RESEARCH ARTICLE

Application of Membrane Permeability Evaluated in *In Vitro* Analyses to Estimate Blood–Retinal Barrier Permeability

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ABSTRACT: The relationship between the *in vitro* membrane permeability and systemic blood–retinal barrier (BRB) permeability of drugs was investigated. To determine membrane permeability trend lines in this relationship, the apparent permeability (P_{app}) and initial uptake rate (V) of 23 compounds were evaluated in a parallel artificial membrane permeability assay and the uptake study with a rat retinal endothelial cell line (TR-iBRB2 cells) for comparison with their retinal uptake index (RUI). The RUI values of compounds undergoing passive diffusion across the BRB were correlated with a log of the P_{app} [RUI = 7.93 × 10 × exp (0.994 × log P_{app}), $r^2 = 0.660$] and a log of the V [RUI = 26.5 × exp (1.55 × log V), $r^2 = 0.581$]. The RUI values of compounds undergoing carrier-mediated transport across the BRB were correlated with a log of the V [RUI = 26.5 × exp (0.887 × log V), $r^2 = 0.559$]. These results showed that the membrane permeability trend lines derived from the RUI and V values reflect the transport of drugs at the BRB, suggesting that an *in vitro* analysis-based estimation of the BRB permeability can be obtained using TR-iBRB2 cells and membrane permeability trend lines. © 2012 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

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INTRODUCTION

In drug discovery investigations involving retinal diseases, the design of drug delivery systems to target the retina from the circulating blood is a major problem. The determinant of drug transport to the retina is the membrane permeability at the blood–retinal barrier (BRB) because the paracellular transport is prevented by the complex tight junctions of the retinal capillary endothelial cells (inner BRB) and the retinal pigment epithelial cells (outer BRB).[1–3] Thus, it is important to investigate the transport and barrier function of the BRB for the design of efficient drug delivery systems and to prevent the unfavorable entry of drugs into the neural retina.

Physiologically, retinal homeostasis is maintained by the supply of nutrients and the removal of xenobiotics and endobiotics at the BRB.[4] It is known that nutrients, such as glucose, vitamins, and amino acids, cross the BRB by carrier-mediated transport, and recent studies of the BRB have revealed the expression of influx and efflux transporters, such as glucose transporter (GLUT1/solute carrier (SLC)2A1),[5,6] L-type amino acid transporter (LAT1/SLC7A5),[7] nucleoside transporters (ENTs/SLC29A subfamily),[8,9] glycine transporter (GlyT/SLC6A9),[10] cationic amino acid transporter (CAT1/SLC7A1),[11] carnitine/organic cation transporter (OCTN2/SLC22A5),[12] organic anion transporter 3 (OAT3/SLC22A8),[13] P-glycoprotein (P-gp/MDR1/ABCB1),[14–17] multidrug resistance-associated protein 4 (MRP4/ABCC4),[18] and breast cancer resistance protein (BCRP/ABCG2),[19] in the BRB.

Although the molecular features of the BRB have been significantly clarified, it still remains difficult to predict drug transport to the retina from the circulating blood because the systemic BRB permeability varies depending on the properties of the drugs, such as their lipophilicity and the availability of carrier-mediated transport. It is known that the BRB permeability can be directly evaluated using *in vivo*

Additional Supporting Information may be found in the online version of this article. Supporting Information

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analyses, such as the retinal uptake index (RUI) and integration plots, that require technical proficiency and enormous financial cost and include ethical questions about animal care.[7,9–12,20–23] Thus, it will not be efficient to apply *in vivo* analysis of the BRB permeability to industrial high-throughput screenings of candidate compounds and this highlights the need to estimate the BRB permeability based on convenient *in vitro* analysis. Achieving this will be helpful for accelerating the development of new drugs to treat retinal diseases.

The use of *in vitro n*-octanol/Ringer distribution coefficient (*DC*)-based estimations of *in vivo* drug distribution to the ocular tissues has been proposed by Kadam and Kompella.[24] Regarding compounds undergoing passive diffusion, our previous study showed a significant relationship between BRB permeability and lipophilicity, allowing the lipophilicity trend line to be estimated from the RUI and *DC* values obtained from *in vivo* and *in vitro* analyses, respectively.[25] A similar relationship has also been described in the recent research by Toda et al.[26] By contrast, compounds undergoing carrier-mediated transport, such as glucose, exhibit greater RUI values than the lipophilicity trend line, strongly suggesting that the lipophilicity trend line constructed from RUI and *DC* is not applicable to the estimation of the BRB permeability of relatively hydrophilic nutrients and drugs. In addition, the difference in properties between the BRB and blood–brain barrier (BBB) was shown by the greater RUI value of verapamil, a substrate of P-gp, than the lipophilicity trend line,[25] emphasizing the BRB-specific study of barrier permeability.

