Blood-Brain Barrier and Effectiveness of Therapy Against Brain Tumors

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1. Introduction

The challenge to comprehend the physiology as well as cell biology of the blood-brain barrier (BBB) began with Ehrlich and Goldman’s experimental observations that the central nervous system (CNS) is not stained by intravascular vital dyes. These studies provided the first evidence of the presence of an obstructing barrier between blood and brain. Later on, researchers like Friedemann (1942) used basic highly lipid soluble dyes to cross the BBB in order to show the brain penetration of the dyes by direct transport across the cerebral microvasculature. In 1941, Broman presented his observations upon the existence of two different barrier systems within the brain: the BBB at the cerebral microvasculature, and the blood-CSF barrier at the choroid plexus. It is now clear that in fact three main barrier layers at the interface between blood and tissue protect the CNS: the endothelium of brain capillaries, and the epithelia of the choroid plexus (CP) and the arachnoid (Abbott, 2005; Engelhardt, 2006).

In 1941, Broman proposed that it was the cerebral capillary endothelial cells that contribute the physical barrier function of the BBB, and not the astrocytic end feet. The argument concerning whether the astrocytes or the capillary endothelium constitute the BBB was supported by electron microscopic cytochemical studies performed in 1967 by Reese and Karnovsky. They used horseradish peroxidase (HRP), ~40 kDa, to visualize the BBB by systemic injections of HRP which failed to reach the brain extracellular fluid, whereas intracerebroventricular injection into the CSF stained the brain extracellular fluid positive for HRP (Reese & Karnovsky, 1967).

It is now evident that the BBB is a unique membranous barrier, which restrictively isolates the brain parenchyma from the circulating molecules/compounds within the blood. The permeability of BBB is regulated by transport machineries of the brain capillary endothelial cells that are modulated by autocrine and paracrine secretions from several types of cells, such as the pericyte, the astrocyte, and neurons (Rubin & Staddon, 1999).

The pericyte cells share the capillary basement membrane with the endothelium and physically supports endothelial cells (Allt & Lawrenson, 2001). It has been revealed that there is approximately one pericyte for every three endothelial cells (Pardridge, 1999). It is deemed that the pericyte cells play a regulatory role in brain angiogenesis, endothelial cell...
tight junction formation, BBB differentiation, and also contribute to the microvascular vasodynamic capacity and structural stability.

The astrocyte cells invest approximately 99% of the abluminal surface of the brain capillary and induce endothelial cells to differentiate directly through cell to cell communication or indirectly by secreting astrocytic factors (Pardridge, 1999). Brain capillary endothelial cells display different features in comparison with peripheral endothelial cells. The BBB can be thought of brain capillary endothelial cells (BCECs) with the physical and paracrine interactions between the endothelial cells (ECs), the pericyte, and the astrocyte (Abbott et al., 2006; Pardridge, 1999).

The ability of BCECs to form a restrictive barrier between blood and brain is not completely intrinsic to the brain microvascular endothelial cells, but instead is induced by the brain environment itself. Induction of BBB may be categorized as "directive" and "impressive" events. The latter term means that the inductors functions upon a tissue that is already determined toward its final fate but still needs an exogenous stimulus for the expression of its full phenotype. Of note, with the lack of a brain neuronal environment, the selective restrictiveness characteristics of the BCECs disappear, and as a result appropriate incitement(s) ought to be continuous at the BBB microenvironment to maintain its functionalities (Abbott, 2005).

The astrocyte inductive effects upon endothelial cell differentiation have been examined by Stewart & Wiley (1981). They transplanted avascular tissue from 3-day-old quail brain into the coelomic cavity of chick embryos, the chick endothelial cells then vascularized the quail brain grafts formed a competent BBB. In contrast preformed microvessels growing in embryonic quail muscle, which were implanted in chick brain were leaky and lacked BBB enzymes (Stewart & Wiley, 1981).

With regard to the complexity of the BBB, basically, other differentiating factors apart from astrocytes may play a role on the formation of the BBB. However, we discuss the most important features of BBB in relation to drug delivery and targeting for brain tumors. Fig. 1 represents the schematic illustration of BCECs.

2. BBB junctional complexes and cell-to-cell interactions

Stable cell-to-cell interactions are required to keep the structural integrity of tissues. Dynamic changes in cell-to-cell adhesion will participate in the morphogenesis of developing tissues. Adhesion mechanisms are highly regulated during tissue morphogenesis and related to the processes of cell motility and cell migration. Cell junctions, basically, can be classified into three functional groups, including: 1) tight junctions (TJs), 2) anchoring (adherent) junctions (AJs), and 3) gap (communication) junctions (GJs). Of these junctions, the TJs seal cells together in cell sheet, the AJs attach cells to their neighbors or to the extra-cellular matrix mechanically, and the GJs mediate the passage of chemicals or electrical signals from one interacting cell to its partner (Engelhardt, 2006; Omidi & Gumbleton, 2005). Because of crucial role of TJs in restrictive function of BBB, they are briefly discussed.

Fig. 2 shows the diagrammatic representation of TJs and its complexity with other proteins at the BBB. TJs of the BBB generate a rate-limiting restrictive barrier to paracellular diffusion of solutes between endothelial cells. They are the most apical element of the junctional
complex that includes both tight and adherens junctions. In terms of morphology, TJs form a continuous network of parallel, interconnected, intra-membrane strand of various proteins arranged as a series of multiple barriers.

Fig. 1. Schematic demonstration of part of cortex and brain capillary endothelial cells. Star shape astrocytes are in communication with both brain capillaries and neurons via end-foot. The ultrastructural characteristics (in particular presence of tight junction) of the brain capillary endothelial cells differ them from other peripheral capillaries. Some of important specialized transporters are illustrated on luminal section which represents vesicular trafficking machineries for traverse necessary macromolecules from blood to brain, adapted with permission (Omidi & Barar, 2012).

2.1 Tight junctions of BBB

Using freeze-fracture technique, it has been shown that the tight junctions of the BBB are characterized by the highest complexity found in the vasculature, so that protoplasmic fracture face (P-face) association of the BBB compare to the peripheral endothelial cells is 55% and 10%, respectively. The altered particles distribution implies the presence of a strong interaction between TJs of the BBB and cytoskeleton. TJs of the BBB, morphologically, are more comparable to TJs of the epithelial cells than to TJs of the endothelial cells in
peripheral blood vessels. Although the tight junction of BBB discloses many characteristics of epithelial TJ$s, there are distinctive differences between them, e.g. in terms of particle density and distribution, in relation to response to ambient factors.

Fig. 2. Illustration of the brain capillary endothelial cells (BCECs) morphology and architecture. A) Schematic elongated two brain capillary endothelial cells with some apical transporters. B) Vesicular membrane of BCECs showing coated and uncoated vesicles. C) Transmission electron microscopy micrograph of BCECs showing tight junction (TJ). D) Tight junctional interactions of two endothelial cells. The TJ is embedded in a cholesterol-enriched region of the plasma membrane. Claudins comprise a multigene family with 20 isoforms currently identified and form the backbone of TJ strands by making dimers and binding homotypically to claudins on adjacent cells to produce the primary seal of the TJ.
Claudin 1, 3 and 5 are present at the BBB. Occludin functions as a dynamic regulatory protein causing increased electrical resistance across the membrane and decreased paracellular permeability. BBB endothelial cells in vivo reveal a P-face/E-face ratio of about 55%/45%, and as claudin-3 and claudin-5 are well expressed, it can be suggested that the degree of association with one or the other leaflet roughly reflects the stoichiometry of claudin expression in the TJs of BBB. However, in the non-BBB endothelial cells, tight junctions are almost completely associated with the E-face and claudin-3 is rarely or not expressed, adapted with permission (Omidi & Barar, 2012).

Many identified ubiquitous molecular components of junctional complexes in the epithelia such as claudins, occludins, zonula (ZO-1, ZO-2, ZO-3), junctional adhesion molecules (JAMs), cingulin, and 7H6 have been detected at the BBB. Both tight and adherent junctions are composed of multiple protein complexes, which communicate with the actin cytoskeleton of the cells (Kniesel & Wolburg, 2000).

The TJ contains various proteins, which are necessary to form structural support for the tight junction such as zonula occludens proteins that belong to a family of membrane associated guanylate kinase-like proteins and serve as recognition proteins for tight junctional placement and as a support structure for signal transduction proteins. Junctional adherent molecules (JAMs) are localized at the TJ and are members of the immunoglobulin superfamily, which can function in association with platelet-endothelial cellular adhesion molecule 1 (PECAM1) to regulate leukocyte migration. Cingulin is a double-stranded myosin-like protein that binds preferentially to ZO proteins at the globular head and to other cingulin molecules at the globular tail. The primary cytoskeletal protein, actin, has known binding sites on all of the ZO proteins, and on claudin and occludin.

In terms of TJ regulation, phosphorylation of both transmembrane and accessory proteins plays an important role in establishing and regulating the TJs. The Occludin and ZO1, as the primary regulatory proteins of the TJs, are phosphorylated on serine, threonine and tyrosine residues. The increased phosphorylation of serine/threonine correlates with decreased extractability of occludin and TJs assembly. Regulation of TJs is also dependent on tyrosine phosphorylation of proteins at cell to cell contacts. Development of TJ barrier functions has been correlated with decreased tyrosine phosphorylation of proteins at the TJ complexes. Protein kinase C (PKC) also is a major regulator of TJs formation and regulation. It plays an important role in ZO1 migration to the plasma membrane and there are PKC phosphorylation consensus sequences in the ZO1 protein, suggesting that ZO proteins serve as scaffolding for PKC signal transduction pathways on the cytoplasmic surface of intercellular junctions. It has also been shown that TJs are generally localized at cholesterol-enriched regions or rafts within the plasma membrane. Caveolin-1, an integral protein within caveolae membrane domains may associate with TJ components. Caveolin-1 itself interacts with and regulates the activity of several signal transduction pathways and downstream targets. Several cytoplasmic signaling molecules are concentrated at TJ complexes and are involved in signaling cascades that control assembly and disassembly of TJs (Krizbai & Deli, 2003).

The structure and function of TJs in primary cultures of bovine brain endothelial cells were directly analyzed, using quantitative freeze-fracture electron microscopy, and ion and inulin permeability (Wolburg et al., 1994), and it was shown that the cultured brain endothelial
cells tend to lose the TJ-dependent BBB characteristics such as macromolecular impermeability and high electrical resistance with conditioned culture. The tight junction complexity index (CI), as the number of branch points per unit length of tight junctional strands, was decreased 5 hr after culturing the primary bovine brain microvessel endothelial cells. However, the association of TJ particles with the cytoplasmic leaflet of the endothelial membrane bilayer (P-face) decreased steadily during culture with a major drop between 16 hr and 24 hr. Wolburg et al. showed that the CI could be increased by elevation of intracellular cyclic adenosine monophosphate (cAMP) levels, while phorbol esters had the opposite effect - the endothelial cells P-face association of TJ particles was enhanced by elevation of cAMP levels and by astrocyte coculture (ACC) or exposure to astrocyte conditioned-medium (ACM). The authors also highlighted that astrocytes induced the latter effect on P-face association; and also showed that elevation of cAMP levels together with ACM increased trans-endothelial/epithelial electrical resistance (TEER) synergistically and decreased inulin permeability of primary cultures. They, thus, concluded that P-face association of TJ particles in brain ECs may be a critical feature of the blood-brain barrier functionality that can be specifically modulated by astrocytes and cAMP levels.

