REVIEWS

In Vivo, In Vitro and *In Silico* Methods for Small Molecule Transfer Across the BBB

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ABSTRACT: The inability of molecules to permeate the BBB is a significant source of attrition in Central Nervous System (CNS) drug discovery. Given the increasing medical drivers for new and improved CNS drugs, small molecule transfer across the BBB is attracting a heightened awareness within pharmaceutical industry and medical fields. In order to assess the potential for small CNS molecules to permeate the BBB, a variety of methods and models, from *in silico* to *in vivo* going through *in vitro* models are developed as predictive tools in drug discovery. This review gives a comprehensive overview of different approaches currently considered in drug discovery to circumvent the lack of small molecule transfer through the BBB, together with their inherent advantages and disadvantages. Particularly, special attention is drawn to *in silico* models, with a detailed and contemporary point of view on prediction tools and guidelines for rational design. © 2009 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 98:4429–4468, 2009

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INTRODUCTION

The blood–brain barrier (BBB) is a specialized system of capillary endothelial cells that protects the brain from harmful substances such as toxins and viruses circulating in the blood stream (Fig. 1). Moreover, this barrier also keeps out many would-be central nervous system (CNS) therapeutic agents,[1] while supplying the brain with the required nutrients for proper function.

Unlike endothelial cells in the systemic (peripheral) circulation, a minimal level of pinocytosis and lack of membrane fenestrations characterizes the endothelial cells forming the BBB.[2,3] Due to this restriction in the paracellular (tight junctional)

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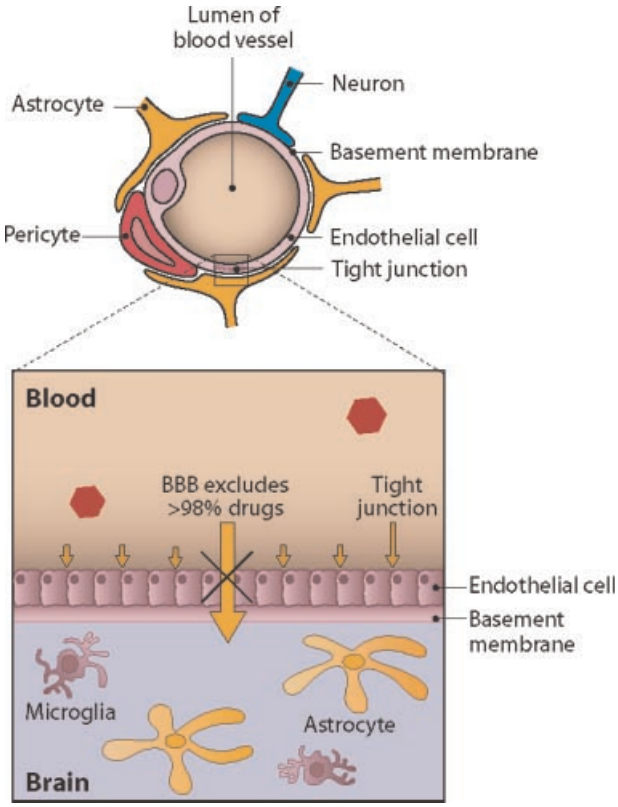

Figure 1. Schematic representation of the blood–brain barrier (modified from Francis et al.[316]).

pathway, exchange between the blood and the brain is dominated by the transcellular route, making the endothelial cells the "gatekeepers" of the brain. Therefore passive diffusion through the BBB is the primary process of translocation from the blood stream to the brain for the large majority of therapeutic compounds. Furthermore, the BBB includes an enzymatic barrier at the cerebral endothelia, capable of metabolizing drug and nutrients.[4–6] Enzymes such as glutamyl transpeptidase (GTP), alkaline phosphatase, and aromatic acid decarboxylase are present at elevated concentration in cerebral microvessels, yet often in low concentration or absent in non-neuronal capillaries. The BBB also expresses various efflux transporters that are involved in another significant transport mechanism: carrier mediated efflux. By this mechanism, drugs are extruded from the brain with the ABC transporter P-glycoprotein (P-gp) being the principle efflux mechanism.[7–12] Other efflux transporters at the level of the BBB are the multidrug resistance proteins (MRP)[13,14] and breast cancer resistant proteins (BCRP).[15–17] Although it is assumed that passive diffusion through the BBB is the most important permeability process, more and more scientists support the idea that carrier-mediated transport and active influx/efflux of drugs may be more important then is generally assumed.[18–20] However much work is still needed to fully characterize the drug transporters at the BBB before a reconsideration of the present view on transport to the brain can be suggested.

CNS-targeting drugs need to cross the BBB in order to reach their therapeutic receptors inside the brain. Over 98% of small molecules intended for therapeutic use in the CNS never reach the market because of their inherent inability to cross the BBB.[21,22] The BBB effectively restricts delivery of valuable pharmaceuticals to the brain thereby presenting major therapeutic limitations toward the treatment of CNS diseases.[23,24] It is reported that 12% of all drugs are active in the CNS, but only 1% of all drugs are active in the brain for diseases other than affective disorders.[25] However, diagnostic imaging agents, anti-infective, antiviral and anticancer drugs targeting the brain are, nowadays, an unmet medical need. For example, cerebral metastases are clinical significant in 10–30% of patients with neoplasia.[26] Successful treatment has been limited by difficulties in delivering therapeutic agents to the central nervous system. Specifically, drug penetration of the blood–brain barrier (BBB) poses a unique and challenging problem in brain tumors therapy.[27] In addition, the trend toward increasing life expectancy emphasizes the need to develop new drugs for age-related neurodegenerative conditions such as Alzheimer and Parkinson's disease.

The entry of a CNS drug candidate from the blood stream into the brain depends on many physicochemical factors, including lipophilicity, total polar surface area (TPSA); charge state, molecular size, flexibility and hydrogen-bonding potential. Further confounding factors include plasma protein binding, active uptake into the CNS and efflux out of the CNS. In today's drug discovery research, many models are used to assess the transport characteristics of drug candidates across the BBB, including *in silico*, *in vitro*, and *in vivo* methods.

The recent developments of combinatorial chemistry call for systems that can be used for high throughput screening. Given the increasing number of compounds to test in early discovery, costly and labor-intensive *in vivo* measurements and traditional, low throughput, *in vitro* assays of