Available online at www.sciencedirect.com
SciVerse ScienceDirect
www.elsevier.com/locate/brainres

Research Report

A detailed method for preparation of a functional and flexible blood–brain barrier model using porcine brain endothelial cells

Adjanie Patabendige[a,*,1], *Robert A. Skinner*[b], *Louise Morgan*[c,2], *N. Joan Abbott*[a]

[a]*Institute of Pharmaceutical Science, Franklin Wilkins Building, King's College London, London, UK*
[b]*Faculty of Life Sciences, University of Manchester, Manchester, UK*
[c]*Eisai Limited, European Knowledge Centre, Hatfield, UK*

ARTICLE INFO

Article history:
Accepted 9 April 2013
Available online 17 April 2013

Keywords:
Blood–brain barrier
Brain endothelium
In vitro model
Transendothelial electrical resistance
Tight junction
Permeability

ABSTRACT

The blood–brain barrier (BBB) is formed by the endothelial cells of cerebral microvessels and forms the critical interface regulating molecular flux between blood and brain. It contributes to homoeostasis of the microenvironment of the central nervous system and protection from pathogens and toxins. Key features of the BBB phenotype are presence of complex intercellular tight junctions giving a high transendothelial electrical resistance (TEER), and strongly polarised (apical:basal) localisation of transporters and receptors. *In vitro* BBB models have been developed from primary culture of brain endothelial cells of several mammalian species, but most require exposure to astrocytic factors to maintain the BBB phenotype. Other limitations include complicated procedures for isolation, poor yield and batch-to-batch variability. Some immortalised brain endothelial cell models have proved useful for transport studies but most lack certain BBB features and have low TEER. We have developed an *in vitro* BBB model using primary cultured porcine brain endothelial cells (PBECs) which is relatively simple to prepare, robust, and reliably gives high TEER (mean~800 Ω cm^2); it also shows good functional expression of key tight junction proteins, transporters, receptors and enzymes. The model can be used either in monoculture, for studies of molecular flux including permeability screening, or in co-culture with astrocytes when certain specialised features (e.g. receptor-mediated transcytosis) need to be maximally expressed. It is also suitable for a range of studies of cell:cell interaction in normal physiology and in pathology. The method for isolating and growing the PBECs is given in detail to facilitate adoption of the model.

This article is part of a Special Issue entitled Companion Paper.

*Corresponding author. Fax: +44 151 7955 529.
E-mail addresses: adjanie@liverpool.ac.uk, akpadjanie@yahoo.co.uk (A. Patabendige).
[1]Current address: Institute of Infection & Global Health, Brain Infections Group, The Ronald Ross Building, University of Liverpool, 8 West Derby Street, Liverpool L69 7BE, UK.
[2]Current Address: Institute of Pharmaceutical Science, King's College London, London, UK.

0006-8993
http://dx.doi.org/10.1016/j.brainres.2013.04.006

1. Introduction

The blood–brain barrier (BBB) is formed by the endothelial cells of cerebral microvessels under the influence of associated cells of the neurovascular unit (NVU), chiefly pericytes and the end-feet of perivascular astrocytes (Abbott et al., 2006; Neuwelt et al., 2011; Wolburg et al., 2009). The BBB is the protective interface regulating molecular, ionic and cellular traffic between the blood and the central nervous system (CNS). The barrier has several key features (Abbott et al., 2010). The 'physical barrier' results from the nature of the lipid membranes and presence of particularly tight intercellular *zonulae occludentes* (tight junctions); the junctions help to segregate apical and basal membrane proteins, conferring strong cellular polarity, and significantly restrict permeability of small hydrophilic solutes through the intercellular cleft (paracellular pathway), giving rise to the high transendothelial electrical resistance (TEER) (Abbott et al., 2010; Tsukita et al., 2001; Wolburg et al., 2009). The 'transport barrier' applies to transcellular flux of small and large molecules: solute transporter proteins (SLCs) and ATP-binding cassette (ABC) efflux transporters regulate traffic of small molecules (nutrients, substrates, waste products) (Begley, 2004; Mahringer et al., 2011; Miller, 2010), while specific vesicular mechanisms regulate permeation of peptides and proteins needed by the CNS (Bickel et al., 2001; Hervé et al., 2008; Jones and Shusta, 2007). The 'enzymatic' or 'metabolic barrier' function of the BBB results from the presence of a number of ecto- and endo-enzymes including cytochrome P450s (CYPs) that add a further level of protection (Ghosh et al., 2011). Finally the 'immunological barrier' restricts and regulates the entry of circulating leucocytes, maintaining a low level immune surveillance of the CNS, and with the potential for concerted response in conditions of pathology (Greenwood et al., 2011; Hawkins and Davis, 2005; Persidsky et al., 2006; Stanimirovic and Friedman, 2012).

