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REVIEW ON THE BLOOD BRAIN BARRIER AND DRUG THERAPEUTICS

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ABSTRACT

The relationship between the BBB permeability and the lipid solubility of anions transported solute follows exactly the relationship predicted by studies with artificial lipid bilayers. Thus, highly polar compounds such as mannitol penetrate the barrier very slowly, whereas lipid-soluble compounds such as butanol penetrate so rapidly that they are completely extracted from the blood by the brain during a single pass through the cerebral circulation. The foregoing discussion emphasizes that the BBB is formed by a cell layer with different properties of the membranes on either side. These cells also have an intracellular cytoplasm and organelles containing specific enzymes that play an important role in BBB function. It is necessary to transport therapeutic agents across the specialized vascular system of the brain, the blood–brain barrier (BBB), which can present formidable challenges It is necessary to transport therapeutic agents across the specialized vascular system of the brain, the blood–brain barrier (BBB), which can present formidable challenges to the drug therapeutics.

Keywords: BBB, Permeability, Brain, Permeation, Nanoparticle

INTRODUCTION

The existence of a permeability barrier between the blood and the brain has been known for more than a century; however, during the past two decades there has been a tremendous expansion in knowledge of the multiple functions of the blood-brain barrier (BBB). Besides forming a permeability barrier that limits the movement of some solutes

between blood and brain, the BBB is now seen as a metabolically active tissue that facilitates and controls the brain uptake of certain solutes while helping to maintain homeostasis within the central nervous system (CNS).

German scientist Paul Ehrlich was the first to describe the existence of a permeability barrier between the blood and the brain [1]. Following the injection of a vital dye such as trypan blue into the bloodstream, he observed that virtually all organs of the body stained blue except for the brain. Ehrlich believed that the dye did not stain the brain because the brain lacked the ability to bind the dye, his associate; Edwin Goldmann later disproved this hypothesis by showing that trypan blue, when injected into the cerebrospinal fluid (CSF), did indeed stain the brain. He also demonstrated the presence of a brain-blood barrier and, in addition, showed that there was no permeability barrier between CSF and brain. Although several investigators later hypothesized that the blood vessels in the brain were the site of the BBB [2, 3] this view was not widely held. Later Reese & Karnovsky and Brightman & Reese [4, 5] repeated the Ehrlich-Goldmann experiments at the ultrastructural level using electron microscopy to observe the distribution of the protein tracer horse radish peroxidase following intravenous and intrathecal administration. These experiments conclusively identified the brain capillary endothelial cell as the site of the BBB as well as the brain-blood barrier.

In this manuscript, the attempts that have been made to use either chemical modifications of drugs or the development of drug-nanoparticulate systems to reach these objectives will be reviewed and their advantages and drawbacks discussed, to define what should be the chemical, biophysical, and biological characteristics of an optimized system.

The formation of a permeability barrier by these cells is the presence of continuous tight junctions that seal together the margins of the endothelial cells. Furthermore, in contrast to endothelial cells in many other organs, brain capillary endothelial cells contain no direct transendothelial passage such as fenestrations or channels. Recent ultrastructural studies suggest that there may be an endocytotic process that is capable of moving protein tracers from the blood to the brain through the lysosomal or Golgi compartments of the brain capillary endothelial cell [6]; however, the flux mediated by this pathway is substantially less than the transvascular permeability observed in most other vascular beds.

REGULATION OF BBB FUNCTION

The relationship between the BBB permeability and the lipid solubility of anions transported solute follows exactly the relationship predicted by studies with artificial lipid bilayers[7,8] Thus, highly polar compounds such as mannitol penetrate the barrier very slowly, whereas lipid-soluble compounds such as butanol penetrate

so rapidly that they are completely extracted from the blood by the brain during a single pass through the cerebral circulation. Uptake of very lipid-soluble compounds is so high that it is only limited by blood flow [7], and these substances are useful both clinically and experimentally for measuring the cerebral blood flow rate.

