DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 20(4), 469-483 (1994)

RATIONAL DELIVERY STRATEGIES FOR THE DESIGN OF PEPTIDES WITH ENHANCED ORAL DELIVERY

Ronald T. Borchardt, Department of Pharmaceutical Chemistry,
The University of Kansas, Lawrence, KS 66045

ABSTRACT

One of the most significant biological barriers to delivery of peptides and peptide mimetics is the intestinal mucosa, which is a cell monolayer with tight intercellular junctions representing both an anatomical and an enzymatic barrier to the permeability of most polar molecules. In order to properly evaluate strategies for enhancing membrane permeability of peptides and peptide mimetics, our laboratory has developed an *in vitro* model of the intestinal mucosa, which consists of human adenocarcinoma cells (Caco-2) grown onto microporous membranes. This cell culture system, as well as an *in situ* intestinal perfusion model, has been employed in our laboratory to evaluate strategies for enhancing membrane permeability of peptides. The strategies that will be discussed in this article include: (1) designing conjugates of peptide mimetics targeted to endogenous transporter systems (e.g., bile acid) so as to enhance their intestinal permeability by a carrier-mediated pathway; and (2) optimizing the lipophilicity, hydrogen-bonding potential and conformation of peptide mimetics so as to enhance their intestinal permeability by passive diffusion.

INTRODUCTION

A major challenge confronting pharmaceutical scientists in the future will be to design peptides and peptide mimetics having permeability characteristics adequate for the development of oral dosage forms (1–3). Through rational

drug design, synthetic medicinal chemists have prepared many very potent and very specific peptidic drugs (4–10). These peptidic drugs are developed with molecular characteristics that permit optimal interaction with a specific macromolecule (*e.g.*, receptor, enzyme) which mediates the desirable therapeutic effect. However, rational drug design does not necessarily mean rational drug delivery, which strives to incorporate into a molecule the molecular properties necessary for optimal transfer between the point of administration and the first target site in the body.

As with conventional organic-based drugs, the success of oral delivery systems for peptide drugs will depend on physical-chemical factors and biological factors (1–3). The physical-chemical factors include formulation factors (*e.g.*, dosage form size and shape, rates of disintegration, deaggregation and dissolution, and rates of release from polymeric dosage forms) and drug factors (*e.g.*, solubility, chemical and enzymatic stability, lipophilicity, hydrogen bonding potential, conformation, pK_a, molecular size and shape, and affinity for endogenous transporters). The biological considerations that will determine the success of a peptide oral delivery system include stomach emptying, intestinal motility, the composition (*e.g.*, pH, enzymes, food) of the intestinal lumen, the intestinal mucosal barrier (*e.g.*, mucus layer, apical and basolateral cell membranes), the liver (*e.g.*, clearance by uptake and/or metabolism), and the production of antibodies (1–2).

Perhaps the most significant obstacle to oral absorption of peptide mimetics are the intestinal mucosal cells, which constitute both a physical and a metabolic barrier (11). The intestinal epithelial cells of primary interest, from the standpoint of drug absorption and metabolism, are the villus cells, which are the fully differentiated, polarized cells located at the upper two-thirds of the villi. These cells have tight intracellular junctions, which precludes paracellular flux of solutes, and they have high metabolic capacity. This metabolic capacity is particularly refined for peptides, since these types of molecules are abundant nutrients in the diet which need to be metabolized to amino acids prior to oral absorption (12).

One of the questions most often asked by pharmaceutical scientists is "can peptides be absorbed orally?". The answer to this question is probably both yes and no! Yes, because there is good evidence that peptide transporters exist within the intestinal mucosa (13,14). These transporters move dipeptides and tripeptides into intestinal enterocytes where they are metabolized or released into the systemic circulation. The evidence to support this concept is very strong and it is primarily based on the nutritional literature (12–14). Recently, investigators have been able to observe expression of an intestinal peptide transporter in *Xenopus oocytes*, a system widely used by molecular biologists for expression cloning of proteins (15). Thus, it will be only a matter of time before the structure of this protein is determined and this information is used to rationally design peptide mimetics having affinity for this transporter in order to enhance oral peptide absorption. Various laboratories have already shown that, in addition to nutrient peptides, drugs including cephalosporins, angiotensin-converting enzyme inhibitors and peptidic prodrugs have affinity for this intestinal peptide transporter (16).

