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Various *in vitro* models to assess drug permeability across the blood brain barrier

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ABSTRACT

Delivery of drugs to the brain is still a major challenge. Much research has focused on the development of novel therapeutic agents to target various central nervous system disorders, however less attention has been given to determine the potential of such agents to permeate the Blood Brain Barrier (BBB). Inorder to assess the potential for novel compounds to permeate the BBB, various invitro, invivo and insilico methods may be employed. Although invitro models (such as primary cell culture and immortalized cell lines) are useful as a screening method and can appropriately rank compounds inorder of BBB permeability, they often correlate poorly to invivo brain uptake due to down regulation of some BBB specific transporters. This paper reviews current invitro models used for assessing BBB permeability.

Key words: Blood brain barrier, *Invitro* Models, Tissue culture, brain endothelial cells and artificial membranes

INTRODUCTION

The Blood Brain Barrier represents a formidable obstacle for a large number of drugs, including anti cancer agents, peptides and nucleic acids. As a consequence this barrier presents effective treatment for many severe and life threatening diseases such as Brain tumours, Alziemours disease, Parkinsons disease and other neurological disorders[1]. Targeting drug to this organ is really a challenging tasks were it has to convince a variety of constraints. Thus a clear and indepth knowledge of anatomy of brain would be required to design a proper drug delivery system.In order to cater the research of brain targeting it is the need of the hour to design proper *invitro* models which could simulate brian *invivo* mileu. The whole *invivo* testing protocols invovlve various constraint such as time factor , animal species, psychological status of the animal, drug properties etc. This would potentially reduce the number of drugs towards screening for brain delivery. In this shortlist candidate there are chance for a potential drug for *invivo* study. Thus less time consuming and technicall based *invitro* study would be a right chance for proper design of drug delivery. This review article covers some updated *invitro* study for brain delivery which would potentiate the research activities.

Blood brain barrier

The blood brain barrier in its original meaning is formed by a complex of endothelial cells,astroglia and pericytes as well as basel lamina interconnecting the cellular system[2]. The structural basis of this barrier consists of endothelial cells with tight junctions as special features that seal the intercellular cleft[3]. Astrocytes, pericytes and extracellular matrix(ECM) components are believed

to control the integrity of this barrier. Most of these barrier properties characteristic of brain capillary endothelial cells are partly induced and maintained by the close association of astrocytic foot processes with the endothelium[4]. In addition to its physical barrier properties the blood brain barrier is considered to be a metabolic barrier[5] while immunological properties are displayed by endothelial cells together with the surface molecules, which play a key role in pathological conditions such as inflammation , tumour,angiogenesis and wound healing.

The permeability of most compounds through BBB to gain assessment into brain is possible for compounds to circumvent the BBB and still reach the brain parenchyma. One such mechanism is nose to brain route, where a compound may be directly transported to the brain via an olfactory pathway following absorption across the nasal mucosa[6]. Alternatively compounds may permeate from the blood into the cerebrospinal fluid (CSF) and subsequently permeate into the brain interstitial fluid. However, transport into the CSF is controlled by the choroids plexus(the epithelial barrier separating the blood from the CSF),and the capillaries perfusing the choroids plexus are quite porous, allowing normal access of compounds into the CSF[7]. Therefore transport across the choroids plexus is not an accurate measure of transport across the BBB .As these barriers are anatomically different even if a compound enters the CSF, its availability in the brain interstitial fluid (ISF) should not be assumed, since a functional barrier between these compartments exists resulting in the difference between the bulk flow properties of CSF through the CSF flow tracks and diffusional flow rates in the brain parenchyma[8].

A summary of the invitro methods, together with their inherent advantages and disadvantages is presented in this review, with a particular focus on methods that are suitable for moderate to high throughout screening of potential CNS drug candidates.

***Invitro* models**

The advantages associated with any invitro BBB model include lower compound requirement, the ability to assay compound directly in physiological buffer, greater throughout relative to invivo models, ability to assess transport mechanisms, the identification of early signs of cell toxicity and generally lower cost. However, inorder to appropriately mimic the BBB invivo there are some basic characteristics that an invitro model must possess. The invitro model that is chosen should possess as many of these characteristics as possible, while at the same time remaining practical and feasible for moderate to high throughout screening.

Isolated brain capillaries

It has been possible to isolated brain capillaries from various animal sources, however these are not well suited for permeability screening purposes. This is because of the inability to assess the luminal surface of the isolated micro vessels and consequently, only drug loss from the albuminal (brain) compartment can be monitored. This involves aseptic separation of precise brain capillaries which involves expertise hands .These capillaries can be used as an invitro model to test the drug delivery to brain which would face the same scenario of tight junctions.

Primary or low passage brain capillary endothelial cell cultures

Primary or low passaged brain capillary endothelial cell cultures provide the closest phenotypic resemblance to the invivo BBB phenotype[8]. Although some features such as BBB transporters and enzymes, can be down regulated when the endothelial cells are removed from the brain and grown in culture[9]. These capillary endothelial cells can be obtained from bovine, porcine, rat or human sources, although most researchers use bovine or porcine endothelial cells for the purposes of assessing drug transport because of the availability of such cells.

