El-Asmar, 2002; Fasano et al., 2000).

BIOAVAILABILITY

Bioavailability is a key pharmacokinetic parameter representing the fraction of an orally administered drug that reaches the systemic circulation in a no-load molecular form:

F = fabs X (1 - Eg) X (1 - Eh)

Here f is the bioavailability, and eg and eh are the extracted fractions in the intestinal wall and liver, respectively. The fraction of the absorbed dose (fabs) and the rate of absorption are largely determined by the following biopharmaceutical factors: dissolution, solubility, lumen stability (chemical and/or enzymatic), intestinal transit time and intestinal permeability component (API) of the active pharmaceutical substance. To achieve a sufficiently high systemic bioavailability, most drug products require pharmaceutical development to produce a plasma concentration-time profile that provides optimal pharmacodynamic response and acceptable side effects. These are formulated release (MR) products specifically designed to improve the pharmacodynamic response. In general, oral products with poor bioavailability (below f 25%-35%) are considered to have wider interindividual and interindividual variability in plasma exposure. (C.V. > 60%–120%) In 1996, Helriegel et al. He reported an inverse relationship between the bioavailability of oral drug products and the total variability of the bioavailability parameter. Now, more than two decades later, we know a little more about the causes of poor and highly variable bioavailability values for oral pharmaceuticals. However, for these dynamic processes to occur, we need to understand significantly more about the interactions between the advanced oral dosage forms of healthy subjects and patients of all ages, from newborn to elderly, and the complex and dynamic gastrointestinal (GI) physiology that is adequately understood. (Sjogren et al., 2014)

It is crucial that this information can be incorporated into complex software that can then be applied to decision-making in drug development and regulation studies.To achieve high bioavailability and low variability for oral pharmaceutical products, the API must be soluble and stable in the GI lumen and also adequately absorbed at relevant sites in the small and large intestine. Regional intestinal effective permeability (PE) is an important biopharmaceutical parameter that determines the absorption potential of the API from any dosage form (Dahlgren et al., 2016).

Information on the extent of drug absorption from the human large intestine is important for accurately estimating the production potential of a dosage form. The colon, which is the last main organ in the GI tract, plays a key role in the administration of drugs intended for long-term release and administered once a day, as well as in diarrhea, constipation and regulation of the composition of microflora. Although regional intestinal PE is an important biopharmaceutical parameter, the final drug absorption profile for a drug in the intestinal tract is determined by the interaction of various processes such as motility, transition, solubility, dissolution, precipitation and stability. The Biopharmaceutical Classification System (BCS) of drugs provides information on understanding and predicting GI drug absorption and bioavailability in general, which is also related to the absorption potential for the colon. Recently, eleven major pharmaceutical companies responded to a survey on the use of in vitro and in silico biopharmaceutical tools to predict outcomes in vivo. Companies use these

predictive models at various stages of drug development, for example during regulatory contact for scientific advice, and for various types of drug applications (Lennernas et al., 2017).
Bio-linked dissolution-absorption physiologically based pharmacokinetic (PBPK) modeling and simulation was used by 88% of responding companies in early drug development processes. Biopharmaceutical models are particularly useful to investigate the effect of API particle size on drug absorption in the gut and to investigate different pharmaceutical dosage forms. (Figure 1)

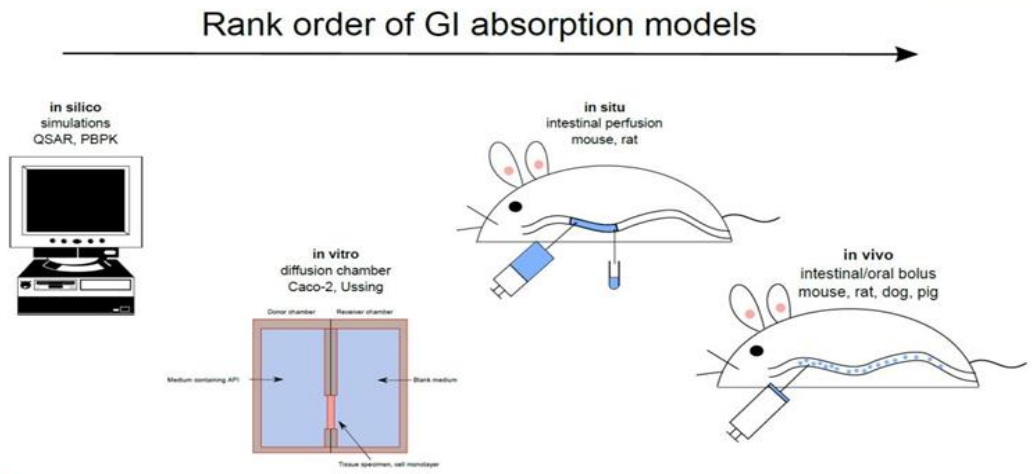

Figure 1. (Lennernas et al., 2017).

Extensive early human research has shown a good correlation between Pe. The main purpose of this review is to discuss recent developments in general research.It was determined using the SPIP model and Fabs in the fast-release dosage form.

GI In Vivo Estimation of Drug Absorption
Intestinal Peff is commonly known as Pharmacokinetics/mass balance clinical trials are the best way to determine the fraction absorption rate and extent of intestinal absorption of orally administered drugs in humans. Between for a drug that is administered orally. However, these mass balance studies are very complex and expensive, various biopharmaceutical processes are discussed, the focus of the study will be on the gut (Roffey et al., 2007).