2.2 Astrocytes

Astrocytes are glial cells that envelop >99% of the BBB endothelium (Hawkins & Davis, 2005). Astrocytes and endothelial cells reciprocally affect each other’s structure and functions - their interactions induce and modulate the development of the BBB. Such interaction enhances the TJs and reduces the gap junctional area of BCECs, while increases the number of astrocytic membrane particle assemblies and astrocyte density. Astrocytes are essential for proper neuronal activities, for which the close proximity of astrocytes and BCECs appear to be essential for a functional neurovascular unit (Abbott et al., 2006). Based on in vitro investigations, the coculture of BCECs with astrocytes can improve the BBB functionality such as permeability and cellular transport functions (Omidi et al., 2008).

The nature of the astrocyte-derived factors (ADFs) is not fully understood, inductive effect of ADFs on brain microvascular endothelial cell differentiation and BBB formation has been well reported. Investigation upon the modulatory effects of astrocyte on BCECs have revealed that the rat astrocyte cells are able to modulate the chick peripheral ECs to make them less permeable to large molecules. On the molecular level, increased expression of barrier-relevant proteins (e.g., tight junction proteins) has so far been documented in the presence of ADFs. Many studies have shown the improvement of physiological parameters (e.g., increased TEER and decreased paracellular permeability) in different in vitro models of the BBB treated with ADFs. Moreover, it should be evoked that the interaction of BCECs and astrocytes is bidirectional and that the other cell types surrounding the brain microvasculature also contribute to BBB function or dysfunction. From many kinds of different experimental designs, it is quite clear that astrocyte factors are able to modulate TJ restrictiveness under perhaps certain defined conditions. To test the astrocytic factors effect on endothelial cells, Shivers et al. (1988) aimed to find out whether these factors can initiate development of non-CNS microvessel endothelial cells by culturing and passaging bovine aorta and pulmonary artery endothelial cells in the presence of 50% ACM. The investigators reported the endothelial cells maintained in ACM displayed complex tight junctions as well as gap junctions, but the cells plated onto plastic or fibronectin-coated substrates without
ACM showed no tight or gap junctions. This finding directed them to conclude that CNS astrocytes generate soluble factor(s), which can provoke formation of tight junction components in non-CNS endothelial cells. Astrocyte inductive effect(s) on the expression of the BBB characteristic enzymes including γ-glutamyl transpeptidase (γ-GTP) and alkaline phosphatase have been well presented (Allt & Lawrenson, 2001; Haseloff et al., 2005). Using an astrocyte coculture system with endothelial cells Rauh et al (1992) showed that a possible direct cell-to-cell contact between BCECs and astroglial cells is probably needed for astrocyte inductive effects. However this is a controversial issue as secreted ADF effects on endothelial cells are well recognized. Shivers et al (1988) and Tio et al. (1990) also reported that both ACC and ACM (isolated rat brain astrocyte cells) can induce the alkaline phosphatase activity and tight junction generation in the human umbilical cord vein endothelial cells (Shivers et al., 1988; Tio et al., 1990).

In 1999, Igarashi et al. examined the relationship of glial-derived neurotrophic factor (GDNF), which maintains the dopaminergic system and motor neurons in vivo, with BBB using isolated primary porcine brain capillary endothelial cells (PBCECs) and reported that GDNF at concentrations of 0.1 and 1 ng per ml can significantly enhance the barrier functionality (tight junction integrity) with increases in TEER values and decreases in mannitol permeability (Igarashi et al. 1999). It can be deduced that GDNF is able to seal tightly the paracellular pathway in addition to its homeostasis role on the CNS. Moreover, it appears that factors secreted by brain endothelial cells including leukaemia inhibitory factor (LIF) can induce astrocyte differentiation. ADFs also influence the functionality of BBB carrier-mediated transport systems (Abbott, 2005). The use of BBB transports will be discussed later in this chapter.

2.3 Pericytes

Pericytes are an imperative cellular constituent of the BBB, which also play a regulatory role in terms of brain angiogenesis and tight junction formation within BCECs. These cells also contribute to the microvascular vasodynamic capacity and structural stability. They are actively involved in the neuroimmune network operating at the BBB and confer macrophage functions. The pericyte and endothelial cell interaction occurs via cytoplasmic processes of the pericyte indenting the EC and vice versa. This contact process is called "peg and socket"—an interdigitation process (Wakui et al., 1989). Larson et al. (1987) studied intercellular relationship in the microvasculature using fluorescent Lucifer yellow CH dye/radiolabeled nucleotide and freeze-fracture methodology, and showed that cultured pericytes presented gap junctions in freeze-fracture replicas and extensive nucleotide and dye transfer. Observing low dye transfer and high nucleotide transfer between bovine brain microvessel endothelial cells and pericytes, they proposed a possible junctional contact between these cells that promotes their differentiation (Larson et al., 1987).

Basically, some biomolecules including adhesive glycoprotein and fibronectin have been found localized at the BCECs and pericytes junctional sites adjacent to "adhesion plaques" at the plasma membrane. The adhesion plaques implied the existence of a mechanical linkage between pericytes and ECs, i.e. a linkage that allowed mechanical contraction or relaxation of the pericyte to influence vessel diameter. This later process may assist the endothelial cells to reduce their size. Pericytes have specific and localized distribution in different tissues displaying a granular morphology reflecting lysosomal enrichment. Using an in vivo...
rat model, it has been reported that blood-brain barrier disruption, e.g. by hyperosmotic mannitol with adriamycin, causes an increase of pericyte lysosomes (Kondo et al., 1987). Pericytes are rich in plasmalemmal and cytoplasmic vesicles as well as microfilament bundles, but interestingly only about 10% of vesicles locate to the surface facing ECs. Most importantly pericytes modulate endothelial cells phenotype not only by physical action but also via secreting epidermal growth factor (EGF). Indeed, it has already been shown that EGF is an effective endothelial cell mitogen that enhances angiogenesis, and is concentrated at and during pericytes-ECs cross-talk and interdigitation processes. The expression of γ-GTP has been shown in the pericyte as well as the brain microvessel endothelial cells. Using γ-GTP as the sole marker for the primary isolated brain capillary endothelial cells seems not to be right and proper due to possible pericytic contamination. The transforming growth factor-beta (TGF-b), produced by pericytes, plays an important role in reducing lymphocyte infiltration into the CNS in inflammatory demyelinating diseases. Pericytes can stabilize capillary-like structures formed by endothelial cells in coculture with astrocytes. This latter process can be driven by TGF-b1, which is one of the TGF-b isoforms (Allt & Lawrenson, 2001; Ramsauer et al., 2002). The cultured pericytes in the endothelial cell conditioned-medium (ECCM) allowed the cerebral pericyte aminopeptidase N (pAPN) to be re-expressed, while purified pericytes deprived of endothelial cells even in the presence of ACM showed no reexpression. This indicates that endothelial cells constitute an essential requirement for the in vitro re-expression of pAPN, but not astrocytes. Pericytes are involved in amino acid and peptide catabolism of the brain. This suggests that pericytes play a key metabolic role aside from structural role in relation to the BBB maintenance and homeostasis. In addition, based on the reciprocal pericytes-ECs interactions and cross talk, it can be suggested that the two-way interdigitations may stimulate not only the BBB endothelial cell activities but also promote pericyte functions. Pericytes may have a role in the possible cross-talk between pericytes, astrocyte and endothelial cells, a triple intercommunication process that may bring us to consider the notion of pericyte positive contribution in the physiopathology of the BBB as well as certain diseases such as Alzheimer. For example, β-amyloid peptide 42 can be uptaken by the phagocytic pericytes and astrocytes. This process helps to clear the exogenous peptide from the brain extracellular space and deliver it to the blood circulation system that shows also the contribution of pericytes in the BBB transport functionality (Allt & Lawrenson, 2001; Balabanov & Dore-Duffy, 1998; Pluta et al., 2000).

3. Bioelectrical properties and permeability

Due to the association of the TEER with permeability, TEER values commonly are exploited to describe the permeability of the BBB. The $^{14}$C sucrose permeability is used to assess the restrictiveness of BBB in relation to in vivo $(1.2 \times 10^{-7} \text{cm.sec}^{-1})$. In vivo TEER values vary in the epithelial and endothelial cells. For instance human placental endothelium shows TEER 22–52 $\Omega$.cm$^2$ that permits rapid paracellular exchange of nutrients and waste between the mother and fetus (Jinga et al. 2000), whereas urinary bladder epithelium has a very high TEER of 6000–30000 $\Omega$.cm$^2$, necessary for preserving urine composition (Powell 1981). The BBB possesses TEER vales of ~2000 $\Omega$.cm$^2$, which helps to maintain brain homeostasis (Engelhardt, 2006).
Efforts to generate an *in vitro* BBB model, in fact, have been based upon measurement of the TEER, assessment of the sucrose permeability and expression of the specific enzymes and markers of the BBB. The higher TEER and the lower sucrose permeability confer the better characteristics. To achieve this aim different techniques have been recruited, e.g. utilizing of the hydrocortisone and serum free medium in order to increase the TEER (up to 1000 Ω.cm²) by stimulating the formation of barrier properties (Hoheisel *et al.* 1998).

### 4. Modulation of BBB permeability

#### 4.1 Extracellular matrix

The influence of extracellular matrix on the BBB properties has been investigated by several researchers using cell lines and primary isolated BCECs. Shivers *et al.* (1988) showed that the local control of tight junction biogenesis in brain capillary endothelial cells depends on astrocyte-produced factors and extracellular matrix. The ECs in general do not express their final destination-specific differentiated features until those features are induced by local environment-produced conditions including extracellular matrix. Using primary cultures of PBCECs, Tilling *et al.* (1998) examined the effect of collagen IV, fibronectin, laminin and a secreted acidic protein and rich in cysteine alone or one-to-one mixtures of them. They showed that these proteins are involved in tight junction formation between cerebral capillary endothelial cells by presenting increased TEER (Robert & Robert, 1998; Tilling *et al.*, 1998).

#### 4.2 The role of cyclic AMP (cAMP)

The effect of cAMP on BBB function has been studied by several researchers. Using combination of astrocyte conditioned-medium and cAMP elevators, Rubin *et al.* (1991) reported a cell culture *in vitro* BBB model that generated high resistance tight junctions and exhibited low rates of paracellular permeability. Hurst *et al.* (1996) showed that a coculture BBB model of the immortalized human umbilical vein endothelial cells ECV304 (reassigned later as T24 bladder epithelial carcinoma cell) with rat C6 glioma cells can generate a BBB model with high TEER (~400-600 Ω.cm²). They demonstrated bioelectrical alterations by vasoactive agonists and cAMP elevators (i.e. decreased TEER by histamine, bradykinin, and serotonin and increased TEER by cAMP, such as forskolin elevators). The researchers also showed formation of inositol triphosphates (IPs) that can induce the release of calcium ions from cellular storage sites and a subsequent rise in intracellular calcium which can activate diacylglycerol (DAG) and accordingly the PKC that could increase the permeability of the endothelial cells (Hurst & Clark, 1998). Investigation on the effects of elevated intracellular cAMP and astrocyte derived factors on the F-actin cytoskeleton and paracellular permeability of RBE4 cell monolayers have revealed that the cAMP effects on the TEER appear likely to be independent of new gene transcription (Rist *et al.*, 1997).

