Review Article

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A RATIONALIZED DESCRIPTION ON STUDY OF INTESTINAL BARRIER, DRUG PERMEABILITY AND PERMEATION ENHANCERS

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ABSTRACT

Per oral delivery of hydrophilic drugs is one of the most challenges in biopharmaceutical research. Hydrophilic drugs show low bioavailability following oral administration because of their poor intestinal permeation. In the last few years great interest has focused on search of different intestinal permeation enhancers for the oral delivery of BCS class iii drugs, small polar molecules, vaccines, hormones, peptides and proteins, which are well suitable for the delivery of the above products to give enhanced bioavailability by increasing intestinal permeability. This review sets out to discuss about anatomy and physiology of the intestinal barrier, drug absorption from intestinal tract, mechanism of intestinal drug permeability, detail information about intestinal permeation enhancers and its mechanism of action, invitro methods for studying drug permeability, advantages and applications of intestinal permeation enhancers.

KEYWORDS: Intestinal Permeation Enhancers, BCS, Tight Junctions, Oral Bioavailability

1. INTRODUCTION:

Oral administration is the most common method of drug delivery used today. Optimizing bioavailability of orally administrated drug is one of the most important aim for the pharmaceutical industry. Transport across mucosal membranes is a fundamental step for oral absorption and systemic availability. The drugs which are small and lipophilic in nature are easily permeated through the intestinal barrier where as oral administration of macromolecules are restricted by the intestinal epithelial barrier which results in greatly reduced

bioavailability .A great number of currently available drugs fall under the class III of the biopharmaceutical classification system[1], possess high therapeutic potential but cannot be delivered by oral route because of its poor permeation across the GIT epithelia. In general, these are hydrophilic compounds, of medium to high-molecular weight, and sometimes containing strongly charged functional groups implying that transport across the intestinal barrier occurs essentially via the paracellular pathway. The contribution of the latter to intestinal absorption is considered to be small, since this pathway occupies less than 0.1% of the total surface area of the intestinal epithelium, and the presence of tight junctions (TJ) between the epithelial cells limits drug absorption. These drugs have low intrinsic membrane permeability, probably because of their low lipophilicity and zwitterionic character at physiological pH or act as a substrate to drug efflux pumps like p-glycoprotein, ionic charge and high molecular weight. WHO listed out the BCS class III drugs, they are shown in below Table-1

Drugs	**Solubility**	**Permeability**	**Therapeutic activity**
Abacavir	High	Low	Antiretroviral
Acyclocir	High	Low	Antiherpes
Amoxicillin	High	Low	Antibacterial
Atenolol	High	Low	Antianginal,
Benznidazole	High	Low	American tripanosomiasis
Chloramphenicol	High	Low	Antibacterial
ChlorpromazineHcl	High	Low	Psychotherapeutic
Codeine phosphate	High	Low	Opionid analgesic
Didanosine	High	Low	Antiretroviral
Enalapril	High	Low	Antihypertensive
Ergocalciferol	High	Low	vitamin
Ethambutal Hcl	High	Low	Antituberculosis
Folic acid	High	Low	Antianaemia
HydralazineHcl	High	Low	Antihypertensive
Hydrochlorthiazide	High	Low	Diuretic
Levothyroxin Na salt	High	Low	Thyroid hormone
Mannitol	High	Low	Osmotic diuretic
α-Methyldopa	High	Low	Antihypertensive
Metoclopramide Hcl	High	Low	Anti emetic
Neostigmine bromide	High	Low	Muscle relaxant
Penicillamine	High	Low	Antibacterial

2. PHYSIOLOGY OF BARRIERS:

The barrier is composed of a single layer of columnar epithelial cells, primarily enterocytes and goblet cells, joined at their apical surfaces by tight junctions.[2]

2.1 Tight junctions and epithelial barrier function:

Tight junctions restrict epithelial cells immediately below the brush border forming a seal between neighboring epithelial cells. This seal acts as a gate to restrict passage of small molecules in a charge specific manner and completely occludes diffusion of molecules with molecular radii larger than 0.1nm. In addition, the tight junction acts as a fence that separates components of the apical and basolateral domains of the epithelial plasma membrane [3].

