REVIEW ARTICLE

Influence of excipients on drug absorption via modulation of intestinal transporters activity

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One of the major factors affecting oral drug bioavailability is the activity of intestinal transport proteins, particularly for the drugs that undergo absorption by active transport mechanism. Many of the active pharmacological agents and the excipients used in their formulation are reported to modulate the activity of these transporters thereby either enhancing or decreasing the drug absorption and its systemic availability. These excipients are considered pharmacologically "inert" and have been used since years in pharmaceutical formulations. Appreciable interest is developing on the data demonstrating the role of excipients in altering the drug absorption across the intestine. Careful selection of the excipients thus is very important. A correctly chosen excipient can enhance the drug bioavailability and thus its therapeutic efficacy without increasing its dose. For locally acting drugs having systemic side effects, a proper excipient could lead to a decrease in its systemic absorption, thus reducing its side effects. This review focuses on the current findings of the excipients identified to modulate the activity of transporters, their mechanism of modulating the transporter's activity and various formulation strategies using these excipients to enhance drug absorption.

Key words: *Caco-2 cell monolayers, drug transport, intestinal transporters, pharmaceutical excipients*

INTRODUCTION

Oral route has remained most preferred route of administration for drug delivery due to its convenience of administration, ease of production as well as considerable patient safety. Upon the oral administration of three major factors as can be seen in Figure 1, govern the drug absorption: Dissolution rate of drug, solubility of the drug in luminal fluid and drug permeability across biomembrane. Hence, a drug has to permeate through the intestinal barrier, which is accomplished by passive paracellular (through intercellular space between the cells), passive transcellular (through the cells), and carrier-mediated transport or by transcytosis. Passive route of absorption is governed by diffusion, the driving force for which is the concentration gradient. Carrier mediated transport, on the other hand, depends on transport proteins and is either energy dependent or independent.[1-3] Various such transport proteins are discovered that affects the absorption of many drugs and their metabolites through the intestinal barrier, blood brain barrier, and other organs like liver and kidney. These transport proteins include P-glycoprotein (P-gp), multi-drug resistant protein, oligo-peptide transporter and others. Transcriptionally, a higher expression of transport proteins could change its activity. After protein transcription, stabilization or destabilization of mRNA could also play a role. Amount of proteins at any particular time may also vary by translation, posttranslation events or trafficking of proteins at the membranes. Once inside the membrane, activity of proteins could be altered by competitive, noncompetitive inhibitors or allosteric effectors.[4,5] Most of these transporters are secretory type (efflux back their substrates to the luminal side) thus impeding the drug permeability through intestinal epithelium producing lower pharmacological response.

Many drugs are already known to inhibit these transport proteins activity. For example, drugs like

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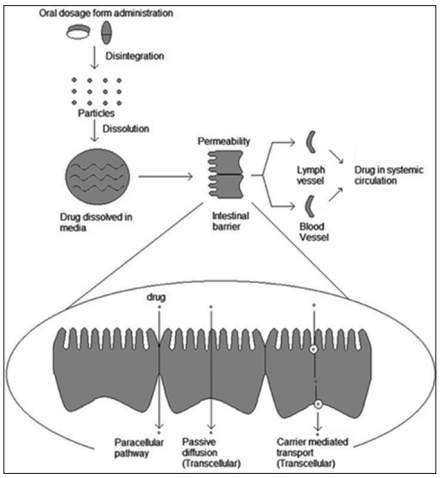

Figure 1: Factors influencing oral drug absorption: Dissolution rate of drug, solubility of the drug in luminal fluid and drug permeability across biomembrane

quinidine,[6] verapamil[7] and vinblastine[8] are recognized as P-gp inhibitors. Hence, co-administration of such P-gp inhibitors with the P-gp substrates is expected to increase the absorption of the substrate drugs. Excipients, which are considered pharmacologically inert components of a dosage form, have also been found to modulate the activity of the transport proteins. Thus, use of excipient having an inhibitory effect on the secretory transporters could increase the absorption of drugs found to be substrates of these transport proteins. Careful selection of the excipient for development of formulation of a new drug is thus an important parameter in achieving enhanced bioavailability. For existing formulation, unexpected pharmacokinetic performance may be prevented. Moreover, various novel drug delivery systems like self-microemulsifying drug delivery system, micelles, liposomes and nanoparticles incorporates these excipients, and thus possess inherent properties of modulating transporters activity. Such formulations are listed in this review.

This review focuses on the various intestinal transporters, different categories of excipients which are found to modulate their activity and the novel drug delivery systems reported to change the activity of these transport proteins. The knowledge of these properties of excipients could be useful from the regulatory point of view to evaluate postapproval changes in the formulation.

INTESTINAL TRANSPORTERS

Membrane-associated proteins (or simply transporters) are integral transmembrane proteins existing permanently within cells and span the membrane across which they transport substances. These membrane proteins govern the transport of solutes (for example, drugs and other xenobiotics) into and out of the cells. The transporters can play an important role in the level of drug concentrations in the systemic circulation and in cells. Furthermore, transporters on either side of the membrane can affect the uptake and/or efflux of drugs. Influx transporters act by facilitating the translocation of substances into the cell while efflux transporters pump materials out of the cell.[9] Their location on apical or basolateral side of the membrane determines the transport of substances on either side. For example, on apical membrane, efflux transporters prevent the entry of undesirable, potentially toxic materials into the systemic circulation.[10] Transporters are categorized into two super families: ATP-binding cassette (ABC) transporters and Solute carrier (SLC) transporters.

ATP-BINDING CASSETTE TRANSPORTERS

The ABC genes represent the largest family of transmembrane proteins. There are 49 ABC genes recognized in humans. Out of these, functioning of 16 ABC genes has been identified and of them, 14 ABC genes are known to be associated with definite human disease. They are involved in physiological processes like transport of ions, lipids, amino acids, peptides and toxic compounds. They are also known as nucleotide binding folds consisting of characteristics motifs (called as walker A and B) separated by approximately 90–120 amino acids. ABC genes contain an additional segment, known as signature C motif, located just upstream of walker B side.[11,12] These transporters efflux their substrate back using energy from ATP-hydrolysis and are mostly unidirectional. Various drugs, most importantly chemotherapeutics, are substrates of these transporters. Hence, ABC transporters are major reasons for causing multi-drug resistance (MDR) affecting the oral bioavailability of its substrates drugs.[13] ABC transporters, apart from intestinal epithelium, are also found in normal tissues and are highly expressed in cancerous cells. This superfamily is divided into seven subfamilies: A–G and each subfamily contains many transporters.[14,15] Those ABC transporters found to be expressed in the intestinal epithelium and will be of concern in later review of excipients effect on them are described in Table 1.