The protein GDNF can activate the barrier functions of the BCECs in the presence of cAMP. It has been reported that GDNF not only can promote the barrier restrictiveness but also support the survival of neurons in the presence of cAMP. The role of other factors on brain ECs signaling and the BBB formation is uncertain (Igarashi *et al.*, 1999). However, some other factors such as vascular endothelial growth factor (VEGF) appear to increase the permeability of BCECs because of loss of occludin and ZO-1 from the endothelial cell
junctions (Wang et al., 2001), while cAMP acts against such phenomenon. All of these processes, somehow, play a role in modulating the full BBB characteristics. By means of an unpassaged primary culture of rat BCECs, it has been shown that certain cell-surface receptors may fulfill a role in BBB regulation. For example, brain endothelial regulation was shown by P2Y2 receptors coupled to phospholipase C, Ca\(^{2+}\) and MAPK; and by P2Y1-like (2MeSATP-sensitive) receptors that are linked to Ca\(^{2+}\) mobilization. It should be stated that differential MAPK coupling of these receptors appear to exert fundamentally distinct influences over brain endothelial function in terms of cAMP modulation (Albert et al., 1997).

4.3 Inflammatory mediators

Basically, the brain endothelium forming the BBB can be modulated by a range of inflammatory mediators. Given that the main routes for penetration of polar solutes across the BBB include the paracellular tight junctional pathway and vesicular machineries, inflammatory mediators appear to influence both pathways, while such impacts can also be seen in other closely associated cells such as pericytes, astrocytes, smooth muscle, microglia, mast cells, and neurons. Various inflammatory agents are able to increase both endothelial permeability and vessel diameter, and these together can result in significant leakiness of BBB and accordingly cerebral edema. Of these agents, the bradykinin (Bk) is able to increase the permeability of BBB by acting on B2 receptors, perhaps via elevation of Ca\(^{2+}\), activation of phospholipase A2, release of arachidonic acid, and production of free radicals. Serotonin (5HT) can also increase the permeability of BBB through a calcium-dependent mechanism. Histamine as nervous system neurotransmitters possesses capability of consistent blood-brain barrier opening mediated by H2 receptors and elevation of Ca\(^{2+}\), but the H1 receptor coupled to an elevation of cAMP can decrease the permeability of BBB. Elevation of arachidonic acid may also cause gross opening of the BBB to large molecules such as peptides/proteins. There exist a number of studies showing purposed recruitment of such mechanisms for deliberate opening of the BBB for drug delivery to the brain; readers are directed to see (Abbott, 2000).

5. BBB enzymes and other differentiation markers

Several markers are most commonly exploited and include γ-GTP and alkalin phosphatase (ALP) enzymes expression, or antigenic endothelial cell markers such as: Factor VIII, von Willebrand Factor (vWF). Additionally the uptake of acetylated low density lipoprotein (LDL) i.e. dioctadecyl-indocarbocyanine (Di-I) labeled acetylated LDL (Di-I-Ac-LDL) and the binding of lectin, i.e. biotinylated lectins (BL) Ultex europaeus I (UE I) and Bandeiraea simplicifolia isolation B4 (BSA IB4) have been exploited. Further, a high glucose transporter (GLUT-1) density has been shown as a marker of brain microvascular endothelia. The γ-GTP, ALP and membrane peptidases (aminopeptidase N, peptidyl dipeptidase and dipeptidyl peptidase IV) have been used as marker for the BBB. The γ-GTP is of special importance due to its high expression in the BCECs, therefore it is used as a marker for ECs of the BBB. By contrast, this marker is absent from brain ECs of paraventricular nucleus, where the BBB characteristics and properties are lacking. The induction of γ-GTP by astrocyte has been reported, even though the high expression of this enzyme in the pericytes makes it a non-specific marker of the BBB. The ALP is highly expressed in the BCECs and used as a marker. Like γ-GTP, its expression can be modulated by astrocytes. The
metabolism of neuropeptides is mediated by membrane peptidases at the BBB. Three members of the membrane peptidases (dipeptidyl peptidase A; aminopeptidase N; dipeptidyl peptidase IV) have been found in the BCECs, but many more vessels are positive for them, in particular more positive for dipeptidyl peptidase A than other two. The expression of the BBB enzymes depends on the different parameters such as direct cell-to-cell communications or cell-derived factors. Enzymatic activities of the γ-GTP and ALP are taken as indicators for the expression of the BBB phenotype; reader is directed to see (Allt & Lawrenson, 2000; Allt & Lawrenson, 2001; Orte et al., 1999).

6. Transportation of exogenous/endogenous compounds across BBB

The capability of a particular substance to cross the BBB and enter the CNS is dependent upon a number of parameters, including physicochemical properties such as molecular weight (MW), lipophilicity, pKa, hydrogen bonding as well as biological factors. The BBB transportation, nevertheless, may generally be classified into different categories, including: 1) passive diffusion that depends on physicochemical properties and mainly on the lipophilicity of the molecule, 2) facilitated transport via carrier-mediated transporters (Glut1, LAT1), 3) paracellular pathway (small hydrophilic compounds), 4) receptor-mediated endocytosis/transcytosis, and 4) Liquid-phase (adsorptive) endocytosis/transcytosis. Fig. 3 represents schematic illustration of BBB transport systems.

Brain drug delivery and targeting requires overcoming the limited access of drugs to the brain. Different methods have been developed to achieve BBB penetration, including: opening of BBB TJs by means of osmotic or biologically active agents (such as bradykinin and histamine); exploiting of various specific transport mechanisms. The last methodology includes: conjugation of a drug with a targeting protein, or to a monoclonal antibody that gains access to the brain by receptor-mediated transcytosis, or to a small peptide-vectors to enhance brain uptake of several therapeutic drugs. Further the use of drug delivery devices such as liposomes has also been reported. As shown in Fig. 4, the BBB carrier-mediated transporters comprise two different classes, including the efflux and the influx pump transport systems.

6.1 Efflux transporters

The BBB represents a major hindrance to the entry of many therapeutic drugs into the brain and efflux pumps are part of this protection. P-glycoprotein (P-gp; MDR1/ABCB1) is an ATP-binding cassette (ABC) drug transport protein that is predominantly found in the apical membranes of a number of epithelial cell types in the body as well as the brain microvessel endothelial cells. The putative transmembrane structural organization of human MDR1 P-gp is primarily found in the cell plasma membrane as 12 transmembrane segments that are thought to fold together and form a three-dimensional barrier like structure in the cell plasma membrane. The latter polypeptide chain consists of two similar halves. Each half contains of six putative transmembrane segments and intracellular ATP-binding site. The hydrolysis of ATP provides the energy for active drug export. Schinkel et al (1995) showed that mouse MDR1a and the human MDR1 P-glycoprotein actively transport ivermectin, dexamethasone, digoxin, and cyclosporin A and, to a lesser extent, morphine across a polarized kidney epithelial cell layer in vitro. The investigator reported that injection of the radiolabeled substrates of P-gp in MDR1a knockout and wild-type mice resulted in 20-
50-fold higher levels of radioactivity in the MDR1a knockout mice brain for digoxin and cyclosporin A (Schinkel et al., 1995b). These researchers generated mice with a genetic disruption of the drug-transporting MDR1a P-gp and showed that the P-gp knockout mice were overall healthy but they accumulated much higher levels of substrate drugs in the brain with markedly slower elimination. For the drugs (e.g., anticancer agents) that are P-gp substrates, this can lead to dramatically increased toxicity (Schinkel et al., 1995a). Thus, drugs inhibiting the MDR1 P-gp activity should be co-administered during chemotherapy of the brain tumors. The authors concluded that P-gp is the major determinant for the pharmacology of several medically important drugs apart from anti-cancer agents, especially in the BBB.

Fig. 3. Schematic illustration of transportation systems for shuttling of endogenous and/or exogenous substrates at the BBB. 1) Lipid-soluble small substrates (<500 Da) are able to diffuse across the membrane – they may be effluxed back into the blood circulation through efflux transporters (e.g., P-gp, MRP4). 2) Carrier-mediated transport machineries (e.g., Glut1, LAT1) are responsible for transport of small endogenous molecules (e.g., amino acids, nucleosides, and glucose). 3) Some small hydrophilic molecules can be transported via paracellular route. 4) Larger molecules (e.g., insulin, transferrin) are transported through receptor-mediated endocytosis/transcytosis using vesicular trafficking towards the brain parenchyma. 5) Some large proteins (e.g., albumin) are transported across the BBB by adsorptive-mediated endocytosis/transcytosis. Of the carrier-mediated transporters, glucose transporters (Gluts) are responsible for traverse of glucose from blood to brain and between different cells within the brain parenchyma. Adherens junctions provide a path for cell-to-cell intercommunication within endothelial cells of the BBB, adapted with permission (Omidi & Barar, 2012).
Multidrug-resistance associated protein (MRP) (Zhang et al. 2000) actively transports a broad range of anionic compounds out of the cell in the BBB and is known as another member of the ABC superfamily of transport proteins. It has approximately 15% amino acid sequence homology to P-gp, and the characteristics ATP binding sites that allow for the active transport of a diverse array of compounds out of the cell. In contrast to P-gp, MRP transports organic anions. Zhang et al (2000) reported that the MRP1, MRP4, MRP5, and MRP6 were consistently expressed in both the capillary-enriched fraction of the brain homogenate and the BBMEC monolayers. The expression of MRP2 has also been shown in the isolated primary porcine microvessel endothelial cells (Fricker et al. 2002). The presence of several different MRP homologues at the BBB highlights the MRP role in controlling the permeability of the BBB to organic anions. In the brain, MRP4 was shown to be expressed on the luminal membrane of BCECs as well as the basolateral membrane of the choroid plexus. The chemotherapeutic drug topotecan was shown to be accumulated in the brain and the CSF in an MRP4 knockout mouse model. This clearly highlights the important role that MRP4 plays in determining the CNS distribution of this drug (Leggas et al., 2004). It is likely that multiple efflux drug transporters including MRP4 govern the brain penetration and activity of this anticancer agent. Readers are directed to see (Tsuji, 2005; Urquhart & Kim, 2009).