2.2 Biochemical composition of the tight junctions:

The biochemical composition[4] of tight junctions is still being elucidated, but many of the key components have been identified. It is currently recognized that the tight junctions primarily are complex multicomponent protein structures. Identification of the principal transmembrane component has only recently come to light and it is now known that the tight junction is composed of a homotypic protein termed occludin. Other tight junctional complex proteins which have been identified are ZO-1[5], claudin. All of these proteins are oriented peripheral to the cytoplasmic surface of the tight junction complex and are thought to be involved in the stabilization and/or regulation of tight junction integrity.

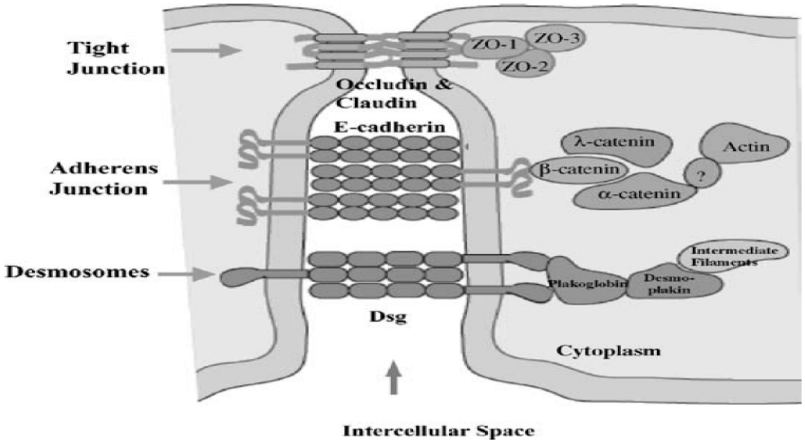

Figure 1: Biochemical composition of tight junction

2.3 Regulation of tight junction permeability:

The tight junction complex is not a static structural component as once was thought, but slightly resembles a dynamic and elaborate protein signaling complex. Regulation of tight junction paracellular permeability by various physiological, pathological, and experimental agents has been extensively examined in a number of in situ and in vitro culture models, particularly Caco-2[6], brain endothelial, and MDCK cells. Peptide hormones, cytoskeleton perturbing agents, oxidants, Ca++ chelators and ionophores has all been shown to alter paracellular permeability by disrupting tight junctions. In addition, it has been determined that tight junction permeability is influenced by nearly all second messenger and signaling pathways, such as tyrosine kinases, Ca++, protein kinase C (PKC), protein kinase A (PKA), G proteins, calmodulin, CAMP, and phospholipase C.. Two factors which appear to play a prominent role in the regulation of paracellular permeability by absorption enhancers are contraction of the perijunctional actinmyosin ring, and protein kinase or phosphatase mediated changes in tight junction protein phosphorylation.

2.3.1 Role of the perijunctional actin-myosin ring:

Adjacent to the tight junction in the cytoplasm is an actin-myosin ring which restricts the cell. This ring is associated with both the tight and intermediate junctional complexes and can contract exerting an inward force on the lateral plasma membrane. Such contractions are ATP-dependent and have been correlated with a loosening of the tight junctions indicating that contractions of the perijunctional ring pull on tight junction components and induce changes in paracellular permeability between neighboring cells. Further evidence for a physical link between the perijunctional actin-myosin ring and tight junctions has been inferred by direct observation of tight junction-associated actin and by observations showing disruption in the structure and integrity of tight junctions by agents which disrupt actin filaments (e.g. cytochalasin D) possible to visualize the perijunctional actin-myosin ring by staining actin filaments with fluorescent-labeled phalloidin[7]. Using this approach global changes in actin distribution have been documented with some tight junction disrupting agents including oxidants , protein kinase C activators , Ca++ depletion , and cytoskeleton disrupting

agents while more subtle changes have been observed with other tight junction disrupting agents (i.e. interferon-y) .

2.3.2 Role of calcium:

Extracellular calcium levels play a prominent role in the formation and regulation of tight junctions and paracellular permeability. Adhesion at the adherens junction is mediated by cadherins[7] which are Ca' ' -dependent, cell-cell adhesion molecules that interact homotypically. Removal of Ca' + has been known for many years to lead to an increase in tight-junction permeability and cause a redistributionof tight junction proteins. It appears that it is the disruption of cadherin adhesiveness by removal of Cat+ rather than a direct effect on the tight junction, which leads to the increase in paracellular permeability.

