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Guest Articles

Role of medium chain triglycerides in paracellular permeation

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Introduction

Medium chain triglycerides (MTCs) consists of three fatty acids chains of carbons ranging from 6 to 12 and a glycerol molecule. MCTs are easily absorbed into the bloodstream unlike other lipid molecules that retains long period of time in the stomach for a complex digestion process. Since these class of lipid are fully digested and absorbed into the body, there are no any safety issues identified. (Pouton CW, *et al,* 2008) These features confer unique benefits of MCTs. At the same time MCTs have a potential to reduce obesity, neurological diseases and cardiovascular diseases. (Roger E, *et al* 2011) This article provides a comprehensive summary on role of MCTs in paracellular permeation, its proposed mechanisms and other factors which effect paracellular permeation.

Some physicochemical properties of compounds restrict or reduce the absorption through the intestine. Properties such as presence of strong charged functional group, low octanol/aqueous partition, a substantial number of hydrogen-bonding functional groups, high molecular weight and high polar surfaces affect the poor membrane permeability. (Sunil P, *et al*, 2005) Compounds which have any of these properties may benefit most from the property of MCTs in the paracellular permeation. Nowadays pharmaceutical industry focuses on excipients which can carry the drug molecules to target sites that are poorly absorbed through active transport. Generally, absorption enhancers or absorption excipient are related to its concentration at the site of drug absorption. For this purpose, both the excipient and the drug molecule should be intestinal site dependent. The basic property which benefits as a solvent for drugs is decides by its effective ester group concentration. (Cao Y, *et al*, 2004). MCTs have higher solvent capacity when compared with long chain triglycerides at the same time they are less prone to oxidization. (Kaukonen AM, *et al*, 2004) Different vegetable sources have different compositions of fatty acids. Some of them are listed in the Table 1. Pure triglycerides are available in refined vegetable oils. Coconut oil and palm seed oil are rich sources of MCTs. (Collnot EM, *et al* 2004 and Collnot EM, *et al*, 2007).

Hydrophilic compounds which contain strongly charged functional groups and that are of high molecular weight confer the importance of paracellular pathway as these molecules are refrain from passing through the intestinal barrier by intracellular permeation.(Hayashi,

M, *et al*, 1999).When considering the total surface area of intestinal epithelium, 0.1% occupies paracellular pathway along with the tight junctions. (Pappenheimer, J.R. J, 1987). Apart from MCTs as enhancers of paracellular permeation, in literature there are various other compounds such as chelators like Ca^{2+}, steroidal detergents, chitosan and mucoadhesive polymers were tested on their potential to act as a paracellular enahancer.(Humberstone AJ, *et al*, 1996). According to Aungst *et al*, 1993 there are critical issues when selecting compounds as paracellular permeation enhancers like possible toxicities, degree of bioavilability enhancement achieved and influence of physiological variables are some of them. The objective of this article is to review the possibilities of MCTs to enhance the paracellular permeation and to overview the other enhancers similar to MCTs.

Overview of intestinal structure

The intestinal surface is an important entry of nutrients and fluids. Epithelial structure comprises of different cell types connected by intercellular junctions to facilitate diverse functions. Glycocalyx which consists of a luminal layer secrete water, ions and mucus while subepithelial layer consists of lymphatics, immune cells, nerves and vessels. (Odenwald MA *et al*, 2017) Rather than acting as a complete barrier, specialized cells and intercellular junctions in the epithelium actively regulate the absorption of nutrients, vitamins, ions and microbial metabolites while preventing the entering of harmful components to the body. (Pearce SC, *et al*, 2018). Different molecules and structures are transported through intestinal epithelium with the use of surface receptors, ion channels and membrane transporters and they are regulated by neurohormonal signals and bioactive molecules. The intestinal surface mucus layer is responsible to restrict the entering of intact bacteria and large particles through the membrane. (Kim YS *et al*,2010). Metals like Fe^{2+} and Ions such as Na^{+}, K^{+}, H^{+} and Cl^{-} are transported through channels, antiporters and symporters. (Singh AK *et al*, 2013). Amino acids, saccharides, vitamins and divalent metals are transported by variety of integral membrane transport proteins like Organic ion transporters (OAT) s and glucose transporters SGLT1 (Karasov WH *et al*, 2017). In the distal small intestine there are specialized M cells which facilitate the transport of antigens, intact bacteria and other particulate materials along with the goblet cell associated antigen pathway (GAPs) .(Knoop KA *et al*, 2018) With the use of receptor mediated endocytic mechanisms macromolecules like proteins are absorbed through the intestine (Williams IR, *et al*, 2015)