It should be stated that most of anticancer agents are substrate to ABC transporters. Of these, the MDR1/ABCB1 can pump out a wide range of compounds (e.g., Acebutolol, Actinomycin D, Amprenavir, Azidopine, Betamethasone, Calcein-AM, Cepharanthin, Cerivastatin, Chloroquine, Cimetidine, Clarithromycin, Colchicine, Cortisol, Cyclosporin A, Daunorubicin, Dexamethasone, Digitoxin, Digoxin, Dipyrindamole, Docetaxel, Domperidone, Doxorubicin, Eletriptan, Emetine, Epinastine, Erythromycin, Estradiol-17b-D-glucuronide, Estrone, Ethynyl estradiol, Etoposide, Fexofenadine, Grepafloxacin, Imatinib, Indinavir, Irinotecan, Ivermectin, Lansoprazole, Levofloxacin, Loperamide, Losartan, Lovastatin, Methylprednisolone, Mitoxantrone, Morphine, Neostigmine, Omeprazole, Pantoprazole, Prazosin, Prednisolone, Puromycin, Quinidine2, Ramồsetron, Ranitidine, Reserpine, Ritonavir, Saquinavir, Somatostain, Sparfloxacin, Talinolol, Taxol, Terfenadine, Trimethoprim, Vecuronium, Verapamil, Vinblastine, Vincristine), while the MRP4/ABCC4 is able to efflux a narrower spectrum (e.g., cAMP, cGMP, Dehydroepiandrosterone-3-sulfate, Estradiol-17b-D-glucuronide, Folate, Methotrexate, Prostaglandin E1, Prostaglandin E2). The breast cancer resistance protein (BCRP/ABCG2) have been reported to be expressed as active efflux drug transporters at the BBB that can display efflux functionality similar to that of P-gp for a wide range of compounds (e.g., Azidodeoxythymidine, Bisantrene, Cerivastatin, Doxorubicin, Daunorubicin, Dehydroepiandrosterone-3-sulfate, Etoposide, Estrone-3-sulfate, Estradiol-17b-D-glucuronide, Folate, Flavopiridol, Imatinib mesylate, Mitoxantrone, Methotrexate, Prazosin, Pantoprazole, Pravastatin, Rhodamine 123, Topotecan), for more details readers are directed to see (Ohtsuki & Terasaki, 2007).

Many of these chemicals are important anticancer agents (e.g., Doxorubicin, Vinblastine, Vincristine, Methotrexate, Docetaxel, Etoposide, Idarubicin, and Taxol), thus it is vital to inhibit these efflux machineries to reach suitable concentration in brain during brain tumors chemotherapy. Various compounds were reported as inhibitors for these efflux transporters such as colchicine, phenothiazines and quinacrine.
6.2 Influx transporters

BBB influx transporters can be divided into different groups as follows:

1. the energy transport systems for transport of glucose and mannose (Glut1); lactate, short-chain fatty acids, biotin, salicylic acid and valproic acid (MCT) and creatine (CRT),
2. the amino acid transport systems such as small (LAT2/4F2hc) and large (LAT1/4F2hc) neutral amino acid transporter systems for transport of neutral amino acids and L-dopa; acidic amino acid transporter for aspartate and glutamate (ASCT2); basic amino acid transporter (BAAT) for arginine and lysine; the β-amino acid transporter for β-alanine and taurine (TAUT); System A (ATA2) for small neutral amino acids; System ASC/system B0+,
3. the organic anion transport system such as OATP2 for digoxin and organic anions,
4. the nucleoside transport systems such as ENT2 and CNT2,
5. the peptide transport systems such as oligopeptide transporters (PepT1, PepT2), polypeptid transport systems such as OAT3 for PAH, HVA, indoxyl sulfate; OATP14 for thyroid hormones,
6. the neurotransmitter transport systems such as GAT2/BGT1, SERT and NET respectively for transport of γ-aminobutyric acid, serotonin and norepinephrine, and
7. the choline transport system for choline and thiamine (Ohtsuki & Terasaki, 2007).

Given that the main properties of the BBB that differ the brain capillary endothelia from other blood microvessel because of the presence of restrictive high-resistance tight junctions between blood and brain parenchyma, the BBB forming BCECs almost completely prevents the uptake of potential CNS drugs via the paracellular pathway. Thus compounds passing the BBB almost exclusively have to exploit the transcellular pathway, but this is not always the case since there are increasing evidences that a broad variety of transport machineries are involved, including both carrier-mediated transport and receptor-mediated transcytosis for transporting compounds into the brain (the so called active influx) and multidrug transport pumps for actively effluxing unwanted chemicals out of brain (the so called active efflux). In the case of transport into the brain, numerous systems have been discovered, including transport proteins for amino acids, monocarboxylic acids, organic cations, hexoses, nucleotides and peptides. Several of these proteins have successfully been used in prodrug strategies to enable or at least enhance brain uptake of neurotherapeutic agents. Classic examples are l-dopa and progabide. Of these influx transport machineries, both pyrimidine and purine nucleoside analogs are currently used clinically as anti-metabolite drugs. Cytarabine, an analog of deoxycytidine (1-β-d-arabinofuranosylcytosine, araC, Cytosar-Us), is used as combination chemotherapy in the treatment of chronic myelogenous, leukemia, multiple myeloma, Hodgkin’s lymphoma and non-Hodgkin’s lymphomas; Gemcitabine (dFdC, 2',2'-di.uorodeoxycytidine, Gemzars), a broad spectrum agent, which is used for treatment of a variety of cancers including pancreatic and bladder cancers. Capecitabine (5'-deoxy-5-N-[(pentoxy) carbonyl]-cytidine, Xelodas) is used, as a prodrug, in treatment of metastatic colorectal cancer. Two purine nucleoside anti-metabolite drugs, Fludarabine (9-β-d-arabinofuranosyl-2'-uoroadenine), and Cladribine (2-chloro-2'-deoxyadenosine, CdA, Leustatins) are used for treatment of low-grade lymphomas and chronic lymphocytic leukemia (Omidi & Gumbleton, 2005).
Astrocytes, neurons and microglial cells are intercommunication with brain capillary endothelial cells. P-gp: P-glycoprotein. MRP1: multidrug resistance associated protein 1. MRP4: multidrug resistance associated protein 4. MRP5: multidrug resistance associated protein 5. BCRP: breast cancer resistance protein. Glut1: glucose transporter 1. MCT1: monocarboxylate transporter 1. LAT1: large neutral amino acids transporter 1. ASCT2: neutral amino acid transporter 2. EAAT: excitatory amino acid transporters. CNT2: centratractive nucleoside transport 2. ENT: equilibrative nucleoside transport 2. SERT: serotonin transporter. NET: norepinephrine transporter. CRT: creatine transporter. TAUT: taurine transporter. OATP2: organic anion-transporting polypeptide. OAT3: organic anion transporter 3. OATP1A2: organic anion-transporting polypeptide. OAT2: organic anion transporter 2.

6.3 Endocytosis, transcytosis and exocytosis

Cell membranous vesicular machinery domains comprise numerous components including lipid rafts, caveolae and clathrin-coated pits. All of them appear to participate in endocytosis and probably transcytosis of macromolecules.

Caveolae are flask-shaped invaginations of the plasma membrane coated by a 22 kDa structural protein caveolin. Initially detected in endothelial cells, caveolae tend to mediate
the selective uptake of molecules as small as folate to full size proteins such as albumin and lipoproteins. These micro-domains are highly enriched in glycosphingolipids, cholesterol, sphingomyelin, and lipid-anchored membrane proteins. Caveolae have been implicated in a wide range of cellular functions including transcytosis, receptor-mediated uptake, stabilization of lipid rafts and compartmentalization of a number of signaling events at the cell surface. Several studies have also shown that caveolae-mediated uptake of materials is not limited to macromolecules; in certain cell-types, viruses (e.g. simian virus 40) and even entire bacteria (e.g. specific strains of E. Coli) are engulfed and transferred to intracellular compartments in a caveolae-dependent fashion. Clathrin-mediated endocytosis is the most widely studied vesicular membrane internalizing system, and participation of clathrin-coated vesicles has also been investigated in terms of receptor-mediated transport in the BBB. Clathrin forms a non-covalently bound triskelion structure composed of three heavy chains (192 kDa each) and three light chains (Omidi & Gumbleton, 2005; Smith & Gumbleton, 2006). Fig. 5 represents schematic illustration of the clathrin coated vesicles (CCV) and its main protein “triskelion”.

![Fig. 5. TEM micrograph (A) and schematic representation of molecules involved in assembly of the clathrin coated pit (B) and its main protein triskelion (C). TEM: transmission electron microscopy; CCV: clathrin coated vesicle (Omidi & Barar, 2012).](image)

**7. BBB cell culture models**

A simple, applicable, and robust *in vitro* cell based BBB model can provide a useful tool for screening of permeability to central nervous system-acting drugs. Thus, in the 1970s, techniques for isolation of brain microvessels were introduced using mechanical or enzymatic homogenization. In the late 1970’s, BCECs were plated in tissue culture and this led to the early *in vitro* BBB model establishment (DeBault et al., 1979). The primary BCECs cultures are very well-characterized systems used as *in vitro* models; nevertheless they have the disadvantage of being very laborious and variable and must be repeated often to provide an adequate stock of cells. Therefore various immortalized endothelial cell lines have been developed and examined such as CR3, EC219, RBE4 b.End3, ECV304, and even epithelial cell line MDCK.
Basically, an appropriate cell culture model confers a useful platform for not only transcellular and paracellular drug diffusional processes, but also metabolism and active transport processes. Such model can be used for investigating the nondefined interactions between a drug and cellular material that may impact upon a membrane’s overall permeability profile. Thus, ideally the *in vitro* BBB cell model should represent a restrictive paracellular barrier functionality, physiologically realistic cell architecture and functional expression of key transporter mechanisms. Such model should also confer ease of culture to meet the technical and time constraints of a screening program. Based upon these facts, immortalized cell based BBB models often fail to meet all these essentialities.

Despite many benefits of immortalized cell based BBB models, the lack of restrictive barrier function has limited their use for permeability screenings and therefore the primary BCECs have been isolated to be used as a cell culture BBB *in vitro* model. The main advantage from pharmaceutical perspective is that these primary models, practically the bovine and porcine models, possess a restrictive paracellular pathway. Nevertheless, BCECs grown in tissue cultures probably lack many of the characteristics of the BBB *in vivo*. In an attempt to improve such models, endothelial/astrocyte and endothelial/pericyte cocultures have been examined, even attempts to generate a 3 dimensional (3D) model have been made. For instance aortic cells cocultured with astrocytes (C6 cells) in the presence of flow within hollow fiber tube develop a selective barrier with an estimated electrical resistance of 2,900 $\Omega\cdot cm^2$ (Stanness et al., 1996). The same group later reported that the previously introduced 3D dynamic *in vitro* BBB model can be successfully used for the coculture of differentiated serotonergic neurons in the presence of a BBB (Stanness et al., 1999). A number of less-complicated *in vitro* coculture BBB models have also been established and developed. To produce continuous BBB *in vitro* models, several academic groups have reported the generation of immortalized brain microvessel endothelial cell lines. An immortalized human brain capillary endothelial BB19 cell line was transformed with the E6E7 genes of human papilloma virus and retained their endothelial nature. It was used to study the cytoadherence of Plasmodium falciparum-infected erythrocytes (Prudhomme et al., 1996). Human cerebral microvascular endothelial cells SV-HCEC, transfected with the plasmid pSV3-neo coding for the SV40 large T antigen, was utilized to serve as a human BBB *in vitro* model showing the expression of factor VIII-related antigen, the uptake of acetylated low-density lipoprotein, the binding of fluorescently labeled lectins, the expression of transferrin receptor, and high activities of the ALP and $\gamma$-GTP (Muruganandam et al., 1997).