In addition, sensitivity of cadherin adhesiveness to Ca' ' can be modulated by intracellular signaling events, such as. Tyrosine phosphorylation. Whereas extracellular Ca-- is required for formation and maintenance of tight junctions, intracellular Ca*' may be involved in regulation of tight junction permeability. In isolated hepatocyte couplets (another cell model commonly used to investigate tight junction regulation), A calcium channel blocker has been shown to increase paracellular permeability with an accompanying inhibition of intracellular calcium.

2.3.3 Role of CAMP:

Intracellular CAMP[8] alters paracellular permeability by reducing NaCl diffusion potentials and increase passive permeability to Cl- as well as Cl: Na permeability ratios in intestinal and gall bladder epithelium. CAMP may also decrease tight junction resistance, but this effect may be masked by the increased resistance that accompanies collapse of the lateral spaces. The exact role of CAMP in regulation of tight junction is not yet clear.

2.3.4 Role of ATP depletion:

Under normal physiological conditions, the tight junction is maintained by an energy-dependent (ATP) process involving the actin cytoskeleton and tight junctions. Alteration in cellular energy status, a decrease in adenosine triphosphate (ATP) levels, has been shown to disrupt epithelial barrier function and increase permeability. Energy depletion results in net loss of phosphorylation of brush border, and possibly junctional, proteins.

3. DRUG ABSORPTION FROM THE GASTRO-INTESTINAL TRACT:

Drug absorption following oral administration is a fairly complex sequential series of events outlined in Figure shown below.

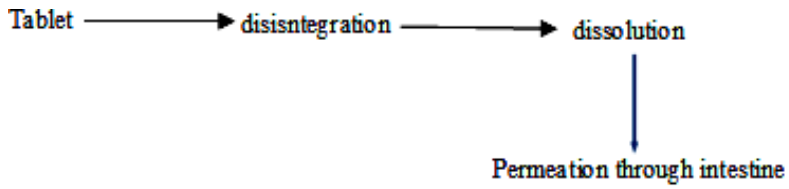

Figure- 2: Drug absorption from the GI tract

Depending on the physico-chemical properties of the drug, either the dissolution rate or the transport rate across the intestinal epithelium may be the rate-limiting step for drugs to enter the systemic circulation.

3.1 Mechanisms of intestinal drug permeability:

The intestinal mucosa can be considered as a system of sequential barriers to drug absorption, the outermost barrier being the mucus layer and the unstirred water layer. The gel-like structure of the mucus is thought to be a barrier to absorption of highly lipophilic drugs and some peptides because of the restricted diffusion in this matrix. The absorptive epithelium lining the GI tract follows the folds and villi that increase the anatomical surface area of the mucosa several-fold in the small intestine. The villi are interspaced with crypts in which the regeneration of intestinal cells occurs. In between the crypts and the tips of the villi are the basal parts of the villi. The properties which are relevant for drug absorption differ between the cells along the crypt-villus axis[9].

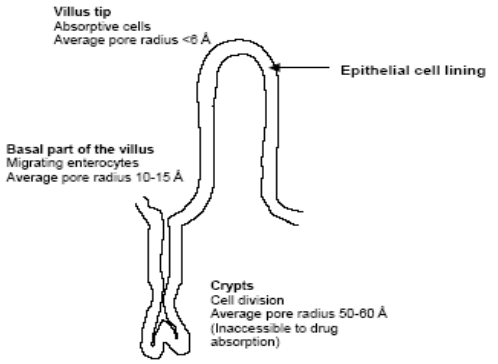

Figure- 3: The absorptive epithelium lining of the GI tract

The main purpose of the intestinal epithelium is not only to restrict access and in this way protect the body from harmful agents, but also, to allow selective absorption of nutrients and secretion of waste products and xenobiotics

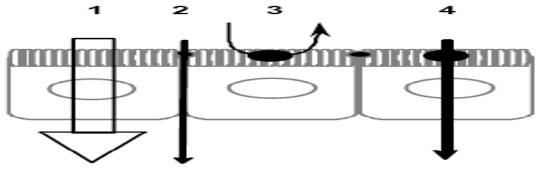

Figure -4: Mechanisms of intestinal drug permeability

Schematic illustration of the different transport routes that are relevant for drug absorption. 1. Passive transcellular and 2. Parcellular transport, 3. Carrier-mediated efflux and 4. Carrier-mediated active transport.

