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Pathways and Progress in Improving Drug Delivery through the Intestinal Mucosa and Blood-Brain Barriers

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Abstract

One of the major hurdles in developing therapeutic agents is the difficulty in delivering drugs through the intestinal mucosa and blood-brain barriers (BBB). The goal here is to describe the general structures of the biological barriers and the strategies to enhance drug delivery across these barriers. Prodrug methods used to improve drug penetration via the transcellular pathway have been successfully developed, and some prodrugs have been used to treat patients. The use of transporters to improve absorption of some drugs (e.g., antiviral agents) has also been successful in treating patients. Other methods, including (a) blocking the efflux pumps to improve transcellular delivery and (b) modulation of cell-cell adhesion in the intercellular junctions to improve paracellular delivery across biological barriers are still in the investigational stage.

1. Introduction

One of the major challenges in developing therapeutic agents is the difficulty in delivering them to the site of action. Both the intestinal mucosa barrier and the blood-brain barrier have roles in the absorption of nutrients, electrolytes, and water while restricting the entry of toxins and pathogens (e.g., virus and bacteria) [1]. The intestinal mucosa barrier limits oral drug absorption into the systemic circulation to prevent reaching the target tissue. If the target organ is the central nervous system (CNS), the drug has to penetrate the blood-brain barrier (BBB). One important function of the BBB is to help maintain a separate pool of neuroactive agents between the central nervous system (CNS) and the peripheral tissues, preventing signal crosstalk [2]. The BBB can be an obstacle in developing therapeutic agents to treat brain diseases. If ways can be found to improve the delivery of drugs to the brain, many available drugs that are unable to cross the BBB can be used to treat patients with brain tumors and Alzheimer's and Parkinson's diseases.

Studies have been carried out to determine the relationships between the physicochemical properties of a drug and its ability to cross these barriers. In addition, the physiology of the intestinal mucosa and the BBB have been studied to understand how best to modulate these barriers to enhance drug delivery. In general, drugs can penetrate these barriers through cell membranes (transcellular pathway) or in between the cells of the barrier (paracellular pathway) (Figure 1). To improve drug delivery through these biological barriers, efforts

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have been made to alter the physicochemical properties of drugs as well as to alter the properties of the barriers via formulation design. This review is focused on the characteristics of these biological barriers and ways to improve the delivery of drugs through these barriers.

2. Structure and Function of Intestinal Mucosa and Blood-Brain Barriers

2.1. Structure of Biological Barriers

There are some similarities and differences between the intestinal mucosa barrier and the BBB. The intestinal mucosa barrier is formed by an epithelial cell layer while the BBB is formed from endothelial cells. The intestinal mucosa barrier is composed of microvilli at the luminal side followed by an epithelial cell layer. The epithelial cells are anchored to basement membrane at the top lamina propria. The lamina propria below the epithelial cells has nerves, blood and lymph vessels. The drug that is absorbed through the intestinal mucosa barrier will be transported by the blood vessels throughout the body, including the brain, if the drug can cross the BBB. Immune cells (e.g., lymphocytes and eosinophils) are also found in the lamina propria. The epithelial cells of the intestinal mucosa and endothelial cells of the BBB are connected by intercellular junctions that are divided into (a) tight junctions (*zonula occludens*), (b) adherens junctions (*zonula adherens*), and (c) desmosomes (*macula adherens*) (Figure 1). The capillaries of the BBB have very few pinocytotic vesicles for substance transport [3]. The endothelial cells are anchored to a basal lamina containing extracellular matrix proteins (ECM) such as fibronectin, collagen, and laminin. The basal lamina of the endothelium is surrounded by pericytes, astrocytes, and neuron endfeet.

The tight junctions or *zonula occludens* are found in most apical regions of the intercellular junctions; the extracellular domains of cell membranes show close cell-cell contact (kiss region) mediated by protein-protein interactions. The cell surface proteins interact with proteins in the cytoplasmic domain to control intercellular junction cell-cell adhesion. Occludins, claudins (claudins-3, -5, and -12), and junctional adhesion molecules (i.e., JAM-A, -B, and -C) are transmembrane proteins anchored at the tight junction membranes. Each protein type interacts in a homotypic fashion (i.e., occludin-to-occludin or claudin-to-claudin) at the extracellular domain of opposing cells (Figure 1) [4]. These tight junction proteins can also interact in a heterotypic manner with leucocyte receptors to facilitate leukocyte extravasation through the biological barriers [5].

Although occludin and claudin have a similar general structure, these proteins have some distinctive differences related to their roles and selectivity in tight junctions. Both occludin and claudin have four transmembrane domains (i.e., tetraspanin member), three cytoplasmic domains, and two extracellular loops. Both loops of occludins are approximately the same length (44–45 amino acids (aa)) [4,6,7], while claudins have long (55 aa) and short (14 aa) loops [7–10]. For occludins, the N- and C-termini have 149 aa and 254 aa residue lengths, respectively, and they are located in the cytoplasmic domain. In contrast, claudins have a short C-terminus (21–42 aa) and a very short or no N-terminus domain [4]. The extracellular loop-1 of occludins contains several Tyr and Gly residues, and loop-2 has several Tyr residues while claudins have multiple charged amino acids that are important for cell-cell adhesion. Although the function of occludin has been not fully elucidated, occludin

knockdown in Caco-2 cell monolayers and mouse intestinal mucosa increases the paracellular permeation of marker molecules through these biological barriers [11]. This result suggests that occludin is important in maintaining the integrity of the tight junctions.

There are five conserved amino acid residues in claudin-4 that are important for its correct folding. Mutation of any one of these residues can cause incorrect protein folding. The incorrectly folded protein is normally trapped in the endoplasmic reticulum and the incorrectly folded claudin cannot migrate to the cell border of the tight junctions [12]. Phe147, Tyr148, and Tyr158 residues mediate interactions between the extracellular domains of claudin-5 from the opposing cells. The structure and functions of tight junction proteins have been reviewed previously in more detail [5,8,13,14].

Occludins and claudins form cytoskeletal complexes with zonula occludin-1 (ZO-1), ZO-2, and ZO-3. The C-terminus of occludin interacts with the guanylate kinase-like (GUK) domain of ZO-1, ZO-2, and ZO-3 while claudin interacts with PDZ1 of ZO-1. JAMs bind to ZO-1 via the PDZ3 domain [10,15,16]. ZO-1 is connected to ZO-2 and ZO-3 proteins via the PDZ2 domain of ZO1. Finally, the C-termini of ZO-1 and ZO-2 are anchored to actins to build the cell cytoskeleton [5]. The integrity of tight junctions is regulated through phosphorylation of the cytoplasmic domains of occludin and claudin by serine/threonine kinases (i.e., PKC, MAPK, PKA) and protein tyrosine kinases (i.e., c-Src) [17]. Dephosphorylation of these proteins loosens the tight junction integrity due to the relocalization of occludin and claudin away from the cell surface into the intracellular compartments.

