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The Grasshopper: A Novel Model for Assessing Vertebrate Brain Uptake

Olga Andersson, Steen Honoré Hansen, Karin Hellman, Line Rørbæk Olsen, Gunnar Andersson, Lassina Badolo, Niels Svenstrup, and Peter Aadal Nielsen

EntomoPharm R&D, Medicon Village, Lund, Sweden (O.A., K.H., G.A., P.A.N.); Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark (S.H.H., L.R.O.); and Division of Discovery Chemistry and Drug Metabolism and Pharmacokinetics, H. Lundbeck A/S, Copenhagen, Denmark (L.B., N.S.)

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ABSTRACT

The aim of the present study was to develop a blood-brain barrier (BBB) permeability model that is applicable in the drug discovery phase. The BBB ensures proper neural function, but it restricts many drugs from entering the brain, and this complicates the development of new drugs against central nervous system diseases. Many in vitro models have been developed to predict BBB permeability, but the permeability characteristics of the human BBB are notoriously complex and hard to predict. Consequently, one single suitable BBB permeability screening model, which is generally applicable in the early drug discovery phase, does not yet exist. A new refined ex vivo insect-based BBB screening model that uses an intact, viable whole brain under controlled in vitro-like exposure conditions is presented. This model uses intact brains from desert locusts, which are placed in a well containing the compound solubilized in an insect buffer. After a limited time, the brain is removed and the compound concentration in the brain is measured by conventional liquid chromatography-mass spectrometry. The data presented here include 25 known drugs, and the data show that the ex vivo insect model can be used to measure the brain uptake over the hemolymph-brain barrier of drugs and that the brain uptake shows linear correlation with in situ perfusion data obtained in vertebrates. Moreover, this study shows that the insect ex vivo model is able to identify P-glycoprotein (Pgp) substrates, and the model allows differentiation between low-permeability compounds and compounds that are Pgp substrates.

Introduction

The vertebrate blood brain barrier (BBB) is composed of capillary endothelial cells that control the entry of nutrients and xenobiotics to the brain, and it ensures an optimal environment for proper neural function (Abbott et al., 2006, 2010). However, the BBB creates a great obstacle for the medical treatment of diseases related to the central nervous system (CNS) and is recognized as a major obstacle in the discovery of new drugs against CNS-related diseases (Geldenhuys et al., 2012). Therefore, a number of cell based in vitro models have been developed and used as tools in the drug-discovery screening process (Polli et al., 2001; Weiss et al., 2003; Mensch et al., 2009). Two commonly used systems are the renal cell lines LLC-PK1 and MDCK. Both are easy to grow, and this makes them attractive for industrial use. However, these cells are of epithelial origin, and compared with barrier endothelial cells, epithelial cells display differences in morphology, tight junction organization, and transporter expression (Garberg et al., 2005; Cecchelli et al., 2007; Abbott et al., 2008; Liu et al., 2008). Recently, we presented an insect-based BBB screening model that uses an intact whole brain under controlled in vitro–like exposure conditions (Nielsen et al., 2011). As in vertebrates, the protection of the insect CNS requires a tight brain barrier containing influx and efflux transporter proteins, which control elements entering the brain (Banerjee and Bhat, 2007; Bundgaard and Abbott, 2008; Stork et al., 2008; DeSalvo et al., 2011). The insect brain barrier consists of glia cells, which are the most abundant cell type in the vertebrate CNS. Paracellular diffusion in insects is controlled by septate/tight junctions that are homologous to tight junctions controlling paracellular diffusion in vertebrates (Wu and Beitel, 2004; Daneman and Barres, 2005; Freeman and Doherty, 2006).

Transporters are present in the insects, and it has been shown that the human MDR1 antibody (c219) binds to the MDR65 (i.e., an insect MDR1 homolog present in the insect brain barrier) (Mayer et al., 2009). Functionally, we have shown that the Pgp inhibitor verapamil blocks the efflux of Pgp substrates (Nielsen et al., 2011). This study concluded that insect models can be used as models to identify Pgp substrates. The structural and functional similarities of the vertebrate and invertebrate BBB enable the use of invertebrates as advanced screening models for BBB permeability determinations.

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ABBREVIATIONS: BBB, blood-brain barrier; CNS, central nervous system; Ctot, total brain concentration; KO, knockout; LC-MS, liquid chromatography-mass spectrometry; MDCK, Madin-Darby canine kidney; MDCK-MDR1, MDR1-transfected MDCK; MDR1, multidrug resistance protein-1; P_{app}, apparent permeability coefficient; PS, permeability-surface area; Pgp, P-glycoprotein.