3.1.1 Passive transcellular transport:

Drug transport via the passive transcellular route requires that the solute permeates the apical cell membrane. Cell membranes are made up of phospholipids arranged in bilayers that are intermingled with membrane proteins. The composition of phospholipids and proteins varies from cell type to cell type and may theoretically give rise to different permeability properties depending on the cell type. In addition, the intestinal enterocytes have a polarized cell membrane with distinct differences in membrane composition in the apical and the basolateral membrane. It is generally believed that the apical membrane has a lower permeability than the basolateral membrane and the former is therefore considered to be the rate-limiting barrier to passive transcellular drug transport.

3.1.2 Active transport:

Transport proteins embedded in the apical cell membrane actively shunt nutrients such as peptides, amino acids and sugars across the phospholipid bilayer. In order to restrict access of unwanted solutes via this pathway, these transporters display substrate specificity. Therefore, in order to utilize this pathway to increase absorption, the drug has to share some structure similarity with the normal substrate. A limited number of drugs are substrates for uptake carrier proteins. These include some cephalosporin antibiotics, cytostatics and angiotensin-converting enzyme (ACE) inhibitors that are substrates for oligopeptide transporters. The oligopeptide transporters have unusually broad substrate specificity, are abundantly expressed in the small intestine [38] and have

therefore been the deliberate target for redesigning pharmaceuticals such as antiviral drugs to make them substrates for this transport protein. Common to all absorption processes involving transport proteins is that they are saturable. Drugs that are substrates for an active transport protein can therefore display a non-linear dose-response relationship resulting in a decreasing absorbed fraction with an increasing dose. In addition, these proteins are transporters of nutrients, and therefore their capacity is likely to be influenced by food intake. These factors may complicate the oral delivery of drugs that are absorbed by active mechanisms. In contrast to transport proteins acting in the absorptive direction, the active efflux proteins secrete certain drugs that are substrates for these efflux proteins. The most well-studied efflux proteins belong to the adenosine triphosphate (ATP)-binding cassette (ABC) super-family of membrane transporters. These include the multi-drug resistance 1 (MDR1; ABCB1) gene product P-gp and the multi-drug resistance protein family (MRP; ABCC). More recently the breast cancer related protein (BCRP, ABCG2) has been identified as potential contributor in actively limiting oral bioavailability of some drugs. The function of the efflux proteins in the intestine may be to prevent the uptake of toxic substances and also, to eliminate such substances from the blood.

3.3.3 Paracellular transport:

Drugs of small to moderate molecular weights (MWs) can permeate the intestinal epithelium through the water-filled pores between the cells. This process is known as paracellular transport, and is generally considered to be a passive process, even if this pathway appears to be selective for cationic rather than anionic and neutral drugs. The paracellular pathway has also been shown to be saturable, by at least two separate mechanisms, one of which involves an intracellular process. The paracellular permeability is dynamically regulated and varies both along the path of the intestine and along the crypt-villus axis. The average pore radius of the human small intestine is 8–13 Å, which will limit the paracellular permeability of drugs >4 Å and restrict those >15 Å. The colon is even more size-discriminating since the paracellular pathway restricts drugs <3.5 Å[10].

4. INTESTINAL PERMEATION ENHANCERS:

These are the exepients which increases the intestinal permeability of poorly absorbed drugs in the small intestine and improve the oral bioavailability. These substances promote

the permeability of poorly permeable drugs mainly by opening the tight junctions, leading to the increased paracellular permeability.

4.1 Classification of intestinal permeation enhancers:

Surfactants	**Ionic:** Sodium lauryl sulphate Sodium dodecylsulphate Dioctyl sodium sulfosuccinate **Nonionic:** Polysorbitate Nonylphenoxypolyoxetyylenes Tween80
Bilesalts & its derivative	Sodium glycholate Sodium deoxycholate Sodium taurocholate Sodium dihydrofusidate Sodium glycodihdro fusidate
Fattyacids & its derivatives	Oleic acid Caprylic acid Lauric acids Sodium caprate Acyl carnites Acyl choline
Chelating agents	EDTA Citric acid Salicylates
Chitosans & derivatives	N-sulfanto-N,O-carboxymethylchitosan N-trimethylated chloride(TMC) Chitosan glutamate
Other enhancers	Zonula occludens toxin (Zot) polycarbophyl-cysteine conjugate(PCP-Cys)

Table-2: Classification of intestinal permeation enhancers

4.2 Mechanism of permeability enhancers:

4.2.1 Surfactants:

Disruption of intestinal epithelial cell membrane leads to increase in the permeability of drugs that cross the intestinal barrier through transcellular mechanism.

