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Invited Review-pharmacology across disciplines

Zebrafish: A promising *in vivo* model for assessing the delivery of natural products, fluorescence dyes and drugs across the blood-brain barrier

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ABSTRACT

The blood brain barrier (BBB) is the network of capillaries that controls the passage of substances from the blood into the brain and other parts of the central nervous system (CNS). As this barrier is the major obstacle for drug delivery into CNS, a credible BBB model is very necessary to assess the BBB permeability of novel neuroactive compounds including thousands of bioactive compounds which have been extracted from medicinal plants and have the potential for the treatment of CNS diseases. Increasing reports indicated that zebrafish has emerged as a timely, reproducible model for BBB permeability assessment. In this review, the development and functions of the BBB in zebrafish, such as its anatomical morphology, tight junctions, drug transporters and enzyme expression, are compared with those in mammals. The studies outlined in this review describe the utilization of the zebrafish as a BBB model to investigate the permeability and distribution of fluorescent dyes and drugs. Particularly, this review focuses on the use of zebrafish to evaluate the delivery of natural products and nanosized drug delivery systems across the BBB. Due to the highly conserved nature of both the structure and function of the BBB between zebrafish and mammals, zebrafish has the potential to be developed as a model for assessing and predicting the permeability of BBB to novel compounds.

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Abbreviations: BBB, blood brain barrier; CNS, central nervous system; PD, Parkinson's disease; BBMEC, bovine brain microvessel endothelial cells; HBMEC, human brain microvascular endothelial cells; TEER, transendothelial electrical resistance; JAMs, junctional adhesion molecules; TJs, tight junctions; CYP, cytochrome P450; UDP, uridine diphosphate; UGT, UDP-glucuronosyltransferases; ABC, ATP-binding cassette; MDR1, multidrug resistance protein 1; P-gp, P-glycoprotein; BCRP, breast cancer resistance protein; MRPs, multidrug resistance-associated proteins; hpf, hours post fertilization; EGFP, enhanced green fluorescent protein; CtA, central arteries; dpf, days post fertilization; TEM, transmission electron microscopy; GFAP, glial fibrillary acidic protein; GFP, green fluorescent protein; MAGAK, membrane associated guanylate kinases; BECs, brain endothelial cells; R123, rhodamine 123; Rho-HRP, rhodamine conjugated horseradish peroxidase; FI, fluorescence intensity; CVO, circum ventricular organ; GSTs, glutathione-S-transferase; FD4, fluorescein isothiocyanate-dextran; HRP, horseradish peroxidase; VOLT, vascular organ of the lamina terminalis; ACeV, anterior cerebral vein; PrA, prosencephalic artery; MsV, mesencephalic vein; PHBC, primordial hindbrain channel; MtA, metencephalic artery; MCeV, middle cerebral vein; MaA, mesencephalic artery; PMsA, primitive mesencephalic artery; IDs, indoline derivatives; OT, optic tectum; CBV, cerebral blood vessels; PTZ, pentylenetetrazole; PNIPAM, poly N-isopropyl acrylamide; AD, Alzheimer's disease; C-QDs, carbon quantum dots.