Silico Gastrointestinalabs Absorption Estimates
In silico methods are now widely used by the pharmaceutical industry and regulatory agencies to support decisions regarding dosage form development, bioequivalence, and other bridging processes.Pharmaceutical properties such as particle size and coating layer of the API, which affect drug dissolution and subsequent intestinal absorption, and plasma drug concentration-time profile are frequently applied (Heimbach et al.2019). However, due to the increase in the computer structure-activity relationship (QSAR), the power with which these computer programs correlate various molecular identifiers, drug penetration studies can now be performed using complex molecular simulations. And it can simulate the interaction between the physicochemical properties of the drug molecule and important biopharmaceutical processes (Gozalbes et al., 2011).

This improves our understanding of mechanical membrane transport, ensuring the pharmaceutical success of a computational approach to predicting membrane permeability at early high throughput.

Gastrointestinal Experimental Absorption Models
Before reliable models can be developed, we need to improve our understanding of the interaction between pharmaceutical, biopharmaceutical, biochemical and physiological factors in determining absorbed fraction and bioavailability. Currently, our knowledge of gi-secretion, gi-motility, and regional intestinal permeability in both healthy subjects and patients with gi-disease is limited by the relative inaccessibility of some human intestinal segments A new class of GI sampling capsules is available based on an intralumen technique that offers te possibilities of spatial and temporal information of GI samples.

In situ

The future use of these clinical techniques in oral biopharmaceuticals awaits our better understanding of the GI processes involved in oral drug administration. In particular, our understanding of the complex and highly dynamic physiology of the region, from the middle jejunum to the sigmoid colon, can be significantly improved. One approach to assessing intestinal permeability is to use animal models that allow for detailed investigation of these intestinal regions, and then compare the results with those obtained from simple human permeability models, such as cell cultures.The SPIP model is typically used after the drug discovery phase and in the early formulation development phase of drug development when more relevant biopharmaceutical data are needed. An important advantage of the SPIP model is that it allows for the relevant mechanistic research of drug absorption and predicts the effects of various physiological processes. Some of the advantages over the spip in vitro models include intact gut morphology, the presence of blood flow, the presence of neural and hormonal feedback mechanisms, and the possibility of controlling lumen conditions (Dahlgren et al., 2017).

The rat SPIP model is often used to investigate the physiology of the GI, membrane drug transport, and the potential for a novel drug candidate to be formulated in oral MRI dosage form. In this model, the potentially adverse effects of abdominal surgery are reduced by simultaneous treatment of mice with parecoxib, a selective cyclo-oxygenase-2 inhibitor that has been shown to positively affect certain bowel functions such as GI motility, epithelial permeability, fluid, etc. Flux and ion transport However, in a recent SPIP study, treatment with parecoxib had only minimal effects on membrane permeability and water flow. It was also found that the permeability of the intestine to poorly penetrating drugs is best determined by the appearance of the parent drug in the plasma, rather than the disappearance of the drug from the perfused intestinal segment.

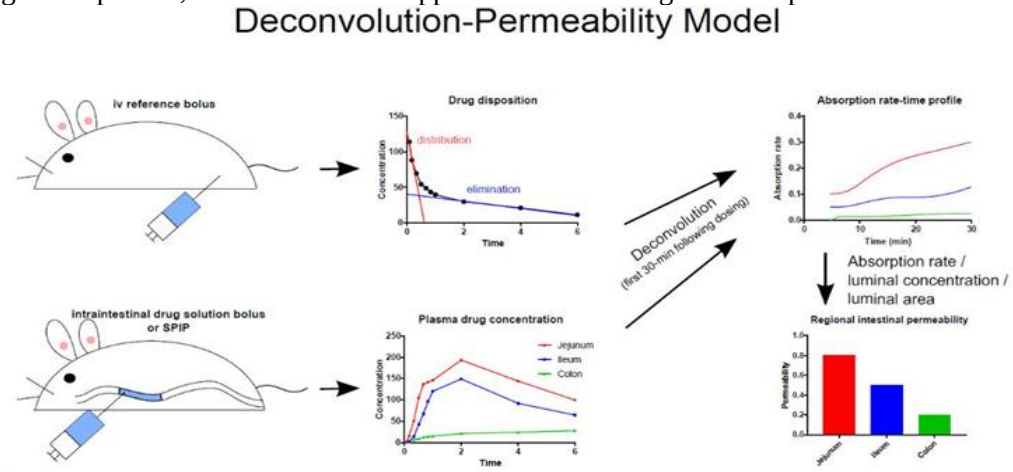

Figure 2. (Dahlgren et al., 2017)

In vivo: These in vivo animal models are the most clinically most physiological factors, such as gastric emptying time, lumen water content, and drug degradation, and are related to physiological factors such as gastric emptying time, lumen water content, and medication.First-pass metabolism after absorption affects the established parameters and the predicted result. First-pass metabolism after degradation and absorption affects the established parameters, and such models are clearly less applicable to the mechanistic studies of intestinal absorption as the predicted result. Such models are obviously less applicable to mechanical work .These mixing and transition processes are located both in the lumen and in the area adjacent to the intestinal mixture, and the transition processes are located both within the lumen and in the space adjacent to the intestinal epithelium, and are coordinated and regulated through a complex circuitous interaction between them. It is an epithelium and is coordinated and regulated through a complex cyclical interaction between enteric, autonomic and a set of physiological systems, including but not limited to the number of central physiological systems, autonomic and enteric. Central nervous systems. It has been suggested that there are long-distance and short-distance motor activities in the nervous systems. It has been suggested that long-distance and short-distance motor activities in the GI tract may interact to push undigested luminal bone along the canal, while regional mixing may interact to push undigested luminal thyme along the channel, with regional mixing stimulating the gut (Baker et al., 2019). If disorders occur in any of these systems, the intestine can accelerate absorption.