The utilization of this intestinal peptide transporter to achieve oral delivery may be limited because of its substrate specificity (12–14). Therefore, the pathway of primary interest to most pharmaceutical scientists is passive diffusion (17). To date, most of the success in achieving oral delivery of peptide mimetics by passive diffusion has resulted from serendipity. Is it possible today, with our current knowledge base, to rationally design peptidic drugs or formulations of these molecules with enhanced membrane permeability? With only a few exceptions (18), the answer to this question is probably no. Unfortunately, we lack sufficient knowledge to allow us to rationally design peptidic drugs or delivery systems to achieve this objective. In recent years, pharmaceutical scientists have begun to employ sophisticated cell culture techniques to improve our knowledge about the barrier function of the intestinal mucosa and to evaluate strategies to enhance the intestinal permeability of peptide mimetics. Since I feel that these techniques will be extremely helpful in the efforts to enhance the oral permeability of peptides and peptide mimetics, a review of the recent literature in this area has been provided below.

CELL CULTURE SYSTEMS OF THE INTESTINAL MUCOSA

In the late 1980s, our laboratory pioneered the use of cell culture systems grown onto microporous membranes as an *in vitro* model of the intestinal mucosa (19). Subsequently, this cell culture system has been shown by many academic and industrial laboratories worldwide to be an accurate model of the intestinal mucosa and very useful for conducting drug transport and drug metabolism studies *in vitro* (20–26).

In order to successfully mimic a biological barrier like the intestinal mucosa with an *in vitro* cell culture system, the selection of the cell line becomes particularly important (23). The transport and metabolic properties of cultured cells can vary depending on (a) whether the cells are primary cultures, passaged lines or transformed lines; (b) the number of times the cells have been passaged; (c) the phenotypic stability of the cell line; (d) the heterogeneity of the cells line; and (d) the inherent ability of the cell line to undergo differentiation. Once the cell line has been selected, the properties may vary depending on (a) the cell seeding density; (b) whether the cells have reached confluency; (c) the stage of cellular differentiation; and (d) the presence or absence of essential nutrients, growth factors or associated cells that produce trophic factors (23). During transport experiments, the properties may change depending on (a) the composition of the transport media (*e.g.*, concentration of the solute, temperature, pH, presence or absence of a metabolic source of energy or ions, presence or absence of proteins that might bind the solute, presence or absence of competing solutes); and (b) whether the solute is added to the apical or basolateral side of the monolayer (23). All of these factors need to be carefully optimized and regulated so as to best mimic the biological barrier *in vivo*.

The development of a cell culture system that will mimic a specific biological barrier like the intestinal mucosa requires not only an appropriate cell line and appropriate cell culturing conditions but also a microporous membrane, which by itself or after treatment with an appropriate matrix material (*e.g.*, collagen) will support cell attachment and cell growth (23). Ideally, the microporous membrane should also be (a) sufficiently translucent so that the

development of the cell monolayer can be verified by microscopic techniques; (b) readily permeable to hydrophilic and hydrophobic solutes; and (c) readily permeable to both low and high molecular weight solutes.

Finally, a particularly critical factor in the study of the transport of lipophilic molecules is the selection of the diffusion apparatus (23). Whether the diffusion apparatus is stagnant or stirred can influence the thickness of the aqueous boundary layer on the surface of the cell monolayer and, thus, the permeability of lipophilic solutes. Two types of diffusion apparatus are currently employed for studying transport across intestinal mucosal monolayers. These include the unstirred cell-insert system (Figure 1A) and the side-by-side diffusion system stirred by gas lift (Figure 1B) (26). The gas lift system was recently developed in our laboratory specifically for conducting transport studies on cell cultures (27,28). The stirring provided by the O_2/CO_2 gas lift system produces minimal damage to the cell monolayer and also minimizes the thickness of the aqueous boundary layer.

