SAPONIN AS ABSORPTION ENHANCER

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ABSTRACT

One of the important aim for the pharmaceutical industry is to optimize bioavailability of orally administered drug. Drug transport across mucosal membrane is a fundamental step for oral absorption. Class III and IV drugs of biopharmaceutical classification system, show low membrane permeability. Strategies such as, structural modification of the drug molecule to increase lipophilicity, prodrug method, formulating with permeation enhancer, ion pairing and complexation have been reported to enhance membrane permeation. In this article, we have reviewed the use of saponin as absorption/permeation enhancer. Saponin contains a steroidal or triterpenoid aglycon attached to one or more sugar chains. They exhibit membrane permeabilizing property. Saponin acts mainly by solubilising cholesterol and leaves much of membrane structure intact. Saponin can affect the integrity of biological membrane. By many examples of saponin viz. Gypsophylla saponin, saponin from Aralia elata, saponin from Phytolacca americana, saponin from Tribulus terrestris it may described.

KEY WORDS: absorption, drug permeability, absorption enhancer, saponin.

INTRODUCTION

Absorption enhancer

Oral administration is the most common method of drug delivery from ancient time and still it is the most preferred route for drug administration. The important aim for the pharmaceutical industry is to optimize bioavailability of orally administrated drug. Drug transport across mucosal membranes is a fundamental step for oral absorption and systemic availability. The drugs with small molecular weight and lipophilic in nature are easily
permeated through the intestinal barrier whereas macromolecules are restricted by the intestinal epithelial barrier which causes reduced bioavailability. Mostly available drugs fall under the class III of the biopharmaceutical classification system (BCS), possess high therapeutic potential but cannot be effectively delivered by oral route because of its poor permeation across the gastrointestinal epithelia. Drugs show low intrinsic membrane permeability, because of their low lipophilicity and zwitter ionic character at physiological pH or substrate to drug efflux pumps like p-glycoprotein, high molecular weight and ionic charge. [1, 2]

The oral delivery of class III and IV drugs present in BCS classification is partially or completely decreased due to their poor intestinal permeability. Most of the drug molecules show poor permeability due to their physicochemical and chemical properties which are difficult to change so to enhance permeation transiently, an excipient may be added externally. [2]

Drug delivery by oral route is preferred for its convenience. Tablets and capsules can be formulated in large quantity at low price as compared to other dosage form. Therefore, in lead optimization step of drug discovery, oral bioavailability is important. It depends on various factors like intestinal permeability, solubility during gastrointestinal transit, liberation from dosage form, liability to efflux and metabolism. The solubility and permeability property is especially reflected in the adoption of Amidon’s BCS by the FDA in 2000, devised as a scientific basis to grant biowaivers for in vivo bioavailability and bioequivalence studies. [3, 4]

Poor membrane permeation is most commonly due to either poor partitioning into the lipid membrane or low membrane diffusivity. Some strategies, such as pro-drug method, structural modification of the drug molecule to increase lipophilicity, formulating with permeation enhancers, ion pairing, and complexation have been reported in the literature. Pro-drug or Structural modification methods to enhance membrane permeation involve extensive chemistry, toxicological, and efficacy studies and also needs additional regulatory approval procedures. But, formulations with permeation enhancers are safe and non-toxic and also provide an easier and faster solution. [5-8]

Drug molecules across the intestinal epithelium transported by passive diffusion through transcellular or paracellular routes, through carrier-mediated active or facilitated transport.
Among all, the paracellular route is a dominant pathway for the passive transepithelial transport of hydrophilic drug molecules in the small intestine. Mostly hydrophilic drugs are not absorbed by the intestinal epithelium because of the presence of junctional complexes and their physicochemical characteristics such as hydrophilicity and molecular weight. Many approaches used to increase the intestinal absorption of hydrophilic drugs, use of absorption enhancers, such as surfactants, fatty acids, medium-chain glycerides, N-acetylated α-amino acids, N-acetylated non-α-amino acids, steroidal detergents, acylcarnitines, alkanoylcholines, mucoadhesive polymers, and secretory transport inhibitors is one of them. In the present study, we reviewed the use of saponins as absorption enhancers. [9-12]