Underneath the tight junctions reside the adherens junctions or *zonula adherens*. The adherens junctions are mediated by cell-cell adhesion proteins, including calcium-dependent cadherins as well as calcium-independent nectin and platelet endothelial cell adhesion molecule-1 (PECAM-1) [18]. PECAM-1 and nectin both belong to immunoglobulin family, and PECAM-1 is found in the intercellular junctions of vascular endothelial cells [19,20]. One of the functions of nectin is to recruit JAM-1 in the early stages of barrier formation prior to segregation of JAM-1 into the tight junction [5]. PECAM-1 and JAM-A are involved during leukocyte extravasation through the vascular endothelium [21,22]. The adherens junctions of the intestinal mucosa barrier are mediated by E-cadherin [23]. VE-cadherin-5 and E-cadherins have been found in the primary cell culture of bovine brain microvessel endothelial cells (BBMEC) [24–26] and rat brain vascular endothelial cells (RBE4) [27,28]. The cytoplasmic tail of VE-cadherin is connected to α -, β -, γ -catenin, p120, and cytoskeletal proteins. The associations of VE-cadherin with p120 and ZO-3 proteins connect the adherens junctions to the tight junctions. Thus, modulation of the adherens junctions may affect the integrity of the tight junctions and *vice versa* [13]. Finally, N-cadherin found in the BBB also connects endothelial cells to surrounding cells [29].

Desmosomes or *macula adherens* are located below the adherens junctions; they also mediate cell-cell adhesion with desmocollins (i.e., Dscs: Dsc-1, -2, and -3) and desmogleins (i.e., Dsgs: Dsg-1, -2, 3, and -4) [30]. Dscs and Dsgs belong to the cadherin family, and they interact with cadherins at the adherens junctions using protein complexes at the cytoplasmic domain. The extracellular domains of Dscs and Dsgs have a structural motif similar to that

of cadherins. Dscs are more related to classical cadherins such as N- and E-cadherins; in contrast, Dsgs have a slightly different cytoplasmic domain structure than classical cadherins. The cytoplasmic domains of Dscs and Dsgs have an intracellular anchor (IA) domain and an intracellular cadherin-like sequence (ICS) domain. However, only Dsgs have an additional proline-rich linker (IPL) domain, repeat unit domains (RUD), and a desmoglein terminal domain (DTD) [30]. The number of repeats in RUD is different in different Dsg molecules; for example, Dsg-1 has 5 repeats while Dsg-3 has 2 repeats. The C-terminal cytoplasmic domains of Dscs and Dsgs are associated with plakoglobins and plakophilins. Desmoplakins (DP) connect both plakoglobins and plakophilins to keratin intermediate filaments [30]. In addition to their homophilic interactions, Dsgs and Dscs mediate cell adhesion using heterophilic interactions; for example, heterophilic interactions of Dsg2-to-Dsc2 [31] and Dsg2-to-Dsc1a [32] have previously been observed.

The basal laminae of biological barriers play an important role in the cellular integrity of the barriers. Both epithelial cells of the intestinal mucosa barriers and endothelial cells of the BBB interact at the basal lamina ECM through integrins. For example, the intestinal mucosa epithelial cells utilize β_1 and β_4 integrins (e.g., $\alpha_2\beta_1$, $\alpha_3\beta_1$, $\alpha_7\beta_1$, and $\alpha_6\beta_4$) to bind laminin and collagen [33]. Interruption of basal lamina interactions reduces claudin-5 expression, and this interruption increases BBB permeability [34]. Matrix metalloproteinase-9 (MMP-9) regulates the composition of the basal lamina protein, and the increased fibrinogen blood level activates MMP-9 to digest type-IV collagen. Collagen digestion causes the internalization of VE-cadherin to the cytosol, reducing the barrier function of the BBB [35]. Elevation of TNF- α level during inflammation induces the secretion of MMP-9 by pericytes around the BBB [36]. The MMP-9 secretion is induced upon activation of kinases such as MAPK (p42/p44, JNK, and p38) and PI3K/Akt [37]. TNF- α also induces NF κ B to suppress claudin-5 promoter, followed by suppression of claudin-5 expression at the tight junctions [38]. In contrast, IFN- γ , IL-6, LPS, and IL-1 β do not induce secretion of MMP-9 by pericytes [37].

2.2. Physical and Metabolic Barriers

The intestinal mucosa or BBB impose physical barriers that prevent the drug from reaching the site of action. To cross via the transcellular pathway, drug molecules must partition into cell membranes at the apical side to enter the cytoplasmic domain. Then the drug molecules partition into another cell membrane at the basolateral side for access to the systemic circulation or brain (Figure 1). Thus, the physicochemical properties of the drug (i.e., size, lipophilicity, and hydrogen-bonding potential) become important factors for membrane partition. Normally, the small hydrophobic drugs favor the transcellular pathway because they can partition into the cell membranes (Figure 1). In contrast, it is difficult for hydrophilic molecules to partition into cell membranes; thus, they cannot cross through the transcellular pathway (Figure 1) [3]. The hydrophilic drugs can diffuse via paracellular pathways; however, depending on the size of the drugs, this diffusion is limited by the presence of tight junctions. Alternatively, the hydrophilic drugs can penetrate via the transcellular pathway using receptor-mediated transport or transporters.

In addition to the physical barriers, biochemical barriers such as enzymes, transport receptors and efflux pumps can prevent drug molecules from crossing the biological barriers. For example, both the intestinal mucosa and BBB have enriched peptidases and other metabolism enzymes (e.g., cytochrome P450) that degrade small and macro molecules before or during passage through these barriers [39–41]. The efflux pumps (e.g., multidrug-resistant related proteins (MRP), P-glycoproteins (Pgp), and breast cancer-related proteins (BCRP)) can expel a wide variety of drug molecules from the membranes to prevent them from crossing through the transcellular pathway (Figure 1) [42–44]. They are found in cancer cells [45,46] and biological barrier cells such the BBB [44,47–49], and intestinal mucosa [50–52]. The activities of these pumps are ATP-dependent activity with broad substrate selectivity; therefore, it is difficult to determine the substrate structural recognition [44,53–55].

2.3. Executive Summary

- The intestinal mucosa barrier is formed by epithelial cells and is an impediment to oral drug absorption.
- The blood-brain barrier (BBB) is the major barricade to delivering drugs to the brain.
- Drug molecules can penetrate the biological barriers via the transcellular and paracellular pathways of the biological barriers.
- The transcellular penetration of drugs depends on drug physicochemical properties and can be inhibited by efflux pumps.
- Transporters can aid receptor-mediated transcellular crossing of drug molecules.
- The presence of tight junctions limits drug transport through the paracellular pathway.