In vitro: Common in vitro models for studying membrane permeability include single layers of cells grown in cell culture filters (e.g., Caco-2 cells) and cut gut tissue samples mounted in a di usion (Ussing) chamber. Apparent permeability (Papp) is calculated by correlating the mass of the drug appearing at multiple time points (dm/dt) in the recipient chamber with the barrier (A) area and the drug concentration in the donor chamber (Cdonor). Limitations associated with these models include high variability between laboratories and between

laboratories and the sensitivity of the cell/tissue to the preparation setup and the room environment. For permeability investigations in drug discovery, therefore, it is recommended to use relative Papp values (compared to reference standards) instead of absolute Papp values BCS can also be used to predict drug absorption in vivo based on in vitro drug dissolution data. One of the goals of this in vitro approach is to improve organ development and, accordingly, improve its relevance in vivo. Gut organoids are expected to be a useful drug development technology for a variety of biopharmaceutical and pharmacokinetic analyses and in vivo predictions.

Intestinal Membrane Transport

The movement of ions, donor compounds, nutrients and other endogenous substances across various biological membranes is a central dynamic molecular process necessary for life in mammals. Selective permeability is a fundamental property of biological membranes and is determined by the physicochemical properties of the lipid bilayer and the physicochemical properties and molecular structure of the drug molecules together with the channel-forming membrane proteins. These transport processes along biological membranes with a different composition occur through carrier-mediated (CM) mechanisms that require direct and indirect energy even against a concentration gradient Simplified membrane resolution, passive membrane resolution, and paracellular distribution occur along a concentration gradient. It encapsulates cells and their contents to optimize the various functions for which cells are responsible in a living organism. At the core of any biological membrane is a lipid bilayer, which in vivo can consist of hundreds of different lipid molecules. Membrane lipids have amphiphilic molecular properties with a polar head group and a non-polar tail containing esterified fatty acids. These lipid molecules vary greatly in size, chemical structure, and polarity, and can be combined and combined to provide a wide variety of physical properties and functions. The movement of drugs through various membranes is necessary for many pharmacokinetic and pharmacodynamic processes. The basic nature of drug transport is divided into intracellular and intracellular processes, where the intracellular pathway is the most common.

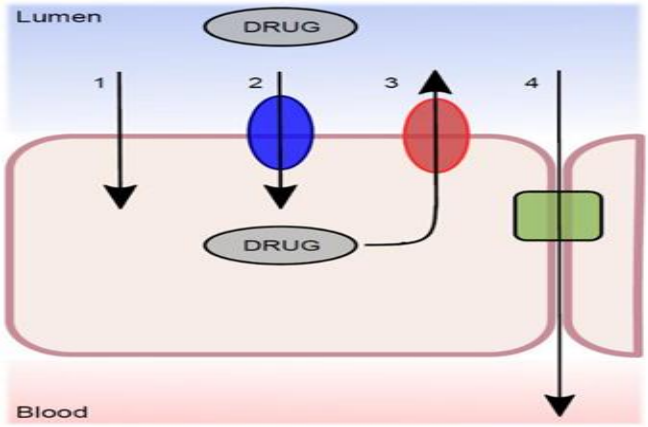

Figure 3. Mechanisms of transport from the lumen through the intestinal epithelium, which determine the net permeability of a dissolved drug molecule from the lumen. (1) Passive transcellular diffusion; (2) absorbent carrier-mediated transport; (3) carriage mediated by the flow carrier; and (4) passive paracellular diffusion.

Figure 3. (Wang et al., 2015)

Passive diffusion, or CM transcellular transport, occurs throughout the intestinal cell (enterocyte) via both octanols. The penetration of these molecules depends largely on the pH at the surface.

Apical and basolateral membranes. Paracellular transport takes place between epithelial cells.For example, mechanisms that are rapidly absorbed underneath the mechanisms absorbed across human paracellular and databases, such as intestinal formulacular drugs of barriers (Wang et al., 2015) that despite the roles of dependent individual cladins, increases size selectivity, while smaller molecules, smaller molecules, permeability less known permeability ammonium compounds. In addition, the processing of the different degrees of lipoids thanks to the lipoidals of the loaded branesanions recharge thousands of times faster, although their membranes are permanent . It is more controlled than expected. Thus, the membrane is an obvious transport of atphall's division of vivo-related theory or hiphhall.

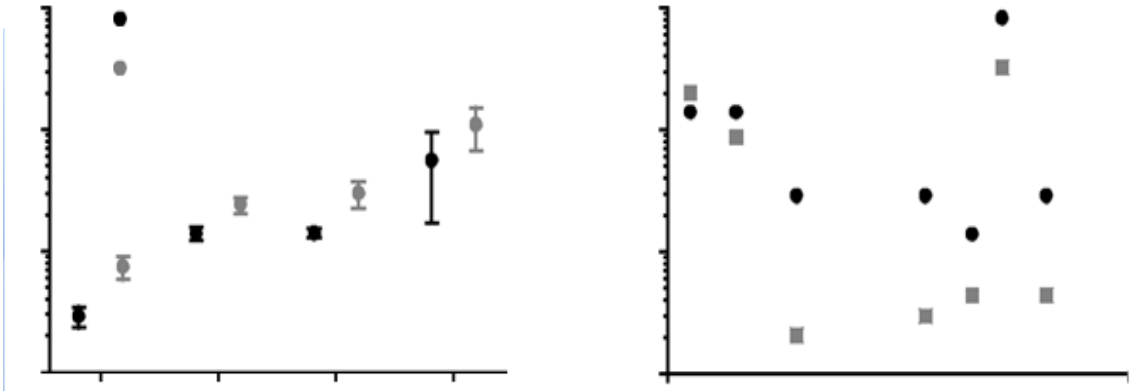

Figure 4. (Roos, C., 2018).