SOLUTE CARRIER TRANSPORTERS

Solute carrier transporters are generally located in the cell membranes and use ion gradients as driving force or functions by the principle of facilitative transport. They contain many hydrophobic transmembrane alpha helicles connected by hydrophilic intra-and extra-cellular loops. The SLC gene products include passive transporters, symporters, antiporters, mitochondrial and vesicular transporters located in all cellular and organelle membranes, except perhaps the nuclear membrane.[19] SLC superfamily is categorized into 55 families covering about 386 members,[20] those found in the intestinal epithelium and are discussed in later review are described in Table 2.

EXCIPIENTS AND TRANSPORTERS ACTIVITY

Excipients play vital and differential role in a formulation like solubilization of drug in the formulation, ease of

Table 1: ABC transporters expressed in the intestinal epithelium

Transporter	Abbreviation	Location	Function	Reference
MDR P-gp	ABCB1	Apical membranes in various tissue membranes like intestine, liver, blood brain barrier, kidney and placenta	Work to efflux many xenobiotic compounds with broad substrate specificity. Causes decreased drug resistance in MDR cells. Particularly effluxes hydrophobic substrates	[12,16]
MRP1	ABCC1	Basolateral side in various tissues like intestine, lung, testes and peripheral blood mononuclear cells	Transports organic anions, oxidized glutathione, cysteinyl leukotrienes and activated aflatoxin B1, drugs in the presence of glutathione	[16]
MRP2	ABCC2	Apical side in tissues like intestine, liver and kidney	Biliary transport of mainly anionic conjugates with sulfate or with glucuronosyl. Also many drugs like anticancer drugs	[12]
MRP3	ABCC3	Basolateral side in intestine and kidney	Not clearly known but may transport organic anions like bile salts	[16]
BCRP	ABCG2	Apical side in small intestine, colon, hepatocytes, breast and placental barriers	Transports xenobiotics, organic anions and steroids (like cholesterol and progesterone)	[17,18]

BCRP: Breast cancer resistant protein, P=gp: P-glycoprotein, MDR: Multi-drug resistance, MRP: Multi-drug resistance associated protein, ABC: ATP-binding cassette

Table 2: SLC transporters expressed in the intestinal epithelium

Transporter	Abbreviation	Location	Function	Reference
PepT1 - Proton dependent oligopeptide transporter	SLC15 A1	Apical membrane in intestinal epithelium and bile duct epithelium	Absorptive function. Transports di-and tripeptides, uptake and digestion of dietary proteins and absorption of peptidomimetic drugs	[21]
MCT1 - Proton linked monocarboxylate transporter	SLC16A1	Apical side in intestinal epithelium and in erythrocytes and cardiac muscle	Absorptive function. Uptake of weak organic acids (like benzoic acid and pravastatin) and atorvastatin	[22]
OCT1 - Organic cation transporter	SLC22A1	Basolateral side of small intestinal enterocyte, renal proximal tubular cell and at lower levels in some neurons, the heart, skeletal muscle, tumor cells and basophilic granulocytes	Mediates sodium independent transport of type 1 organic cations (like choline) and type II cations (like quinidine)	[23,24]

SLC: Solute carrier, MCT: Monocarboxylate transporter

compressibility of tablet, encapsulation of drug, modifying drug release, coating the drug or the formulation, fillers, flavoring agent, coloring agent or for stabilizing the formulation. There are few significant differences in the physiology of the patients suffering from particular diseases like HIV infection, cancer etc., In such cases, varieties of excipients may affect the pharmacokinetic profile of the formulation differently in healthy and diseased personals. Hence, these effects should be preassessed. Excipient, which are found to modulate transporters activity are broadly classified as given in Table 3.

SURFACTANTS

Surfactants are added in formulation for varied reasons including solubilization of drug, stabilization of formulation, modification in surface properties of semi-solid formulations or for alteration in the floability of granules in order to ease during compression of tablets. Surfactants may carry anionic, cationic or nonionic charge. Nonionic surfactants like esters of fatty alcohols are commonly used in pharmaceutical formulations since these surfactants show lesser cytotoxicity in comparison to charged surfactants.

Cremophor®

These are nonionic surfactant. Two grades of Cremophor® have been identified to affect the activity of intestinal transporters: Cremophor® EL and Cremophor® RH 40, both being ester derivatives of castor oil. Both have hydrophilic lipophilic balance (HLB) value in the range of 12–14.

Cremophor® EL

Cremophor® EL (a polyethoxylated castor oil) is used as a solubilizer and as emulsifier particularly in liquid preparations. It is a good vehicle for increasing the solubility and thus bioavailability of various poorly soluble drugs like paclitaxel and saquinavir.[25] Being nonionic in nature, it exhibits lesser toxicity *in vivo* in comparison to ionic surfactants. However, it has been postulated that in addition to the solubilizing effect of Cremophor® EL the bioavailability enhancement of P-gp substrates might also be due to its P-gp inhibitory effect.

Table 3: Classification of excipients that are found to modulate activity of intestinal transporters

Category	Example
Surfactants	Cremophor, Tocopheryl-polyethyleneglycol-1000-succinate, Polysorbate 80, Poloxamers, Solutol HS15, Softigen 767, PEG and its derivatives
Polymers	Thiolated poly (acrylic acid), Thiolated chitosan derivatives, Eudragit
Glycerides and its fatty acid derivatives	Labrasol, oleic acid, Peceol®
Lipids	Phospholipids, rhamnolipids
Miscellaneous	Cyclodextrin, glucose

In a study conducted by Shono *et al*.,[26] the effect of Cremophor® EL on the uptake of rhodamine 123 (RHO 123) as a P-gp substrate, was evaluated. The study was conducted in a rat jejunal intestinal tissue and used Cremophor® EL concentrations ranging from 0.005 to 1% w/v. They found that Cremophor® EL at concentration as low as 0.05% w/v caused increased transport of RHO 123 from apical-to-basolateral side and decreased transport from basolateral-to-apical side of tissue. The effect was found to diminish at high concentrations. This result might be due to the fact that at higher concentrations, the amount of RHO 123 available for transport is reduced and so would be absorption. MDR associated protein 1 (MRP1) and MRP3 are present on the basolateral side of the membrane as secretory transporters. RHO 123 is also known to be a MRP substrate. Hence, to evaluate whether the enhanced absorptive transport of RHO123 by Cremophor® EL was a consequence of the common action of these transport proteins (P-gp, MRP1 and MRP3), Shono *et al*. further added Cremophor® EL to basolateral side of the cell tissue (since MRP1 and MRP3 are present on this side). No inhibition of RHO 123 transport was observed and the authors suggested that MRP1 and MRP3 either does not alter RHO 123 transport in rat tissue or that Cremophor® EL do not play role in the transport activity through these transporters. Cremophor® EL also increased transport from apical-to-basolateral side of P-gp substrate drugs like saquinavir,[27] digoxin[28] and docetaxel.[29] The effect of Cremophor® EL on MRP2 activity was studied by Hanke *et al*.[30] and they found that it moderately inhibited P-gp but, showed a strong inhibition on MRP2. Li *et al*. showed that Cremophor® EL led to an increased permeability of scutellarin (a MRP2 substrate) from apical-to-basolateral side and decreased basolateral-to-apical permeability. This led to a conclusion that Cremophor® EL probably inhibited MRP2 activity.[31] Malingré *et al*. reported a different aspect of Cremophor® EL in altering the absorption of paclitaxel when administered in cancer patients. They found a decreased absorption of paclitaxel in presence of Cremophor® EL as compared to Polysorbate 80. The authors suggested that at high concentration, Cremophor® EL forms micelles, paclitaxel gets incorporated into these micelles and the availability of paclitaxel for uptake decreases resulting in its decreased absorption.[32] Cremophor® EL (at 0.01-1 mM concentrations) did not alter transport of gly-sar (substrate of PePT1 - Proton dependent oligo-peptide transporter) and benzoic acid (substrate of monocarboxylate transporter [MCT] - Proton linked MCT) across Caco-2 cell monolayers[33] suggesting that it might not interfere with these transport proteins activity.