Immortalized brain capillary endothelial cell lines (TM-BBB1-5) were established from 3 transgenic mice harboring temperature-sensitive simian virus 40 large T-antigen gene displaying the expression of Glut-1 and P-gp. The TR-BBB cell line has also been established using same approach from a transgenic rat (Hosoya et al., 2000). An immortalized rat brain endothelial cell line, RBE4, was immortalized by transfection with a plasmid containing the E1A adenovirus gene (Roux et al., 1994). The RBE4 cell line appears to be most commonly used cell line to be exploited for investigation of different features of BBB, e.g. the transport of insulin, the expression of P-gp, the uptake of L-dopa and the expression of transferrin receptor (Balbuena et al., 2011; Hulsermann et al., 2009; Yu et al., 2007). The MBEC4 cells, established from BALB/c mouse cerebral microvessel endothelial cells, were used to investigate the high-affinity efflux transport system for glutathione conjugates (Homma et al., 1999). The CR3 cells, established by genomic introduction of the immortalizing SV40 large T gene under the control of the human vimentin promoter, displayed endothelial
morphological and biochemical characteristics for up to 30 passages. The b.End3 cells was used to study the uptake and efflux of transferrin (Tf) and Fe showing the expression of Tf receptor and suggesting a receptor-mediated endocytosis for the uptake (Lechardeur et al., 1995; Lechardeur & Scherman, 1995). Given that the immortalized continuous cell lines tend not to generate a restrictive barrier property, isolated primary BCECs have been used from a variety of sources including human, bovine, porcine, monkey, rat, canine, and murine. For more details, readers are directed to see following citations (Gumbleton & Audus, 2001; Lacombe et al., 2011; Ribeiro et al., 2010).

8. BBB permeation and in-silico prediction models

Undoubtedly, the BBB is designed to protect the brain from entering of toxic compounds. As outlined above, the main underlying concept seems to be to force the compounds to take the transcellular route, where nutrients are actively transported into the brain and possibly toxic compounds are expelled via active efflux pumps. Thus, BBB permeation is a multifactorial, complex issue which requires advanced computational methods for proper modeling.

Computational models, in general, exploit two different approaches. The passive diffusion-controlled permeability is dependent upon the inherent physicochemical characteristics (e.g., logP, solubility and surface area) of the compounds, and basically molecular descriptor based methods are used to generate predictive models. The ligand-receptor (i.e., influx/efflux transporters, or receptor mediated endocytosis) active/facilitated transport can be considered for carrier/receptor mediated trafficking. Given these, predictive in-silico models suitable for both the lead identification and the lead optimization processes should include both categories. The most commonly used type of data appear to be the logBB values that are described as the ratio of the steady-state concentration of a designated compound in the brain to that of the blood which can describe by Equation (1).

\[
\text{LogBB} = \log\left(\frac{[C_{\text{Brain}}]}{[C_{\text{Blood}}]}\right) \quad (1)
\]

The most commonly used in vitro model for BCECs culture is based on Transwell™ system, which consists of a porous membrane support submerged in culture media. This system is normally characterized by two direction diffusion, i.e. apical to basal (A to B) or basal to apical (B to A). Given existence of large number of drug-like compounds (e.g., ChemNavigator), a very small number of molecules have been drawn to carefully monitor the main permeation driving/limiting force (i.e. passive diffusion, active influx or active efflux), however the data available mostly represent both passive and active transport phenomena. For detailed information, reader is directed to see (Wolburg, 2006).

8.1 Passive diffusion

Having assumed that the rate of drug release from the formulation is not rate-limiting, the absorption of drug molecules across BBB depend upon: 1) the rate of drug dosing which takes into account the administered dose (mass) and the dosing interval (τ ; time), 2) the interactions of drug molecules with circulating biomolecules in blood (protein binding), 3) drug biostability and clearance, 4) the apparent absorption rate constant for the drug (K_a; time^{-1}). Clearly the stability of drug during the absorption process and importantly the intrinsic permeability of the BBB to the drug are critical factors in determining logBB.
Passive diffusion involves the movement of drug molecules down a concentration or electrochemical gradient without the expenditure of energy. Firstly, if we consider only the transport of drug molecules across BBB via passive diffusional processes then the overall flux (J) of a drug in one dimension (i.e. the net mass of drug that diffuses through a unit area per unit time) can be described by Equation (2).

\[ J = -D \cdot K_p \cdot A \cdot \left( \frac{dC}{dx} \right)_t \]  

(2)

Where \( J \) is the flux of drug; \( D \) is the diffusion coefficient of drug across the cellular barrier; \( K_p \) is a global partition coefficient (cell membrane/aqueous fluid); \( A \) is the surface area of the barrier available for absorption; \( x \) is the thickness of the absorption barrier, and \( (dC/dx)_t \) is the concentration gradient of drug across the absorption barrier. The negative sign in Equation (2) indicates that diffusion proceeds from high to low concentration and hence the flux is a positive quantity. In fact, the greater this concentration gradient, the greater the rate of diffusion of a drug across the cell membrane.

The apparent permeability coefficient (\( \rho \)) of an epithelial/endothelial barrier to a given drug approximate \( D \cdot K_p \). The processes of drug partitioning with the biomembrane (including partitioning between extracellular fluid and plasma membrane, partitioning between plasma membrane and cell cytosol, and other organelle interactions etc.) and of drug diffusion across the biomembranes (including a range of organelle and macromolecule interactions that will influence the diffusion process) are largely dependent upon the molecular properties of the drug, i.e. molecular size/shape, ionic properties (hydrogen bonding potential, pKa), and hydrophobic properties. Basically, such molecular properties will determine if passive diffusional transport across an epithelial/endothelial barrier involves either a predominantly paracellular (between cells) pathway negotiating a tortuous intercellular route via the aqueous channels formed by the anastomosing tight junctional fibrils between adjacent cells, or predominantly (but not exclusively) transcellular (across the cell) pathway requiring partitioning of drug into the plasma membrane bilayer. In fact, it should be stated that if a drug’s molecular properties afford partitioning into biomembranes (i.e. nonionized form of the drug predominates and is of a sufficient hydrophobic nature) then the membrane surface area available for transcellular diffusion will be considerably greater, by many orders of magnitude, than the surface area available for diffusion via the paracellular route. As a corollary, the transcellular diffusion can potentially result in a higher permeability and a higher rate and extent of absorption. For detailed information, reader is directed to see (Omidi & Gumbleton, 2005; Wolburg, 2006)

### 8.2 Macromolecular permeation

With respect to in-silico methods, the passive diffusion seems to be well described by physicochemical parameters of the respective solutes (e.g., lipophilicity, H-bond acceptor/donor features, molecular weight, polar surface area, number of rotatable bonds). These descriptors can be simply calculated and are thus a versatile tool for high-throughput in-silico screening of large compound databases. Basically, small hydrophilic molecules exploit the paracellular pathway to diffuse into organs (Fig. 3).
The impact of the molecule’s steric, ionic and hydrophobic properties upon passive membrane transport and epithelial/endothelial permeability are equally applicable for peptides, proteins and nucleic acids, as they are for traditional low molecular weight drugs. Nevertheless, their permeation across biological barriers will be limited by the presence of a significant number of hydrogen bond acceptor and donor groups, i.e. requiring considerable desolvation energy for the molecule to leave the aqueous environment and partition into a biological membrane. The diffusion of macromolecules (peptides, proteins and nucleic acids) across biological membranes appears to be through endocytosis/transcytosis route or in some cases via the paracellular pathway (Fig. 3). For example, to pursue the binding, uptake and transcytosis of 60 nm porous nanoparticles (NPs) differing in their surface charge and inner composition, Jallouli et al studied their trafficking at the BBB. Having used maltodextrins with/without a cationic ligand, they showed that the cationic NPs were accumulated mainly around the paracellular area, while neutral NPs were mainly on the cell surface and the dipalmitoyl phosphatidyl glycerol (DPPG) NPs were at both paracellular areas and on the surface of the cells. It was shown that filipin can increase the binding and uptake (sterols may entail in their efflux), while decrease the transcytosis of neutral NPs. They concluded that the neutral NPs, like LDL, exploit the caveolae pathway and suggested the neutral and cationic 60 nm porous NPs as potential candidates for drug delivery to the brain (Jallouli et al., 2007). It is believed that Tf receptor, as a molecular part of vesicular trafficking, can facilitate brain delivery of NPs \textit{in vivo}. To explore the attributed mechanism of this process, Chang et al evaluated the endocytosis of poly(lactic-co-glycolic acid) (PLGA) NPs coated with transferrin using an \textit{in vitro} coculture of BCECs and astrocytes. Using solvent diffusion method, they prepared PLGA NPs by means of DiI as a fluorescent marker and coated with Tween 20, BSA and Tf. Depending upon DiI incorporation and surface coating, the size of NPs varied from 63 to 90 nm. In comparison with BSA NPs, the Tf-NPs were found to be highly adsorbed BCECs through an energy-dependent process. Having used specific inhibition, these researchers showed that the Tf-NPs can interact with BCECs in a specific manner and enter the cells via the caveolae endocytic pathway (Chang et al., 2009). Having used a cross-reacting material 197 (CRM197) which is a non-toxic mutant of diphtheria toxin, Wang et al reported that the apical-to-basal transcytosis of CRM197 can involve the caveolae-mediated pathway in the hCMEC/D3 endothelial cells as the caveolin-1 mRNA and protein expression levels were significantly increased by CRM197. These researchers speculated that the upregulation of caveolin-1 may be mediated via a PI3K/Akt dependent pathway and reduction of the phospho-FOXO1A (forkhead box O) transcription factor. Based upon such findings, it was proposed that carrier protein CRM197-mediated delivery across the BBB is involved in the induction of FOXO1A transcriptional activity and upregulation of caveolin-1 expression (Wang et al., 2010). In fact, the BCECs exploit a variety of endocytic pathways (i.e., clathrin-mediated endocytosis, caveolar endocytosis, and macropinocytosis) for the internalization of exogenous materials. It is deemed that the properties of drug delivery vehicles can direct the intracellular processing in brain endothelial cells. Using fixed-size NPs, it has been shown that surface modifications of nanoparticles (e.g., charge and protein ligands) can affect their mode of internalization by BCECs and thereby the subcellular fate (Georgieva et al., 2011).

It should be also evoked that the diffusion coefficient of a drug is inversely proportional to its molecular weight, and while for traditional low molecular weight drugs (100-500 Da) the
diffusion coefficient varies little between drugs. Further, the epithelial permeability of biotechnology products, like that of traditional low molecular drugs, are subjected to the extent of degradation occurring within the barrier itself, in which the role of proteases and nucleases will be important for biotechnology drugs, readers are directed to see (Wolburg, 2006).