A drug usually loses its degree of freedom when it dissolves in the membrane, and this penetrates similarly to tetracycline and labetalol, suggesting that there may be this>5 HBD drugs. To a significant extent it is absorbed by passive lipoidal diffusion. To explain why some drugs are absorbed by passive lipoidal di use, regardless of their negative physicochemical properties, it is necessary to find more complex definitions of molecular interaction with the lipoidal membrane. For example, permanently charged molecules vary in their degree of passive penetration according to their ability to spread the charge over various ring structures. Several experimental studies have shown that intramolecular hydrogen bonding can mask polar structures and therefore increase membrane transport. The principle is that intramolecular hydrogen bonding reduces the thermodynamic damage of dissolving in the membrane nucleus. This has also been shown in various molecular dynamics simulations of the transport of solutes along a lipid bilayer. The following detailed discussion of the intestinal membrane transport of atenolol has been gathered from data from a variety of sources, from theoretical calculations to human pharmacokinetic data.

Intestinal metabolism and flow transport

After the drug molecules enter the cytosol of enterocytes, they can undergo metabolism. The most important metabolizing enzymes are CYP enzymes 5, 6. CYP3A4 is the most abundant isoform in the gut and makes up about 70% of the total CYP content 90. It is estimated that about 50-60% of all drugs given orally occur. It will be subjected to oxidative metabolism by clinically demonstrating CYP3A4. The importance of this enzyme 91. Despite the relatively low expression of CYPs in the gut compared to the liver, etabolism in the gut can have an impact on it. ioavailability of drug compounds. This is thought to be partly attributed to a substrate overlap between CYP3A4 and flow carrier P-glycoprotein (P-gp) 92 in general and between CYP3A4 in particular. Such a synergistic interaction reduces the drug compounds in enterocytes and thus increases the fraction of the drug compound, which can undergo metabolism 92. The magnitude of this apical recycling has not been shown to be clinically relevant in vivo, but P-gp and other flow carriers may have a role in limiting the bioavailability of xenobiotics containing drug compounds 90,93. As a result, inhibiting the activity of metabolizing enzymes or flow carriers can increase the bioavailability of a drug compound. (Roos, C., 2018).

Atenolol: The delivery mechanisms for a low-molecular-mass drug are often interpreted based on more than one technique. Atenolol controls the passage of substances from the intestine to the blood through transcellular, paracellular and various CM processes.. Therefore, atenolol is suitable for demonstrating the complexity of classifying the transport mechanisms of a drug, as data from various in silico, in vitro, in situ and in vivo models are needed. Following oral administration to humans, the plasma pharmacokinetics of atenolol are linear for area below the concentration-time curve (AUC) for doses of 25 to 200 mg and for oral doses of 0.1 to 200 mg (1.4–2857 g/kg). For the maximum concentration (Cmax) (Figure 5) (Mahajan et al., 2009).

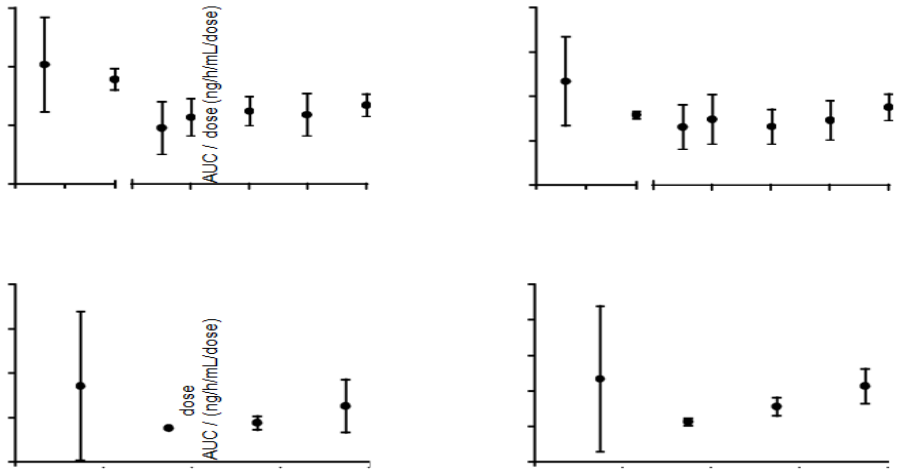

Figure 5. (Dahlgren et al., 2019)

There is a 1.5- and 1.6-fold higher AUC for doses of 0.03 and 0.1 and a 1.6-fold higher Cmax for an oral dose of 0.03 mg respectively than the average values in the clinical oral dose range. These data from microdosing studies (0.03 and 0.1 mg) mean that atenolol at lower oral doses/luminal concentrations contribute some CM to intestinal permeation.