The availability of isolated brain capillaries as a model system for studying properties of the BBB has led to the discovery that brain capillary endothelial cells contain many neurotransmitter- and hormone-binding sites, like ß-Adrenergic, Adrenergic, Dopamine, Histamine, Adenosine, Muscarinic cholinergic, Prostaglandin, Leukotriene C4, Insulin, Transferrin, Vasoactive intestinal peptide, Parathyroid hormone, Atrial natriuretic peptide, Vasopressin, Angiotensin II, Bradykinin. Beyond the changes in second messengers, however, little is known about the effects of hormones or neurotransmitters on endothelial cell function.

The brain uses glucose for its metabolism, the BBB is richly endowed with glucose transporters [9-11] The BBB glucose carrier recognizes D-glucose, D-galactose, D-mannose, and synthetic hexoses such as 2-deoxy-D-glucose and 3-O-methyl-D-glucose. Transport is half saturated at a plasmaglucose concentration of 5 to 10 mM, which is within the normal physiologic range. BBB glucose transport is not energy dependent; therefore, it cannot move glucose against a concentration gradient. It is also symmetrical and transports glucose out of as well as into the brain. Thus, it is a facilitative-diffusion type of transporter that facilitates the equilibration of blood and brain glucose concentrations. In contrast to glucose transport in certain other cells, BBB glucose transport is not regulated by insulin. Recent studies of the quantity and distribution of glucose transporters in brain indicate a very rich endowment in brain capillary endothelial cells, consistent with their role in providing glucose for all the cells in the brain [12, 13]. Another prominent transport system in the BBB enhances the brain uptake of large neutral amino acids, which are important precursors for neurotransmitter and protein synthesis. This carrier is similar [14] to the L-system for amino acid transport described in other cells in that it has high affinity for large but not small neutral amino acids and is not sodium dependent [14]. In contrast to the glucose transporter for which glucose is the only naturally occurring substrate present in the blood at a significant concentration, the L-system amino acid carrier has affinity for at least 10 different amino acids that are present in the blood [15]. Indeed, the carrier seems to be quite insensitive to the size of the hydrophobic side chain. The presence in blood of multiple substrates for the same carrier means that they compete with each other for transport into brain. Consequently, when the plasma concentration of one amino acid is increased, it can reduce the uptake of other amino acids, which may play a role in the neurologic damage that occurs in certain

metabolic diseases such as Phenylketonuria [16].

THE BBB AS A METABOLIC BARRIER

The foregoing discussion emphasizes that the BBB is formed by a cell layer with different properties of the membranes on either side. These cells also have an intracellular cytoplasm and organelles containing specific enzymes that play an important role in BBB function. One of the first demonstrations of the enzymatic BBB was made by Bertler and colleagues, 1966 [17] who observed intraendothelial histofluorescence of dopamine after intravascular administration of its precursor, Levodopa during Parkinsonism. In later studies, they found that rat brain capillary endothelial cells were richly endowed in enzymes involved with neurotransmitter synthesis (e.g., decarboxylase) and degradation (e.g., monoamineoxidase [MAO]) [18]. Since neurotransmitter precursors such as Levodopa can enter the endothelial cell from the blood via the L-system for large neutral amino acid transport, the subsequent intraendothelial metabolism provides an effective mechanism for preventing neurotransmitters from moving beyond the endothelial cell and into the brain. Neurotransmitter degrading enzymes present in the endothelial cells also may play a role in inactivating neurotransmitters released during neuronal activity since, transport systems for uptake of catecholamines by the endothelial cell appear to be present on the abluminal membrane. Besides the enzymes being involved in energy metabolism [19], these cells also contain acid hydrolyses typically found in lysosomes [20] and aminopeptidase [21]. Similar to other endothelial cells, they contain Angiotensin-converting enzyme [22] xanthine oxidase [9], and enzymes that protect cells from peroxidative damage such as superoxide dismutase, catalase, and glutathione peroxidase [23]. MAO present in brain capillaries may serve to provide protection of the brain from circulating. Indeed, brain capillary endothelial cells are well equipped to handle circulating drugs since they contain drug-metabolizing enzyme systems such as cytochrome P-450-linked monooxygenases, epoxide hydrolase, NADPH:cytochrome P-450 reductase, and 1-napthol UDP-glucuronosyl transferase, which are typically found in the liver [24]; they also contain a multidrug transport protein, P170[25]