3. Strategies for Improving Drug Transport through the Biological Barriers

In many cases, the general principles for improving drug delivery through the intestinal mucosa and the BBB are similar; however, many drugs that are orally bioavailable cannot cross the BBB. This is partly due to the many processes (i.e., clearance, degradation, protein binding) that the drug must overcome before it can reach the BBB. Furthermore, large molecules (i.e., peptides and protein) have problems in crossing the intestinal mucosa and BBB [56]. Many methods to improve the delivery of drugs through the biological barriers have been investigated, including (a) changing the physicochemical properties of the drug to improve partitioning to the cell membranes, (b) modifying the formulation as well as the use of carriers such as liposomes and nanoparticles [57], (c) utilizing receptor-mediated endocytosis processes (e.g., antibody or protein drug conjugates) [58–62], and (d) disrupting the biological barriers [63].

3.1. Improving Drug Delivery through Transcellular Pathways

One way to enhance drug delivery through the intestinal mucosa and BBB is by aiding passive diffusion of drugs through the transcellular pathways. For crossing via transcellular

pathways of the intestinal mucosa, the drug molecules have to partition into the cell membranes at the apical side (the mucosa layer) followed by entering the intracellular space after escaping from the membranes due to the concentration gradient. While residing in the intracellular space, the molecule partitions into cell membranes at the basolateral side (blood side) followed by entrance into the blood in the systemic circulation. The drug molecule undergoes a similar process during crossing the BBB from the blood side to the brain tissues. Unfortunately, besides partitioning into the membranes, there are other competing processes or factors that contribute to the difficulty in crossing the biological barriers. These competing mechanisms are imposed by the presence of efflux pumps, metabolizing enzymes, and intracellular sequestration mechanisms. Ideally, all the different factors that influence molecular transport of a particular molecule should be investigated simultaneously. Realistically, it is difficult to simultaneously evaluate the contribution of each factor that is involved in the transport of a drug molecule. Therefore, most studies focus on evaluating only one or two factors that influence the drug transport properties.

3.1.1. Passive Diffusion—One way to improve passive diffusion of drugs via transcellular pathway is by changing the physicochemical properties of the drug to favor membrane partition [64,65]. Therefore, one way to transiently alter the physicochemical properties of a drug is by forming a prodrug using a promoiety that can be removed after crossing the biological barriers [66–68]. The lipophilicity and complexity of cell lipid bilayers embedded with proteins contribute to the barriers to drug transport [69]. The optimal membrane partition and transport properties for a drug molecule are influenced by its size, charge, lipophilicity, and hydrogen-bonding potential [56,64,65,70,71]. In addition, drug solubility and dissolution properties can also influence the intestinal absorption of the drug [67]. For peptides and proteins, other additional factors such as their conformation and dynamic properties should be taken into consideration. In many cases, balancing all the favorable physicochemical properties of a drug to favor transport properties without changing its biological activity can be difficult and/or impossible.

A promoiety is added to a drug to temporarily alter the undesirable physicochemical properties to the desired ones (e.g., low hydrogen-bonding potential, lipophilicity) for partitioning to membranes (Figure 2A). In other words, the prodrug formation transiently modifies the physicochemical properties of the drug to characteristics favorable for delivery. The prodrug formation could also be used to improve drug solubility to increase drug bioavailability [67]. Often time, the formation of prodrug could lower or eliminate the drug biological activity; therefore, it is necessary to convert the prodrug to the drug at the appropriate time and site of action. The prodrug-to-drug conversion can be achieved by chemical and/or enzymatic reaction; this depends on the type of promoiety. The chemical conversion can be triggered by pH change, hydrolysis reaction, or structural rearrangement. The enzymatic reaction can be produced by enzymes such as esterase, phosphatase, and peptidase. The ultimate goal is to have a sufficient amount of prodrug to be converted to drug for its intended biological activity.

Prodrugs of small molecules have been designed to increase drug lipophilicity for enhancing drug absorption via partition to cell membranes. This is done by simple esterification and amidation of the carboxylic acid or acetylation of the amine and alcohol groups. Heroin is a

classic example of a prodrug for morphine, in which the free hydroxyl groups of morphine are acetylated. This causes a 100-fold brain uptake of heroin compared to morphine (Figure 2B) [72]. S-acetylthiorphan prodrug [73] and *m*-nitrophenyl nipecotic ester [74] are other examples of ester prodrugs that are designed to improve brain delivery.

Because dissolution of a solid drug can influence bioavailability, prodrug formation has been utilized to improve drug solubility for oral and parenteral delivery [67]. Although drug solubility can be improved by formulation design, there is a limitation to formulation methods to improve solubility. Frequently, the formulation method cannot provide the needed amount of soluble drug in plasma [67]; in such cases, the prodrug method can be utilized to overcome the solubility problem. For example, formation of phosphate prodrug has been shown to increase the solubility of camptothecin; in this case, a phosphate acyloxy ester is formed on the tertiary alcohol group of camptothecin (Figure 3A). The prodrug can be converted to parent camptothecin by alkaline phosphatases that are found in the brush border of the intestinal mucosa [67] as well as on the BBB [39]. Irinotecan is a similar prodrug of a camptothecin derivative with a diamine promoiety linked via an ester group, and the diamine groups can form salts to increase the drug solubility (Figure 3B). After delivery, esterase enzyme(s) can convert irinotecan to the parent drug.

One of the major problems in developing peptides for oral drugs or for treating brain diseases is their inability to cross the intestinal mucosa and BBB. This is due to their physicochemical properties (e.g., size, hydrogen-bonding potential, size, and conformation) that are not favorable for partitioning into cell membranes for transcellular transport. Due to their size, peptides also cannot readily cross via paracellular pathways due to the presence of tight junctions. To overcome peptide delivery problems, cyclic peptide prodrugs have been developed (Figure 4) to alter the physicochemical properties of the peptide for cell membrane partition for transcellular passive diffusion. The cyclic peptide prodrug method has been applied to improve oral bioavailability and/or brain delivery of linear opioid peptides [75–85] and RGD peptidomimetics [86–90]. Four different promoieties have been developed, including acyloxyalkoxy- [79,80,82,91,92], phenylpropionic acid- [75,77,81,93,94], coumarinic acid- [75–77,84,85], and an oxymethyl-modified coumarinic acid (OMCA)-based cyclic peptide prodrugs [52,95,96] (Figure 4).