Xenopus laevis oocyte transport studies suggest ***that OATP1A2*** may be a plausible absorptive carrier for atenolol. However, it should be noted that there was no statistical difference in AUC between 0.1 and 50 mg in one of the microdosing studies, suggesting that passive and unsatiable transmembrane transport may apply to atenolol in vivo. In addition, the difference in plasma exposure was not associated with the elimination of 100% renal (the parent drug) atenolol in both humans and rats, and was not affected by oral doses in the range of 0.3-80 mg/kg. [86]. In conclusion, the dose of atenolol does not affect renal clearance, which has been shown to be partially mediated by ***the eux*** transporters ***OCT2*** and ***MATE 1 and 2***. This is also in line with Km values (280, 32 and 76 μm, respectively), which are significantly higher than the maximum plasma concentration of 2 μm following an oral dose of 100 mg. In humans, co-administration of oral atenolol with apple or orange juice on an empty stomach reduces the plasma exposure of atenolol to 20-50%, compared to water. This interaction may be a result of inhibition of absorptive transporters, as observed for fexofenadine and celliprolol. However, given the large amounts of apple juice (600-1200 ml) or orange juice (200 ml) used and the known high osmolarity of the juices, the reduced exposure is likely the result of increased intestinal transit time. Similar results have been observed when administered orally together with atenolol. In cell unilayer studies (Caco-22 and IPEC-J2) for atenolol 2,3 and 3,5

The ratios E ux (B – A : A – B) were observed; With the concomitant administration of PGP inhibitors, these were reduced to 1.7 and 1.1 and IPEC-J2); These were reduced to 1.7 and 1.1 respectively by co-administration of the Pgp inhibitors verapamil and zosuquidar This suggests that atenolol may be a Pgp substrate. This suggests that atenolol may be a Pgp substrate. However, other Caco-2 studies (atenolol concentrations between 30 μm and 3.8 mm) have shown. Other Caco-2 studies (atenolol concentrations between 30 M and 3.8 mm) have shown that the outflow rate of atenolol is 1, independent of concentration, and differs between laboratories. The E ux ratio of atenolol is 1, is independent of concentration and differs between laboratories (ranging from 0.18 to 3.76) and between batches in the same laboratory. The Papp of Atenolol ranges from 0.18 to 3.76. And among the batches in the same lab, Papp of atenolol was also unaffected after verapamil and knockout of the Pgp gene in the mouse SPIP model. Also the mouse is not affected by verapamil in the SPIP pattern and after knockout of the Pgp gene. Similarly, atenolol absorption rate increased in rat in situ jejunal cycle Similarly, co-administration of cyclosporine, another Pgp inhibitor, increased atenolol absorption rate in rat in situ jejunal cycle study. In addition, atenolol has linear linear pharmacokinetics (AUC, Cmax) in mice following oral administration of doses between 0.55 μg and 5.5 mg (0.167-1670 μg/kg) in rats followed by oral administration of doses between 0.55 μg and 5.5 mg (AUC, Cmax), and oral co-administration of the Pgp inhibitor itraconazole 5.5 mg (0.167-1670 g/kg) and oral co-administration of the Pgp inhibitor itraconazole to humans did not affect its pharmacokinetics.

In humans, regional intestinal Peff was important for atenolol (Figure 6A). However, when the Peff value was corrected for the regional intestinal difference in surface area, this difference almost disappeared. These results suggest that passive membrane permeability is the predominant transport mechanism of atenolol. In humans, regional intestinal Pe was important for atenolol. These results give passive membrane permeability the dominant transport mechanism of atenolol.

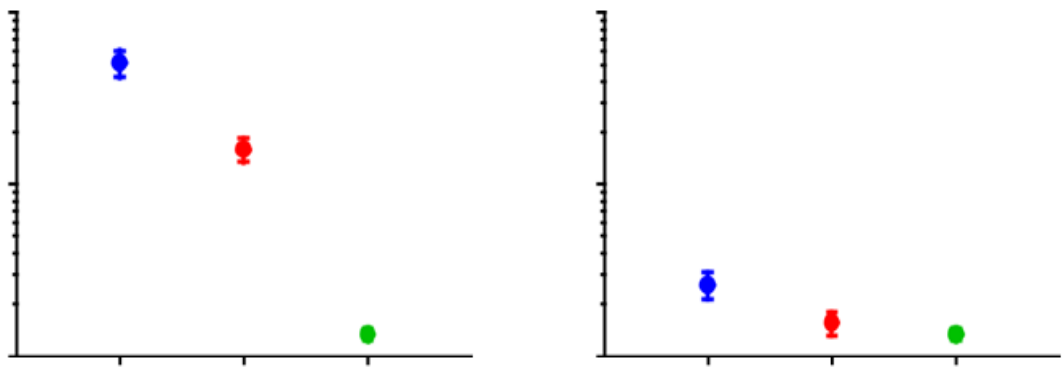

Figure 6. (Dahlgren et al., 2019)

Papain-Cyclodextrin Complexes: Papain (cysteine protease enzyme) has a wide range of uses in pharmacological applications. Due to its debridement properties and stimulating the penetration of drugs into the skin, papain improves its biological stability to its complexes with cyclodextrins. A single-layer Caco-2 system was used to assess drug permeability and apparent permeability factor, and in vitro cytotoxicity analysis was performed on (CHO-K1, Hep). Genotoxicity of G2 cell lines And(Caco-2) and(CHO-K1) and (Hep G2) cell lines were also performed, Papine cyclodextrin complexes did not show any cytotoxicity higher than 31 µg/ml , No significant genotoxic damage was observed. Papain and cyclodextrin complexes led to an almost 2.5-fold increase in the penetration of furosemide, and the preservation of the integrity of cells adjacent to monolayer Caco-2 cells was confirmed. Papain complexes can be safely applied in pharmaceutical formulations, can be administered not only as a therapeutic agent, but also as a strategic pharmaceutical adjuvant , ilicing the penetration of drugs with low oral permeability. As a method in research:

- Cells and reagents
- Papain-cyclodextrin complexes and their enzymatic activity
- Biological assays (Cell culture, Cytotoxicity tests, Genotoxicity tests)
- Permeability tests and TEER
- Statistical analysis

Enzymatic activity: The proteolytic activity of papain becomes key, when it comes to its effects on adherent cell lines, its cytotoxic effect is known to occur during the binding of cell lines and inhibits the growth of cells. In these experiments, the result of complexation with cyclodextrin revealed that it led to no corresponding changes in the proteolytic activity of papain, which can be traced using a specific substrate.