Paracellular pathway

To get an idea about paracellular permeation, understanding of structure and function of tight junction (TJ) is important. But since it is beyond the scope of this review and there are already made reviews regarding TJ and paracellular pathway (PW), (Claude, P. & Goodenough, *et al* 1973, Staehelin, L. A., *et al*, 1969, Morita, K, *et al*, 1999) only a brief summary will be given here

Table 1. Soluble absorption enhancers used in oil based oral formulations

Water insoluble excipient	Triglycerides	Surfactants used
• Propylene glycol esters of fatty acids. • Medium chain (C8/C10) mono and diglycerides • Corn oil mono-di-triglycerides • Beeswax • Soy fatty acids • D-α-Tocopherol (vitamin E)	Medium chain triglycerides • Caprylic acid • Capric acid • Triglycerides derived from coconut oil or palm seed oil Long chain triglycerides • Sesame oil • Soybean oil • Peanut oil • Corn oil • Olive oil • Hydrogenated vegetable oil	• PEG 400 caprylic/capric Glycerides (Labrasols) • PEG 1500 lauric glycerides (Gelucires 44/14). • Polysorbate 20 (tween 20) • Polyoxyl 35 castor oil (cremophor EL) • PEG 300 oleic glycerides (Labrafil s M-1944CS)

Basically, there are two major pathways in which compounds are absorbed through the intestinal epithelium. They are intracellular pathway in which transport occur across the basolateral and apical plasma membrane of the cells and paracellular pathway (PW) where transport occur in between cells. (Frömter E,*et al*,1972) Intracellular pathway is a well-studied and known route while PW was studied deeply during sixties when it was discovered large variety of molecules transported through PW. (Lindeman *et al*, 1962). Paracellular transport can be described as the aqueous pathway in which small solutes move between adjacent epithelial cells which is restricted by TJ at the most apical part of the cells. (Diamond JM, *et al*, 1969). Intercellular junctions comprises 0.01% of the total intestinal surface area (Pappenheimer JR,*et al*,1987) through which significant amount of water and solutes transport. (Pappenheimer JR, *et al*, 1987). There are several factors which influence the permeability and selectivity of the intercellular junctions and it can be regulated. (Cerejido, M,*et al*,1992) Size of the permeant, its charge and the type of the permeant are some of them. There are three distinct areas in the junctional complex namely tight junction or zonula occludens, desmosome and zonula adherens. (Figure 1) Among these three areas, TJ or zonula occludens are responsible to regulate paracellular permeation as it is the rate limiting boundary of the transport pathway. (Madara, J *et al*,1998)