9. Targeting brain tumours

The integrity of the BBB in metastatic cancerous tumors appears to be different from the normal ones. In addition to the various morphological alterations of BBB in metastatic cancerous tumors (e.g., compromised tight junction structure and increases in the perivascular space, fenestrated BCECs, increased number and activity of pinocytic vacuole), the expression of transporters has also been reported to be altered in the microvasculature of the brain tumors. This implies that the BBB is less intact in primary and metastatic brain tumors compared with the normal brain vasculature, and accordingly the pharmacotherapy of brain tumors demands specific strategies. So far, to enhance the amount of therapeutic agent to a brain tumor, a number of strategies have been exploited including: 1) increasing drug plasma concentration (e.g., intraarterial infusion), 2) physicochemical modification to increase drug permeability, 3) design of inactive drug precursors (the so-called prodrugs) that could more easily cross the blood–brain barrier before conversion to a drug with active formulation, and 4) osmotic disruption of the blood–brain barrier using osmotic-disruptive agents such as mannitol (Provenzale et al., 2005). Of these methodologies, liposomal formulations seem to be promising since they can passively target tumors in which there is disorganized vasculature. This appears to be also related to higher permeability than to disorganization per se; nonetheless specific features (e.g., vesicle size, chemical affinity, and thermal/pH sensitiveness) may affect the targeting potential of liposomes. The hyperthermia looms to be an important means for modifying the target environment and increasing liposome delivery to tumors. For example, in animal models, it was observed that the heating to temperatures up to 41–43°C can increase the tumor microvascular pore size and accordingly increases its permeability to various substances (e.g., ferritin, antibodies, and liposomes), perhaps hyperthermia can disaggregate the endothelial cell cytoskeleton. For instance, in a human tumor xenograft murine model (SKOV-3), the extravasation of 100 nm liposomes was not observed at 34°C, but it was seen after heating to 40°C (Kong et al., 2001). In 2010, Bellavance et al reported on development of a novel cationic liposome formulation composed of DPPC:DC-Chol:DOPE:DHPE Oregon Green, which was shown to possesses efficient internalization and intracellular delivery to F98 and U-118 glioblastoma (GBM) cells in pH-sensitive manner. At which point, they suggested such liposomal formulation as a novel potent and efficient vehicle for cytosolic delivery of intracellular therapeutics such as chemotherapy agents to the glioblastoma (Bellavance et al., 2010). So far, various liposomal and polymeric formulations have been introduced as smart (thermo-sensitive and/or pH sensitive) nanomedicines that can provide a controlled drug release in target tumors. In particular, the pH-sensitive nanosystems have been given greater attention since the pH targeting approach is regarded as a more general strategy than conventional specific tumor cell surface targeting approaches. In fact, the nanosystems display greater potential to overcome multidrug resistance of various tumors when they are combined with triggered release mechanisms by endosomal or lysosomal acidity plus endosomolytic capability – this important domain has been well reviewed recently, see (Lee et al., 2008).
In addition, little is known about impacts of metastatic disease on the BBB. Although the brain metastases respond to chemotherapy modalities, such responses are largely dependent upon condition of patients. For example, 14 patients with brain metastases from small cell lung cancer (SCLC) were treated with combination therapy of cyclophosphamide, doxorubicin, vincristine, and etoposide. Of the treated patients, 9 of 11 patients (82%) showed responses in their brain lesions, whereas 9 of 12 evaluated patients (75%) had responses in their extracranial lesions (Lee et al., 1989). In another study, patients with SCLC and brain metastases were treated with cisplatin, ifosfamide, and irinotecan with rhG-CSF support. The response rate was 50% in brain lesions and 62% in extracranial primary or metastatic lesions (Fujita et al., 2000). The most studies for assessing leakiness of the BBB in human brain tumors appear to be the T1-weighted techniques (in particular, T1-weighted dynamic contrast-enhanced imaging). In fact, researchers implement this methodology using a 3D spoiled gradient acquisition steady-state technique that monitors contrast material accumulation over a few minutes rather than observing the first-pass phenomenon. The main advantages of this technique seem to be the relatively short imaging time, a need for only a single dose of contrast material, and availability of a large number of user-friendly analysis programs (Provenzale et al., 2005).

**10. Recent advancements for crossing BBB**

Selective functional presence of BBB makes brain delivery and targeting very challenging issue, for which various strategies have been developed, including different classes of nanomedicines. These novel strategies are largely dependent upon biological characteristics of BBB, and accordingly nanomedicines exploit endocytic pathways. Unfortunately, patients with primary brain tumors and brain metastases have a very poor prognosis. This is often exacerbated with low responses to chemotherapy attributed to BBB selective control on permeation of cytotoxic agents. Nanomedicines represent great promise in glioma therapy as they protect therapeutic agent and allow its sustained release even though tumor specific targeting paradigms with extensive intratumoral distribution must be developed for efficient delivery.

Paclitaxel as an active agent against gliomas and various brain metastases is substrate of P-gp efflux transporter, and thus it is pumped out of brain parenchyma. To tackle this issue, Kozia et al. developed novel cetyl alcohol/polysorbate NPs for encapsulation and delivery of anticancer agents such as paclitaxel (PX) to brain. They showed significant increase of paclitaxel in brain using such NPs because of the limited binding of PX to p-gp (Kozia et al., 2004). With an effort to evaluate the characteristics of an ultrasmall superparamagnetic iron oxides (USPIO) agent in patients with brain tumors and to correlate changes on MRI with histopathologic data collected systematically in all patients, Taschner et al. examined 9 patients with brain tumors before and 24 hr after administration of a USPIO at a dose of 2.6 mg Fe/kg. They witnessed USPIO-related changes of signal intensity in gadolinium-enhancing brain tumors in 7 patients. Upon such findings, they suggested that USPIO agents can offer complementary information useful to differentiate between brain tumors and areas of radiation necrosis (Taschner et al., 2005). Interestingly, in 2006, Wu et al. reported construction of a drug delivery vehicle with ability to target the epidermal growth factor receptor (EGFR) and its mutant isoform EGFRvIII. In their work, the EGFR targeting monoclonal antibody, cetuximab, was covalently linked to a Polyamidoamine (PAMAM) dendrimer containing the cytotoxic drug methotrexate. Using
the EGFR-expressing rat glioma cell line F98(EGFR), they showed that the bioconjugate retained its affinity for F98(EGFR) cells and the IC50 of the bioconjugate was 220 nmol/L. The bioconjugate in rats bearing i.c. implants of either F98(EGFR) or F98(WT) gliomas was determined 24 hr following convection enhanced delivery of (125)I-labeled complex, showing specific molecular targeting of the tumor. Based on such findings, they concluded that the antibody-drug bioconjugate is therapeutically useful approach in brain tumors (Wu et al., 2006). In 2009, Veiseh et al reported on development of an iron oxide nanoparticle coated with polyethylene glycol-grafted chitosan with ability to cross the BBB and target brain tumors in a genetically engineered mouse model. The nanoprobe was conjugated to a tumor-targeting agent, chlorotoxin, and a near-IR fluorophore. Using in vivo magnetic resonance, biophotonic imaging, and histologic and biodistribution analyses, they showed an innocuous toxicity profile induced by the nanoprobe, while it showed a sustained retention in tumors and suggested its application for the diagnosis and treatment of a variety of tumor types in brain (Veiseh et al., 2009). Using novel quaternary ammonium beta-cyclodextrin (QAbetaCD) NPs (with 65-88 nm diameter and controllable cationic properties), Gil et al reported successful delivery of doxorubicin (DOX) across the BBB. They showed that QAbetaCD NPs are not toxic to bovine brain microvessel endothelial cells (BBMVECs) at concentrations up to 500 μg/mL. They also showed that the DOX/QAbetaCD complexes can kill U87 cells as effectively as DOX alone, while the QAbetaCD NPs completely protect BBMVECs from cytotoxicity of DOX. And as a result, it was suggested that the QAbetaCD NPs as safe and effective delivery system for anticancer agents such as DOX for brain tumors (Gil et al., 2009). Upon the of note tropism of mesenchymal stem cells (MSCs) for brain tumors, Roger et al exploited the MSCs as NP delivery vehicles, in which they used two types of NPs loaded with coumarin-6, i.e. poly-lactic acid NPs (PLA-NPs) and lipid nanocapsules (LNCs). They showed efficient internalization of the NPs into MSCs that were able to migrate toward an experimental human glioma model. They suggested MSCs as potential cellular carriers for delivery of NPs into brain tumors (Roger et al., 2010). In 2011, A dual-targeting drug carrier (PAMAM-PEG-WGA-Tf) was developed based on the PEGylated fourth generation PAMAM dendrimer with Tf and wheat germ agglutinin (WGA) on the periphery and DOX loaded in the interior (He et al., 2011). Having nanoscaled size (~ 20 nm), the PAMAM-PEG-WGA-Tf efficiently inhibited the growth rate of the C6 glioma cells, while it reduced the cytotoxicity of DOX to the normal cells. These researchers reported significantly increase and accumulation of DOX in the tumor site (due to the targeting effects of both Tf and WGA) and suggested that it could be used as a BBB penetrating agent with tumor targeting properties (He et al., 2011).

11. Chapter summary

Entry of blood circulating agents into brain is highly controlled by selectively functional presence of BBB. This makes brain drug delivery and targeting very intricate. Owing to unique biology of brain capillary endothelial cells, carrier and/or receptor mediated transport machineries of BBB can be exploited using smart pharmaceuticals. In the case of tumors such as glioma, use of intelligent molecular Trajan horses appears to provide a combined imaging-therapy as “theranostic” to ease brain drug delivery and targeting by simultaneous imaging techniques such as positron emission tomography (PET). In near future, it is expected new multifunctional “all in one” therapeutic to be translated into clinic to cure brain tumors in much more efficient manner. Such therapeutics may consist of homing device for targeting, imaging moiety for sensing/imaging, and therapeutic itself in a
vehicle that could be activated by outside/inside stimulation (pH, temperature, enzyme). To translate such fascinating molecular therapy into clinical use, however, we need to recruit several disciplines such as nanotechnology, biotechnology, biophotonics, engineering, biopharmaceutics and clinical expertise.