In vivo studies continue to provide valuable information about the physiology and pathology of the BBB and operation of the NVU; however, for detailed molecular and functional understanding, *in vitro* models can give particular additional insights (Deli et al., 2005; Naik and Cucullo, 2012). Moreover, *in vitro* models allow rapid conduct of complex experiments involving parallel manipulation of bathing media, addition of inhibitors and calculation of transport kinetics while minimising the use of animals. For studies of transendothelial flux, including drug permeability assays, it is important to use models with well-developed tight junctions (high TEER) and well preserved apical: basal polarity of transporters and receptors (Abbott et al., 2008; Deli et al., 2005; Tóth et al., 2011).

The key features of the adult BBB result from a sequence of cell:cell interactions during development between the ingrowing vessel sprouts and the associated cells of the NVU (Liebner et al., 2011). When brain microvessels are isolated from adult mammalian brain and brain endothelial cells are cultured from these vessel fragments, they retain many key features of the BBB phenotype. In 1969, Siakotos and colleagues described for the first time a method to successfully isolate bovine and human brain endothelial cells (Siakotos et al., 1969). Nearly a decade later, Panula et al. demonstrated the migration of rat brain endothelial cells from isolated capillaries. These cells were able to grow in culture and had strong alkaline phosphatase activity (Panula et al., 1978). Tontsch and Bauer (1989) simplified the culture methods for isolating murine and porcine brain endothelial cells (e.g. avoiding sieving steps, gradient centrifugations) and optimised the culture medium to increase cell yield. They also found that when proliferative factors such as endothelial cell growth supplement (ECGS) and heparin were removed from culture medium, the morphology of cells changed from spindle-shape to cobblestone phenotype. Through a series of experiments, DeBault and Cancilla gave evidence for the influence of astrocytic factors on BBB phenotype of brain endothelial cells (DeBault and Cancilla, 1980a, 1980b; DeBault, 1981). These studies led to the development of co-culture models of the BBB (Joó, 1985).

We chose to develop a porcine BBB model for several reasons: (1) A single pig brain gives a high yield of cells compared to that from rat or mouse. (2) Porcine brains are relatively easy to obtain as they are a by-product of the meat industry; there is no need to have animal breeding facilities on site to maintain a continuous supply of brain tissue. (3) Porcine brain endothelial cells (PBECs) generally retain many key features of the BBB following isolation, and the rate of loss of BBB phenotype in culture is less than for rodent or bovine BBB models (Deli et al., 2005), therefore co-culture with astrocytes is not essential to induce functional expression of tight junctions (i.e. high TEER) (Patabendige et al., this issue). (4) The porcine genome, anatomy, physiology and disease progression reflect human biology more closely than many established laboratory animals (Walters et al., 2011). (5) The availability of miniature pigs and novel porcine transgenic disease models make the pig the most suitable animal model to study human disease (Bendixen et al., 2010; Lunney, 2007). The miniature pig is now a well established 'large' mammalian model for pharmacokinetics/toxicology studies (Bode et al., 2010) and is also used for surgical studies to generate organs for xenotransplantation (Vodicka et al., 2005). Transgenic pig models have been established for studying several diseases, including Alzheimer's disease, Huntington's disease, cardiovascular disease, cystic fibrosis and diabetes mellitus (Aigner et al., 2010).

We have developed and validated a cell culture model of the BBB using PBECs with functional tight junctions (Patabendige et al., this issue). This model reliably gives high TEER (mean TEER~800 $\Omega\,cm^2$) with good expression of tight junction proteins claudin-5, occludin and ZO-1, and shows expression of functional BBB transporters (P-glycoprotein, breast cancer-resistance protein), receptors (interleukin-1 receptor) and enzymes (alkaline phosphatase) (Patabendige et al., this issue; Skinner et al., 2009). The strengths of this model are that it is relatively simple and straightforward to generate compared to other published porcine BBB models and is able to give high TEER reliability even without co-culture with astrocytes. For certain specialised studies, BBB features can be further upregulated by exposure to astrocytes or astrocyte-conditioned medium (ACM). The model has been validated in studies of basic functions of the BBB at the cellular and molecular level, screening of drug entry into brain for pharmaceutical purposes, and examination of mechanism(s) for CNS entry of 'biologicals' (large organic molecules) (Patabendige et al., this issue; Skinner et al., 2009). It is highly suitable for a range of further studies including cell:cell interaction.