As another indicator of BRB permeability, the membrane permeability has been examined using uptake studies, wherein TR-iBRB2 and ARPE-19 cells were used as *in vitro* models reflecting the properties of the inner and outer BRB, respectively.[27–29] In particular, TR-iBRB2 cells, a conditionally immortalized retinal capillary endothelial cell line, have a spindle-fiber-shaped morphology and express von Willebrand factor, as an endothelial cell marker, and a scavenger receptor for acetylated low-density lipoprotein,[27] and are thought to be a key *in vitro* tool for studying the BRB permeability because two-thirds of the retina is nourished by the inner BRB.[2,30,31] In industrial drug screening, parallel artificial membrane permeability assay (PAMPA), a cell-free experimental system, is used for the evaluation of the membrane permeability of candidate compounds.[32–35]

Although there are many reports about the *in vitro* membrane permeability and the *in vivo* BRB permeability,[2,30,31] their relationship has not been examined. As described above, the lipophilicity trend line estimated from the RUI and *DC* values is only applicable to the estimation of the BRB permeability of compounds undergoing passive diffusion, and any significant trend lines have not been available for compounds undergoing carrier-mediated transport,[25,26] exhibiting the necessity to investigate the significance of different trend lines such as the membrane permeability trend line estimated from the relationship between membrane permeability and BRB permeability. Therefore, the purpose of this study was to determine the membrane permeability trend line estimated from the BRB permeability and membrane permeability of a series of compounds. The uptake study with TR-iBRB2 cells and PAMPA was performed to evaluate the membrane permeability of 23 compounds in terms of the initial uptake rate (V) and the apparent permeability (P_{app}), which were compared with regard to their lipophilicity and BRB permeability.

MATERIALS AND METHODS

Reagents

[N-methyl-^{3}H]Acetyl-L-carnitine hydrochloride ([^{3}H]acetyl-L-carnitine, 85 Ci/mmol); [N-methyl-^{14}C]antipyrine ([^{14}C]antipyrine, 55 mCi/mmol); L-[2,3-^{3}H]arginine ([^{3}H]L-arginine, 50.6 Ci/mmol); [4-^{14}C]creatinine ([^{14}C]creatinine, 55 mCi/mmol); [N-methyl-^{14}C]diazepam ([^{14}C]diazepam, 55 mCi/mmol); L-3,4-[ring2,5,6-^{3}H]dihydroxyphenylalanine ([^{3}H]L-DOPA, 60 Ci/mmol); D-[1-^{3}H]mannitol ([^{3}H]D-mannitol, 14.2 Ci/mmol); [1,2,6,7-^{3}H]progesterone ([^{3}H]progesterone, 101.3 Ci/mmol); [N-methyl ^{3}H]verapamil hydrochloride ([^{3}H]verapamil, 80 Ci/mmol); and [^{3}H(G)]vincristine sulfate ([^{3}H]vincristine, 10 Ci/mmol) were purchased from American Radiolabeled Chemicals (St. Louis, Missouri). [2,8-^{3}H]-Adenosine ([^{3}H]adenosine, 39.2 Ci/mmol); [2-^{3}H(N)]D-glucose ([^{3}H]D-glucose, 8.4 Ci/mmol); [4,5-^{3}H]valproic acid ([^{3}H]valproic acid, 51.0 Ci/mmol); and (RS)-[phenyl-4-^{3}H]warfarin, ([^{3}H]warfarin, 17.4 Ci/mmol) were purchased from Moravek Biochemicals (Brea, California). *p*-[glycyl-2-^{3}H]-Aminohippuric acid ([^{3}H]PAH, 4.04 Ci/mmol); [1,2,6,7-^{3}H(N)]-corticosterone ([^{3}H]corticosterone, 70.0 Ci/mmol); [^{3}H(G)]-digoxin ([^{3}H]digoxin, 35.4 Ci/mmol); 3,4-[ring-2,5,6-^{3}H]-dihydroxyphenylethylamine ([^{3}H]dopamine, 54.2 Ci/mmol); L-[4,5-^{3}H(N)]-leucine ([^{3}H]L-leucine, 59.2 Ci/mmol); [1,2,6,7-^{3}H(N)]-testosterone ([^{3}H]testosterone, 70.0 Ci/mmol); and [5,6-^{3}H]-uracil ([^{3}H]uracil, 31.9 Ci/mmol) were purchased form PerkinElmer Life Science (Boston, Massachusetts). [U-^{14}C]Glycine ([^{14}C]glycine, 109 mCi/mmol) and L-[2,6-^{3}H]phenylalanine ([^{3}H]L-phenylalanine, 54.0 Ci/mmol) were purchased from GE Healthcare (Piscataway, New Jersey). All other chemicals were of reagent grade and available commercially. According to a previous study,[25] these compounds were classified into passive diffusion and carrier-mediated transport types. The classification