Linear opioid and Arg-Gly-Asp (RGD) peptides are hydrophilic and/or charged in nature and have a random conformation with high hydrogen-bonding potential to water. Furthermore, some of these peptides have open N- and C-termini, which make them prone to enzymatic degradation by exopeptidases (i.e., amino- and carboxy-peptidases). For the RGD peptidomimetics, the presence of guanidine and carboxylic acid groups, which are necessary for biological activity, causes them to have low membrane partition coefficients. The cyclic peptide prodrugs were designed to have (a) low hydrogen-bonding potential, (b) increased conformational rigidity and intramolecular hydrogen bonding, (c) reduced hydrodynamic radii, (d) increased hydrophobicity for membrane partition, and (e) improved enzymatic stability to exopeptidases by protecting the N- and C-termini. The cyclic prodrug can be converted to the parent linear peptide by esterase enzymes (Figure 4). The cyclic prodrugs of opioid peptides were transported more effectively than the parent linear peptides in cell culture monolayers. Compared to the parent linear peptides, the cyclic peptide

prodrugs have smaller hydrodynamic radii, lower hydrogen-bonding potential, and higher octanol/water partition coefficients.

There are some disadvantages of cyclic peptide prodrugs; therefore, there is a need to pay careful attention to some of their potential drawbacks. First, the formation of a cyclic prodrug can enhance recognition by efflux pumps as well as metabolism by cytochrome P450 enzyme. These recognition and metabolism processes have been observed in cyclic prodrugs of opioid peptides [48,52,95,97,98]. Second, the promoiety in the cyclic prodrugs can be chemically unstable during a pH change in formulation, which contributes to short shelf-life. Third, the prodrug may be converted to drug by enzymes before and during diffusion across the cellular barriers into the blood stream or brain tissue. This premature conversion of the prodrug inside the cells can cause trapping of the drug in cells of the barrier and prevent the drug from crossing the barrier.

3.1.2. Passive Diffusion Modified by Efflux Pumps or Intracellular Sequestration

3.1.2.1. Efflux Pumps: The presence of polarized efflux pumps (e.g., Pgp) can create a problem for effective delivery of drugs through the intestinal mucosa and BBB (Figure 1). It has been suggested that these pumps recognize molecules with hydrophobic aromatic and/or tertiary amino groups. Normally, Pgp substrates have log P octanol/water > 1.0. In essence, even though the drug molecules can effectively partition to cell membranes, the efflux pumps expel the molecules from the membranes, preventing them from crossing the biological barriers. Using the X-ray structure of Pgp, several potential mechanisms of action of efflux pumps have been proposed, including (a) hydrophobic vacuum cleaner, (b) flippase, and (c) pore generation mechanisms [99]. Several methods have been proposed and investigated to elude the activity of Pgp, including (a) designing competitive inhibitors for Pgp, (b) modifying drugs to avoid Pgp recognition, and (c) developing inhibitors that completely shut down the function of Pgp [100].

Verapamil, cyclosporine A (CsA), and LY335979 have been identified as competitive inhibitors of Pgp [100,101]. In contrast to cyclosporine A (CsA), cyclosporine D (SDZ PCS833) inhibits Pgp without immunosuppressive activity [102,103]; therefore, SDZ PCS833 has been used to enhance intestinal mucosa delivery of some molecules [103,104]. Although inhibiting Pgp with verapamil improves drug transport, it creates unwanted side effects because it blocks the activity of calcium channels. Dexverapamil was also developed to inhibit Pgp without blocking calcium channel activity [103]. Several pharmaceutical excipients such as surfactants (i.e., polysorbate 80, cremophore) and polymers (i.e., polyethylene glycols, pluronic) have also been found to inhibit Pgp and enhance drug transport [105–107].

The formation of a dimer prodrug has been investigated to inhibit Pgp and enhance drug delivery to the brain. Compared to the parent monomer, galantamine-2 (Gal-2) and abcavir dimer (NBD-abcavir) prodrugs have higher binding affinities and lower the off-rate to Pgp receptors (Figure 5) [49,108]. The hypothesis is that the dimer can fill multiple binding sites on Pgp to effectively block Pgp activity while simultaneously enhancing prodrug transport through the BBB. In a test of Pgp inhibition activity, Gal-2 was shown to increase cellular

uptake of rhodamine-123 into MCF-7/DX1 cells [108]. As a prodrug, Gal-2 can be converted to galantamine monomer by esterase (Figure 5A). It is anticipated that both Gal-2 and NBD-abcavir prodrugs will inhibit the activity of Pgp while simultaneously enhancing prodrug permeation through the BBB.

The motivation for developing brain delivery of abcavir is to eliminate reservoirs of HIV in the brain [49,108]. NBD-abcavir was constructed by linking two abcavir molecules with a disulfide bond promoiety (Figure 5B) [49]. The NBD-abcavir prevents HIV replication in 12D7 cells and inhibits cell death of HIV-infected MT-2 cells in a concentration-dependent manner [49]. The uptake of NBD-abcavir is higher than abcavir in rat brain microvessel endothelial cells; this is presumably due to its effectiveness in inhibiting Pgp [49]. The release of abcavir from the NBD-abcavir prodrug is initiated by reduction of the disulfide bond followed by the cyclization of the promoiety (Figure 5B).

Finally, the drug structure can be modified to avoid Pgp recognition while maintaining its intended biological activity; however, this strategy can be very challenging because Pgp has broad structural recognition. The proposal to design Pgp inhibitors that completely shut down the function of Pgp may have toxic implications for the brain. These types of inhibitors could block the activity of Pgp altogether by using very tight binding inhibitors (e.g., antibodies) or covalently linked inhibitors at the active site(s). Shutting down the efflux mechanisms completely may allow toxic molecules to enter the brain.

3.1.2.2. Passive Diffusion and Intracellular Sequestration and Trapping: During the process of passing through the biological barriers, one of the potential fates of the molecules is becoming trapped in the intracellular space. This is due to sequestration of drug molecules in the cellular compartment(s). Membrane-bound compartments (i.e., endosomes, lysosomes) occupy over 50% of the intracellular space [109,110]. Krise *et al.* have shown that many amine-containing drugs can be sequestered or trapped in the acidic compartment (i.e., lysosomes). This is because there is a higher population of positively charged molecules than neutral molecules in ionic equilibrium in the lysosomes at acidic pH (4.5) compared to the cytoplasmic domain at neutral pH [109]. Thus, the positively charged molecules cannot effectively partition into the membranes to escape the lysosomes. In contrast, neutral (free base) molecules in the cytoplasmic domain can readily partition and cross the membranes into the lysosomes. Therefore, there is a buildup of amine drugs in the lysosomes. Even though amine-containing drugs are transported via receptor-mediated endocytosis, they can also be sequestered in lysosomes. The sequestration properties of amine drugs are correlated with the pKa of the weak base [109]. Comparison of transport properties of propranolol, ibuprofen, and neutral testosterone in Caco-2 cell monolayers showed that the weakly basic propranolol was sequestered inside Caco-2 cells, preventing some propranolol molecules from crossing the monolayers [111]. Similarly, the transport of amodiaquine across Caco-2 cell monolayers and intestinal rat tissue was prevented by its sequestration in the lysosomes [112]. The sequestration mechanism should also be considered when evaluating drug transport across the biological barriers.