Cytotoxicity tests: In vitro safety experiments are often performed in the initial molecular screening and upon cell proliferation to assess the activity of these molecules and the possible direct toxic effects that eventually cause cell death. In this regard, cytotoxicity experiments are considered an important tool for determining or accessing the biological safety of many compounds when conducting non-clinical studies.
Initially, two different approaches were applied to the evaluation of cell viability, aimed at understanding the toxicity of natural papain and papain-β-cyclodextrin complexes.

An MTT test to confirm possible toxic effects on the mitochondrial respiratory chain CHO – K1, HEP G2 and Caco-2 cells

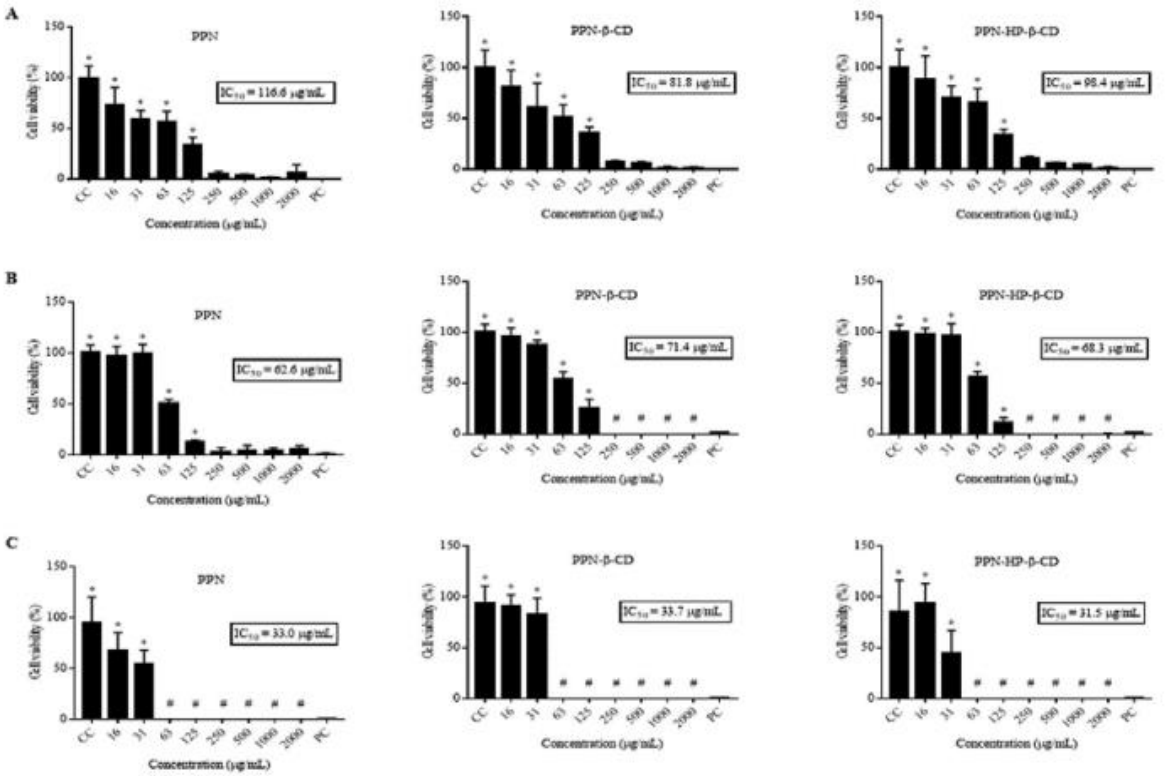

Figure .7 (Corazza et al., 2020)

An assessment of LDH release after possible damage to the plasma membrane of Caco-2 cells.

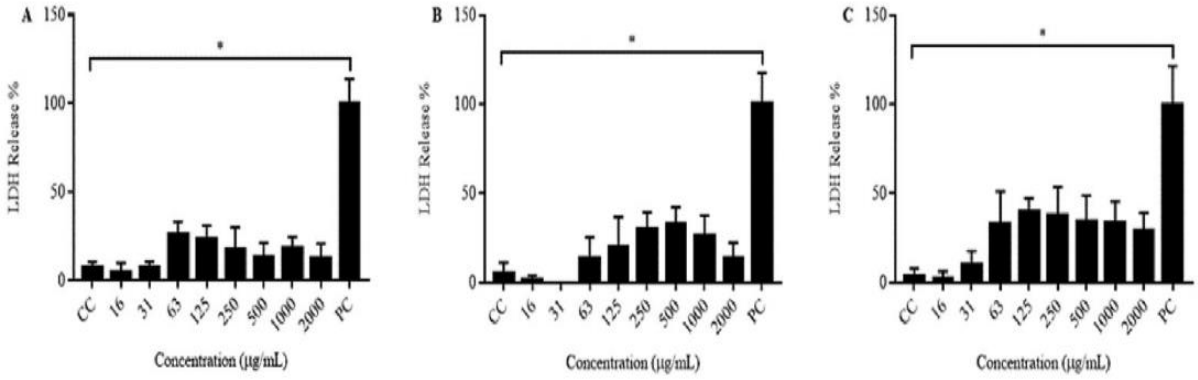

Figure 8. (Corazza et al., 2020)

