

Effect of Permeability Enhancers for Polar Drug Transport Across Caco-2 Cell Monolayers

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Abstract

Purpose: Polar drugs exhibit poor permeability across the intestinal mucosa in the absence of facilitated transport by multiple possible transporter proteins. Exogenous permeability enhancers in some cases can act to increase polar drug absorption through either the paracellular or transcellular pathways. Multiple permeability enhancers targeting either the paracellular or transcellular pathways were tested with a drug compound having an absolute bioavailability in the absence of enhancers of about 2% in Caco-2 cell assays. **Methods:** Caco-2 cells were plated at a seeding density of 90,000 cells per 12 mm Costar #3401 Transwell membranes and cultured in DMEM medium supplemented with 20% fetal bovine serum, glutamine, pyruvate, EGF, and ITS for 21 days prior to use in permeability assays. The transepithelial electrical resistance (TEER) of the monolayers was tested and wells having TEER readings $>650 \Omega\text{cm}^2$ were used in permeability assays. Transwells were washed with Hank's Balanced Salt Solution (HBSS) and placed in 12-well plates containing HBSS in the lower chamber and drug with or without permeability enhancer in HBSS in the upper chamber. The drug transport rate was determined by LC/MS assays of drug present in the lower chamber after a minimum of eight 30-minute time points. **Results:** Caco-2 cell transport assay results were collected from 24 potential permeability enhancer compounds and mixtures composed of 14 differing medium chain mono- and diacylglycerol derivatives or mixtures, 5 differing surfactants, and 5 miscellaneous compounds. Enhancements to drug permeability varied from zero to about 40-fold depending on the enhancer compound used. The best results were obtained using either Capmul MCM-L8 composed of mono- and diglycerides of medium chain fatty acids (mainly caprylic) which primarily impacts the paracellular transport mechanism, and 3-(N,N-dimethylpalmityl-ammonio)propane-sulfonate (PPS) which primarily affects cell membrane permeability and a transcellular absorption pathway. These results demonstrate the utility of a Caco-2 cell assay for initial screening of permeability enhancers for pairing with differing polar drugs. **Conclusion:** The results presented demonstrate that a variety of permeability enhancer compounds and compositions can be successfully applied in Caco-2 cell assays to define optimal conditions for a polar drug having very poor inherent intestinal permeability.

Introduction

Polar drugs exhibit poor permeability across the intestinal mucosa in the absence of facilitated transport by multiple possible transporter proteins. Exogenous permeability enhancers in some cases can act to increase polar drug absorption through either the paracellular or transcellular pathways. Such increases in absorption can be of particular use when attempting to develop oral formulations of low permeability drugs currently only available as parenteral formulations. The Caco-2 cell model is well-established method of evaluating intestinal permeability for orally-delivered drugs. Enhancers were tested with a zwitterionic drug compound and an absolute bioavailability in the absence of enhancers of about 2% in Caco-2 cell assays. The enhancement of observed permeability by glycerol was particularly striking and unexpected.

Method

Caco-2 Cell model

To evaluate the effectiveness of permeability enhancers, data was obtained to demonstrate the ability to increase drug transport using Caco-2 cell permeability assays. The assays were performed according to the methods described by Artursson P, Palm K, Luthman K. (2001) *Adv Drug Deliv Rev.*;46:27-43, and by Shah P, Jogani V, Bagchi T, Misra A. (2006), *Biotechnol Prog.*, 22:186-98. Caco-2 cells were plated at a seeding density of 90,000 cells per 12 mm Costar #3401 Transwell membranes and cultured in DMEM medium supplemented with 20% fetal bovine serum, glutamine, pyruvate, EGF, and ITS for 21 days prior to use in permeability assays. The transepithelial electrical resistance (TEER) of the monolayers was tested and wells having TEER readings $>650 \Omega\text{cm}^2$ were used in permeability assays. Transwells were washed with Hank's Balanced Salt Solution (HBSS) and placed in 12-well plates containing HBSS in the lower chamber and drug with or without permeability enhancer in HBSS in the upper chamber.