In conclusion, Cremophor® EL can be used at appropriate concentrations to increase absorption of transporters substrate drugs. On the other side, attempt of enhancing solubility of the drug through micellization can counteract the effect produced by inhibition of transporters activity. At appropriate low concentrations, enhanced absorption of the drug can be obtained through dual effect of micellar solubilization as well as transporters inhibition.

Cremophor® RH40

Cremophor® RH40 (polyoxyl 40 hydrogenated castor oil) is used as a solubilizer for various hydrophobic drug moieties. In a study performed using transfected Madin-Darby canine kidney (MDCK) II cell models stably expressing human ABCC2 (MRP2) transporter, Cremophor® RH40 was found to inhibit MRP2 in a concentration-dependent manner.[30] The concentration range used was 0.005–0.04% where it did not alter cell viability up to the concentration of 0.05% upon incubation for 7 h. Transport of calcein (MRP2 substrate) without MRP2 inhibitor was taken as a negative control and in the presence of probenecid (MRP2 inhibitor) was taken as a positive control. Increase in Cremophor® RH40 concentrations resulted in a significant reduction in secretory transport of calcein indicating that activity of MRP2 was inhibited by Cremophor® RH40 and probenecid in the cell line model taken. Cremophor® RH40 affected activity of MRP2 in lesser extent in comparison to Cremophor® EL as was seen during a study in Caco-2 cell monolayers. Scutellarin was taken as a MRP2 substrate in the study, and the efflux sequence of scutellarin for surfactants was obtained as: Cremophor® EL > Cremophor® RH40 > Pluronic® F68.[31] The result indicates that Cremophor® EL strongly inhibited the performance of MRP2 as compared to other two surfactants. The transepithelial electrical resistance (TEER) value during efflux study remained stable, and the authors concluded that the membrane barrier maintained its integrity during experiments. Safe concentrations of surfactants calculated from cytotoxicity study in Caco-2 cells were used in this study to nullify the other effects of surfactant.

Apart from MRP2, Cremophor® RH40 also inhibits P-gp activity as was seen in a clinical trial using digoxin (a P-gp substrate). It was found that Cremophor® RH40 increased digoxin absorption resulting in its increased bioavailability by up to 22%.[34] Taking this inhibitory effect on P-gp, Cremophor® RH40 has also been used as a surfactant to increase bioavailability of tacrolimus (a poorly water soluble

P-gp substrate) by formulating SMEDDS.[35] Thus, Cremophor® RH40 can be used for P-gp and MRP2 substrate drugs. Its effect on MRP2 can be further justified well by *in vivo* models while its effects on P-gp has already been proved by human studies and thus it could be a useful excipient particularly for enhancing bioavailability of P-gp substrates.

Tocopheryl-polyethylene glycol-1000-succinate

Tocopheryl-polyethylene glycol-1000-succinate (TPGS) is polyethylene glycol (PEG) ester of d-α-tocopheryl succinate. TPGS-1000 (PEG chain 1000) is a water-soluble form of Vitamin E used to treat Vitamin E deficiency. TPGS-1000 is used widely as a solubilizer to increase bioavailability of many drugs like amprenavir[36] and cyclosporine A.[37] It is listed as an excipient in USP24 and is used as an embedding agent for formulation of soft-gelatin capsules.

Permeability of paclitaxel (a P-gp substrate) across Caco-2 cell monolayers was found to be enhanced in the presence of TPGS-1000 in an *in situ* rat study. Below its critical micelle concentration (CMC) also, the absorptive transport of paclitaxel increased (in comparison to control-absence of TPGS). Above the CMC, absorption of paclitaxel decreased which might be due to micelle formation where the availability of free drug and free TPGS is low. Membrane integrity was found to be maintained during the study indicating that permeability enhancement by TPGS was not due to compromise in membrane integrity. This confirmed the P-gp inhibitory action of TPGS-1000.[38] This inhibitory effect on P-gp activity was further proved by clinical studies done using talinolol (metabolically stable and a P-gp substrate). The study was further controlled to exclude effect of surfactants (like solubility, dissolution and wetting) using non P-gp inhibiting excipient, Poloxamer 188, as a control and nasogastric tube administration route. Possible metabolic effects on bioavailability were also excluded using talinolol as substrate as it can be considered as metabolically stable. The area under the curve $(AUC)_{0-\infty}$ of talinolol was found to be increased by 39% through use of TPGS-1000 as compared to control group.[39] Hanke *et al*.[30] reported that TPGS-1000 also interacted with MRP2 transporters along with P-gp, causing its inhibitory action.

With respect to PEG chain length, it was found that TPGS-1000 (PEG chain 1000 Da) had optimal chain length (chain length of TPGS was varied from 200 to 6000 Da) required for enhancement of RHO 123 transport while maximum efflux inhibitory effect was found with TPGS having PEG molecular weight 1300 Da. Moreover, it was found that PEG 1000 alone had no effect on P-gp activity. Membrane integrity was found to be intact during study from TEER values and the concentration of TGPS was found to be noncytotoxic hence these effects could be excluded and it could be postulated that increase in absorption of RHO 123 was probably due to inhibition of P-gp activity.[40]

The marketed formulation Agenerase® soft gelatin capsules of 50 mg and 150 mg amprenavir contains 23% of TPGS. TPGS forms micelles at concentrations higher than 0.2 mg/mL in water and improves the aqueous solubility of amprenavir from 36 to 720 μg/L. It not only improves the gastro-intestinal absorption of amprenavir by improving its aqueous solubility, but also by increasing its permeability through inhibition of the intestinal efflux.[41]

In end, there is pure evidence that TPGS can enhance the bioavailability of various P-gp substrates like paclitaxel and talinolol. TPGS increased the solubility of these substrates as well as enhanced absorption by P-gp inhibitory action. The evidence came from *in vitro*, *in situ* and clinical trials. Moreover, its effect on MRP2 has also been found and clinical trial is needed to further prove its inhibitory effect on MRP2.