Currently, there is considerable interest in developing cell culture systems that would mimic the intestinal mucosa in order to evaluate strategies for enhancing peptide oral delivery. The epithelium of the small intestine, which is the major site of drug absorption, consists of a monolayer of cells having considerable cellular heterogeneity with respect to both morphology and function. The intestinal epithelial cells of primary interest, from the standpoint of drug absorption and metabolism, are the villus cells, which are the fully differentiated cells located at the upper two-thirds of the villi. An *in vitro* cell culture system consisting of a monolayer of viable, polarized and fully differentiated villus cells, similar to that found in the small intestine, would be a valuable tool in the study of drug and nutrient transport and metabolism. Attempts to culture intestinal epithelial cells (*e.g.*, crypt cells) or to establish cell lines derived from enterocytes have not been successful.

Recently, alternative approaches have been considered, which include the utilization of some transformed cell lines. Several human colon carcinoma cell lines (*e.g.*, Caco-2, HT-29, SW116, LS174T, SW-480) have been reported to undergo varying degrees of enterocytic differentiation in culture. The most

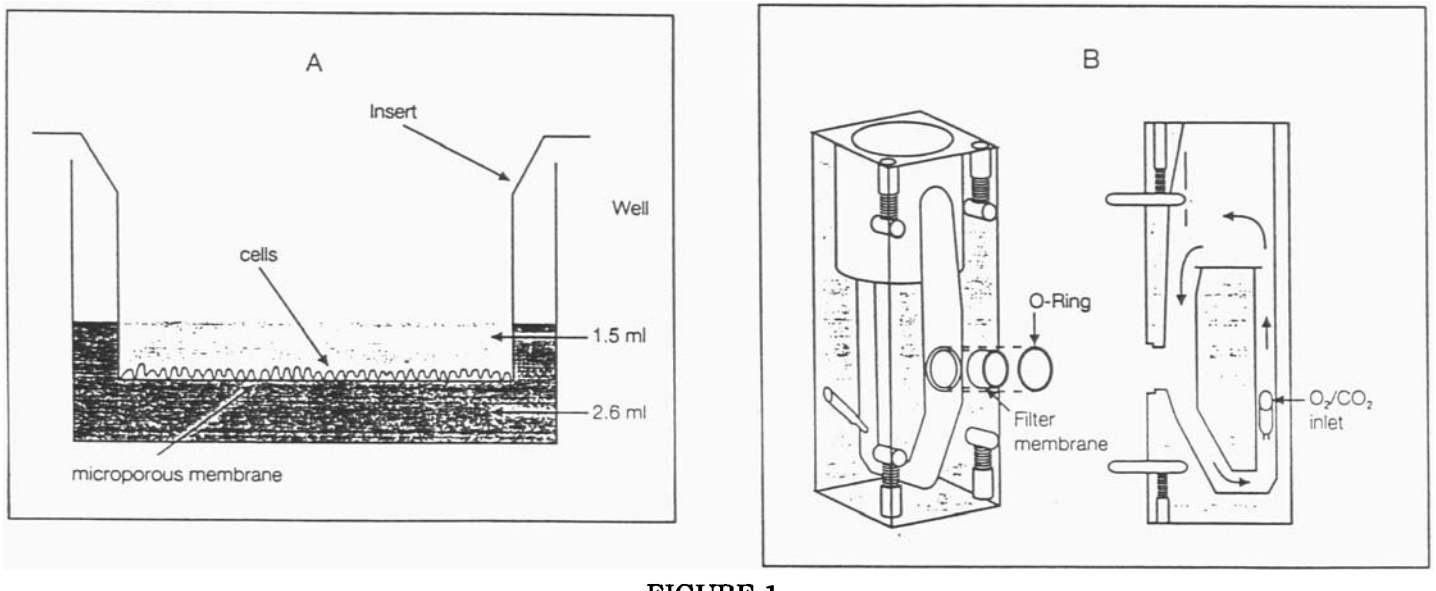

FIGURE 1

Diffusion cells for conducting drug transport studies with cell culture system.
A. Unstirred cell-insert system. B. Gas lift system.

extensively studied cell lines have been the HT-29 and Caco-2. These cell lines have received a great deal of attention in recent years because of their ability to express the morphological features of mature enterocytes or goblet cells.