Evaluation of Permeability Enhancers

Caco-2 cell transport assay results were collected from 24 potential permeability enhancer compounds and

mixtures. The drug transport rate was determined by LC/MS assays of drug present in the lower chamber after a minimum of eight 30-minute time points. The LC/MS/MS system consisted of an Agilent 6410 LC/MS Triple Quadrupole system with an Agilent 1200 LC system equipped with an Agilent Zorbax SB-C18 column (2.1 x 30mm, 3.5 μm). The mobile phase comprised 20% methanol in deionized water with 0.1% formic acid and was delivered isocratically at a flow rate of 0.25 mL/min. Autosampler with Agilent 96 well plates injected 15 μL of samples. Three broad categories of enhancers were evaluated: medium chain mono- and diacylglycerol fatty acid derivatives or mixtures, known surfactants, and miscellaneous compounds with potential enhancement capabilities. Medium chain length fatty acids and salts and esters thereof evaluated included mono-, di-, and triglycerides, such as sodium caprylate, sodium caprate, glycerides (CAPMUL®, Abitec, Columbus, OH; Gattefossé SAS, Saint Priest, Cedex, France), and GELUCIRE® 44/14 PEG-32 glyceryl laurate EP (Gattefossé). Known surfactants and other compounds examined included polysorbate-80, phosphatidylcholine, N-methylpiperazine, sodium salicylate, melittin, and palmitoyl carnitine chloride (PCC). These compounds were selected for their potential interactivity with the charged groups on the drug compound or their potential effects on membrane fluidity.

Results and Discussion

Enhancements to drug permeability varied from zero to about 40-fold depending on the enhancer compound used. The best results were obtained using glycerol [Fig 1], Capmul MCM-L8, largely composed of mono- and diglycerides of medium chain caprylic fatty acids [Fig 2], and 3-(N,N-dimethylpalmityl-ammonio)propane-sulfonate (PPS) anticipated to also modify membrane fluidity properties [Fig 3]. The results demonstrate that glycerol provides substantially increased permeability at concentrations approaching 5%, a concentration likely achievable *in vivo* in the proximal GI tract using amounts within the acceptable limits of the FDA approved inactive ingredients list. This unexpected result can potentially be explained by observations that glycerol disrupts tight junction organization (Wiebe JP, Kowalik A, Gallardi RL, Egeler O, and Clubb BH, 2000. *J. Androl*, 21, 625-635). Both the safety profile of glycerol and its *in vivo* permeability enhancement capability make it an attractive candidate for oral formulation.