Absorption rate controlling step [13-14]

Table: BCS Classification

<table>
<thead>
<tr>
<th>Class</th>
<th>Permeability/Solubility</th>
<th>Absorption rate control step</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>High/High</td>
<td>Gastric emptying</td>
</tr>
<tr>
<td>II</td>
<td>High/Low</td>
<td>Dissolution</td>
</tr>
<tr>
<td>III</td>
<td>Low/High</td>
<td>Permeability</td>
</tr>
<tr>
<td>IV</td>
<td>Low/Low</td>
<td>Case by case</td>
</tr>
</tbody>
</table>

Potential absorption barriers [15-20]

The location of potential absorption barriers may be in the unstirred water layer, the mucous layer, the apical and basal cell membrane and cell contents, the tight junctions and the wall of lymph and capillaries.

Mucous

A mucous layer consisting of water glycoproteins (mucins), electrolytes, proteins and nucleic acids covers the epithelial cells of the entire intestine. This membrane layer is bound to the apical surface by the glycocalyx which is covalently linked to lipids and proteins of the brush border membrane. The unstirred water layer is composed partially of the mucous layer, and the minimal thickness of the unstirred water layer should be 50-100µm. This layer maintains the pH of the epithelial surface near 6 by acting as a buffer.

Apical cell membrane

The apical cell membrane is like a 1µm thick brush border, and it consists of a 10nm thick double layer of polar lipid molecules arranged as hydrophilic phosphate head points ‘out’ to the water on each side of bilayer and the hydrophobic tails point ‘in’ to the core of the bilayer. Lipid constituents are phosphatidylcholine, phosphotidyl ethanolamine,
sphingomyline (zwitterionic), phosphatidylinositol, phosphatidyl serine, phosphatidic acid (anionic), cholesterol and lipids. Proteins are entrenched in hydrophobic segments of lipid bilayer. For the optimal activities of membrane bound enzymes, fluid state of the membrane is required; cell preserves the membrane transition temperature below environmental temperature. Cholesterol having regulating action is employed on membrane structure, increasing fluidity of gel-state membrane and decreasing fluidity of liquid crystalline membrane. Sphingomyline enhance the assemblong influence of cholesterol. Natural fatty acids also influenced membrane because their cis-double bonds distracting phospholipid organisation. For this reasons fluidity of fluid state membranes may increase with decreasing cholesterol/phospholipid molar ratio or increasing total lipid/protein ratio and double bond index thus increasing permeability. The transport of molecules across the phospholipid bilayer is commonly correlated with lipid-water coefficient.

**Basal cell membrane**

The basal cell membrane is 9 nm thick phospholipids bilayer which contains proteins. Because of lower content of glycosphingolipids, the lipid fluidity of the basolateral membrane surpasses apical membrane fluidity. Hence the barrier function of the basal membrane is possibly less prominent than that of apical membrane.

**Tight Junctions**

Tight junctions are regions of close communication between apical ends of epithelial cells. They are constructed of a network of strands. Tight junction permeability increases with the decreasing strand number, thus determining the ‘leakiness’ of epithelium. The medium sized solutes (e.g. disaccharides), ions and water thus establishing route for passive ion permeation. Tight junctions are cation selective and they have been suggested for cations with a molecular weight higher than 350 nm or a diameter exceeding 0.8 nm which are impermeable. Alternatively, it is conceivable that a distribution of pore sizes exists, with a large number of small pores and a few large ones. The tight junction structure is destabilized by exposure to hypertonic solutions and by Ca$^{2+}$ depletion.

**Capillary wall**

Capillary wall is located in 500 nm underneath the basal membrane. The endothelial cell membrane of capillary wall contains small perforations of 0.4-1 nm radius and the blood capillary wall is fenestrated, fenestrate radius up to 20-30 nm while lymphatic capillaries are provided with an intracellular junction of larger size, permitting passage of particles having...
radius up to 300 nm. Particles having radius less than 6 nm are not retained by basement membrane surrounding fenestrated capillaries. Due to the existence of large pores, the intestinal blood and lymph capillaries are not considered to execute an important barrier for drug absorption.