When HT-29 cells are cultured in the presence of glucose, they grow as a multilayer of unpolarized, undifferentiated cells and do not express any characteristics of enterocytes. However, when the glucose in the medium is replaced with galactose, the cells express moderate enterocytic differentiation. Caco-2 cells, on the other hand, undergo spontaneous enterocytic differentiation in culture, which starts as soon as the cells achieve monolayer density (*i.e.*, 7 days) and is completed within 20 days. That Caco-2 cells form numerous domes spontaneously after reaching confluence is consistent with their ability to undertake transepithelial ionic transport. The ability of Caco-2 cells to achieve a higher degree of enterocytic differentiation than that expressed by the HT-29 cell line and their spontaneous dome formation make this cell line a more relevant *in vitro* model for the investigation of intestinal differentiation and transport processes associated with intestinal cells.

Recently, our laboratory reported that Caco-2 cells could be grown onto collagen-coated polycarbonate membranes. Caco-2 cells reached monolayer density 6–7 days after seeding onto collagen-coated polycarbonate membranes. Close examination of cell morphology between days 3 and 15 showed that as the length of time in culture and cell number increased, changes in cell dimensions also occurred. For example, from day 3 to day 16, cell height increased by 489% and cell width decreased by 42%. Electron microscopic examination revealed that by day 3 in culture, adjacent cells had formed occluding junctional complexes; however, intercellular spaces were prominent. By day 6 in culture, the lateral membranes of neighboring cells were strongly interdigitated and contained numerous desmosomes. Occluding junctional complexes were present only at the apical surface of the now functionally polarized cell monolayer. Concomitant with these observations was the progressive formation of a brush border with microvilli becoming more numerous and more organized. After 16 days in culture, Caco-2 cell monolayers consisted of cells ~30 μm in height that possessed a morphology

similar to that described for the simple, columnar epithelium of the small intestine. Thus, the cells appeared to undergo differentiation from "crypt-type cells" to "villus-type cells."

Caco-2 cell monolayers grown onto polycarbonate membranes for 10 days exhibited a formidable barrier function, as judged by the leakage (<0.25%/hr) of lucifer yellow CH (mol. wt. 453), polyethylene glycol (mol. wt. 4,000), inulin (mol. wt. 5,000) and dextran (mol. wt. 70,000). Moreover, the lack of apical-to-basolateral or basolateral-to-apical diffusion of horseradish peroxidase (40,000 daltons), despite its ability to penetrate the intercellular space, illustrates a low level of vesicular trafficking.

In support of the morphologic and transport studies, monolayer integrity also was evaluated by measuring transepithelial electrical resistance (TEER). TEER values increased from 96.6 ± 22 at day 3 to 173.5 ±10.9 Ωcm^2 at day 6 and remained constant through 30 days. From these studies it was concluded that Caco-2 cells grown onto collagen-coated polycarbonate membranes form a tight monolayer of polarized epithelial cells and thus represent a potential transport model system for the small intestinal epithelium.

The cell culture systems afford the opportunity (a) to rapidly assess the potential permeability and metabolism of peptide drugs; (b) to elucidate the mechanism(s) of drug metabolism; (c) to rapidly evaluate strategies for enhancing drug transport and minimizing drug metabolism; (d) to employ human rather than animal tissue; and (e) to minimize the use of time-consuming, expensive and sometimes controversial animal studies.