Polysorbate 80

Polysorbate 80 is a widely used nonionic surfactant added as an emulsifier in O/W emulsions as well as solubilizing agent. Now-a-days, it is also used with other excipients in many formulations with self-emulsifying properties like Rapamune® (oral solution containing sirolimus).[42]

Polysorbate 80 was found to increase apical-to-basolateral permeability and decrease basolateral-to-apical permeability of RHO 123 in Caco-2 cell monolayers at concentrations ranging from 0.01 mM to 1 mM in a dose-dependent manner.[33] In an another study, P-gp inhibition by Polysorbate 80 was found for a model peptide drug $(Acf(NMef)_2NH_2)$ in Caco-2 cell monolayers. Basolateral-to-apical permeability of the drug was decreased maximally to below CMC of Polysorbate 80 (~50 μM) which indicated that monomers of this surfactant were responsible for its activity and not its micellar forms. Apical-to-basolateral permeability was enhanced when concentration of Polysorbate 80 was increased up to 30 μM. It fluidized the membrane at even very low concentrations of 0.025 mM. The authors thus suggested the potential role of this surfactant on the transporters activity.[43] Recently Hanke *et al*. studied the influence of Polysorbate 80 on P-gp activity and MRP2 activity. They found that Polysorbate 80 inhibited activity of both transporters slightly.[30] It also decreased absorptive transport of gly-sar suggesting that it might also inhibit peptide transporter. Another mechanism of enhanced absorption for lipophilc drugs was addressed by Seeballuck *et al*. The authors stated that Polysorbate 80 gets digested by intestinal cells to liberate oleic acid, which in turn is used by cells to enhance basolateral secretion of triglyceride rich lipoproteins including chylomicrons. Increase in chylomicrons secretion leads to enhanced lymphatic uptake of lipophilic drugs through bypassing the first pass hepatic metabolism.[44] This feature has led to increased use of Polysorbate 80 for lipophilic drug candidates which are P-gp and MRP2 substrates, e.g. furosemide[45] and ganciclovir.[46] Although no *in vivo* and clinical studies has been carried out

to describe the effects of Polysorbate 80 on P-gp, *in vitro* results strongly prove it. Thus, Polysorbate 80 might be a potential excipient for lipophilic drugs candidates and P-gp substrates. Its multiple effects like enhancement of lymphatic transport, P-gp inhibition and enhanced solubility render it a valuable excipient for bioavailability improvement of lipophilic drugs. Its effect on MRP2 and peptide transporter need to be explored in a more detailed manner.

Poloxamer

Poloxamers are ABA block copolymers built by a hydrophobic polyoxypropylene chain (B) centrally flanked between two polyoxyethylene chains (A) on its either side. Poloxamers are widely used as O/W type emulsifying agents, viscosity increasing excipients and for surfactant gels. Pluronics P85 was found to inhibit both MRP1 and MRP2 in a study performed on MDCK cells resulting into increased intracellular accumulation of vinblastine and doxorubicin (MRP substrates).[47] In the same study, vinblastine transport was not affected by poloxamer-181 in MDCK II-MRP transfected cells suggesting that this surfactant might not affect MRP1 and MRP2 activity.[48] Pluronic® PE 10300 has also been found to cause a strong inhibition of P-gp whereas, it had no effect on MRP2 transport proteins.[30] Pluronic F127 also had no effect on MRP2 transport activity.[31]

Pluronic P85 inhibited apically located transporter activity as was seen in a study done on Caco-2 cells using RHO 123 as substrate. The transport of RHO 123 was increased on the basolateral side, but only when P85 was added on apical cell side. This indicated the inhibitory action of P85 on the transporters present on the apical side like P-gp and MRP2. This activity was observed at concentration of 22 μM that is lesser than its CMC (~67 μM) indicating that like other nonionic surfactants, here also monomers are the active moiety.[49] The polymeric content also affects the pluronic activity on transport proteins. It was found that with increasing polypropyleneoxide content and higher hydrophobicity, the efflux inhibition potency increased but up to a certain limit only. Higher hydrophobicity (HLB below 10) resulted on lower CMCs. Thus, optimal hydrophobicity (like that of pluronic P85) is required for this action.[50]

Solutol

Solutol HS15 is a nonionic surfactant composed of various molecular entities including mainly PEG-15-hydroxystearate. It is used a solubilizer for various lipophilic drugs. Solutol HS15 has been firstly shown to reverse MDR in MDR cell line KB 8-5-11 (human epidermoid carcinoma cell line) at concentration of 0.001% (10 μg/mL) measured by RHO 123 uptake. In the study, RHO 123 uptake was increased in the absorptive direction in the presence of solutol HS15. CMC of solutol was found to be in the range of 0.005–0.02% due to the fact that solutol is the mixture of many entities and variation in the mixture composition could affect the CMC.[51] Solutol has been used as an excipient in the formulation of paclitaxel loaded lipid nanocapsule to enhance bioavailability of paclitaxel. Solutol HS15 was used here to inhibit P-gp activity and thereby increase the absorptive transport of paclitaxel.[52] It enhanced AUC of a colchicines (a P-gp and cytochrome P450 substrate) by 4-fold as compared to control (without solutol H15). Since, colchicine is a highly soluble drug, its solubility cannot be enhanced by solutol HS15 and hence the effect could be due to P-gp inhibition or inhibition of CYP3A4 metabolism only.[53] Hence, solutol HS15 was able to increase absorptive transport of digoxin and rhodamine *in vitro* as well as enhanced bioavailability of colchicines probably by P-gp inhibition/inhibition of metabolism.

Softigen

Softigen 767 (PEG-6 caprylic/capric glycerides) is also a nonionic surfactant used as a solubilizing vehicle. Sufficient information about its ability to interact with intestinal transporters is not yet available. Cornaire *et al*. has studied its effect *in vivo* on digoxin and celiprolol (P-gp substrates) using rat. They found increased absorption of both drugs as well as their AUC in presence of softigen 767 probably by modulating the activity of P-gp. Paracellular transport as measured by mannitol transport was found to be one parameter affected by softigen 767, but it could not be the sole pathway for lipophilic substrates like digoxin and celiprolol.[28]

Polyethylene glycol and its derivatives

PEG 400 (5% w/v) inhibited digoxin transport in secretory direction in a study done by Johnson *et al*. This inhibitory action was found regardless of addition of PEG 400 on either side of membrane. It was concluded that changes in osmotic pressure at apical side might also result in decreased P-gp activity. While for basolateral side, apart from the partitioning effect, PEG 400 also could have an indirect effect on intracellular ATP levels resulting in efflux inhibition of digoxin.[54] The study done by Ashiru-Oredope *et al*. proved the effect of PEGs on P-gp activity. In this study, a specific screen of different PEGs for P-gp activity was used in order to demonstrate the interaction of PEG 200, 300 and 400 with P-gp activity using Caco-2 cell monolayers. It was found that PEG 300 as well PEG 400 interacted with P-gp transporters resulting in increased flux of ranitidine in the absorptive direction. However, PEG 200 did not showed any effect in the same study.[55]