**Mechanisms of intestinal drug permeability** [21-30]

The transport processes of drug molecule across intestinal epithelia are mediated through one or several of the following: passive transcellular, paracellular, active carrier-mediated and endocytosis as well as carrier-mediated efflux. The endocytosis or vesicular pathway has very limited capacity, and is only of relevance for the transport of small amounts of macromolecules that are excluded from the other pathways. Component passes through vesicular may be oil soluble vitamins, fats and starch.

**Passive transcellular transport**

These transport process do not require energy other than that of molecular motion (Brownian motion) to pass through the lipid bilayer. Drug transport via this route requires that the solute permeates the apical cell membrane. In the absorption process, the permeation of the drug occurs across the apical membrane that requires the drug is sufficiently lipophilic. Therefore moderate size lipophilic drugs are normally transported by the transcellular route. Most of the rapidly and completely absorbed drugs are absorbed by passive transcellular diffusion.

**Active transport**

These transport process require energy from ATP to move drug molecules from extracellular to intracellular milieu. 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.

**Paracellular transport**

In this process transport of drugs takes place through the junction between the gastrointestinal epithelial cells. Drugs having small to moderate molecular weights (MWs) can permeate the intestinal epithelium through the water-filled pores between the cells. Paracellular transport is generally considered to be a passive process, even if this pathway appears to be selective for cationic rather than anionic and neutral drugs.
Saponin as absorption enhancer

Agents that decrease or remove extra cellular layer resistance reversibly and allow the drug to pass through epithelial cells toward blood and lymph are permeability enhancers. Recently, enhancing drugs permeability through cellular membrane becomes one of the main issue in pharmaceutical researches. [31]

A new technique using saponin is described as permeabilize cell membranes. Saponins are secondary metabolites of the plants which contain a steroid or triterpenoid aglycon attached to one or more sugar chains. They exhibit cell membrane permeabilising properties. Saponin properties can affect the integrity of biological membrane. Saponin acts mainly by solubilising cholesterol and leaves much of the membrane structure intact. Their soapy character is due to their surfactant properties. [32]

Like saponin, other surface active agent used as enhancer includes bile salts, glycerides, anionic detergents and lysophospholipids. Morphological and biochemical studies on absorption sites of membrane showed that surfactants enhance membrane transport, followed by acute toxicity, and moreover these effects were reversed after a long time. Permeability enhancing effect of surfactants is not only related to its nature, but also depends on other characteristics like electrical charge, polarity and the membrane. The surfactant may be absorbed and penetrate to the cell membrane, where it affect osmotic phenomenon by altering the permeability of membrane, which in turn causes the cellular lysis. [33-34]

Saponins are glycosides of vegetable source with surfactant (or, surface tension reducing) properties and haemolytic action. They precipitate sterols and exert intestinal and transdermal absorption promoting properties. Absorption supporting properties of saponins may be mediated by their surfactants like properties. A transcellular promoting effect may also be caused by interaction with the membrane stabilizer cholesterol shows that saponins exhibit absorption promoting activity at relatively low concentrations. [23, 35-37]

The naturally occurring saponins molecules composed of sugars conjugated to triterpenes or steroids exert transmucosal permeation enhancing effect. In a previous report, surfactants including saponin, increased the permeability of cell membranes. There are also indications that surfactants can affect not only the cell membrane but also the tight junction (TJ). Saponin shows significant promoting effects on corneal and conjunctival apparent permeability coefficient (Papp) of thyrotropin releasing hormone and its action may be increase in the
permeability in paracellular pathway. Quillaja saponin and the chemically modified compound increased the paracellular permeability induced the dysfunction of the TJ causes opening of the paracellular route. \cite{38-41}

It is clear that saponins having utility as intestinal absorption enhancers at low concentration by affecting the transport mechanisms and permeability in the intestinal epithelium. Saponins also reduce the permeability barrier to sodium at the brush border, thus discharging the electrochemical gradient and removing the driving force for sugar transport. \cite{42-43}