Since it is possible to readily manipulate the experimental conditions in a cell culture system, this *in vitro* model has tremendous potential in the elucidation of the various pathways by which a drug could penetrate a biological barrier (Figure 2). Experiments can be designed to determine whether the permeability of a solute is via passive diffusion (Pathway A), active or facilitated diffusion (Pathway B), and/or paracellular diffusion (Pathway C). For macromolecules, experiments can be designed to determine whether the molecule penetrates the barrier by a paracellular (Pathway C) or transcellular (Pathway D) mechanism (*e.g.*, fluid-phase, absorptive or receptor-

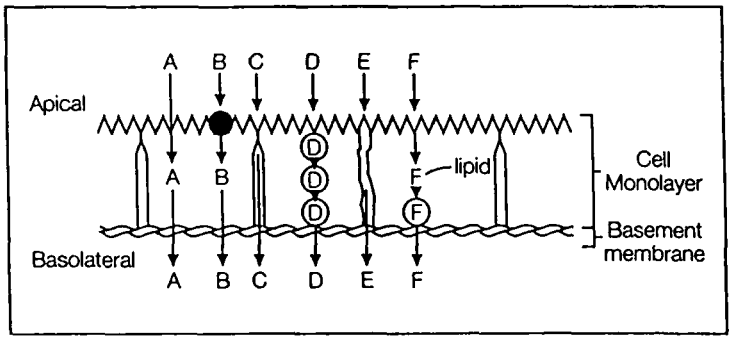

FIGURE 2

Possible pathways for drug transport in a polarized epithelial cell monolayer. Pathways: A, passive diffusion; B, active or facilitated diffusion (carrier-mediated); C, paracellular diffusion; D, vesicular-mediated transcytosis (fluid phase, adsorptive, receptor-mediated); E, "altered" paracellular diffusion; F, passive diffusion with incorporation into lipoprotein particles.

mediated transcytosis). This system is also potentially useful in elucidating the mechanism by which adjuvants enhance intestinal absorption (*e.g.*, Pathway E) and why some drugs partition preferentially into the lymphatic system (Pathway F). Most importantly, this system may provide scientists with new, basic information about transport mechanisms in biological barriers that will permit them to develop novel strategies for enhancing the intestinal permeability of peptide mimetics.

Subsequently, pharmaceutical scientists have used Caco-2 cells extensively as a model of the polarized intestinal epithelium for drug transport and metabolism studies (20–26) and to evaluate strategies to enhance intestinal permeability of peptide mimetics. For example, Caco-2 cells have been used to study: (a) the transport of important nutrients, including bile acids, biotin and large neutral amino acids and peptides via carrier-mediated processes (Figure 2, Pathway B); (b) the transport of bile acid-peptide and biotin-peptide conjugates via carrier-mediated processes (Figure 2, Pathway B); (c) the binding, uptake and transport of proteins, including epidermal growth factor

(EGF) and cobalamin (vitamin B12) via receptor-mediated processes (Figure 2, Pathway D); (d) the production and secretion of lipoprotein particles, a pathway by which lipophilic drugs, perhaps even peptide mimetics, can enter the lymphatic system (Figure 2, Pathway F); (e) the uptake of antibiotics (*e.g.*, defalexin by a dipeptide transport system (Figure 2, Pathway B); (f) the transport of drugs (*e.g.*, α-methyldopa) by the large neutral amino acid transport system (Figure 2, Pathway B); (g) the relative contribution of the paracellular (Figure 2, Pathway C) and transcellular (Figure 2, Pathway A) pathways of absorption of β-adrenergic antagonists and peptide mimetics; and (h) the effects of adjuvants (*e.g.*, palmitoyl carnitine) altering the permeability of drugs. This cell culture system has also been useful in evaluating strategies for enhancing the permeability of peptide mimetics as illustrated below.

STRATEGIES TO ENHANCE INTESTINAL PERMEABILITIES OF PEPTIDES AND PEPTIDE MIMETICS

A number of strategies have been employed in attempts to enhance oral absorption of peptidic drugs These strategies include (a) manipulation of the formulation (*e.g.*, inclusion of penetration enhancers or protease inhibitors); (b) maximizing retention of the delivery system at the site of absorption; and (c) alteration of the molecule so as to optimize affinity for endogenous transporters, build in chemical and metabolic stability, minimize the size and optimize the balance between lipophilicity, hydrogen bonding potential and conformation. To illustrate how these cell culture systems are being employed to evaluate strategies to enhance oral permeability of peptides, I will describe two examples from our laboratory at the University of Kansas.