However, in another study using talinolol (a P-gp substrate), IC_{50} of PEG400 was determined to be 56.25 mM, which was high in comparison to that of Cremophor® EL and Polysorbate 80 obtained as 0.41 and 0.23 mM respectively. It concluded the lesser effectiveness of PEG 400 in transporting talinolol in comparison to these two surfactants.[56] PEG 1000, increased transport of scutellarin from apical-to-basolateral side, reduced efflux from basolateral-to-apical side, and also showed MRP2 membrane vesicles transport assay indicating that PEG 1000 could definitely inhibit MRP2. Moreover, this

inhibition was comparatively greater than the inhibitory activity of PEG 400.[31]

Polyethylene glycol also has been derivatized to form polymer with P-gp inhibitory activity. PEG was grafted to polyethylenimine (PEI) and then thiolated using γ-thiobutyrolactone. This formed thiolated PEG-g-PEI co-polymer in concentration of 0.5% w/v enhanced RHO 123 absorption by 3.3 fold in comparison to control (RHO 123 without any inhibitor). It also decreased efflux of RHO 123 from basolateral-to-apical side across Caco-2 cell monolayers.[57]

Polymers

Polymers have become inevitable excipients in the pharmaceutical preparations because of their versatility of use in all types of dosage forms ranging from solid, semi-solid and liquid preparations to oral and injectable formulations. They are used for wide purposes like rate controlling agents, coating agents, binders, disintegrants and targeted delivery. Semi-synthetic and synthetic polymers have created great interest due to its properties of being modified. Polymers can be tailored for a specific application because of the presence of large chains and functional groups that could be altered by low or high molecular weight materials to obtain a new polymer with desirable properties.

Thiolated poly (acrylic acid)

Recently thiomers (thiolated polymers) have been widely explored as permeation modulators having controlled release capability, improved mucoadhesivity, permeation enhancing and enzyme inhibition properties. Besides chitosan derivatives, other polymers too have been found to modulate transport protein activities.[58,59]

Effect of a thiolated polymer, thiolated poly (acrylic acid)-250 (thiolated PAA_{250}) on MRP2 efflux activity was investigated using an MRP2 substrate, sulforhodamine 101. *In vitro* permeation study across rat intestinal mucosa as well as *in vivo* plasma concentration study inferred that permeation across intestine and absorption of sulforhodamine 101 was increased when thiolated PAA_{250} was co-administered with it in comparison to unthiolated PAA_{250}. Absorption was more from gut where, sulforhodamine 101 does not get absorbed in absence of thiolated PAA_{250} (it gets absorbed only in stomach), thus indicating inhibitory action of MRP2 since expression of MRP2 is reported to be highest in duodenum epithelium and absent in stomach epithelium.[2]

Thiolated chitosan derivatives

Chitosan is known for its widespread use in design of drug delivery systems. Various derivatives of chitosan have been developed to modify its properties. Thiolated chitosan derivatives have been reported to modulate the activity of efflux transporters.

N-octyl-O-sulfate chitosan

N-octyl-O-sulfate chitosan (NOCS) is derived by octylation of the amino group at C-2 and sulfonylation at C-6 position of chitosan. It is a novel amphiphilic polymer and now-a-day widely used in formulating novel drug delivery systems like micelles.[60,61]

N-octyl-O-sulfate chitosan micelles were found to successfully enhance the oral bioavailability of paclitaxel (a P-gp substrate) by 6-fold in comparison to orally dosed Taxol®. The mechanism behind this action of NOCS was studied using Caco-2 uptake studies. It was found that NOCS enhanced absorption of paclitaxel via clathrin- and caveolae mediated endocytosis as well as through inhibition of P-gp activity, which was also proved by enhanced RHO 123 uptake in the presence of NOCS. Further, it showed no effect on intercellular tight junctions confirming the integrity of the membrane during experiment. The mechanism behind P-gp inhibition was revealed to be interference with P-gp ATPase activity. Highest enhancement of P-gp inhibition was found to be at CMC of NOCS. By similar mechanism, NOCS was also found to enhance absorption of another P-gp substrate, etoposide, hence corroborating with the former results.[62,63]

Chitosan-4-thiobutylamide

Another thiolated chitosan derivative, chitosan-4-thiobutylamide, also enhanced RHO 123 uptake and its decreased basolateral-to-apical efflux across excised guinea pig ileum in Ussing chambers. This P-gp inhibitory action was found to be due to inhibition of ATPase activity of P-gp in intestine by this chitosan derivative.[64] The *in vivo* study using rats also supported *in vitro* results. Thiolated chitosan enhanced AUC_{0-12} of RHO 123 by 217% and 58% in comparison to buffer control and unmodified chitosan respectively.[65] In conclusion, both chitosan derivatives NOCS and chitosan-4-thiobutylamide were found to inhibit P-gp activity through inhibition of ATPase activity.

Eudragit

Eudragit L100-55 is a pH sensitive, anionic polymer. It is used as an enteric coating polymer in oral formulations. It has free carboxyl groups that enable pH of the media to be acidic. On the other hand, activity of PePT1 transporter increases by proton gradient and eudragit L-100-55 could activate PePT1.[66,67] Nozawa *et al*. studied intestinal absorption of cefixime, a PePT1 substrate in the presence of this polymer. The study was carried out in rats in an *in situ* ileal closed loops for determining intestinal absorption of drugs and *in vivo* plasma study. There was a significant increase in transport of cefixime and higher drug plasma concentration in the presence of eudragit L-100-55. When cefadroxil, a PePT1 substrate/inhibitor, was co-administered with cefixime, the activity of this polymer was blocked in a concentration-dependent manner that supported the fact that this polymer enhances PePT1 activity. When another

polymer, Eudragit RSPO, a proton-non releasing polymer, was co-administered orally with cefixime, the drug plasma concentration showed no enhancement as was seen with Eudragit L-100-55. This study further confirms the ability of specifically proton releasing polymer for activating PePT1 transport proteins.[68] Thus, eudragit L-100-55 could be effectively used for enhancing absorption of PePT1 substrate drugs.

Glycerides and its fatty acid derivatives

Glycerides and their derivatives are being used since last decade due their wide applications and availability of high purity standards. Majorly, they are employed in the preparation of semi-solid and liquid preparations. Derivatives of fatty acid and glycerides generally have strong surfactant properties and are used as solubilizing or emulsifying agents.

Labrasol

Labrasol is a mixture of mono-, di- and triglycerides and mono and di-fatty acid esters of PEG. It is used as a nonionic surfactant in microemulsion formulation, as a solubilizing agent for lipophilic candidates as well as a permeation enhancer in topical preparations.