Ayyanna et al. studied about the enhancement of the gastrointestinal absorption of poorly absorbable drugs. They used Metformin hydrochloride, a BCS class III drug, having high solubility and poor absorption properties. The intestinal absorption of Metformin HCl is enhanced by using Tribulus terrestris Linn (Gokhru plant) extract, a natural enhancer on intestinal absorption by *everted sac* technique using goat intestine. The drug was absorbed through intestine mainly by passive diffusion mechanism. The absorbed drug was determined by using U.V Visible Spectrophotometer at 234nm. After analyzing the results of all experiments it is found that the Tribulus terrestris Linn plant extract enhance the absorption of Metformin HCl from goat intestine and the absorption enhancement activity of the plant is due to the presence of saponins in the extract. \cite{44}

Johnson et al. investigated the influence of four saponins, three triterpenoid glycosides and one steroidal amine glycoside, upon intestinal transport *in vitro*. The presence of Gypsophylla saponin inhibited carrier-mediated galactose transport, although the uptake of the passively transported L-isomer of glucose increased. The uptake of the polyethylene glycol 4000, extracellular space marker was also higher, indicating the saponin inhibited active transport by increasing the permeability of the enterocytes. Gypsophylla saponin, in contact with the mucosal surface of everted jejunal sacs, induced a rapid decline in glucose-stimulated transmural potential difference. The rate of decline increased as the saponin concentration was increased over the approximate range of 0.3 to 8 mm. Saponaria saponin and a-tomatine also reduced transmural potential difference, but soya saponins were much less effective. The results indicate that some saponins readily increase the permeability of the small intestinal mucosal cells. \cite{42}

Joon Soo Sim et al. investigated the intestinal absorption enhancing effect of the saponins from the root bark of Aralia elata (SRBAE) in Caco-2 cell monolayers and rats. SRBAE at
concentrations of 0.04% and 0.08% (w/v) decreased the transepithelial electrical resistance (TEER) values and increased the paracellular uptake of chondroitin sulfates (CSs) having different molecular weights (MW 500, 4500, and 18000) in a dose-dependent manner. CS (MW 18000) was orally administered with or without SRBAE to rats. The oral administration of SRBAE (250mg/kg) in 1h increased the intestinal absorption of CS, by 4.9-fold versus the control (CS alone). Histological examination of the gastrointestinal tissues showed that SRBAE did not cause any damage to tissues. In conclusion, results suggest that SRBAE acts as an efficient absorption enhancer and makes it easier for hydrophilic molecules to penetrate the intestinal epithelium. [31]

So Yean Cho et al. Studied about the effect of phytolaccosides, saponins from Phytolacca americana, on the intestinal absorption of heparin in vitro and in vivo. The intestinal absorption enhancing property of these compounds (phytolaccosides B, D2, E, F, G and I) was determined by changes in transepithelial electrical resistance (TEER) and the transport amount of heparin disaccharide, the major repeating unit of heparin, across Caco-2 cell monolayer. All of them decreased TEER value and increased the permeability in a dose dependent and time dependent manner except phytolaccoside G. In vitro, phytolaccosides B, D2, and E showed significant absorption enhancing activities, while phytolaccoside F and I showed mild effect. In vivo, phytolaccoside E increased the activated partial thromboplastin time (APTT) and thrombin time, indicating that phytolaccoside E modulated the transport of heparin in intestinal route. Their results suggest that phytolaccosides from Phytolacca americana can be applied to improve the permeability of macromolecules and hydrophilic drugs having difficulty in absorption across the intestinal epithelium. [37]

CONCLUSION
Absorption enhancers are those agents which are used to increase drug transport through intestinal barrier and thereby increase drug’s bioavailability. Above discussion, clearly indicates that saponin can be used as absorption enhancer in pharmaceutical preparations. Saponins are easily available and are comparatively economical than other absorption enhancers. Many useful drugs are found in BCS Class III and Class IV which shows poor absorption property. Hence, saponins can play vital role in this field.
REFERENCES