Certain types of molecules can be trapped inside of cells due to their metabolism or conversion inside the cells. For example, methotrexate (MTX) enters the cells using

transport receptors such as reduced folate receptor (RFC) and membrane-associated folate binding protein (mFBP). Upon cellular uptake, the gamma carboxylic acid of the glutamic acid in MTX undergoes polyglutamation [113–115]. Because of the highly negative charge of polyglutamated MTX, the molecules cannot partition into the membranes and are trapped inside the cells. The polyglutamated MTX has been found trapped in Caco-2 cells, and this trapping mechanism influences its transport across the cell monolayers [114].

There has been limited work carried out to overcome the problem of drug sequestration [109,116–118]. One possible solution is to derivatize the amine group of the drug with a promoiety (e.g., to make an amide prodrug) so the amino group cannot be protonated or charged. Another potential solution is to modify the structure of the drug so it will no longer be protonated and sequestered in the lysosomes. Unfortunately, it is difficult to maintain biological activity when the structure of the drug is modified. The effect of structure on drug sequestration is still under investigation.

3.1.3. Receptor-mediated Transport—Receptor-mediated transport mechanisms have been investigated for delivering small molecules, peptides, proteins, nanoparticles, and liposomes through the biological barriers [57,61,62,119]. Active transporters are present in the biological barriers, including iron transporters (i.e., transferrin receptor), folate receptors (RFC and mFBP), amino acid transporters, di-/tri-peptide transporters, and organic anion and cation transporters. For example, oligopeptide transporters are present in the apical and basolateral membranes of the intestinal mucosa barrier. They are responsible for transport of dipeptide, tripeptide, beta-lactam antibiotic, and renin inhibitors [120,121]. The oral absorption of many dipeptide or dipeptide-like drugs, including captopril, enalapril, lisinopril, and carnosine, is mediated by these transporters [122–126]. The dipeptide transporters are proton-dependent, and their activity is inhibited by dipeptides Gly-Pro and Pro-Gly. Other transporters such as organic anions (e.g., OATP1A1, OATP1B3), organic cations (e.g., OCT1, OCTN2), glucose (e.g., Glut1), and L-amino acid (e.g., Lat-1) have also been exploited to carry drugs across the biological barriers [127].

A combination of prodrug and receptor-mediated transport methods has been used to improve the delivery of small drug molecules. For example, an anti-influenza drug, Zanamivir, has very low oral bioavailability due to its unfavorable physicochemical properties (Figure 6). To overcome this shortcoming, Zan-L-Val prodrug was designed by conjugating Zanamivir with L-valine via an ester-labile acyloxy promoiety (Figure 6) [128]. The cellular transport property of Zan-L-Val, which is similar to that of valacyclovir, allows it to enter the cells by utilizing hPepT1 transporters [129,130]. The receptor-mediated uptake mechanism was confirmed by several experiments. First, Zan-L-Val and valacyclovir were three and five times higher, respectively, in hPepT1-transfected HeLa cells than in wild type HeLa cells. Second, Zan-L-Val inhibited the cellular uptake of ³H-labeled Gly-Sar in Caco-2 cell monolayers. Third, Zan-L-Val can cross the intestinal mucosa barrier while the parent drug had no absorption in the *in-situ* rat intestinal perfusion model [128].

Transferrin receptors (Tf-R) have been utilized in many facets to deliver drugs through the BBB, and this subject has been described in many reviews [131,132]. However, a new Tf-R-targeted method has been developed using phage particles containing homing cyclic

CRTIGPSVC peptides (CRTIGPSVC-phages). These phage particles can penetrate the BBB by indirectly utilizing transferrin receptors [133]. In this case, the peptide on the phage interacts with apo-transferrin (apo-Tf or iron-free Tf) to induce apo-Tf conformation that mimics the conformation of iron-bound transferrin (Holo-Tf). Thus, upon binding CRTIGPSVC peptide to transferrin, the phage-peptide/Tf complex is recognized by the transferrin receptor (TfR) to carry the phage-peptide across the BBB. The CRTIGPSVC-phage particles were detected in the mouse brain as well as in human glioma xenografts in the mouse brain [133]. Intravenous (i.v.) administration of CRTIGPSVC-phages via the mouse tail vein resulted in 100-fold particle brain deposition compared to that of control phages without the peptide, signifying receptor-mediated transport. The results indicate that CRTIGPSVC-phages have the potential to deliver drugs to treat brain tumors or other brain diseases.

3.2. Paracellular Pathways

There are similarities in the physical characteristics of the paracellular pathways of the intestinal mucosa and the BBB. Small hydrophilic molecules and ions can passively penetrate via paracellular pathways of these biological barriers. However, the paracellular penetration of large molecules is limited by the presence of tight junctions. Most peptides and proteins cannot effectively pass through the paracellular pathways; therefore, it is difficult to develop peptides and proteins for oral absorption and for brain delivery [18]. One way to improve the delivery of large hydrophilic molecules is by improving their penetration through paracellular pathways. For this purpose, many researchers have attempted to modulate the intercellular junctions to increase their porosity, thus allowing large hydrophilic molecules to cross the biological barriers. Previously, a hypertonic solution of mannitol has been successfully used to open the intercellular junctions of the BBB to deliver anticancer drugs to patients with brain tumors [134–138]. However, it should be noted that the uncontrolled paracellular opening could allow undesired large molecules (e.g., toxins) to enter the systemic circulation or the brain. The ideal situation is that the porosity of the paracellular opening can be regulated to allow penetration of molecules with a certain size limit.

3.2.1. Modulation of Occludin and Claudin at the Tight Junctions

3.2.1.1. Protein Kinase Modulation: Regulation of phosphorylation and dephosphorylation of cytoskeletal proteins as well as the cytoplasmic domain of cell-adhesion proteins (e.g., occludin and claudin) at the tight junctions alters the paracellular porosity of biological barriers. Phosphorylation of the cytoplasmic domain of occludin or claudin promotes their interactions with actins to produce cell-cell adhesion at the extracellular domain of the tight junctions [17,139,140]. PKC ζ phosphorylates occludin at the Thr438, Thr403, Thr404, and Thr424 residues to activate its cell-adhesion properties. In contrast, inhibition of PKC ζ activity with PKC ζ -PS peptide decreases the cell adhesive function of occludin in Caco-2 cell monolayers [17].