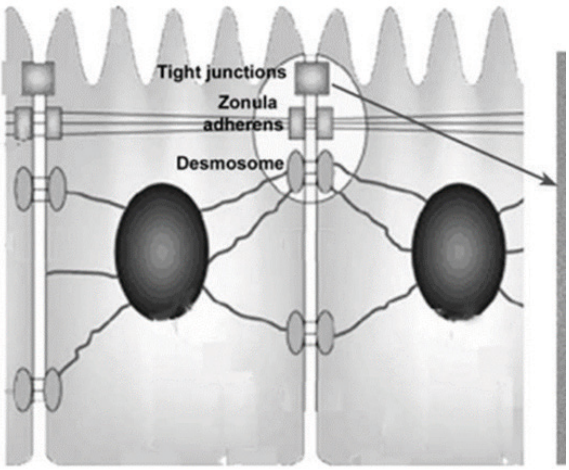

Figure1. Tight junctions, macula adherens and zonula
Medium chain triglyceride

Role of tight junctions in passive paracellular permeation

Tight Junctions or zona occludens can be define as gates which selectively allows the passage of small hydrophilic compounds and as a fence which forms a inter membrane barrier to toxic molecules in contrast to simple fence (Cereijido, L, *et al*, 2000) TJ are anatomically appears as belt like network that surround each cell to connect adjacent cells or neighboring cells to form a continuous seal. (Farquhar MG, *et al*, 1963). Nowadays it's well established that TJ perform the major role in paracellular permeation. Molecules such as hemoglobin, ruthenium red, and colloidal lanthanum which are high electron dense molecules are freely diffuse through intercellular space but it becomes impermeable at the level of TJ representing TJ as the permeability barrier site. (Martinez-Palomo A, *et al*, 1971) There are some occasions in which gate and fence function of TJ dissociate such as depolymerization and transient ATP depletion where it disrupt the gate function but it still preserve the fence function implying that fence function is independent from gate function. (Bacallao R, *et al*, 1994 and Mandel LJ, *et al*,1993). According to the biochemical characterization TJ consists of complex of different proteins including cytoplasmic plaque proteins, transmembrane proteins and signaling protein. Among them transmembrane protein are significant as it perform the basic fence and gate function of TJ. Transmembrane protein of TJ can be divided into three groups. Single transmembrane domain protein, triple transmembrane domain protein and four transmembrane domain protein. Among these three groups four transmembrane domain protein group which includes occludins performe the significant role in the paracellular permeation. (Nichols GE, *et al*, 1986)

Medium chain triglycerides

According to literature MCTs were tested as paracellular permeation enhancers or as an excipient. There are several excipients that can be used in drug industry in order to deliver the drug into target place. Among them triglycerides take a significant place. Triglycerides present in vegetable oils are commonly used excipient in lipid based drug delivery. Since they are fully digested inside the body there are no any adverse

effect in using them as an excipient. (Pouton,C.W, *et al* 2008). Triglycerides can be classified as long chain triglycerides (LCT), Medium chain triglycerides (MCT) and small chain triglycerides (SCT) depending on the number of carbons. The excipient property of glycerides basically depend on effective concentration of ester groups. (Cao C *et al*, 2004) Oxidation of MCT are less when compared to LCT at the same time MCT hold higher solvent capacity than LCT. (Kaukonen A.M *et al*, 2004). MCT term usually used to refer monoglycerides and diglycerides of caprylic acid and capric acid. These are found in general as mixtures which may also contain triglyceriedes as well as monoglycerides and diglycerides. (Morishita M, *et al*, 1998)

Apart from using MCT as excipient in pharmaceutical industry, they are often used as nutritional agents. MCT are less active when compared to diglycerides and monoglycerides hence they are used as membrane permeation enhancers. (Unowsky J, *et al*, 1998) MCT sometimes get digested to fatty acids in the intestine but there are no much evidences which imply that its metabolic products are essential for its membrane permeation enhancement. (Yeh P-Y, *et al*, 1998). According to studies a mixture of MCT and phosphatidylcholine can reduce Caco -2 cells transepithelial electric resistance (TEER) and transepithelial trans-port rate (P_{app}) of mannitol and heparin by tenfold at concentrations ≥4mM (Lohikangas L, *et al* 1994). According to research data irreversible increase in permeability can be obtain from ≥8mM concentration of MCT/ phosphatidylcholine mixture by altering the morphology of the cell monolayer. Apart from that poorly absorbed antibiotics such as moxalactam, penicillin G, cefoxitin, ampicillin, cefamandole, carumonam and cefotaxime were administered along with MCT which results in significant increase in absorption in rats studies. (0.5mL/ rat) (Unowsky J,*et al*, 1988). *In vitro* permeability study implies that distal colon of rabbits were more sensitive to enhancement of absorption of mannitol and cephalexin than ileum when administered along with MCT (Yeh P-Y *et al*, 1994). At the same time in vivo studies safety of MCT was tested by using rats, rabbits and dogs by administrating different concentrations of MCT rectally. According to results there are no any morphological changes to rectal mucosa. (Sekine M, *et al* 1985)