4.2.2 Bile salts & its derivatives:

Bile salt shows the increases the permeability of intestinal barrier mainly by the following mechanisms.

-Denaturation of proteins

-Decrease of mucus viscosity

-Decrease of peptidase activity

-Solubilization of peptides

-Formation of reversed micelles

-Phospholipid acylchain disruption

4.2.3 Fatty acids & its derivatives:

Based on the research conducted in the last decade it has become clear that several sodium salts of medium chain fatty acids are able to enhance the paracellular permeability of hydrophilic compounds Among these MCFAs, sodium caprate is the most extensively studied and the only absorption enhancing agent included in a marketed drug product. It is added in a suppository formulation intended for human use in Sweden and Japan. In Vitro and In Situ Studies of Sodium Caprate produced information regarding its mechanism which is shown below.

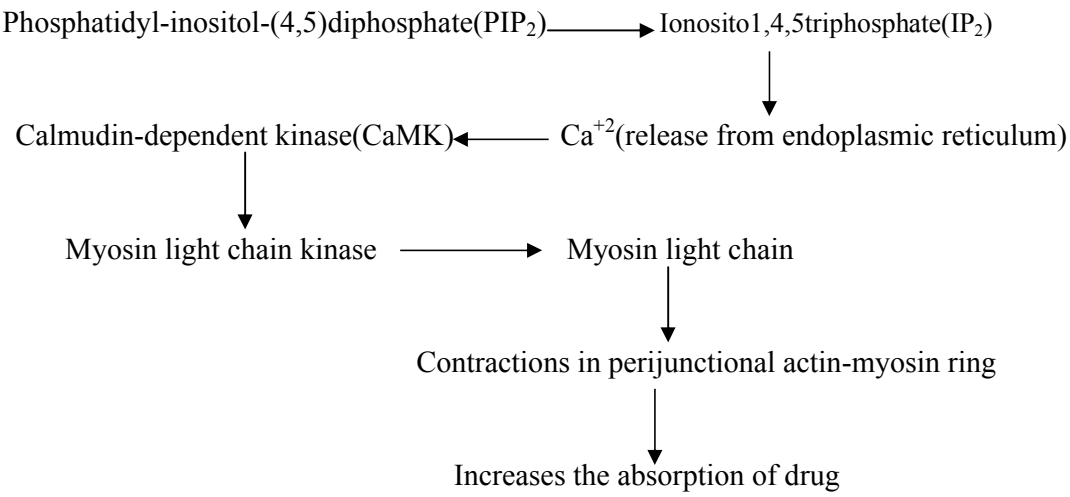

Figure- 5: Mechanism of sodium caprate.

Sodium caprate is able to modulate paracellular permeability by increasing intracellular calcium levels through the activation of phospholipase C in the plasma membrane, as represented in the above Figure. The increase in calcium levels is considered to induce the contraction of calmodulin-dependent actin microfilaments, resulting in increased paracellular permeability.

4.2.4 Chelating agents:

Chelating agent forms complexation of calcium and magnesium ions present in between intestinal epithelial cells and ultimately leads to opening of tight junctions and thereby increasing permeability for exogenous substances.

4.2.5 Chitosans & derivatives:

Chitosan[11] is a cationic polysaccharide obtained by partial alkaline N-deactivation of chitin. Chitin is insoluble in alkaline P^H and neutral values where as its derivatives are soluble at these P^H. High MW

polymers such as chitosan and its derivatives have gained considerable attention as permeation enhancers. Because of their high MW, these polymers are supposedly not absorbed from the gut, and systemic side effects are thus excluded.

These polymers were able to bind tightly to the epithelium and to induce redistribution of cytoskeleton F-actin and the TJ protein ZO-1, this being followed by enhanced transport via the paracellular pathway. Chitosan and its salts also act on tight junction and reduces its integrity and increases intestinal permeability. Chitosan derivatives are especially effective in enhancing the transport of small hydrophilic compounds (e.g., mannitol) though they also improve the transport of large molecules (drugs) such as buserelin, insulin, DGAVP and octreotide acetate.