PKC η is a kinase that is responsible for threonine phosphorylation (i.e., Thr 403 and Thr 404) of occludin, and inhibition of PKC η activity can cause redistribution of ZO-1 protein from the tight junctions [141]. Dephosphorylation of occludin redistributes occludin away from

the tight junctions into intracellular compartments of the cell monolayers. This redistribution causes a decrease in TEER value of the monolayers and an increase in inulin transport through Caco-2 cell monolayers, indicating the increase in porosity of the paracellular pathways. The dephosphorylation process also delays the resealing of the tight junctions of Caco-2 and MDCK cell monolayers when the monolayers are incubated with calcium-deficient medium followed by calcium-sufficient medium [17].

Knocking down the activity of PKC ζ with sh-RNA lowered the integrity of the tight junctions, supporting the idea that PKC ζ maintains the cell-cell adhesion properties of occludin. It is interesting to note that inhibiting PKC ζ activity with PKC ζ pseudosubstrates (PKC ζ -PS, Table 1) can also affect the cellular redistribution of E-cadherin and β -catenin away from the adherens junctions. In contrast, inhibition of PKC η activity with PKC η pseudosubstrates (PKC η -PS, Table 1) does not translocate these two proteins away from the adherens junctions.

3.2.1.2. Tight Junction Modulation by Occludin and Claudin Peptides: Peptides derived from the extracellular loop-1 and -2 of claudin and occludin have been investigated for their ability to modulate tight junctions for enhancing paracellular permeation of drug molecules. The C1C2 peptide (29 aa, Table 1) derived from the first loop of rat claudin-1 lowers the TEER values and increases the paracellular permeation of lucifer yellow (LY)- and fluorescein isothiocyanate (FITC)-labeled 10 kDa dextran (10 kDa) in the cell monolayers [142,143]. The effect of C1C2 peptide on modulation of the tight junctions is due to relocation of the claudin from the cell surface to the cytoplasmic domain upon binding to the peptide [142]. This peptide also enhances the brain delivery and efficacy of analgesics, including opioids [D-Ala², N-MePhe⁴, Gly⁵-ol]-enkephalin (DAMGO)] and tetrodotoxin (TTX, a sodium channel blocker) [143]. The disruption of the BBB *in vivo* can last as long as 3 days, which is considered to be a long-term opening of the BBB. It is still not clear whether the long-term opening of the BBB will introduce side effects; thus, further study is needed.

A small peptide (DFNYNP) derived from extracellular loop-2 of claudins (i.e., claudin-3, 4, 7, and 8) modulates claudin-mediated cell-cell adhesion in mammary epithelial cells [144]. DFNYNP peptide disrupts the tight junctions by triggering the internalization and relocation of claudin-3 and -4 into the cell cytoplasmic domain; some of these proteins are found in vesicles along the tight junction membranes [144]. The peptide activity is attributed to the four conserved residues at Phe147, Tyr148, Asn149, and Pro150 in different claudins. Mutation of any one of these four residues to a Gly residue makes the peptide inactive. The peptide also induces cell apoptosis via the activation of caspase-3 and -8 pathways [144]. It is not known whether the peptide can induce apoptosis on the cells of biological barriers.

The OCC2 peptide from loop-2 of chick occludin (Table 1) decreases the TEER values and enhances the transport of inulin or dextrans (3 and 40 kDa) across *Xenopus* kidney epithelial A6 cell monolayers; this effect can be attributed to the modulation of occludin-mediated tight junctions [145]. Another peptide called OCC2R that is derived from rat occludin increases the paracellular permeability of the blood-testis barrier [146,147]. The loosening of the tight junctions was proposed to be due to the induction of fast turnover of the occludin

at the cell surface by the peptide and was not due to the suppression of occludin synthesis. It is indicated that the occludin peptide does not alter the distribution of ZO1, ZO2, and cadherins at the intercellular junctions.

It is interesting that OCC1 peptide from loop-1 chick occludin does not disrupt the tight junctions of A6 cell monolayers [145]. Similarly, OP₉₀₋₁₃₅ peptide derived from loop-1 of human occludin has no ability to modulate the tight junctions of Caco-2 cell monolayers when added from the apical (AP) side [148]. In contrast, OP₉₀₋₁₃₅ peptide decreases TEER values and improves the permeation of ¹⁴C-mannitol in Caco-2 cell monolayers when added to the basolateral (BL) of the cell monolayers [148]. One explanation of why peptide addition was not effective is that the peptides (i.e., OP₉₀₋₁₃₅ and OCC1) were enzymatically unstable. In other words, they were digested by metabolic enzymes before reaching the tight junctions. The second possible explanation is that these peptides are too large to penetrate the tight junctions to display their modulatory activity. A third possible explanation is that the peptides can readily form oligomers, and the oligomer formation protects the active site of the peptide from the target receptors. After systematic size reduction, a smaller OP₉₀₋₁₀₃ peptide can disrupt the tight junctions; however, it is still prone to dimer and oligomer formation. To solve the oligomerization problem, a hydrocarbon chain (C14) was conjugated with the N-terminus of OP₉₀₋₁₀₃ to generate LIP-OP₉₀₋₁₀₃. This conjugation prevents oligomer formation, and the modified peptide has higher modulatory activity than does the parent peptide.

3.2.1.3. Junction Modulation by Zonula Occludens Toxin (Zot): Tight junctions of the BBB and intestinal mucosa barrier can be disrupted by zonulin and zonula occludens toxin (Zot). Zot is secreted by *Vibrio cholera* bacteria, which causes diarrhea in cholera patients [149–151]. Zot also enhances permeation of small and large hydrophilic compounds (i.e., mannitol, 4 kDa polyethylene glycol (4000-PEG), and inulin) as well as small hydrophobic molecules (paclitaxel and doxorubicin) through Caco-2 cell and/or BBMEC monolayers [149]. Zot delta-G (12 kDa fragment of Zot) with a mixture of enzyme inhibitors (i.e., bestatin, captopril, and leupeptin) increases intestinal mucosa and brain absorption of ritonavir about 57-fold compared to control [149,152,153]. The need for enzyme inhibitors suggests that Zot delta-G is enzymatically unstable. Reduction of Zot protein to a hexapeptide FCIGRL (AT-1002) can disrupt the tight junctions of Caco-2 cell monolayers and enhance the intestinal absorption of paracellular marker molecules *in vivo* [154]. AT-1002 improves the oral and lung delivery of low molecular weight heparin and calcitonin, respectively. It was suggested that Zot and zonulin are recognized by Zot receptors in the brain, and that these proteins modulate the tight junctions by inhibiting PKC. This inhibition initiates rearrangement of protein-protein interactions and/or induces actin polymerization in the tight junctions [151,155]. As mentioned above, inhibition of PKC prevents the phosphorylation of occludin and claudin and causes these tight junction proteins to relocate away from the cell surface, disrupting the tight junction integrity.