When comparing most of the research work in the past decade it clearly reflex several sodium salts of medium chain fatty acids like caprylate C8, caprate C10 and laurate C12 have the ability to enhance the paracellular permeation of hydrophilic compounds. (Lindmark, T, *et al*, 1995). This research was further extended to compare these compounds with small chain fatty acid, sodium caproate C6 which make it clear that medium chain fatty acids have the pronounced effect in the enhancement of absorption. It showed MCT which contain C8, C10 and C12 structures enhance the mannitol transport through cell monolayer by dose dependent enhancing effect. Among these three MCTs C12 exhibit the most effective enhancement while small chain triglycerides (C6 compounds) has no any effect in the enhancement of absorption. Interestingly, lowest concentration needed to increase the transport of marker molecule were in the vicinity of the critical micellar concentration and it vary depending on the size of the compound. Among the medium chain fatty acids sodium caprate is one of the most studied agent that can be used in drugs for human use as an absorption enhancer. In Japan and Sweden Caprate is most commonly used in suppository formulation in drugs. (Takahashi, K, *et al*, 1994). Since MCT has lower molecular weight, it can easily absorbed through the cell layers than drug itself. (Aungst, B.J.2000). Apart from that different studies have conducted to identify the enhancing effect of the permeability of compounds by MCT depend on their concentration, molecular weight, mechanism action , toxicity and time dependent effect. (Sakai, M, *et al*, 1997)

Permeability of markers

When comparing permeability of different compounds, C10 MCT can be easily used since it is convenient to differentiate it between marker molecule and drugs. Most of the studies have performed in Caco-2 cells with the use of low molecular weight marker molecules like mannitol, sodium fluorescein and phenol red have shown C10 MCT can enhance the permeability of substances through paracellular route in the intestine. (Chao, A.C, *et al*, 1999 and Anderberg, E.K *et al*, 1993). According to Sakai *et al*,1997 sodium caprate can enhance the permeability of rhodamine 123

hydrate (hydrophobic molecule) in both paracellular and transcellular pathway. When considering the high molecular weight compounds such as polysucrose (MW 15000) (Söderholm, J.D, *et al*, 1998), insulin (Uchiyama, T *et al*,1999) and FITC dextrans (average MW 4000) (Quan, Y.S, *et al*,1998), MCT have a pronounced effect in the transport. This was tested for long range of high molecular weight compounds in which all the cases C10 MCTs enhance the transport effect. But anyhow author conclude that this enhancement effect is significant when the molecular weight of the substance is ≤ 1200g/mol in case of large molecules. (Artursson, P, *et al*, 1999)

Drug permeability

A poorly absorbed antibiotics, cefmetazole showed increase in the absorption in the jejunal when it is given with sodium caprate which is medium chain fatty acid at a concentration 13mM. (Tomita, M, *et al*, 1992). At the same time similar experiment was done by using ebitaride (ACTH analog) and insulin. In this experiment authors found that drug permeability has improved in the colonic, not at the jejunal. (Yamamoto, A, *et al*, 1997). These regional differences cannot be identified clearly when Caco-2 cell monolayers were used but property of C10 as a multidrug resistance modulator can be tested. (Lo, Y.L, *et al*, 2000) In this study it was observed that C10 can reduce basolateral to apical secretion of ebitaride. (Kamm, W *et al*, 2000). Taken as whole C10 MCT enhance the transport of paracellular marker compounds whether it is small or large molecular weight at the same time drugs like antibiotics and peptide drugs transportation also enhanced by MCT. It is important to mention that according to a study, C10 at a concentration of 10mM does not have the ability to enhance the absorption of bisphosphonate drug clodronate through Caco-2 cell monolayers. (Raiman, J, *et al*, 2003)