Genotoxicity tests: In this study, the three lowest non-cytotoxic PPN concentrations (8 µg/ml, 16 µg/ml and 31 µg/ml) as well as the PPN-β-CD and PPN HP-β-CD CHO–K1 and Hep G2 cell lines were tested using OECD 487. Micronucleus frequency, effects of papain and its complexes on the CHO-K1 and Hep G2 cell lines were evident after an incubation period of 4 hours. The number of micronuclei formed when CHO–K1 cells were exposed to positive controls was crucial (COLCH $p < 0.01$ and MTMC $p < 0.0001$), indicating that the use of these agents was efficient for the performance of these assays. Furthermore, no significant genotoxic damage was observed in CHO-K1 cells at natural and complex papain concentrations of 8, 16 and 31 µg/ml. With regard to Hep G2 cells, no significant genotoxic damage was observed in these cells upon stimulation with any of the natural and complex papain concentrations. The number of micronuclei that formed when cells were exposed to positive controls was very significant (COLCH and MTMC $p < 0.0001$), which confirmed the functionality of the test.

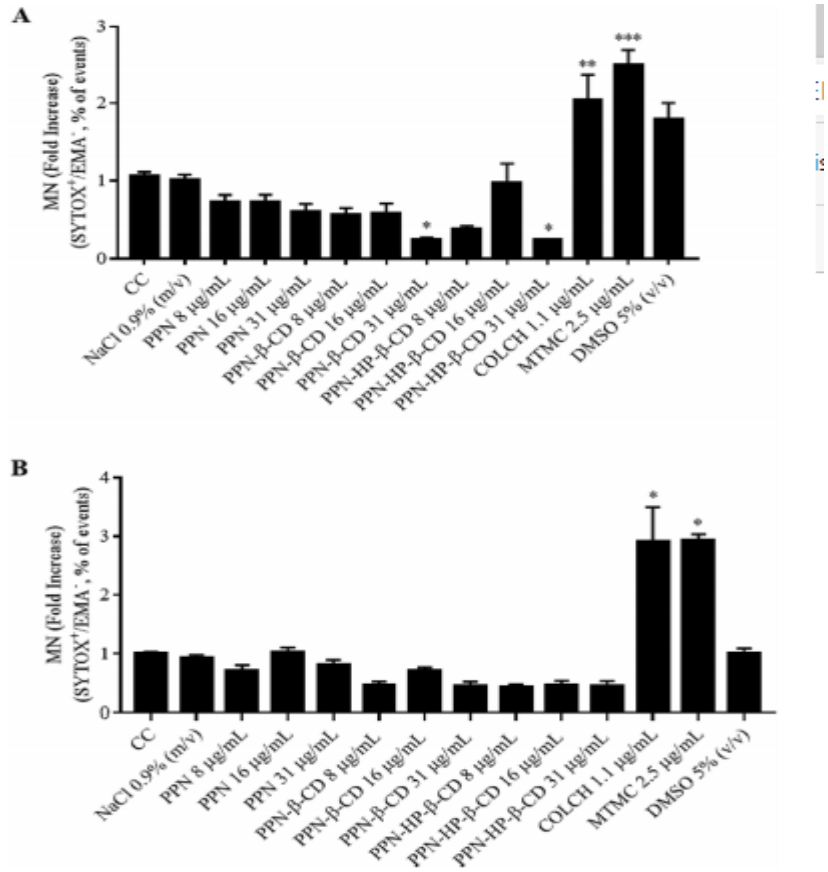

Figure 9. (Corazza et al., 2020)

Permeability tests: HPLC was used to determine the amount of furosemide and metoprolol in the apical and basolateral compartments. The calibration curves were performed by linear regression of the peak area versus concentration. The calibration curves for furosemide and metoprolol were linear for concentrations between 1 and 1000 ng/ml (R = 0.996 for both curves). The method was considered specific and met all analytical specifications for the quantification of both drugs. The biophysical integrity of membranes formed by Caco-2 cells cultured in thincert ™ membranes during permeability tests was assessed by determination of TEER in the initial periods and after 72 hours of the experiment. Based on the lowest non-cytotoxic concentration of samples containing natural papain or complexed with β-cyclodextrins (16 µg/mL), a permeability test was performed. Papp value of furosemide (alone)

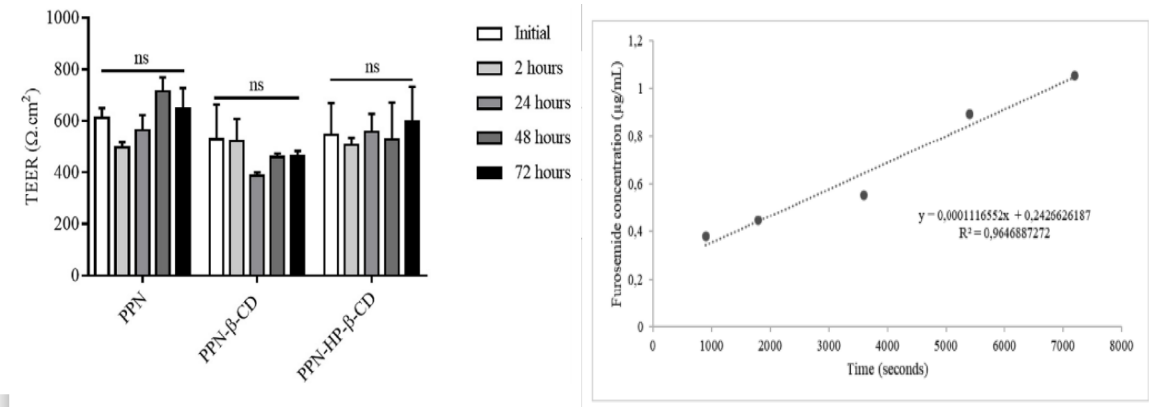

Figure 10-11. (Corazza et al., 2020)

Compared to the control containing only furosemide, it was possible to notice an almost 2.5-fold increase in the permeation of furosemide with papain and its complexes.