12. References

Abbott, N.J. (2000). Inflammatory mediators and modulation of blood-brain barrier permeability. *Cell Mol.Neurobiol.*, Vol.20, No.2, (April 2000), pp. 131-147, ISSN 0272-4340

Abbott, N.J. (2005). Dynamics of CNS barriers: evolution, differentiation, and modulation. *Cell Mol.Neurobiol.*, Vol.25, No.1, (February 2005), pp. 5-23, ISSN 0272-4340

Abbott, N.J.; Ronnback, L. & Hansson, E. (2006). Astrocyte-endothelial interactions at the blood-brain barrier. *Nat.Rev.Neurosci.*, Vol.7, No.1, (January 2006), pp. 41-53, ISSN 1471-003X

Albert, J.L.; Boyle, J.P.; Roberts, J.A.; Challiss, R.A.; Gubby, S.E. & Boarder, M.R. (1997). Regulation of brain capillary endothelial cells by P2Y receptors coupled to Ca2+, phospholipase C and mitogen-activated protein kinase. *Br.J.Pharmacol.*, Vol.122, No.5, (November 1997), pp. 935-941, ISSN 0007-1188

Allt, G. & Lawrenson, J.G. (2000). The blood-nerve barrier: enzymes, transporters and receptors--a comparison with the blood-brain barrier. *Brain Res.Bull.*, Vol.52, No.1, (May 2000), pp. 1-12, ISSN 0361-9230

Allt, G. & Lawrenson, J.G. (2001). Pericytes: cell biology and pathology. *Cells Tissues.Organs*, Vol.169, No.1, (2001), pp. 1-11, ISSN 1422-6405

Balabanov, R. & Dore-Duffy, P. (1998). Role of the CNS microvascular pericyte in the blood-brain barrier. *J.Neurosci.Res.*, Vol.53, No.6, (September 1998), pp. 637-644, ISSN 0360-4012

Balbuena, P.; Li, W. & Ehrich, M. (2011). Assessments of tight junction proteins occludin, claudin 5 and scaffold proteins ZO1 and ZO2 in endothelial cells of the rat blood-brain barrier: cellular responses to neurotoxicants malathion and lead acetate. *Neurotoxicology*, Vol.32, No.1, (January 2011), pp. 58-67, ISSN 0161-813X

Bellavance, M.A.; Poirier, M.B. & Fortin, D. (2010). Uptake and intracellular release kinetics of liposome formulations in glioma cells. *Int.J.Pharm.*, Vol.395, No.1-2, (August 2010), pp. 251-259, ISSN 0378-5173

Chang, J.; Jallouli, Y.; Kroubi, M.; Yuan, X.B.; Feng, W.; Kang, C.S.; Pu, P.Y. & Betbeder, D. (2009). Characterization of endocytosis of transferrin-coated PLGA nanoparticles by the blood-brain barrier. *Int.J.Pharm.*, Vol.379, No.2, (September 2009), pp. 285-292, ISSN 0378-5173

DeBault, L.E.; Kahn, L.E.; Frommes, S.P. & Cancilla, P.A. (1979). Cerebral microvessels and derived cells in tissue culture: isolation and preliminary characterization. *In Vitro*, Vol.15, No.7, (July 1979), pp. 473-487, ISSN 0073-5655
Engelhardt, B. (2006). Development of the Blood-Brain Interface, In: Blood-Brain Barriers, Dermietzel, R., Spray, D. C., and Nedergaard, M., pp. 11-40, WILEY-VCH Verlag GmbH & Co. KGaA, ISBN 3-527-31088-6, Weinheim

Fujita, A.; Fukuoka, S.; Takabatake, H.; Tagaki, S. & Sekine, K. (2000). Combination chemotherapy of cisplatin, ifosfamide, and irinotecan with rhG-CSF support in patients with brain metastases from non-small cell lung cancer. Oncology, Vol.59, No.4, (November 2000), pp. 291-295, ISSN 0030-2414

Georgieva, J.V.; Kalicharan, D.; Couraud, P.O.; Romero, I.A.; Weksler, B.; Hoekstra, D. & Zuhorn, I.S. (2011). Surface characteristics of nanoparticles determine their intracellular fate in and processing by human blood-brain barrier endothelial cells in vitro. Mol. Ther., Vol.19, No.2, (February 2011), pp. 318-325, ISSN 1525-0016

Gil, E.S.; Li, J.; Xiao, H. & Lowe, T.L. (2009). Quaternary ammonium beta-cyclodextrin nanoparticles for enhancing doxorubicin permeability across the in vitro blood-brain barrier. Biomacromolecules., Vol.10, No.3, (March 2009), pp. 505-516, ISSN 1525-7797

Gumbleton, M. & Audus, K.L. (2001). Progress and limitations in the use of in vitro cell cultures to serve as a permeability screen for the blood-brain barrier. J.Pharm.Sci., Vol.90, No.11, (November 2001), pp. 1681-1698, ISSN 0022-3549

Haseloff, R.F.; Blasig, I.E.; Bauer, H.C. & Bauer, H. (2005). In search of the astrocytic factor(s) modulating blood-brain barrier functions in brain capillary endothelial cells in vitro. Cell Mol.Neurobiol., Vol.25, No.1, (February 2005), pp. 25-39, ISSN 0272-4340

Hawkins, B.T. & Davis, T.P. (2005). The blood-brain barrier/neurovascular unit in health and disease. Pharmacol.Rev., Vol.57, No.2, (June 2005), pp. 173-185, ISSN 0031-6997

He, H.; Li, Y.; Jia, X.R.; Du, J.; Ying, X.; Lu, W.L.; Lou, J.N. & Wei, Y. (2011). PEGylated Poly(amidoamine) dendrimer-based dual-targeting carrier for treating brain tumors. Biomaterials, Vol.32, No.2, (January 2011), pp. 478-487, ISSN 0142-9612

Homma, M.; Suzuki, H.; Kusuhara, H.; Naito, M.; Tsuruo, T. & Sugiyama, Y. (1999). High-affinity efflux transport system for glutathione conjugates on the luminal membrane of a mouse brain capillary endothelial cell line (MBEC4). J.Pharmacol.Exp.Ther., Vol.288, No.1, (January 1999), pp. 198-203, ISSN 0022-3565

Hosoya, K.I.; Takashima, T.; Tetsuka, K.; Nagura, T.; Ohtsuki, S.; Takanaga, H.; Ueda, M.; Yonai, N.; Obinata, M. & Terasaki, T. (2000). mRNA expression and transport characterization of conditionally immortalized rat brain capillary endothelial cell lines; a new in vitro BBB model for drug targeting. J.Drug Target, Vol.8, No.6, (2000), pp. 357-370, ISSN 1061-186X

Hulsermann, U.; Hoffmann, M.M.; Massing, U. & Fricker, G. (2009). Uptake of apolipoprotein E fragment coupled liposomes by cultured brain microvessel endothelial cells and intact brain capillaries. J.Drug Target, Vol.17, No.8, (September 2009), pp. 610-618, ISSN 1061-186X

Hurst, R.D. & Clark, J.B. (1998). Alterations in transendothelial electrical resistance by vasoactive agonists and cyclic AMP in a blood-brain barrier model system. Neurochem.Res., Vol.23, No.2, (February 1998), pp. 149-154, ISSN 0364-3190
Igarashi, Y.; Utsumi, H.; Chiba, H.; Yamada-Sasamori, Y.; Tobioka, H.; Kamimura, Y.; Furuuchi, K.; Kokai, Y.; Nakagawa, T.; Mori, M. & Sawada, N. (1999). Glial cell line-derived neurotrophic factor induces barrier function of endothelial cells forming the blood-brain barrier. *Biochem. Biophys. Res. Commun.*, Vol.261, No.1, (July 1999), pp. 108-112, ISSN 0006-291X

Jallouli, Y.; Paillard, A.; Chang, J.; Sevin, E. & Betbeder, D. (2007). Influence of surface charge and inner composition of porous nanoparticles to cross blood-brain barrier in vitro. *Int. J. Pharm.*, Vol.344, No.1-2, (November 2007), pp. 103-109, ISSN 0378-5173

Kniesel, U. & Wolburg, H. (2000). Tight junctions of the blood-brain barrier. *Cell Mol. Neurobiol.*, Vol.20, No.1, (February 2000), pp. 57-76, ISSN 0272-4340

Kondo, A.; Inoue, T.; Nagara, H.; Tateishi, J. & Fukui, M. (1987). Neurotoxicity of adriamycin passed through the transiently disrupted blood-brain barrier by mannitol in the rat brain. *Brain Res.*, Vol.412, No.1, (May 1987), pp. 73-83, ISSN 0006-8993

Kong, G.; Braun, R.D. & Dewhirst, M.W. (2001). Characterization of the effect of hyperthermia on nanoparticle extravasation from tumor vasculature. *Cancer Res.*, Vol.61, No.7, (April 2001), pp. 3027-3032, ISSN 0008-5472

Koziara, J.M.; Lockman, P.R.; Allen, D.D. & Mumper, R.J. (2004). Paclitaxel nanoparticles for the potential treatment of brain tumors. *J. Control Release*, Vol.99, No.2, (September 2004), pp. 259-269, ISSN 0168-3659

Krizzbail, I.A. & Deli, M.A. (2003). Signalling pathways regulating the tight junction permeability in the blood-brain barrier. *Cell Mol. Biol. (Noisy.-le-grand)*, Vol.49, No.1, (February 2003), pp. 23-31, ISSN 0145-5680

Lacombe, O.; Videau, O.; Chevillon, D.; Guyot, A.C.; Contreras, C.; Blondel, S.; Nicolas, L.; Ghettas, A.; Benech, H.; Thevenot, E.; Pruvost, A.; Bolze, S.; Kraczkowski, L.; Prevost, C. & Mabondzo, A. (2011). In-Vitro Primary Human and Animal Cell-Based Blood-Brain Barrier Models as a Screening Tool in Drug Discovery. *Mol. Pharm.* (March 2011), ISSN 1543-8384

Larson, D.M.; Carson, M.P. & Haudenschild, C.C. (1987). Junctional transfer of small molecules in cultured bovine brain microvascular endothelial cells and pericytes. *Microvasc. Res.*, Vol.34, No.2, (September 1987), pp. 184-199, ISSN 0026-2862

Lechardeur, D. & Scherman, D. (1995). Functional expression of the P-glycoprotein mdr in primary cultures of bovine cerebral capillary endothelial cells. *Cell Biol. Toxicol.*, Vol.11, No.5, (October 1995), pp. 283-293, ISSN 0742-2091

Lechardeur, D.; Schwartz, B.; Paulin, D. & Scherman, D. (1995). Induction of blood-brain barrier differentiation in a rat brain-derived endothelial cell line. *Exp. Cell Res.*, Vol.220, No.1, (September 1995), pp. 161-170, ISSN 0014-4827

Lee, E.S.; Gao, Z. & Bae, Y.H. (2008). Recent progress in tumor pH targeting nanotechnology. *J. Control Release*, Vol.132, No.3, (December 2008), pp. 164-170, ISSN 0168-3659

Lee, J.S.; Murphy, W.K.; Glisson, B.S.; Dhingra, H.M.; Holoye, P.Y. & Hong, W.K. (1989). Primary chemotherapy of brain metastasis in small-cell lung cancer. *J. Clin. Oncol.*, Vol.7, No.7, (July 1989), pp. 916-922,
Blood-Brain Barrier and Effectiveness of Therapy Against Brain Tumors

Leggas, M.; Adachi, M.; Scheffer, G.L.; Sun, D.; Wielinga, P.; Du, G.; Mercer, K.E.; Zhuang, Y.; Panetta, J.C.; Johnston, B.; Scheper, R.J.; Stewart, C.F. & Schuetz, J.D. (2004). Mrp4 confers resistance to topotecan and protects the brain from chemotherapy. *Mol.Cell Biol.*, Vol.24, No.17, (September 2004), pp. 7612-7621, ISSN 0270-7306

Muruganandam, A.; Herx, L.M.; Monette, R.; Durkin, J.P. & Stanimirovic, D.B. (1997). Development of immortalized human cerebromicrovascular endothelial cell line as an in vitro model of the human blood-brain barrier. *FASEB J.*, Vol.11, No.13, (November 1997), pp. 1187-1197, ISSN 0892-6638

Ohtsuki, S. & Terasaki, T. (2007). Contribution of carrier-mediated transport systems to the blood-brain barrier as a supporting and protecting interface for the brain; importance for CNS drug discovery and development. *Pharm.Res.*, Vol.24, No.9, (September 2007), pp. 1745-1758, ISSN 0253-6269