4.2.6 Other enhancers:

Zonula occludens toxin (Zot):

Zonula occludens toxin (Zot)**,** a protein elaborated by *Vibrio cholera* that is able to reversibly regulate tight junction permeability.

This toxin interacts with a specific intestinal epithelial surface receptor, with subsequent activation of a complex intracellular cascade of events that regulate tight junction permeability. It was also shown that the *invitro* permeabilities of drugs with low oral bioavailability such as paclitaxel, acyclovir, and cyclosporine and enamione anticonvulsants were increased with Zot.

Polycarbophyl-cysteine conjugate(PCP-Cys):

It is a class of permeation enhancers is represented by thiolated polymers[12] also called thiomers. These are polymers in which the thiol groups are covalently bound. It has been shown that polycarbophyl polymers (PCP) display permeation enhancing effects. This property is significantly improved as a result of the covalent attachment of cysteine (Cys) to this polymer (PCP-Cys).

This thiolated polymer (PCP-Cys) is able to significantly increase the transport of marker compounds (sodium fluorescein) and peptide drugs (bacitracin-fluorescein isothiocyanate and insulin-fluorescein isothiocyanate) across the intestinal mucosa of guinea pigs. The thiol groups, covalently attached to the polymer, seem to be responsible for the improved permeation-enhancing properties of these conjugates.

These compounds exert their permeation enhancing effects via glutathione. It seems that PCP-cys can transform oxidized glutathione (GSSG) to reduced glutathione

(GSH), prolonging GSH concentration at the apical membrane. GSH is reportedly capable of inhibiting protein tyrosine phosphatase (PTP) activity by almost 100%, which leads to more phosphorylated occludin and to more open TJ as shown in the below figure.

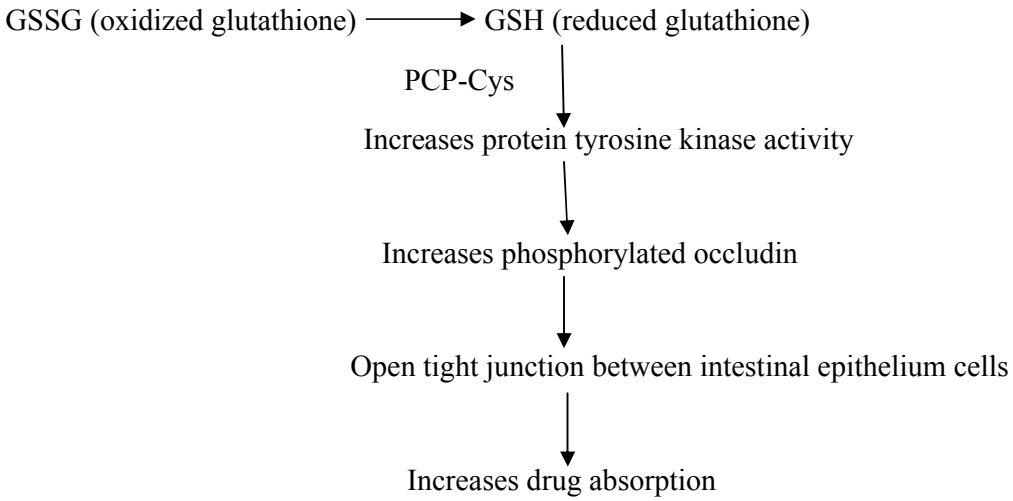

Figure- 6: Mechanism action of polycarbophyl-cysteine conjugates (PCP-Cys)

4.3 Permeability enhancer safety:

The safety of absorption enhancers depends on the mechanism of action. Some enhancers may reversibly 'loosen' tight junctions, or transiently increase membrane permeability - without damage - under 'very controlled' conditions.

Major issues regarding safety are given below

- Ulceration
- immunological issues
- pin-point membrane 'erosions'
- Creating a provision for rapid access of bacteria, virus or even endotoxins to the general circulation.

Several marketed products containing proven absorption enhancers. Yet none have reported in an increased incidence of systemic toxicity. In theory, hyper absorption of a co administered poorly bioavailable drug would be possible during chronic use of an absorption enhancer.