Labrasol increased the paracellular transport across Caco-2 cell monolayers as measured by mannitol transport.[69] It was found that both, increase in paracellular transport and basolateral-to-apical efflux inhibition by labrasol were responsible for permeation enhancement of a poorly permeable drug, ganciclovir.[70] In another study, *in vivo* bioavailability of gentamycin in rats was significantly enhanced when it was co-administered with labrasol. Gentamycin shows high solubility, is metabolically stable and a poorly permeable drug. Polarized transport of gentamycin was not observed and verapamil (a P-gp inhibitor) was found to increase apical-to-basolateral transport and decrease basolateral-to-apical transport of gentamycin in rat intestinal tissue.[71] In both the above studies using ganciclovir and gentamycin, a direct implication of labrasol on the active transport system for increased permeability has not been clearly proved, but also could not be excluded.

Koga *et al*. studied the rat intestinal *in vitro* and *in situ* permeability of cephalexin (a PePT1 substrate). The permeability was enhanced by 10% when labrasol (0.5%) was co-administered with cephalexin in an ATP depleted conditions. Membrane barrier was maintained indicating that labrasol did not change membrane fluidity and authors concluded that labrasol enhanced absorptive transport of PePT1 substrate like cephalexin.[72] Moreover, in a study done on permeation of scutellarin as an MRP2 substrate in Caco-2 cell monolayers, scutellarin concentrations measured in the presence of labrasol were not higher than that in scutellarin standard group. This indicated that labrasol had no effect on MRP2 activity.[31]

Oleic acid

Oleic acid is a fatty acid used as an emulsifying and solubilizing agent. It is also used as a vehicle for poorly water soluble substances like puerarin[73] and vinpocetine.[74] It enhances triglyceride secretion by the intestinal cells that increase absorption of lipophilic candidates. Recently, oleic acid, on 6 h of exposure, has shown to enhance absorptive transport of mitoxantrone (a breast cancer resistant protein [BCRP] substrate) across the Caco-2 cell monolayers and increased accumulation of drug in the cells without any cell toxicity as measured by TEER and LDH leakage. Oleic acid increased apical-to-basolateral transport of ^{3}H-mitoxantrone in a concentration-dependent manner and BCRP are reported to be present on the apical side. Consequently, authors found that oleic acid at low concentrations (0.5-5 mM) increased the gene expression of transporter BCRP resulting in increased transport of mitoxantrone.[75] Hence, oleic acid may be used as a vehicle for enhancing solubility of a lipophilic drug as well as to enhance transport of BCRP substrate drugs.

Peceol®

Peceol® is composed of mono and diglycerides of oleic acid and closely resembles the end-products of intestinal lipid digestion. It is used as oily vehicle in self-emulsifying liquid formulations and as the bioavailability enhancer for poorly soluble drugs.[76-78] Peceol® (in safe concentration: 0.05–0.5%v/v) has been shown to cause decreased basolateral-to-apical flux of RHO 123 across Caco-2 cells. The TEER values and total cellular protein content were also measured and were found to be unchanged which indicated that the reduced P-gp activity of the transporter was not caused by cell damage or cell death. Peceol® decreased P-gp expression (measured by western blotting) significantly. The study confirmed that Peceol® inhibited P-gp activity not by altering the membrane fluidity or ATPase activity but by down-regulating P-gp expression.[79] In another study, Peceol® was found to decrease expression of both MDR1 and P-gp resulting in decreased secretion of Amphotericin across Caco-2 cell monolayers. Lymphatic transport (mysentric lymph transport measured *in vivo* in rats) of Amphotericin also increased when co-administered with Peceol®. The authors concluded that Peceol® mixed micelles increased gastro-intestinal tract absorption of amphotericin by two mechanisms: Increased lymphatic transport and decreased P-gp and MDR1 mediated drug efflux to the apical side.[80]

Lipids

Lipid-based formulations have created great interest in pharmaceutical preparations and now are widely used to enhance bioavailability of poorly water soluble agents, targeting to lymphatic system, delivery of drug molecules which are substrates of P-gp, and to by-pass hepatic metabolism. Liposomes, solid lipid particle, microemulsifying systems etc., incorporates a range of lipids and are widely used to improve the delivery of existing as well as new upcoming

drugs. Among various classes of lipids, important ones are phospholipids, sterols, lipoproteins, and sphingolipids.

Phospholipids

Phospholipids are widely used to formulate biphasic systems (like liposomes) and to encapsulate drugs like doxorubicin[81] and celecoxib.[82] Liposomes are often used for increasing solubility of lipophilic drugs as these phospholipids can be dissolved in the cell membrane and thus promoting drug absorption from the intestine. Simon *et al*. carried out transport studies in Caco-2 cells using digoxin as a P-gp substrate and performed calcein accumulation assay to assess P-gp expression in the presence of various phospholipids. They found three phospholipids that showed increased digoxin transport and a decreased P-gp expression in an assay study without affecting the cell viability. These lipids consisted of phosphatidylcholine (PC) and either two saturated fatty acid residues of 8 (8:0 PC) or ten carbon atoms (10:0 PC), or of two unsaturated docosahexaenoic acid residues.[83] This P-gp inhibitory activity has also been shown by another two phospholipids: Dipalmitoyl PC or dipalmitoyl phosphatidylethanolamine. Both lipids, in a liposomal formulation, increased absorptive transport of epirubicin (a P-gp substrate) across Caco-2 cell monolayers and also in an inverted rat gut sacs of rat jejunum and ileum. They found that phospholipids could change the composition as well as fluidity of membranes, decreased the P-gp expression and thus inhibition of P-gp activity.[84] A further *in vivo* effect of these phospholipids should be done to elucidate the P-gp inhibitory effect by above phospholipids.

Rhamnolipids

Rhamnolipids are a sub-class of glycolipids and consist of mono-and disaccharides linked by glycosidic bonds to hydroxylated carboxylic acid. Rhamnolipids are bio-surfactants derived from *Pseudomonas aeruginosa*. They are natural surface active agents that can act as emulsifier, foaming agent and anionic complexing agents. They are known to have relatively wide safety profile and strong surface activity but yet not been approved by regulatory bodies like United States Food and Drug Administration for use in pharmaceutical formulations.[85,86]

Recently, Jiang *et al*. compared the effects of rhamnolipids with tween-80 on accumulation and efflux of RHO 123 as a P-gp substrate across Caco-2 cell monolayers. In comparison to control, tween-80 (200 mg/L) and rhamnolipid (20 and 50 mg/L) showed increased intracellular accumulation of RHO 123 by up to 4-folds and also reduced the efflux significantly. This effect was not seen when RHO 123 was replaced by rhodamine-110, which is not a P-gp substrate. Thus, authors concluded that rhamnolipids could inhibit P-gp activity. Regarding the safety, rhamnolipids and tween-80 were found to be safe for Caco-2 cells in the concentration range of 20-150 mg/L and 200–400 mg/L respectively. Apart from P-gp inhibition, it was also found to enhance paracellular and transcellular transport.[87] Thus, rhamnolipids could be effectively and safely administered as P-gp inhibitor. In future, rhamnolipids could emerge as a new class of bio-surfactants which are safe, cost-effective and could be effectively used as excipients to modulate transport protein activities, thereby, facilitating drug transport. However, considerable *in vivo* studies need to be carried out before using such class of lipid as an excipient in pharmaceutical formulations.