It is necessary to transport therapeutic agents across the specialized vascular system of the brain, the blood–brain barrier (BBB), which can present formidable challenges. These include the definition of the properties of the cerebral vascular system during cancer progression and the development of biotechnology to prepare biomarker-targeted delivery of multiple therapeutic agents, coupled to the possibility of avoiding various resistance mechanisms. A great deal of effort, therefore, is presently focused on improving CNS bioavailability, and tumors thereof, of therapeutic drugs that can be specifically

targeted to diseased tissue, improving therapeutic opportunities, efficiency, and patient survival, while decreasing side-effects to normal cells.

The blood–brain barrier (BBB), brain cancers, and therapeutic options and problems The BBB is a system of vascular cellular structures, mainly represented by tight junctions between endothelial cells, and an ensemble of enzymes, receptors, transporters, and efflux pumps of the multidrug resistance (MDR) pathways [26-28] that control and limit the access of molecules to the brain, either by paracellular or transcellular pathways. Since the vascular density in the brain is very high, once molecules have penetrated the BBB, they distribute rapidly to the whole brain tissue. Whereas a few lipid-soluble molecules are able to pass freely by passive diffusion from the blood to the interstitium of the brain, ionic solutes are unable so to do. Chemical modifications of drugs that enhance lipophilicity result in an increased distribution of the drug in all organs. The design of carriers that cross the BBB at sites defined by the properties of the vasculature at that specific localization, using biology-based strategies to target specific transport systems at the BBB must, therefore, be designed. These carriers may consist of drugs or polymeric nanocarriers chemically modified with recognition and transcytosis-enhancing ligands that can allow the release of the active free drug transendothelial transport has been performed. One particular challenge is related to finding those localized changes in the properties and biological change signatures characteristic of a diseased BBB. Functionalized Drugs must structurally resemble the normal transporter substrates, making them recognizable by the transporter; however, maintaining the biological activity of the drug is another challenge.

Drugs targeting brain cancers across the blood–brain barrier: chemical functionalization of therapeutic agents or of nanoparticulate carriers? As previously stated, the limiting factor in the treatment of brain cancers is the delivery of therapeutic agents to the brain across the BBB. A very restricted number of liposoluble small molecules (MW < 400 Da) cross the BBB by free diffusion. All the other molecules must use specific systems to be transported across the BBB [29]. Therefore, the future for treatment of malignant brain cancers relies on the development of therapies targeting the markers and transporters of the tumor-associated cerebral endothelium, not only at the primary tumor sites but also at the invasive areas. Biological targeting involves that a specific marker (a target) is selectively expressed, or is expressed on disease-associated cells at a much higher level than on normal cells. The targeting agents may be antibodies, directed toward an antigen residing on the target tissue, or ligands for receptors or transporters, and may be covalently conjugated via an appropriate chemical bond either directly to the drug or to a vector, such as a

nanoparticulate device. Most of the targets identified and evaluated until now for brain cancer have been related to molecules associated with enhanced angiogenesis or increased nutrient demand of the tumors. Some drugs have been conjugated to ligands or antibodies, or have been incorporated into carriers bearing ligands or antibodies for recognition by cell surface receptors expressed by target cells. Major obstacles include the physiological stability of these structures.