3.2.2. Modulation of Extracellular Cadherin Interactions at the Adherens Junctions—Cadherin peptides (i.e., Ac-SHAVSS-NH₂ (HAV6) and Ac-ADTPPV-NH₂ (ADT6)) derived from the EC1 domain of E-cadherin modulate cadherin-mediated adhesion

in the intercellular junctions of MDCK, Caco-2, and bovine brain microvessel endothelial cell (BBMEC) monolayers [24,156–159]. Compared to control peptides, cadherin peptides enhance the permeation of ^{14}C -mannitol through the paracellular pathways of MDCK cell monolayers [156,158] and through the BBB in the *in-situ* rat brain perfusion model [160]. Although ^3H -daunomycin is an efflux pump substrate, HAV6-peptide improves the permeation of daunomycin across the BBB in the *in-situ* rat brain perfusion model while scrambled HAV peptide (HAVscr) and vehicle do not enhance ^3H -daunomycin transport. A combination of verapamil and HAV6 peptide increases brain delivery of ^3H -daunomycin compared to verapamil or HAV6 peptide alone, suggesting that HAV6 peptide increases the paracellular porosity of the BBB.

Recently, magnetic resonance imaging (MRI) was used to evaluate BBB modulatory activity of cadherin peptides. In this case, HAV6 peptide was used to enhance the brain uptake of gadolinium-diethylenetriaminopentaacetic (Gd-DTPA), an MRI contrasting agent, using the *in-situ* rat brain perfusion model and the *in vivo* rat and mouse models. The amounts of Gd-DTPA in different parts of the brain were quantified using inverse T1 values. In the *in-situ* rat brain perfusion model, the rats treated with HAV6 peptide had higher brain deposition of Gd-DTPA compared to rats treated with vehicle, indicating that HAV6 peptide enhanced the BBB permeation of Gd-DTPA. Compared to vehicle, the HAV6 peptide significantly enhanced the brain deposition of Gd-DTPA at the olfactory bulb, hippocampus, cerebellum, and ventral, deep-rostral, and deep caudal areas. The results indicated that the peptide improves Gd-DTPA delivery at highly vascularized areas in the brain.

The HAV6 peptide also enhanced brain delivery of Gd-DTPA delivery in live adult female Balb/c mice. Different concentrations of HAV6 peptide (0.001 to 0.032 mmol/kg) and a fixed amount of Gd-DTPA (0.4 mmol/kg) were delivered via i.v. injection into anesthetized mice. Saline was used as a control. MRI brain scans were taken every 3 min. MRI imaging of the brain showed that depositions of Gd-DTPA were higher in peptide-treated mice compared to saline-treated mice as determined from T1-weighted MR images [161]. An increase in peptide dose increased the amount of Gd-DTPA in the brain. The Gd-DTPA depositions were observed at the anterior, mid, and posterior regions of the mouse brains. The brain uptake of Gd-DTPA occurred rapidly, within 3 min after peptide delivery, and maximum uptake was seen around 6 min. The amount of Gd-DTPA decreased after 12 min; upon a second administration of Gd-DTPA without peptide at 21 min, the amount of Gd-DTPA increased to a maximum at 27 min. Injection of Gd-DTPA at the 60-min time point did not produce an increase in the uptake of Gd-DTPA, suggesting that the effect of HAV6 peptide on opening of the BBB intercellular junctions lasts less than 60 min [161]. Thus, the peptide has a favorable characteristic in that it opens the tight junctions immediately with a short modulation period. These results suggest that the HAV6 peptide can be used to increase the brain delivery of drug molecules to the brain in the *in vivo* system.

The proposed mechanism of action of HAV- and ADT-peptides in modulating the intercellular junctions is binding to the extracellular (EC) domain of E-cadherin. Using NMR and FTIR spectroscopy, HAV6 peptide has been shown to bind to the expressed EC5 domain of E-cadherin [162]. Recently, HAV- and ADT-peptides were shown to bind the EC1 domain of E-cadherin as determined by circular dichroism (CD) and 2D NMR

spectroscopy. The CD spectra of the EC1 domain were changed upon titration with cadherin peptides, with saturation at high peptide concentrations. Titration of ^{15}N -labeled EC1 domain with HAV- or ADT-peptide affects ^1H and ^{15}N resonances of several amino acid residues of the EC1 domain in ^1H - ^{15}N -HSQC NMR spectra. The affected residues are involved in binding between the peptide and EC1. More details of binding properties of HAV- and ADT-peptides to EC1 are being investigated; the results will be used to design a better modulator of the BBB.

3.3. Nanoparticles to Improve Drug Delivery across Biological Barriers

Nanoparticles have been investigated to improve delivery of drugs across the intestinal mucosa [163] and the BBB [164,165]. The successful delivery of nanoparticles through the intestinal mucosa and the BBB was normally done utilizing receptor-mediated transcytosis [61,163–165]. It has been shown that nanoparticles with peptide ligands can cross the intestinal mucosa using receptor-mediated transport [163]. In contrast, successful delivery of nanoparticles across the BBB was done using particles decorated with large molecules such as apolipoprotein [166], ligands to transferrin (Tf) receptors (i.e., Tf and anti-Tf-receptor antibodies) [167,168], and antibodies to insulin receptors [169]. Although nanoparticles can be internalized via pinocytosis or absorptive-mediated transcytosis (AMP), this process is not efficient because of the low quantity of transported nanoparticles. Furthermore, in the absence of targeting molecules, the nanoparticles are being taken up by other cells non-selectively [163].

Due to their size, nanoparticles cannot cross via the paracellular pathway of either biological barrier [163]. The uptake mechanisms of particles can be via clathrin-mediated endocytosis for small particles (<200 nm) and caveolae-mediated endocytosis for large particles (500 nm) [170]. As mentioned previously, the potential problem of endocytosis processes is that the particle can be trapped in the intracellular space of the cell barriers due to its inability to escape the endosomes or the inefficiency in translocating to the other site of the cell membranes of the biological barriers. Modified liposomes are the most promising particles for delivering anticancer agents through the BBB to treat brain tumors [171,172]. The pegylated liposomes have been used to deliver doxorubicin in combination with prolonged treatment with temozolomide as well as radiotherapy to treat glioblastoma. Unfortunately, the inclusion of pegylated liposomes and temozolomide did not provide any improvement in patients' outcomes [173].

3.4. Executive Summary

- Transcellular transport of drugs can be improved by forming prodrugs that alter drug physicochemical properties and favor partition to membranes.
- Inhibition of efflux pumps can enhance transcellular transport of drugs.
- Receptor-mediated transport has been shown to improve the delivery of drugs.
- Inhibition of cell-cell adhesion molecules in the tight and adherens junctions has been shown to improve paracellular penetration of drug and marker molecules *in vitro* and *in vivo*.