Omidi, Y. & Barar, J. (2012). Impacts of blood-brain barrier in drug delivery and targeting of brain tumours. *BioImpacts*, Vol.2, No.1, (2012), pp. in press, ISSN 2228-5652

Omidi, Y.; Barar, J.; Ahmadian, S.; Heidari, H.R. & Gumbleton, M. (2008). Characterisation and astrocytic modulation of system L transporters in brain microvasculature endothelial cells. *Cell Biochem.Funct.*, Vol.26, No.3, (2008), pp. 381-391, ISSN 0263-6484

Omidi, Y. and Gumbleton, M. (2005). Biological Membranes and Barriers, In: *Biomaterials for Delivery and Targeting of Proteins Nucleic Acids*, Mahato, R. I., pp. 232-274, CRC Press, ISBN 9-780-84932334-8, New York

Orte, C.; Lawrenson, J.G.; Finn, T.M.; Reid, A.R. & Allt, G. (1999). A comparison of blood-brain barrier and blood-nerve barrier endothelial cell markers. *Anat.Embryol.(Berl)*, Vol.199, No.6, (June 1999), pp. 509-517, ISSN 0340-2061

Pardridge, W.M. (1999). Blood-brain barrier biology and methodology. *J.Neurovirol.*, Vol.5, No.6, (December 1999), pp. 556-569, ISSN 1355-0284

Pluta, R.; Misicka, A.; Barcikowska, M.; Spisacka, S.; Lipkowski, A.W. & Januszewski, S. (2000). Possible reverse transport of beta-amyloid peptide across the blood-brain barrier. *Acta Neurochir.Suppl*, Vol.76 (2000), pp. 73-77, ISSN 0001-6268

Provenzale, J.M.; Mukundan, S. & Dewhirst, M. (2005). The role of blood-brain barrier permeability in brain tumor imaging and therapeutics. *AJR Am.J.Roentgenol.*, Vol.185, No.3, (September 2005), pp. 763-767, ISSN 0361-803X

Prudhomme, J.G.; Sherman, I.W.; Land, K.M.; Moses, A.V.; Stenglein, S. & Nelson, J.A. (1996). Studies of Plasmodium falciparum cytoadherence using immortalized human brain capillary endothelial cells. *Int.J.Parasitol.*, Vol.26, No.6, (June 1996), pp. 647-655, ISSN 0020-7519

Ramsauer, M.; Krause, D. & Dermietzel, R. (2002). Angiogenesis of the blood-brain barrier in vitro and the function of cerebral pericytes. *FASEB J.*, Vol.16, No.10, (August 2002), pp. 1274-1276, ISSN 0892-6638

Reese, T.S. & Karnovsky, M.J. (1967). Fine structural localization of a blood-brain barrier to exogenous peroxidase. *J.Cell Biol.*, Vol.34, No.1, (July 1967), pp. 207-217, ISSN 0021-9525

www.intechopen.com
Ribeiro, M.M.; Castanho, M.A. & Serrano, I. (2010). In vitro blood-brain barrier models--latest advances and therapeutic applications in a chronological perspective. *Mini.Rev.Med.Chem.*, Vol.10, No.3, (March 2010), pp. 262-270, ISSN 1389-5575

Rist, R.J.; Romero, I.A.; Chan, M.W.; Couraud, P.O.; Roux, F. & Abbott, N.J. (1997). F-actin cytoskeleton and sucrose permeability of immortalized rat brain microvascular endothelial cell monolayers: effects of cyclic AMP and astrocytic factors. *Brain Res.*, Vol.768, No.1-2, (September 1997), pp. 10-18, ISSN 0006-8993

Robert, A.M. & Robert, L. (1998). Extracellular matrix and blood-brain barrier function. *Pathol.Biol.(Paris)*, Vol.46, No.7, (September 1998), pp. 535-542, ISSN 0369-8114

Roger, M.; Clavreul, A.; Venier-Julienne, M.C.; Passirani, C.; Sindji, L.; Schiller, P.; Montero-Menei, C. & Menei, P. (2010). Mesenchymal stem cells as cellular vehicles for delivery of nanoparticles to brain tumors. *Biomaterials*, Vol.31, No.32, (November 2010), pp. 8393-8401, ISSN 0142-9612

Roux, F.; Durieu-Trautmann, O.; Chaverot, N.; Claire, M.; Mailly, P.; Bourre, J.M.; Strosberg, A.D. & Couraud, P.O. (1994). Regulation of gamma-glutamyl transpeptidase and alkaline phosphatase activities in immortalized rat brain microvessel endothelial cells. *J.Cell Physiol*, Vol.159, No.1, (April 1994), pp. 101-113, ISSN 0021-9541

Rubin, L.L. & Staddon, J.M. (1999). The cell biology of the blood-brain barrier. *Annu.Rev.Neurosci.*, Vol.22 (1999), pp. 11-28, ISSN 0147-006X

Schinkel, A.H.; Mol, C.A.; Wagenaar, E.; van, D.L.; Smit, J.J. & Borst, P. (1995a). Multidrug resistance and the role of P-glycoprotein knockout mice. *Eur.J.Cancer*, Vol.31A, No.7-8, (July 1995a), pp. 1295-1298, ISSN 0959-8049

Schinkel, A.H.; Wagenaar, E.; van, D.L.; Mol, C.A. & Borst, P. (1995b). Absence of the mdr1a P-Glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethasone, digoxin, and cyclosporin A. *J.Clin.Invest*, Vol.96, No.4, (October 1995b), pp. 1698-1705, ISSN 0021-9738

Shivers, R.R.; Arthur, F.E. & Bowman, P.D. (1988). Induction of gap junctions and brain endothelium-like tight junctions in cultured bovine endothelial cells: local control of cell specialization. *J.Submicrosc.Cytol.Pathol.*, Vol.20, No.1, (January 1988), pp. 1-14, ISSN 1122-9497

Smith, M.W. & Gumbleton, M. (2006). Endocytosis at the blood-brain barrier: from basic understanding to drug delivery strategies. *J.Drug Target*, Vol.14, No.4, (May 2006), pp. 191-214, ISSN 1061-186X

Stanness, K.A.; Guatteo, E. & Janigro, D. (1996). A dynamic model of the blood-brain barrier "in vitro". *Neurotoxicology*, Vol.17, No.2, (1996), pp. 481-496, ISSN 0161-813X

Stanness, K.A.; Neumaier, J.F.; Sexton, T.J.; Grant, G.A.; Emmi, A.; Maris, D.O. & Janigro, D. (1999). A new model of the blood--brain barrier: co-culture of neuronal, endothelial and glial cells under dynamic conditions. *Neuroreport*, Vol.10, No.18, (December 1999), pp. 3725-3731, ISSN 0959-4965

Stewart, P.A. & Wiley, M.J. (1981). Developing nervous tissue induces formation of blood-brain barrier characteristics in invading endothelial cells: a study using quail-chick transplantation chimeras. *Dev.Biol.*, Vol.84, No.1, (May 1981), pp. 183-192, ISSN 0012-1606
Taschner, C.A.; Wetzel, S.G.; Tolnay, M.; Froehlich, J.; Merlo, A. & Radue, E.W. (2005). Characteristics of ultrasmall superparamagnetic iron oxides in patients with brain tumors. AJR Am. J. Roentgenol., Vol.185, No.6, (December 2005), pp. 1477-1486, ISSN 0361-803X

Tilling, T.; Korte, D.; Hoheisel, D. & Galla, H.J. (1998). Basement membrane proteins influence brain capillary endothelial barrier function in vitro. J. Neurochem., Vol.71, No.3, (September 1998), pp. 1151-1157, ISSN 0022-3042

Tio, S.; Deenen, M. & Marani, E. (1990). Astrocyte-mediated induction of alkaline phosphatase activity in human umbilical cord vein endothelium: an in vitro model. Eur. J. Morphol., Vol.28, No.2-4, (1990), pp. 289-300, ISSN 0924-3860

Tsuji, A. (2005). Small molecular drug transfer across the blood-brain barrier via carrier-mediated transport systems. NeuroRx., Vol.2, No.1, (January 2005), pp. 54-62, ISSN 1545-5343

Urquhart, B.L. & Kim, R.B. (2009). Blood-brain barrier transporters and response to CNS-active drugs. Eur. J. Clin. Pharmacol., Vol.65, No.11, (November 2009), pp. 1063-1070, ISSN 0031-6970

Veiseh, O.; Sun, C.; Fang, C.; Bhattacharai, N.; Gunn, J.; Kievit, F.; Du, K.; Pullar, B.; Lee, D.; Ellenbogen, R.G.; Olson, J. & Zhang, M. (2009). Specific targeting of brain tumors with an optical/magnetic resonance imaging nanoprobe across the blood-brain barrier. Cancer Res., Vol.69, No.15, (August 2009), pp. 6200-6207, ISSN 0008-5472

Wakui, S.; Furusato, M.; Hasumura, M.; Hori, M.; Takahashi, H.; Kano, Y. & Ushigome, S. (1989). Two- and three-dimensional ultrastructure of endothelium and pericyte interdigitations in capillary of human granulation tissue. J. Electron Microsc. (Tokyo), Vol.38, No.2, (1989), pp. 136-142, ISSN 0022-0744

Wang, P.; Xue, Y.; Shang, X. & Liu, Y. (2010). Diphtheria toxin mutant CRM197-mediated transcytosis across blood-brain barrier in vitro. Cell Mol. Neurobiol., Vol.30, No.5, (July 2010), pp. 717-725, ISSN 1044-7431

Wang, W.; Dentler, W.L. & Borchardt, R.T. (2001). VEGF increases BMEC monolayer permeability by affecting occludin expression and tight junction assembly. Am. J. Physiol Heart Circ. Physiol., Vol.280, No.1, (January 2001), pp. H434-H440, ISSN 0363-6135

Wolburg, H. (2006). The Endothelial Frontier, In: Blood-Brain Barriers, Dermietzel, R., Spray, D. C., and Nedergaard, M., pp. 77-108, WILEY-VCH Verlag GmbH & Co. KGaA, ISBN 3-527-31088-6, Weinheim

Wolburg, H.; Neuhaus, J.; Kniesel, U.; Krauss, B.; Schmid, E.M.; Ocalan, M.; Farrell, C. & Risau, W. (1994). Modulation of tight junction structure in blood-brain barrier endothelial cells. Effects of tissue culture, second messengers and cocultured astrocytes. J. Cell Sci., Vol.107 (Pt 5) (May 1994), pp. 1347-1357, ISSN 0021-9533

Wu, G.; Barth, R.F.; Yang, W.; Kawabata, S.; Zhang, L. & Green-Church, K. (2006). Targeted delivery of methotrexate to epidermal growth factor receptor-positive brain tumors by means of cetuximab (IMC-C225) dendrimer bioconjugates. Mol. Cancer Ther., Vol.5, No.1, (January 2006), pp. 52-59, ISSN 1535-7163
Yu, C.; Kastin, A.J.; Tu, H.; Waters, S. & Pan, W. (2007). TNF activates P-glycoprotein in cerebral microvascular endothelial cells. *Cell Physiol Biochem.*, Vol.20, No.6, (2007), pp. 853-858, ISSN 1015-8987
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