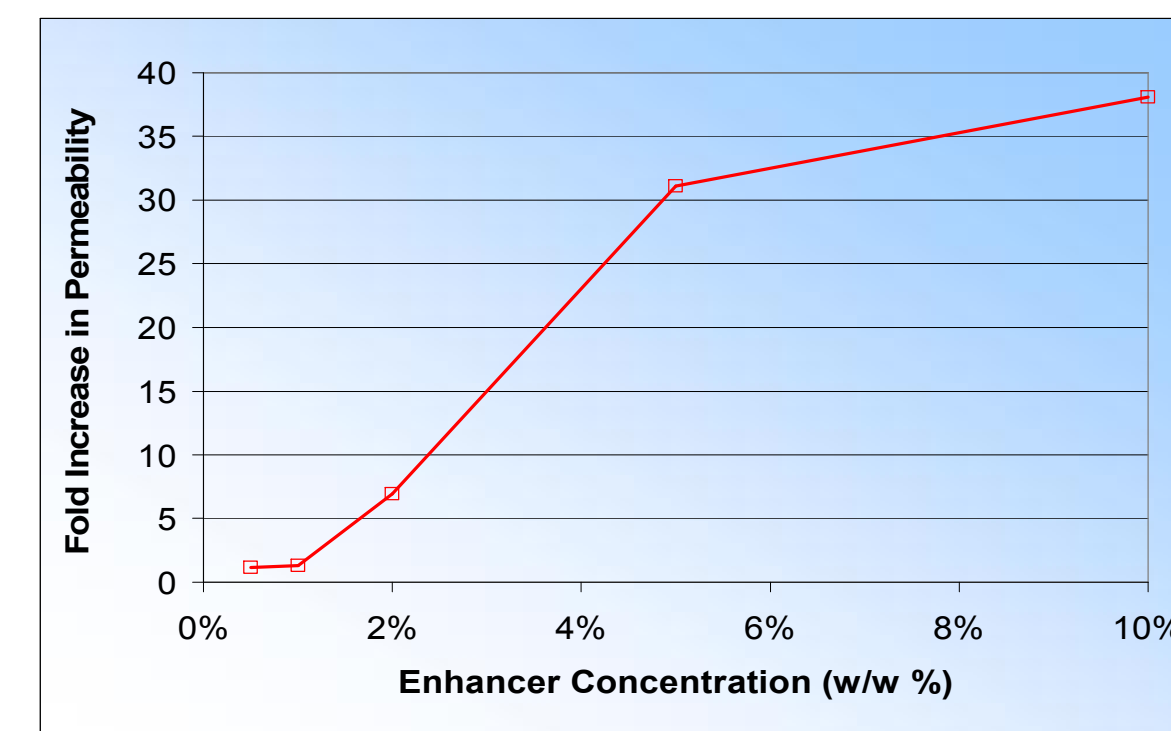


Fig 1: Permeability enhancement properties of Glycerol.

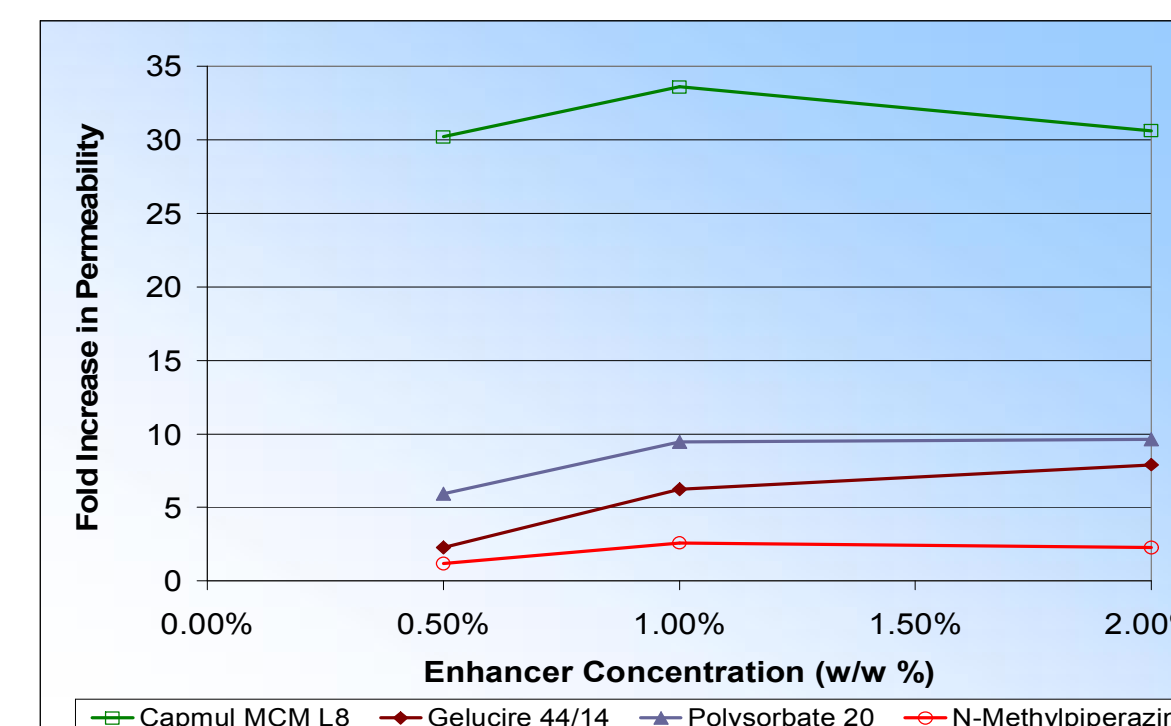


Fig 2: Permeability enhancement properties of Capmul MCM L8, Polysorbate 80, N-methylpiperazine, and Gelucire 44/14