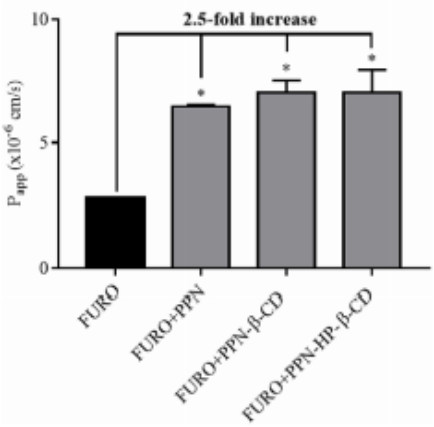

Figure 12. (Corazza et al., 2020)

The permeability coefficient (Papp) of furosemide (Papp) of 100 µg / mL in the presence of compounds containing 16 µg / mL of papain isolated in a single layer of Caco-2 cells after 2 hours of administration (cm / s) . FURO: furosemide; PPN: natural papain; PPN-β-CD: papain complexed with p-cyclodextrin; PPN-HP-β-CD: papain complexed with 2-hydroxypropyl-p-cyclodextrin. The data represent standard deviation ± means (*p <0.05, compared with the negative control (furosemide) followed by One-way ANOVA and Bonferroni test).

Fluorescent confocal microscopy

Sufficient morphology can be obtained by staining cells (by marking actin filaments from the cytoskeleton) and DAPI (by marking nuclei) to observe specific components of Caco-2 cells, making it possible to directly observe the single layer and the resulting network of occlusive junctions.

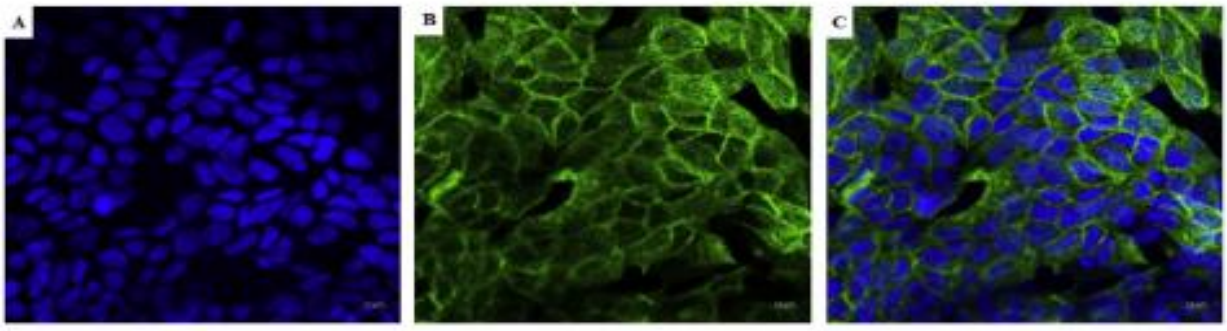

Figure 13. (Corazza et al., 2020)

Fluorescent confocal microscopy of a Caco-2 cell monolayer. (A):DAPI-blue, labeling cores; (B): Alexa Fluor ™ 488 Phalloidin - green, marks actin filaments, preferably located at cell boundaries and microvilli; (C):Combined. Scale bar = 10 µm.

The results of the study showed the efficacy of papain-cyclodextrin in increasing its safety and furosemide permeability in vitro, but the results shown show that b-cyclodextrin alone is not compared to furosemide alone, in light of its physical, chemical and biological aspects. He showed that natural papain, as well as β-cyclodextrinpapain and 2-hydroxypropyl-β-cyclodextrin-papain complexes, had concentration-dependent cytotoxicity, whereas cytotoxicity was not observed at the lowest concentrations examined on CHO–K1, Hep G2 and Caco_2. It was hypothesized that the observed low cell viability values were due to papain proteolytic activity that causes cell separation rather than necrosis caused by direct toxicity or cell death mechanism. In terms of genotoxicity, complexes with natural papain and cyclodextrins did not cause any effect on concentration on the aforementioned cell lines, which highlighted a possible safe use of the enzyme and its complexes in biomedical applications. Compared with the original form of the enzyme, no additives of papain were observed in terms of the effects of the complexes on the formation of micronuclei. As for transport studies, the findings suggested that increased penetration of the intestinal epithelium by papain and papain-b-cyclodextrin complexes may play an important role in increased intestinal absorption of furosemide. shows

that papain-containing complexes can be administered as pharmacotechnical adjuvants to promote the safe penetration of drugs that exhibit low oral permeability (Corazza et al., 2020).

RESULT

The study of intestinal drug transition processes is crucial for the development of oral pharmaceutical products. The current hypothesis for the passage of drugs through the intestine includes several parallel CM and passive passage mechanisms (such as passive lipoidal diffusion, CM uptake transport, CM flow, paracellular diffusion, mucus resistance, endocytosis and transcytosis). The determination of intestinal Peff for a drug is based on the technique, model, and conditions applied and is influenced by multiple interactions between the drug molecule and the biological membrane. Further development of the oral biopharmaceutical system requires the development of new in vitro models and the use of human and animal in vivo techniques. For example, gut organoid technologies that bridge the gap between traditional two-dimensional cellline culture and in vivo models are expected to improve our understanding of mechanics. These innovative and more complex in vitro models need a thorough comparison with high-quality products.
New clinical techniques are expected to provide an improved understanding of biopharmaceutical-related GI processes and high-quality data.

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