Toxicity

When using transport enhancers in drug industry, toxicity level of the compound is one of the major concern. As mentioned earlier sodium caprate is currently used as ampicillin suppository absorption enhancer in some countries but it was tested that its formulation can damage to rectal mucosa. (Lindmark, T, *et al*, 1997) But this damage was reversible and it was not only for sodium caprate but also to other triglycerides. This was further tested and according to those results cytotoxicity can depend on the duration of exposure and concentration (Ando-Akatsuka, *et al*, 1996). Damages to cells can be detected by several methods such as morphological observations, recovery of TEER and release of biological markers. According to reported data concentration of around 13mM of MCT does not cause cytotoxicity and does not affect to the epithelial viability. (Söderholm, J.D, *et al*, 1998). At the same time it's important to mention that intact membrane in the intestine is more resistance to cytotoxic effects of enhancers of permeation than that of cell culture models. As an example dicoyl sodium sulfosuccinate is often used as stool softener at certain doses. Most frequently it is used at a concentration of 1.8mM per 250mL gastrointestinal fluids. But this concentration is toxic to Caco-2 cells.(Anderberg EK, *et al*,1998)

Permeability depend on the concentration and time

Several research groups shown results related to enhancement of absorption by MCT depend on the concentration (Anderberg, E.K *et al*,1993 and sakai *et al*, 1999). When this effect was tested in Caco-2 cell monolayers with an alcoholic antirelapse and acamprosate drugs this was further confirmed. But anyhow when experiments were conducted in situ (Zornoza, T, *et al* 2003) and in vivo with rats, concentration effect on absorption enhancement was disappeared. This effect was also tested on pigs which reflects the same results. According to those results absorption enhancement by MCT is dose independent. (Raoof, A.A *et al*, 2002)

When considering time effect on intestinal permeability, most of the experiments were carried out after a long incubation period like 1 hour with MCT. (Anderberg, E.K, *et al*, 1993). In Caco-2 cell monolayers this was tested as long term and short term effects. According to the results it consists of two phases as initial phase of 10min to 20min which had a rapid enhancement in the absorption and later phase which is a slow but prolonged improvement in the permeability. (kamm, *et al*, 2000)

Mechanisms

Most authors suggest that MCT can modulate the permeability by activating the phospholipase C in the plasma membrane which results in increase in the calcium level in the intracellular spaces. (Madara, J.L, *et al* 2000). This results in the contraction of calmodulin –dependant actin microfilaments which cause for the enhancement of paracellular permeability. (Madara, J.L, *et al*, 1988). Another author revels that C10 could exert enhancement by increasing intracellular calcium level due to alteration in the cytoskeletal actin filaments. (Sakai, M, *et al*, 1998). And also there is another mechanism which explain the enhancement of paracellular permeation by MCT. It explain that C10 paracellular permeation is not due to the tight junction pore dilation but due to increasing the opening of restricted pores as a probability. According to this explanation C10 act by increasing the number of restrictive pores. (Watson, C.J, *et al*, 2001). But this theory was against to Lindmark *et al*, 1998 which have shown experimental results that morphological changes could be observed in TJ structure due to protein ZO-1 and occluding changes after MCT or C10 exposed in both intestinal tissues and cell culture studies.

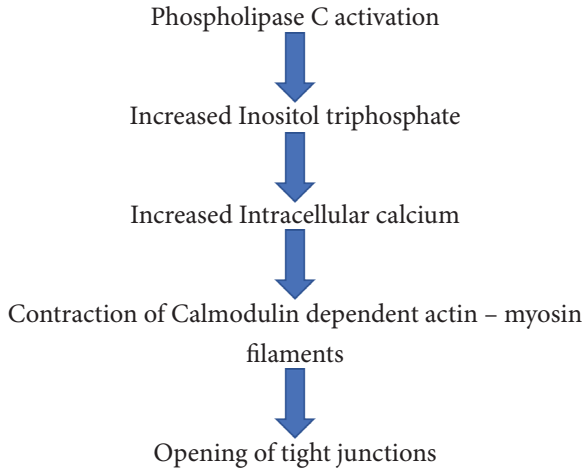

Figure 2. Proposed mechanism for sodium caprate to enhance the paracellular permeation through phospholipase C pathway.