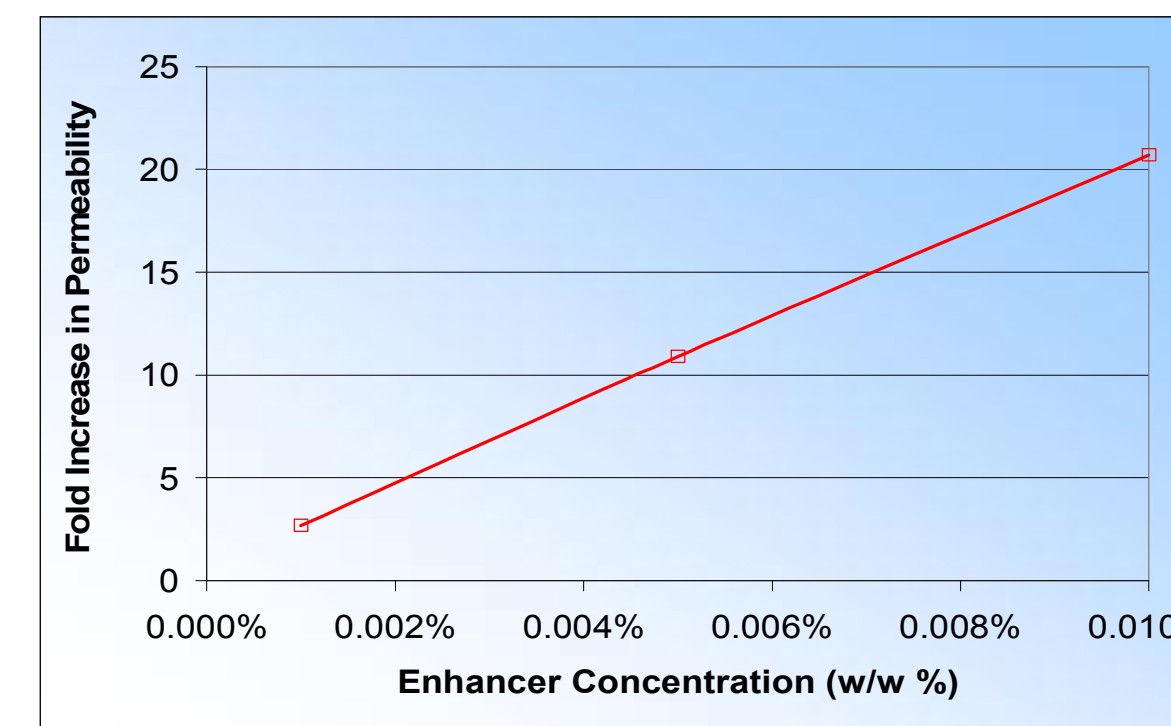


Fig 3: Permeability enhancement properties of 3-(N,N-dimethylpalmityl-ammonio)propane-sulfonate (PPS).