One possible strategy to enhance intestinal permeability of peptides is the conjugation of these molecules with nutrients that are absorbed by endogenous transporters in the intestine. One transporter that could be exploited for this purpose is the bile acid transporter, which is enriched in the ileal intestinal mucosa (29). This concept is based on the rationale that conjugated and non-conjugated bile acids are rapidly and effectively absorbed in the ileum, and that the essential molecular requirement of bile acids for active transport is the retention of the acidic side chain at position 17 on the D ring (30). There-

fore, our laboratory (31) has evaluated the strategy of targeting this bile acid transporter for the enhanced intestinal permeability of a renin-inhibitory peptide ditekiren, which represents an important class of antihypertensive agents that are reported to have poor bioavailability (32). A ditekiren-cholic acid conjugate and a taurocholic acid conjugate were synthesized. Conjugation was through the N-terminus of ditekiren and the 3-position of the bile acid, via a six-carbon spacer. A derivative of ditekiren containing the spacer without the bile acid moiety, was also synthesized. The ability of these ditekiren derivatives to bind to the bile acid transporter and be transported across an epithelial cell monolayer, was evaluated using Caco-2 cell monolayers grown onto microporous membranes. The bile acid transporter in Caco-2 cells was recently characterized by our laboratory and shown to have properties similar to those of the transporter found in the intestinal mucosa (33). Both the ditekiren-cholic acid conjugate ($K_I = 60 \pm 10$ μM) and the ditekiren-taurocholic acid conjugate ($K_I = 19 \pm 5$ μM) were shown to be potent inhibitors of the apical-to-basolateral transport of [^{14}C]-taurocholic acid ([^{14}C]-TA). These ditekiren-bile acid conjugates at concentrations up to 250 μM had no effect on the diffusion of [^{3}H]-PEG (800–1000), which is a marker of the paracellular pathway. The ditekiren derivative, which lacks the bile acid moiety, had no effect on [^{14}C]-TA transport at concentrations up to 250 μM. When the permeability coefficients of the ditekiren-bile acid conjugates were determined using Caco-2 monolayers, they were shown to be six times less than that of [^{3}H]-PEG (800–1000). The transport of the ditekiren-cholic acid conjugate was also investigated in the perfused rat ileum (31), and the disappearance of the peptide-bile acid conjugate from the lumen as well as the appearance in the blood outflowing from the mesenteric vein were measured. The ditekiren-cholic acid conjugate was not detected in blood samples taken from the mesenteric vein, while the concentration of the conjugate in the intestinal perfusate remained almost constant during the perfusion experiment. In conclusion, we have shown in this study that ditekiren-bile acid conjugates retain affinity for the bile acid transporter in a cell culture model and an *in situ* perfusion model of the intestinal mucosa. However, these conjugates do not undergo apical-to-

basolateral transport. Studies are currently in progress to determined whether bile acid conjugates with smaller substituents in the 3 position can undergo transcellular transport. In addition, mechanistic studies are being conducted to determine if uptake and/or efflux of these peptide-bile acid conjugates limit their transcellular transport.

Another pathway which might be exploited to enhance peptide absorption is that of passive diffusion. In an attempt to determine which physicochemical parameters (*e.g.*, lipophilicity, hydrogen bonding potential) are the major determinants of the intestinal permeability of peptides by passive diffusion, a series of model peptides has been synthesized by Dr. Philip Burton and his colleagues at The Upjohn Company, and their permeabilities were determined using the *in vitro* Caco-2 cell culture system (34–37) and an *in situ* perfused rat ileum model of the intestinal mucosa (38).