***In vitro* and *in vivo* correlation in paracellular permeation studies**

Two model drugs griseofulvin and dexamethasone was tested for oral bioavailability *in vitro* and ex vivo intestinal permeability models. In regards to absorption and lipolysis data, both *in vitro* and *ex vivo* results has correlated while permeability results deviate from *in vitro* data to *ex vivo* data. In the digestive media apart from lipid excipient there phospholipids, lipolytic products and bile salts which can trigger paracellulara permeation of griseofulvin which has limited solubility 5mg/mL while *in vitro* studies these factors are absent. (Dahan A, *et al* 2007). Basic importance of *in vitro* and in vivo correlation is to develop more improved formulations of lipid based enhancers which can be commercialized. Period which will take to develop the drug that permeate to the target site can be minimized and product quality also can be improved easily if *in vitro* and *in vivo* results get correlate. MCTs excipients solubility, dissolution, lipolysis and intestinal membrane techniques can be vary in different *in vitro* techniques but it is important to confirm the in vivo performance before commercializing the products since *in vitro* studies provide only the aspects of the formulations. (Seeballuck F, *et al*, 2003)

Caco-2 cells can produce chylomicrons due exposure to lipids just like the in vivo enterocytes. Inorder to develop the prototype for the initial screening of permeation enhancers, rat models are the most suitable and reliable source. But there is a challenge when using rat models to study the paracellular permeation due to absence of gall bladder, different expression levels and different pattern levels of intestinal enzymes when compared to human beings. (Dahan A, *et al*, 2007). There are some experiments which have used dog models (Khoo SM, *et al*, 2003). But there is poor correlation between dog model and human data due to differences in gastric juice pH and enterocyte enzyme profile. There are some similarities between the pig model and the human gastrointestinal tract physiology and in anatomy. (Petri N, *et al*, 2006) According to evidences pig can be used as the most reliable and suitable non primate model basically due to the ability to feed even if needed or can be done fasting studies as well. At the same time pig model permits to administrate the total sized human dose. Therefore paracellular permeation and first pass metabolism in the intestine can be studied using the pig model.(Kararli TT, *et al*, 1995). When studying the paracellular permeation *in vitro* and *in vivo* models, there are some

challenges when correlating the two sets of data. But anyhow in order to establish a reliable commercialize paracellular excipient, *in vitro* and *in vivo* models that correlate *in vitro* and *in vivo* data are important.

Conclusion

MCTs have tested in both *in vitro* and *in vivo* methods for the enhancement of paracellular permeation. Rather than using other excipient in paracellular permeation, MCTs provide more safety platform in drug industry and other industries which provide human consuming products. But there are little information existing regarding the long term safety of compounds which alter the paracellular permeability which is essential to be addressed. At the same time there are some limitations in the technology regarding the manufacturing methods of MCTs mixtures for the paracellular permeability enhancement, lack of database for lipid base formulations and their stability, lack of database considering the solubility of drugs in lipids and proper regulations have to implement for lipid base formulations. New research work should be carried out to find the proper in vivo model that correlates the data of *in vitro* design. This review summarizes the role of MTCs as a paracellular permeation enhancer and mechanisms which explain the absorption in paracellular pathway. At the same time this may be beneficial to address the gaps in the paracellular permeation concept.

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Guest Articles

Chemical modifications of natural rubber latex to produce commercially important raw materials

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Natural rubber (NR) is an important material that is derived from the latex sap of *Hevea brasiliensis* tree. The latex is collected by tapping the trees, where a small incision is made in the bark to allow the latex to flow out and be collected. NR is known for its elasticity, resilience, and durability, making it a popular choice for a wide range of applications. It is widely used in various applications such as tire manufacturing, various industrial products like hoses, conveyor belts, seals, gaskets, toys, gloves, mattresses, medical supplies, footwear, and consumer goods. Despite its benefits, NR has limitations like low heat, oxygen, and oil resistance due to its unsaturated chain structure. To address these drawbacks and expand its uses, chemical modifications can be applied. Main chemical constituent in NR is *cis*-1,4-polyisoprene and hence the chemical alterations made on NR would be similar to that of alkenes. i.e. electrophilic addition at C=C bond or substitution at the allylic position. Thus, the commercially important raw materials could be manufactured via the modifications including hydrogenation, halogenation, grafting, epoxidation, and cyclization, allow for tailored