5. IN VITRO METHODS FOR STUDYING DRUG PERMEABILITY:

For reasons of safety and cost, drug absorption studies[13] in humans are only carried out for a limited number of well-characterized drugs. Studies of drug absorption in the intestine traditionally

been carried out in experimental animals. However, the introduction of combinatorial chemistry and high throughput pharmacological screening in drug discovery has significantly increased the number of compounds entering the pre-clinical phase, and this has made it impossible to assess the absorption properties of all these compounds in experimental animals. This fact has spurred the development and use of *in vitro* methods to assess drug permeability properties in most drug discovery settings. Also, the insight that drug absorption across biological barriers is a complex process involving several pathways that cannot easily be delineated in experimental animals has resulted in the large interest in academic and industrial institutions in these methods .The methods are, cultured cells and artificial membranes.

5.1 Cultured cells:

The human adenocarcinoma[14]cell line Caco-2 model suitable for screening intestinal drug permeability and predicting the oral absorption potential of new drug substances. The Caco-2 cells were grown on permeable supports and spontaneously formed polarized monolayers that resembled that of the intestinal epithelium as shown in below figure.

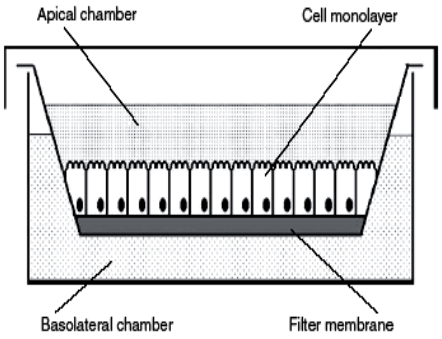

Figure-7: Caco-2 cell

In many respects the Caco-2 cells are therefore functionally similar to the human small intestinal enterocyte, despite the fact that they originate from a human colorectal carcinoma. The methods that are based on cultured cells such as Caco-2 cells are, however, not only useful for drug absorption screening. It is also possible to extract information about specific transport processes that would be difficult to obtain in more complex models such as those based on whole tissues from experimental animals. For instance, the methods enable us to investigate the relative contribution of passive

transcellular and paracellular transport, the effect of charge on paracellular transport and the effect of solvent drug. Caco-2 is the most widely used cell line for drug permeability studies.

5.2 Artificial membranes:

- Epithelial cell cultures.
- Usage of immobilized phospholipids or liposome in organic solvent.
- Chromatographic methods where the stationary phase consists of immobilized phospholipids or liposome.

These methods are attractive for screening purposes since they require very little compound, are easily automated and are adaptable to diverse sets of drugs.

6. ADVANTAGES OF INTESTINAL PERMEATION ENHANCERS:

- Several types of intestinal permeation enhancers are available
- They have diverse mechanism of action
- They are cost-effective
- They are companionable with several drugs

7. APPLICATIONS:

- Enhances oral bioavailability of drug
- Increases pharmacological action of drug
- Complete absorption of poorly permeated drugs and no wastage

8. IDEAL CHARACTERISTICS OF INTESTINAL PERMEATION ENHANCERS:

- They should increase drug shipping across intestinal barrier
- They should be reversible in their action
- They should be non-hazardous to GIT epithelium

9. FUTURE OF ABSORPTION ENHANCERS:

Although many safe and effective permeability enhancers are currently available, the control of their exposure at the epithelium will require a measure of formulation sophistication. Fortunately, such formulation capability is within reach of most pharmaceutical companies. Emerging evidence suggests that multiple cellular signaling pathways are likely to be involved in the modulation of paracellular permeability by these absorption enhancers. As such, they may be directly or indirectly altering the actin microfilament network and/or its association with peripheral proteins of the tight junction complex. Future

research on mechanisms should focus on what concentrations and exposure times result in, 1. Transcellular or paracellular enhancement; 2. Disruption of ion flux, energy and/or tight junction integrity 3. Readily reversible, slowly reversible, and irreversible permeability 4. Foliation of the epithelium. As the time- and concentration-dependent mechanisms of permeability enhancers become fully characterized, their marketing potential will become recognized.

10. CONCLUSION:

It is most promising that absorption enhancers increase drug transport through the intestinal barrier and there by increases the drug bioavailability. Utilization of different intestinal permeation enhancers, many drugs bioavailability can be increased significantly. Fortunately apparently safe and effective intestinal permeability enhancers are currently available, so formulation expertise is within reach of most pharmaceutical companies.

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