The model peptides, which were all blocked on the N-terminal (acetyl, Ac) and the C-terminal (amide, NH_2) ends, consisted of D-phenylalanine (F) residues (*e.g.*, $AcFNH_2$, $AcFFNH_2$, $AcFFFNH_2$). To alter the degree of hydrogen bonding potential, the nitrogens of the amide bonds were sequentially methylated [*e.g.*, $AcFF(Me)FNH_2$, $AcF(Me)F(Me)FNH_2$, $Ac(Me)F(Me)F(Me)FNH_2$, Ac(Me)F(Me)F(Me)FNH(Me)]. These peptides were shown not to be metabolized in the *in situ* perfused rat ileum system or the Caco-2 cell culture system. The results of the transport experiments showed that there were no correlations between the apparent permeability coefficients (P_{app}) determined in the *in situ* perfused rat system (or the $P_{(monolayer)}$ values determined in the Caco-2 cell culture system) and the octanol-water partition coefficients of the peptide mimetics. However, good correlations were observed between the P_{app} values (or $P_{monolayer}$ values) for these peptide mimetics and their partition coefficients in heptane-ethylene glycol and the differences in their partition coefficients between octanol-water and isooctane-water, which are measures of hydrogen bonding potential. These results suggest that lipophilicity may not be the major factor in determining the intestinal permeability of peptides and that hydrogen bonding potential may be a major contributing factor. A good correlation was also observed between the P_{app}

values determined for these peptides in the *in situ* perfused rat ileum model and those $P_{monolayer}$ values determined in the *in vitro* cell culture model (Caco-2). These results suggest that the permeability values determined in the Caco-2 cell culture model are good predictors of intestinal permeability of peptide mimetics.

CONCLUSIONS

In conclusion, I would like to offer the following general thoughts about the design of peptide mimetics with enhanced membrane permeability. In the 1980s, molecular biology and computational chemistry had a significant impact on the development of rational strategies for drug design. These strategies have largely focused on optimization of the interactions of drugs with the targets (*e.g.*, receptor, enzyme) that mediate their pharmacological effects. However, equally important in achieving success in drug design is the ability to deliver drugs to their site of action *in vivo* (*e.g.*, oral bioavailability). The structural features of drugs that provide optimal interaction with their pharmacological targets are not necessarily the same structural features that ensure optimal drug delivery. Therefore, medicinal chemists, in collaboration with pharmaceutical chemists, computational chemists, molecular biologists and cell biologists, need to dedicate more effort to the design of peptide mimetics having structural features which will ensure optimal drug delivery. Only through these types of collaborative interactions will it be possible to rationally design peptide mimetics with adequate oral absorption.

ACKNOWLEDGMENTS

The author's research in this area has been supported by grants from the National Institute of General Medical Sciences, The Upjohn Company, Smith-Kline Beecham, and Glaxo, Inc. The assistance of Nancy Harmony in preparation of this manuscript is greatly appreciated.

REFERENCES

1. V.H.L. Lee, "Peptide and Protein Delivery," Dekker, New York, 1991.
2. B.L. Ferraiolo, C.A. Gloff and M.A. Mohler, eds., "Protein Pharmacokinetics and Metabolism," Plenum, New York, 1992.

3. K.L. Audus and T.J. Raub, eds., "Barriers to Protein Delivery," Plenum, New York, 1993.

4. J.J. Plattner and D.J. Norbeck, in "Drug Discovery Technologies," C.R. Clark and W.H. Moos, eds., Wiley, New York, 1990, pp. 92-126.

5. P.S. Farmer, in "Drug Design," E.J. Ariens, ed., Academic Press, New York, 1980, pp. 267-357.

6. B.A. Morgan and J.A. Gainer, Ann. Rep. Med. Chem., 24, 243 (1989).

7. D.H. Rich, in "Comprehensive Medicinal Chemistry," P.G. Sammes, ed., Pergamon, Oxford, 1990, pp. 391-441.

8. W.J. Greenlee, Med. Res. Rev., 10, 173 (1990).

9. J.R. Huff, J. Med. Chem., 34, 2305 (1991).

10. A.M. Doherty, J. Med. Chem., 35, 2305 (1992).

11. J.L. Madara and J.S. Trier, in "Physiology of the Gastrointestinal Tract," Vol. 2, 2nd ed., L.R. Johnson, ed., Raven, New York, 1987, pp. 1209-1249.