Major obstacles include the physiological stability of these structures and their transport across biological barriers, in particular the blood–brain barrier, for the delivery of therapeutic drugs. In addition, for maximal efficacy, drugs must reach their targets in the appropriate location within tumor cells, that is, the cytosol, cell organelles, or the nucleus. Drug release from the carrier must also be achieved. Therefore, the combination of a drug or drug vector with a molecule recognized by a lumenal blood-to-brain carrier system is mandatory, of which glucose, amino acids, mono- carboxylic acid, oligonucleotides, cationic peptides, or transferrin conjugates represent potential transport systems to the brain [30-32]

Direct conjugation of drugs Most of the approaches attempted has evaluated drug conjugation to a blood-to-brain transporter. To be successful, this approach requires the drug to mimic the endogenous ligand, since most transporters, such as the glucose transporter, are highly selective. The expression of enzymatic activities at the BBB is also important and these activities may be modified in disease. For example, we have shown that in brain cancers, aminopeptidase-A activity is increased, whereas aminopeptidase N activity is decreased [33]. These differences are important factors for delivering intact molecules to the brain and the use of enzymatically stable (pro) drugs may be necessary, using chemical modifications such as cyclization, halogenation, methylation, pegylation or the introduction of un-natural bonds. Approaches previously attempted fell into two categories, namely carrier-mediated and receptor-mediated transport. The former used carrier-mediated transport of small molecules that are brain nutrients. As stated above, the transporters expressed at the luminal and abluminal surfaces of the BBB are structure- specific and rarely transport drug analogs, either drug modified to resemble their normal substrate or drugs chemically bound to their ligands. Therefore, simply coupling drugs to their ligand may not result in their transport and only very few attempts have been successful. Rather, drugs should be modified to mimic the normal ligands, while maintaining bioactivity, which is not an obvious approach for most drugs. For example, the anti-cancer agent melphalan resembles the amino acid phenylalanine and can be transported by the LAT1 carrier [34]. Very recently, the small hydrophilic drug ketoprofen, an anti-inflammatory agent that is not a substrate for

LAT1, was chemically bound via an ester linkage to the phenolic hydroxyl group of the amino acid tyrosine, a LAT1 substrate, and was recognized by the LAT1 transporter [35], opening new possibilities for small anti-cancer drugs. Melphalan has also been conjugated to L-glutamate, with some success [36]. However, for transport by amino acid transporters, the a-amino and a-carboxyl groups of the amino acid must be free, limiting chemical possibilities. The glucose transporter GLUT1 was shown

CONCLUSIONS

A few strategies exist to enhance transport of anti-cancer agents across the BBB for the treatment of high-grade brain tumors: (i) passive permeation of lapidated drugs, however, this strategy is possible only for small molecules; (ii) the development of pro-multiple agents at the same site. Creating a toolbox of molecules that can be assembled hierarchically into ordered structures, spatially and chemically controlled, is, however, essential to make nanoparticles an attractive and efficient means of encapsulating and delivering drugs to the CNS tumors. Some of the chemistry and chemical routes that can be used to achieve biomimetic assemblies comprising the polymer, a linker and a bioactive molecule, have been reviewed in reference [37]. Emphasis must be put on multi-functionality of delivery vectors: these delivery vectors must include positive charges (cations) for enhanced vascular uptake, vascular-targeting, and transcytosis-enhancing agent, and drug(s). Thus, an ideal theoretical therapeutics-delivery nanoparticle system for brain cancer would be one that: (i) selectively targets diseased BBB; (ii) bears an inhibitor of the efflux pump linked by a locally hydrolysable bond, and (iii) transports drugs across the cerebral vasculature and delivers them to their target, that is, the brain cancer cells. Only nanoparticulate systems can offer this diversity, however, the biophysical, biochemical, and biological mechanisms associated with the interaction of nanoparticles with living tissue, will need to be better understood to allow widespread adoption of this technology. Challenges yet to over- come include identification of disease-associated changes of the BBB properties in brain cancers and the modification of drugs or drug- carriers with targeting and transport-enhancing agents. Antibodies have the potential to be selective; however, their size and potential immunogenic properties are a limitation to their diffusion into tissue and use. Protein ligands, such as transferrin and its receptor, which have been widely evaluated for targeting, suffer from similar problems. There will be, in the near future, plenty of possibilities to be explored; however, in my opinion, the most promising vectors are those involving small molecules as BBB targeting or transport enhancing agents, stable in biological media and versatile for synthesis purposes, and able to carry a large drug payload. It is unrealistic, however, to imagine that a general and unique targeting

vector can be designed for all situations and purpose

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