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1. Introduction

More than 1.5 billion people worldwide are suffering from central nervous system (CNS) disorders [1], but few effective therapeutic drugs are used clinically [2]. Natural products have attracted considerable attention for their neuroprotective and therapeutic effects in the treatment of CNS diseases [3]. The neuroprotective activity of bioactive compounds from herbal medicines including ginsenoside Rg1, baicalein, curcumin, gastrodin, resveratrol, tenuigenin, peurarin, quercetin, and schisantherin A were demonstrated by cellular or animal models [4–6]. However, efficient delivery of compounds to the brain remains a major challenge in the discovery and development of new drugs for the treatment of CNS diseases [7,8]. The blood-brain barrier (BBB) is the major barrier to restrict the delivery of compounds into the CNS [9]. Compared with peripheral capillaries that are permeable to small molecules (sometimes even to large molecules, such as proteins), the BBB more strictly limits the penetration of nearly all exogenous chemicals into to the brain by physical and metabolic mechanisms [10]. Essentially, 100% of large-molecules and over 98% of small molecules that should be useful in CNS therapy cannot reach their target because of their poor permeability across the BBB [11]. In the process of developing CNS drugs, appropriate BBB models are essential for assessing and predicting permeability of candidate compounds across the BBB. We can also obtain valuable information about the transport mechanisms and optimize the drug delivery systems for efficient delivery across the BBB [9]. Although the concept of the BBB has been continuously refined for more than a century, an ideal BBB model for drug screening has not yet to be established. As with any model, each comes with its own strengths and weaknesses with cost and reliability [12]. Currently, mice are the most commonly used *in vivo* animal model because the cell types, transporters and restrained permeability properties are similar to those of the human BBB [13,14]. However, animal model is very expensive and time-consuming. *In vitro* cell models are widely used to study BBB properties and have recently become more sophisticated at some level [15–17]. Many cell-based models such as brain endothelial cell lines have been well developed and widely used for bioactivity and mechanistic studies, and some non-cell-based permeability assay such as parallel artificial membrane permeability assay are also developed for high throughput screening of drug permeability [18,19]. Although cell culture models can be utilized quite effectively for screening drug permeability across the BBB, they also have limitations such as downregulated transport systems or enzymes. MDCKII cells have been used for modeling BBB, but they lack BBB transporters and are derived from canine renal epithelial cells, which limits their use in screening BBB-permeable compounds. Another widely used model is bovine brain microvessel endothelial cells (BBMECs), which are isolated from fresh cow brains by enzymatic digestion and density-gradient ultracentrifugation and subsequently grown for up to 14 days [20]. The preparation is complex, and this cell line can only be used once, which is not suitable for large scale screening. Alternatively, immortalized human brain capillary endothelial cells can be used. Human brain microvascular endothelial cells (HBMECs), a type of immortalized brain capillary endothelial cells, could proliferate indefinitely and preserve their differentiating properties after repeated passages. Three major components of adherents and tight endothelial junctions have been detected in this cell line. However, the transendothelial electrical resistance (TEER) values of the monolayers was very low compare to the *in vivo* conditions [21].

In vitro models and *in vivo* animal models have their own disadvantages like unreliable and low-throughput screening. A BBB model which could bridge the gap between *in vitro* models and traditional animal models is required for the CNS drug discovery and development. Accumulating evidence shows similarities between the zebrafish and the mammalian BBB. The zebrafish model could incorporate advantages of animal and cell models and may be more suitable for drug development for CNS diseases. In this article, we will review the BBB structure and function of zebrafish, compare with those of mammals, including human, rat and mouse, and later discuss the potential applicability of zebrafish as an *in vivo* BBB model.

2. Blood-brain barrier

The properties of the BBB are important considerations when developing drugs for CNS diseases. The BBB was discovered in the 1880s by Paul Ehrlich and Edwin Goldman. They observed that the water soluble dyes did not stain the brain when they were injected into the peripheral circulation instead of injecting directly into the brain ventricles [22]. We now understand that the BBB is a selective endothelial interface that controls trafficking between the bloodstream and brain interstitial space [23]. It allows water, oxygen and most lipid-soluble molecules to diffuse freely from the blood to the brain tissue. It is also slightly permeable to ions such as Na^+, K^+, and Cl^- that contribute to ion homeostasis for optimal neural signaling. However, proteins, toxins and most water-soluble compounds with pharmacological activities are excluded by the BBB. Thus, this barrier is very important in regulating drug permeability to the brain [9].

The BBB is composed of cerebral endothelial cells which are lining on the endoluminal surface of the capillaries. Different from the endothelial cells in the rest of the body, these cells in BBB exhibit a specialized phenotype of tight junctions without fenestrations or sparse pinocytotic vesicular transport [24]. The surface of capillaries is covered with pericytes. A unique bilayer basement membrane surrounds the endothelial cells and the pericytes, outer surface of which is then surrounded by protoplasmic astrocytes with protrusions (end feet). This close cellular association is also important in inducing and maintaining the barrier properties [25]. Proteins that span the intercellular cleft such as occludin, claudins and junctional adhesion molecules (JAMs) constitute the tight junctions (TJs) [26]. TJs between the cerebral endothelial cells is the key mechanism for the severe restriction of passage of ions and molecules through the paracellular diffusional pathway between the endothelial cells and effectively block the penetration of