12. W.A. Walker, in "Physiology of the Gastrointestinal Tract," Vol. 2, 2nd ed., L.R. Johnson, ed., Raven, New York, 1987, pp. 1271-1289.

13. D.M. Matthews, Physiol. Rev., 55, 537 (1975).

14. V. Ganapathy and F.H. Leibach, Am. J. Physiol., 249, G153 (1985).

15. H. Saito, T. Ishii and K. Inui, Biochem. Pharmacol., 45, 776 (1993).

16. J.P.F. Bai and G.L. Amidon, Pharm. Res., 9, 969 (1992).

17. P.S. Burton, R.A. Conradi and A. Hilgers, Adv. Drug. Deliv. Rev., 4, 171 (1991).

18. H.D. Kleinert, S.h. Rosenberg, W.R. Baber, H.H. Stein, V. Klinghofer, J. Barlow, K. Spina, J. Polarowski, P. Kovar, J. Cohen and J. Denissen, Science, 257, 1990 (1992).

19. I.J. Hidalgo, T.J. Raub and R.T. Borchardt, Gastroenterology, 96, 736 (1989).

20. P. Artursson, J. Pharm. Sci., 79, 476 (1990).

21. G. Wilson, J.F. Hassam, C.J. Dix, I. Williamson, R. Shah, M. Mackay and P. Artursson, J. Control. Rel., 11, 25 (1990).

22. A.R. Hilgers, R.A. Ferradi and P.S. Burton, Pharm. Res., 7, 902 (1990).

23. R.T. Borchardt, I.J. Hidalgo, K.M. Hillgren and M. Hu, in "Pharmaceutical Applications of Cell and Tissue Culture to Drug Transport," G. Wilson, S.S. Davis and L. Illum, eds., Plenum, New York, 1991, pp. 1-14.

24. W. Rubas, N. Jezyk and G.M. Grass, Pharm. Res., 10, 113 (1993).

25. P. Artursson and J. Karlsson, Biochem. Biophys. Res. Commun.,175, 880 (1991).

26. K.L. Audus, R.L. Bartel, I.J. Hidalgo and R.T. Borchardt, Pharm. Res., 7, 435 (1990).

27. I.J. Hidalgo, K.M. Hillgren, G.M. Grass and R.T. Borchardt, Pharm. Res., 8, 223 (1991).

28. I.J. Hidalgo, K.M. Hillgren, G.M. Grass and R.T. Borchardt, In Vitro Cell Develop. Biol., 28A, 578 (1992).

29. E. Krag and S.F. Phillips, J. Clin. Invest. 53, 1686 (1974).

30. N.F.H. Ho, Ann. N.Y. Acad. Sci., 507, 315 (1987).

31. D.C. Kim, A.W. Harrison, M.J. Ruwart, K.F. Wilkerson, J.F. Fisher, I.J. Hidalgo and R.T. Borchardt, J. Drug Targeting, in press.

32. S. Thaisrivongs, Drug News Perspec., 1, 11 (1988).

33. I.J. Hidalgo and R.T. Borchardt, Biochim. Biophys. Acta, 1035, 97 (1990).

34. R.A. Conradi, A.R. Hilgers, N.F.H. Hu and P.S. Burton, Pharm. Res., 8, 1453 (1991).

35. P.S. Burton, R.A. Conradi, A.R. Hilgers, N.F.H. Ho and L.L. Maggiora, J. Control. Rel., 9, 87 (1992).

36. R.A. Conradi, A.R. Hilgers, N.F.H. Ho and P.S. Burton, Pharm. Res., 9, 435 (1992).

37. M.S. Karls, B.D. Rush, K.F. Wilkinson, T.J. Vidmar, P.S. Burton and M.J. Ruwart, Pharm. Res., 8, 1477 (1991).

38. D.C. Kim, P.S. Burton and R.T. Borchardt, Pharm. Res., in press.