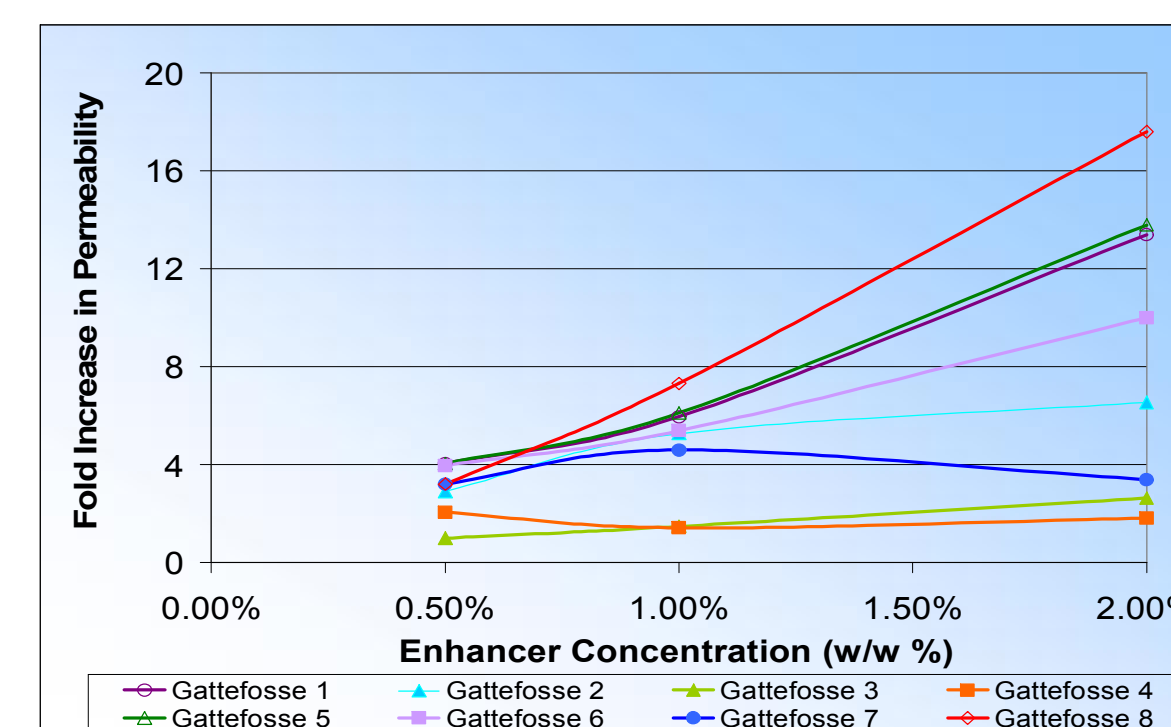


Fig 4: Permeability enhancement properties of select proprietary mixtures of medium-chain fatty-acids.

These results are of significant interest due to the differing modes of action of each enhancer: Capmul MCM-L8 primarily impacts the paracellular transport mechanism and PPS primarily affects cell membrane permeability and a transcellular absorption pathway. Medium chain mono- and diglycerides have been shown to modulate tight junction function through a phospholipase C-dependent pathway (Tomita M, Hayashi M, Awazu S, 1995, *J Pharmacol Exp Ther*, 272: 739-743). In contrast to the other permeability enhancing agents that alter tight-junction behavior, PPS acts through a different mechanism. PPS is a zwitterionic detergent and has membrane fluidizing properties that enhance transport using the transcellular permeability pathway. Caco-2 cell assays with PPS varying from 0.001% to 0.01% final concentration provided substantially enhanced permeability on par to that seen with Capmul MCM L8 or glycerol. This result is especially interesting in comparison to the negative results with polysorbate-80, a non-ionic surfactant. Selected proprietary medium-chain fatty acid compositions (Gattefossé) also showed positive effects [Fig 4], but no trend was observable due to the proprietary nature of their formulations. Not all potential permeability enhancers demonstrated effectiveness for improving the permeability of highly polar drugs in Caco-2 cell assays. For example, polysorbate-80, sodium salicylate, Gelucire 44/14 and N-methylpiperazine showed little effect [Fig 2].

Conclusion

Although improvements in drug permeability as demonstrated in Caco-2 cell models does not always correlate to *in vivo* improvements in bioavailability, these results demonstrate the utility of a Caco-2 cell assay for initial screening of permeability enhancers for pairing with differing polar drugs. Capmul MCM-L8, PPS and glycerol were shown to enhance the permeability of a highly polar zwitterionic drug compound. Such enhancers may be of substantial utility in the reformulation of drugs previously only available as parenteral formulations.

Acknowledgements

The authors would like to acknowledge Dr. Eric Holmes, Director of Research Operations at the University of Hawaii, for his critical contribution to this research.

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