Miscellaneous

Cyclodextrins

Cyclodextrin forms complex with the drug molecule thereby increasing the solubility and hence bioavailability of drugs. Other advantages include improvement in stability of drug, reduction in mucosal irritation effects of drug, odor and taste masking. β-cyclodextrin is an extensively used complexing agent to increase the solubility of poorly soluble drugs like danazol and sparfloxacin.[88] Recently, its potential to modulate transporter activity has been explored.

A study done on dimethyl-β-cyclodextrin using Caco-2 cells demonstrated that it increased cellular concentration and reduced basolateral-to-apical flux of RHO 123 across the cells. The authors found that dimethyl-β-cyclodextrin released P-gp and MRP2 from the apical side and decreased levels of cholesterol, P-gp and MRP2 in caveolae of cell monolayers. The MDR1 and MRP2 levels were found be unchanged and the authors confirmed that the inhibitory effect of dimethyl-β-cyclodextrin on P-gp and MRP2 function could be due to release of these transporters from the apical membranes into the medium as well as through cholesterol depletion in caveolae.[89] It also increased the membrane fluidity that resulted in decreased activity of P-gp.[90] Zhang *et al*. studied P-gp inhibitory effects of β-cyclodextrin on intestinal absorption of a P-gp substrate, berberine. The intestinal absorption of the inclusion complex in rats was found to be significantly higher than the free drug. Solubility enhancement by β-cyclodextrin was found to be one of the mechanisms for its greater absorption. The P-gp ATPase activity, P-gp mRNA expression in rat intestine and P-gp expression in small intestine were also determined after administration of berberine β-cyclodextrin complex. The drug loaded complex showed no effect on P-gp ATPase activity, but inhibited berberine stimulated P-gp ATPase activity. This complex had no effect on ABCB1A and ABCB1B mRNA expression; however, it down-regulated berberine-up-regulated P-gp mRNA expression. In addition, this complex suppressed berberine induced P-gp expression (measured by western blotting). Hence, three mechanisms of P-gp inhibition by β-cyclodextrin were postulated: Inhibition of substrate (like berberine) stimulated P-gp ATPase activity, decrease substrate-induced P-gp mRNA expression and suppression of substrate-induced P-gp expression.[91]

β-cyclodextrin, although showed decreased efflux of scutellarin (MRP2 substrate) across Caco-2 cell monolayers. It,

however, showed lower quantities of scutellarin than control group in MRP2 membrane vesicle transport assay, suggesting that β-cyclodextrin possess no effect on MRP2 and it might increase scutellarin absorption through mechanism other than inhibiting MRP2.[31]

Glucose

Glucose is a widely used excipient in oral formulations especially in liquid formulations like syrup. D-glucose has been examined for modulation of MRP activity. Fluorescein was used as an MRP substrate. It was found that d-glucose significantly increased the efflux of fluorescein from basolateral-to-apical side in the rat jejunum. The effect was observed only when d-glucose was added on mucosal side of tissue. It was found that d-glucose provides the necessary energy in the form of ATP to MRP2 that results in the efflux of fluorescein.[92]

In another study, intestinal epithelium excised from rat jejunum was used and amoxicillin permeability was measured in the presence of d-glucose. It was observed that, when d-glucose was added to the apical side, the amoxicillin was actively transported across the intestine in basolateral-to-apical direction. When glucose was not added, this transport was not seen. This effect of glucose was abolished by MRP inhibitor benzbromarone (0.04 mM), thus confirming the d-glucose activity of modulating MRP activity of MRP present on the apical side.[93]

Mechanisms of excipients explored behind modulating transporter activity

Mechanism behind P-gp inhibition by nonionic surfactants has been found to be modulation of cell membrane fluidity. Change in the membrane fluidity triggers conformational changes in the transporters structure. This ultimately leads to either, hyper-activeness, less activeness or total inactivity of transport protein that gets reflected in its V_{max} or K_m. At V_{max}, the active transport on either side does not change further. Surfactant might result in uncompetitive or noncompetitive inhibition that leads to a reduction in V_{max} and thus a reduction in active transport of substrate. They further result in ATPase inhibition and ATP depletion leading to decreased ABC transporters activity. Cremophor® EL, Cremophor® RH40 and Polysorbate 80 fluidized the cellular lipid bilayers while TPGS-1000 rigidized the lipid bilayers.[33] It has also been reported that surfactant mediated encapsulation in vesicles of particularly MRP2 transporter takes place resulting in a reduction in the active MRP2 in the apical membrane that further leads to a loss of transport activity.[30] As for surfactants, the transport inhibitory activity has been found to be below the CMC of the surfactant. Hence, it inferred that the surfactant monomers, and not its micelle forms increase the drug permeability across physiological membrane. It is due to the fact that all above-cited surfactants, the increase of concentration above CMC did not further increased drug permeability. The concentration of monomer does not change above CMC and thus no further increase in drug permeability. For phospholipids, too, their property of membrane fluidization results in transporters activity inhibition.

On the other hand, for thiomers, their mechanism of P-gp inhibition is well understood. P-gp contains 12 channel forming transmembrane regions through which substrate gets transported. Thiomers seem to enter into these channels forming one or two disulfide bonds with one or both cysteine subunits present in two transmembrane regions of P-gp-namely 2 and 11 subunits. Thiomers forms covalent bond with these disulfide bonds that results in reduction in activity of P-gp. For the rest of excipients like labrasol and rhamnolipids, the mechanism is still unclear while for some excipients, it is well understood. For example, Peceol® has been found to reduce P-gp expression, while β-cyclodextrin down-regulates substrate stimulated P-gp expression, P-gp mRNA expression and P-gp ATPase activity.

Excipients modulated transporter activity for effective drug delivery

Drugs are almost always given in the form of a formulation made with the use of excipients. Hence, excipient have been a vital need for development of a formulation, where they are either added for the purpose of transporting drug in a specific manner to a site in the body or are necessary for the manufacturing of the formulation. Up till now, they were considered as inert material having no pharmacological activity. Since last decade, above-mentioned effects of excipients have been explored, various types of novel drug delivery systems are getting developed like liposomes, lipid nanoparticles and micelles. The delivery systems are formulated incorporating those excipients that have been found to modulate desired transporter activity. Their particle size is advantageous too for enhanced absorption. Thus, these systems possess inherent properties to enhance the delivery of the substrate drug. While developing a formulation, a proper delivery system that incorporates such excipient that will be helpful in getting desirable pharmacokinetic of drug (through modulation of transporters activity) can be chosen. Table 4 reports such formulations where careful selection of excipient has been made that could modify transporter activity and has resulted in enhanced efficacy of the formulation.