4. Conclusions

The presence of biological barriers creates many challenges in delivering drugs orally as well as via the central nervous system (CNS). Changing the physicochemical properties by modifying the drug structure and forming prodrugs has been shown to improve the transport of drug molecules, including peptides, through the biological barriers. Many prodrugs are currently being used to treat patients. Cell surface receptors have also been exploited to improve drug delivery through the biological barriers. Some of the modified drugs (e.g., Val-acyclovir) that utilize cell surface receptors to improve delivery have reached patients. However, some of targeted drug delivery methods have not been successful in treating patients; thus, some of these approaches still need further investigation. Although they are still in the early stages, methods to modulate the intercellular junctions using peptides derived from intercellular junction proteins (e.g., occludins, claudins, and cadherins) have shown promise in improving drug delivery through the biological barriers. There is a need to investigate what size molecules can cross the biological barriers, especially the BBB. The duration of the opening of the biological barriers by these modulators also needs further investigation. Finally, investigations of the long-term effects of opening and closing of the biological barriers, especially the BBB, should be done to ensure patient safety.

5. Future Perspectives

The use of prodrugs and receptor-mediated transport has achieved success in improving drug delivery and treating patients. In the future, inhibition and avoiding efflux pumps can improve drug delivery through the biological barriers; however, this technology still has many challenges because it has the potential to generate side effect(s). Some progress has been made in enhancing drug delivery via the paracellular pathway by modulating the intercellular junction proteins. It is predicted that this method has a future in oral delivery of large molecules such as peptides, proteins, and oligonucleotides. Furthermore, this method has the potential to deliver large molecules to the brain as diagnostic and therapeutic agents for brain diseases such as Alzheimer's and Parkinson's and brain tumors. This method can also be used to deliver molecules to the brain for studying the functions of specific brain cells and their components.

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Defined Key Terms

Blood-brain barrier (BBB)	is microvessel endothelial cells in the brain that are very selective in transporting molecules into the brain.
Intestinal mucosa barrier	is the epithelial cell layers that prevent drugs from orally absorbed and enter the systemic circulation.
Transcellular pathway	is the route by which drugs cross the biological barriers through the cell membranes.

Paracellular pathway	is the path where drug molecules pass between the cell connecting junctions.
Intercellular junctions	are the spaces between cells that are connected by cell-cell adhesion proteins.
Efflux pumps	are receptors on the cell surface that prevent drug molecules from crossing the cell membranes of the biological barriers.
Prodrug	is a molecular conjugate between a drug and a promoiety that changes the physicochemical properties of the drug to increase its solubility, uptake, or transport.
Receptor-mediated	transport is a process in which molecules are shuttled through the cell membranes of the biological barriers by a specific receptor.

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(A)

(B)

Blood-Brain Barrier **Figure 1.**

A diagram of the intestinal mucosa barrier. Drug molecules can pass through the barrier via transcellular and paracellular pathways. The molecules can passively diffuse through transcellular pathways by partitioning into the cellular membranes from the apical side to the basolateral side. The presence of efflux pumps can inhibit the transcellular passive diffusion of drug molecules. Nutrient transporters have also been used to carry drugs or drug conjugates across the biological barriers. Small ions and molecules can cross through barriers via paracellular pathways (intercellular junctions), but the presence of the tight junctions prevents drug molecules from passing through this pathway. Modulation of the protein-protein interactions in the intercellular junctions has been shown to improve paracellular permeation of drug molecules via the paracellular pathways.

Figure 2.

Illustration of drug to prodrug formation by chemical or enzymatic reaction. A prodrug is assembled by conjugating the drug to a promoiety to change the drug physicochemical properties. **(A)** After delivery, the enalapril prodrug can be converted to the parent drug (enalaprilate) by enzymatic reaction. **(B)** Heroin is a classic example prodrug of morphine. The acetyl ester groups protecting the hydroxyl group serve as the promoiety that change the physicochemical properties of heroin to favor brain uptake.

Figure 3.

Formation of **(A)** phosphate and **(B)** ester prodrugs to improve drug solubility. **(A)** The salt of the phosphate prodrug of camptothecin is more soluble than camptothecin, and it can be converted to the parent camptothecin by phosphatase enzymes. **(B)** Irinotecan is an ester prodrug of camptothecin derivative, and the formation of diamine salts enhances drug solubility. The drug is converted to the parent drug by esterase enzymes.

Figure 4.

The formation of cyclic peptide prodrugs using (A) acyloxyalkoxy and (B) phenylpropionic acid promoieties. The cyclic peptide prodrug is converted to the parent peptide by esterase enzymes (slow reaction) followed by a fast chemical reaction.

Figure 5.

(A) Gal-2 and (B) NBD-abcavir are dimer prodrugs of galantamine and abcavir, respectively. Gal-2 can be converted to galantamine monomer by esterase while NBD-abcavir is converted to abcavir monomer upon reduction of the disulfide bond.

Figure 6.
The structures of Zanamivir, Zan-L-Val, and Valacyclovir.

Table 1**Modulators of Biological Barriers**

Peptide Origin	Peptide Name	Sequence
PKC ζ substrate	PKC ζ -PS	Myr-SIYRRGARRWRKL
PKC η substrate	PKC η -PS	Myr-TRKRQRAMRRRVHQING
Claudin-1	C1C2	SSVSQSTGQIQSKVFDSELLNLNSTLQATR-NH ₂
Claudin-1	C2C2	ESATHSTGITQSDIYSTLLGLPADIQAAQ-NH ₂
Claudin-3 & -4	C-term 2 nd loop	DFYNP
ZOT	delta-G	12 kD fragment of ZOT protein
ZOT	AT-1002	FCIGRL
Occludin	OP ₉₀₋₁₃₅	DRGYGTSLGGSVGYPYGGSGFGSGYGSYGYGYGYGGYTDPR-NH ₂
Occludin	OP ₉₀₋₁₃₅	DRGYGTSLGGSVG
Occludin	LIP-OP ₉₀₋₁₃₅	H ₂ N-CH(C ₁₂ H ₂₅)-CO-DRGYGTSLGGSVG
Chick Occludin ₈₁₋₁₂₅	OCC1	DYGYGLGGAYGTGLGGFYGSNYYGSGLSYGYGGYGGVNRQT
Chick Occludin ₁₈₄₋₂₂₇	OCC2	GVNPQAMSSGYYSPLLAMC(Acm)SQAYGSTYLNQYIYHYC(Acm)TVDPQE Note: Acm = Acetamido methyl
Rat Occludin ₂₀₉₋₂₃₀	OCC2R	GSQIYTICSQFYTPGGTGLYVD
E-cadherin	HAV24	Ac-DRERIATYTLFSHAVSSNGNAVED-NH ₂
VE-cadherin	VE-Cad	LDRENISEYHLTA <u>VIVDKDT</u> GENLETP (Bold letters are residues homologous with E-cadherin)
E-cadherin	HAV6	Ac-SHAVSS-NH ₂
E-cadherin	ADT6	Ac-ADTPPV-NH ₂