Bovine Brain Endothelial Cell Culture

The original bovine brain endothelial cell culture (BBEC) model was developed by Audus and Borchardt(1986).To obtain BBEC's,the grey matter of bovine, the bovine brain is isolated and treated either mechanically or enzymatically to yield around 100 million viable cells[10] (from the grey matter of two bovine brains).Once isolated the cells can be stored at -80°C for upto 2months. The BBEC's grown

as primary cultures on standard plates or inserts (transwell), must first be treated with rat tail collagen to improve plating efficiency[11]. Once cells have reached confluence, the permeability of compounds can be assessed in both the apical-to-basolateral and basolateral-to-apical directions and general mechanisms of transport can be elucidated due to the number of cells available, such an approach is well suited to high throughout compound screening.

The most common approach to improve the barrier properties of BBEC cultures is to co-culture the endothelial cells with primary astrocytes isolated from rats. This approach has been shown to maintain the characteristics of the BBB without the use of stimulants, in addition to upregulate p-gp function[12] and significantly increasing transendothelial electrical resistance values. Since astrocytic foot processes invest more than 99% of the cerebral vasculature invivo and many features of the BBB invivo are induced by astrocytes[13]. It is not surprising that a more restrictive invitro BBB model results from the co-culturing of BBEC's with astrocytes. The major limitation associated with this co-culturing technique is that in addition to maintain the growth of BBEC's, there is necessity to grow and culture rat astrocytes. Alternatively C^6 glioma cells may be co-cultured with BBEC's which reduces the need to isolate and culture rat astrocytes. The major disadvantage of C^6 glioma cells is that they may result in a tumour like BBB rather than healthy BBB[(14)] which may lead to poor correlation in brain uptake in a healthy individual. This approach using C^6 glioma cells has been shown to increase the endothelial electric resistance by 75% and reduce sucrose permeability by 50% [15].

Porcine brain endothelial cell culture

Although most research has focused on the development and characterization of BBEC cultures as *invitro* models for BBB, recent studies have shown that porcine brain endothelial cells may also serve as an appropriate model[15]. Some researches have also co-cultured porcine brain endothelial cells with astrocytes in order to improve the restrictiveness of the culture system[17]. However further validation particularly with respect to invitro-invivo correlation may be required before this model becomes extensively utilized for the purposes of high throughout compound screening.

Immortalized brain endothelial cells

Due to problems associated with harvesting and maintaining primary cell cultures, various immortalized cell lines have been developed, most of which are derived from rats. All of these cell lines have one major disadvantage is that although they do form monolayers, they do not form complete tight junctions resulting in a leaky barrier[18,19]. Some of the cell lines have been generated from primary rat endothelial cells include the $RBE_{4,}$ $RBEC_1$ cell line and TR-BBB 13[21,20] cell line. However, the resulting brain endothelial electric resistance values of these cell lines are still fairly low and are therefore not appropriate for BBB permeability screening but more suited to assessing endothelial cell uptake of compounds.

Cells of non-cerebral origin

Because of the insufficient barrier properties of immortalized brain endothelial cell lines some researches have focused on using non-cerebral peripheral epithelial cell lines. One such cell line is the Madin-Darby Canine Kidney(MDCK) cell line, which is easy to grow and can be transfected with the multidrug rresistance gene(MDRI),resulting in the polarized expression of p-gp[22]. This transfected cell line has been used to assess the effect of p-gp on the permeability of various compounds through the BBB[23], and a recent collaborative study found that MDRI transfected MDCK cells were the most representative of *invivo* BBB permeability compared with other *invitro* models including BBEC/astrocytes, human brain endothelial cells/astrocytes and caco-2 cell lines[(24)] . MDRI-transfected MDCK cells have also shown high absorption transport for CNS-positive drugs and low absorption transport for CNS-negative drugs and so may be a suitable model for BBB permeation.

Immobilized artificial membranes

Immobilized artificial membranes are a solid phase model of fluid membranes that have been proposed as an alternative for assessing drug permeability through cell membranes[25,26]. These membranes which are used in a chromatography, consist of phosphotidyl choline residues covalently bound to silica propylamine and mimic a membrane lipid bilayer. There has been some work in attempting to correlate immobilized artificial membrane

retention to brain penetration, however it is only useful for compounds that permeate the BBB via passive mechanisms. In one study, the brain uptake of 26 drugs(basic,neutral and acidic) appeared to correlate markedly to the immobilized artificial membrane retention factors, although an improvement in regression was observed when the effects of ionization and solute size were taken into account[27]. While this method may be useful for predicting solute portioning into membranes,it doesn't mimic diffusion across a membrane and can have poor predictive power when brain uptake is affected by plasma protein binding, active transport, active efflux or metabolism.

CONCLUSION

Various invitro models are available that can be used to assess drug penetration across the BBB. Such model has its disadvantages and there needs to be a compromise between potential and the limitations associated with the chosen model. However inorder to fully assess the brain uptake of new chemical entity and to completely understand the mechanism involved in allowing or hindering BBB transport one should employ both *invitro* and *invivo* techniques and not rely solely on one method of screening.

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