Toxicity profile of the pharmaceutical excipients

Excipients used in the formulation are regarded as pharmacologically inert materials with no effect on the body. However, considering the above effect of excipient on transport properties, it is necessary to understand the toxicity level of the individual excipient. Cremophor® EL and Cremophor® RH 40 are widely used in pharmaceutical formulations. Acute and chronic toxicity study in animals has shown them to be nontoxic and nonirritant. However, several side effects have been reported in animals due to use of these polyoxyethylene derivatives like anaphylactic

Table 4: Examples of developed formulation

Excipient modulating transporter activity	Transport protein	Formulation	Drug/substrate	Reference
Chitosan-4 thiobutylamidine	P-gp	Tablet	RHO 123	[65]
Cremophor® EL	P-gp	Microemulsion	Docetaxel	[29]
Labrasol	Not mentioned	Microemulsion	Gentamicin	[71]
Oleic acid	BCRP	Emulsion	Mitoxantrone	[75]
N-octyl-O-sulfate chitosan	P-gp	Micelles	Paclitaxel	[62]
Peceol®	P-gp	Micelles	Amphotericin B	[80]
Solutol® HS15	P-gp	Lipid nanocapsule	Paclitaxel	[52]
β-cyclodextrin	P-gp	Inclusion complex	Berberine hydrochloride	[91]
Methyl-β-cyclodextrin	P-gp	Inclusion complex	Saquinavir	[94]
TPGS, Cremophor® EL	P-gp	SMEDDS	Tacrolimus	[35]
Cremophor EL	P-gp	SMEDDS	Irinotecan	[95]
Polysorbate 80/Cremophor EL/Cremophor RH40	P-gp	SMEDDS	Etoposide	[96]
DPPC, DPPE	P-gp	Liposomes	Epirubicin	[84]
TPGS 1000	P-gp	Soft gelatin capsule	Amprenavir	[41]

BCRP: Breast cancer resistant protein, SMEDDS: Self-microemulsifying drug delivery system, P-gp: Permeability glycoprotein, TPGS: Tocopheryl-polyethyleneglycol, DPPC: Dipalmotylphosphotidyl choline, DPPE: Dipalmitoylphosphatidylethanolamine

reactions,[97,98] cardiotoxicity[99] and nephrotoxicity.[100,101] The LD_{50} of Cremophor® EL in rat and rabbit by oral route has been reported to be 6.4 and 10 g/kg of body weight respectively. While for Cremophor® RH 40, LD_{50} is reported to be 16 and 12 g/kg of body weight.[102] TPGS has been used in Agenerase product at levels of 280 mg/capsule which is dosed at 8 capsules (2240 mg TPGS) per day. Moreover, it showed no adverse effect in rats at dose of 1000 mg/kg/day.[103] Polysorbate 80 although, a widely accepted excipient, has shown some serious effects like infants' death.[104] WHO has noted 25 mg/kg daily administration as safe limit for use of Polysorbate 80.[102] Poloxamers are not metabolized in the body and are nontoxic and nonirritant materials. No hemolysis of human blood cells was observed in 18 h 25°C upon administration of poloxamer at up to 10% w/v concentrations indicating its safe use in the formulation.[103] Solutol HS15 is relatively, a safe excipient with low toxicity profile. Its LD_{50} in dog is reported to be 3.1 g/kg body weight upon parenteral administration.[103] PEGs have shown few serious adverse effects like hyperosmolarity, metabolic acidosis and renal failure upon topical administration in burn patients[105] and hence should be consciously added in topical preparations and in renal failure patients. LD_{50} of PEG 200, 300 and 1000 in rat on oral administrations is reported to be 28, 27.5 and 32 g respectively per kg of body weight.[102] Low molecular weight PEGs may be absorbed when given orally, and their high dose can give a laxative effect.[106] The WHO has stated 10 mg/kg body weight limit for use of PEGs. Thiolated poly acrylic acid has the benefit of high molecular mass and remains unabsorbed from the intestine and thus excludes systemic side effects.[2] Chitosan is a widely explored excipient due to its biocompatibility and biodegradability and its thiolated form thus incorporates all its advantages. Various grades of eudragit are used as film coating agents. Based on their chronic toxicity study data in rats, a daily intake of 2−200 mg/kg body-weight depending on their grade of Eudragit may be considered safe in humans.[103] LD_{50} of labrasol and oleic acid in rat by oral route is reported to be 22 ml/kg (day) and 74 g/kg respectively. Few side effects like hemolysis are reported with intravenous use of oleic acid, and thus should be carefully added while its oral use is safe. Phosphotidyl choline being component of the cell membrane, is highly biocompatible and has been used up to 80 g daily in the treatment of dyskinesia.[107] Cylodextrins are metabolized by microflora in colon upon oral administration and are finally excreted as carbon dioxide and water and are approved for use in oral pharmaceuticals in number of countries. Specifically, LD_{50} of β-cyclodextrin is reported to be 18.8 g/kg in rat by oral route.[102]

CONCLUSION

Great progress has been made in the development of different classes of excipients resulting into many new delivery systems like surfactant, polymer and lipid-based drug delivery systems. These excipients seem to be safe for long-term therapy too, since they have been used for years in pharmaceutical formulations and are approved for use by responsible government bodies. The above cited work reports interactions of different classes of excipients with at least five different transporters (P-gp, PePT1, MRP, BCRP and monocarboxylate transporter). Thus, excipients used for specific predetermined purposes in formulation like solubility enhancement, taste masking and controlled release, could also be used to enhance therapeutic efficacy of the drug and a dual purpose can be solved with the use of same excipient. Different mechanisms have been proposed for modulation of transporters activity like membrane fluidization, depletion of ATP required for protein activity and interaction with drug binding or ATP binding sites by excipients. For few excipients, their crucial mechanisms have not yet been fully revealed but in the presence of these excipients, the activity of transporters gets altered, on an especially functional level. However, more clinical data are necessary to address *in vivo* correlation of these

interaction activities for few excipients. Result of cellular level studies varies with the cell line model used and the lack of repeatability in results, and there is a need of more accurate cellular studies. Hence, use of correct excipient that can modulate transporter activity, may lead to enhancement in therapeutic efficacy of the substrate drug without need of increasing the dose of drug and thus is an effective and safe way to improve oral bioavailability of therapeutic molecules. As such, selection of excipients during formulation development has been limited to preliminary studies like compatibility of excipient with drug, drug solubility in excipient and desired drug release. Now, as these excipients have been found to modulate transporters activity, the selection criteria for excipients may in the future also incorporate preliminary cellular level investigations prior to formulation development. Unexpected and undesirable changes would thus be minimized and may lead to enhancement in drug delivery.

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