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Case study: adapting *in vitro* blood–brain barrier models for use in early-stage drug discovery

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Several parameters influencing the brain distribution of compounds must be considered when designing potential neuropharmaceuticals in early-stage drug discovery. The blood–brain barrier (BBB) represents an obstacle for drug penetration into the brain. Many *in vitro* BBB models have proven useful for predicting the BBB permeation rate, but do not meet all criteria for use in early-stage drug discovery: feasibility, rapidity, reliability and a low requirement for human resources. To meet this demand, we have developed a robust, higher-throughput, cell-based model exhibiting BBB features (low paracellular permeability, functional efflux pumps and the correct endothelial phenotype). This system comes in a ready-to-use, frozen format, appropriate for in-house use by large pharmaceutical firms and small biotech companies during early-stage drug discovery.

The development of drugs for treating central nervous system (CNS) disorders is a medically challenging and commercially risky field. The blood–brain barrier (BBB) usually hinders a drug candidate's access to pharmacological targets located within the brain parenchyma. Indeed, brain capillaries are the main entry route for the CNS but can also prevent neuropharmaceuticals from reaching the brain parenchyma at an effective dose. Consequently, the therapeutic potential of a drug candidate is not only related to its activity at the target but also to its ability to attain an effective dose at the target site. Ideally, this issue should be addressed early in the drug discovery process.

Pharmacokinetic parameters of CNS drug exposure

The ability of a CNS drug to achieve an adequate concentration–time profile at the target brain site (i.e. sufficient in terms of concentration and duration) depends on a variety of parameters [1,2].

Because it is considered that only the unbound drug brain concentration is available for interaction with most CNS receptors, it is essential to determine this parameter for all candidate CNS drugs. To this end, a brain microdialysis technique [3] can provide useful information by determining the concentration of unbound drug in the brain interstitial fluid. However, this technique is resource-intensive and not broadly applicable, owing to probe recovery problems with lipophilic compounds. Consequently, brain microdialysis is usually reserved for drugs in the development stage. Although the determination of total brain:-plasma ratios in rodents and in the brain unbound fraction (estimated from either brain homogenates or brain slice technology), and the subsequent transformation of total brain concentrations into unbound brain concentrations, can provide a surrogate measure of free drug concentration within the parenchyma, this approach is not practical for routine screening.

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An additional issue relates to the complexity of the processes that determine the brain:-plasma ratio of a compound, making it difficult to derive strategies for the subsequent medicinal chemistry efforts during lead optimization. Indeed, the brain:plasma ratio is a distribution parameter depending on several factors, including the rate of permeation across the BBB and the compound's degree of binding to plasma proteins in the blood [4] and brain tissue in the CNS.

In lead optimization projects, the objective is therefore to identify and successively eliminate the different parameters responsible for a poor brain distribution. Consequently, the rationale for optimizing drug metabolism and pharmacokinetics is generally driven by the use of a cascade of *in vitro* assays and *in vivo* studies of increasing complexity and predictability.

To understand fully brain drug delivery and its consequences for central drug action, it is now commonly accepted that the rate of delivery, which depends on the BBB permeability for the compound in question, must be considered separately from (i) the extent of equilibration across the BBB and (ii) the intra-brain distribution [5]. Accordingly, a high rate of transport across the BBB is not necessarily predictive of a high ratio measured under steady-state conditions. Nevertheless, this parameter is of particular importance for compounds regarding indications such as pain, epilepsy and stroke because they need to reach their peak brain concentration rapidly, which is unlikely to happen if the rate of transport across the BBB is low.

Predicting BBB permeation rate in early-stage drug discovery

Although the *in situ* brain perfusion [6] is considered as the most accurate method for measuring the rate of permeation across the BBB, practical considerations mean that this technique is seldom used in early-stage drug discovery. By contrast, *in vitro* BBB models have the potential to fulfil the practical criteria for drug discovery but choosing one has always involved a compromise between capacity, time, predictability and cost [7].

To the best of our knowledge, none of the available immortalized brain endothelial cell (EC) lines exhibits sufficient tightness to provide a reliable *in vitro* tool for the prediction of BBB permeation rates [8]. Hence, easily cultured non-brain cell lines known to achieve tight, reproducible barriers, such as Caco-2 and Madin–Darby canine kidney cells, have been used to study diffusional transport [9] and to rank drugs in terms of passive diffusion across the BBB. However, their usefulness remains questionable because they exhibit different transporter expression levels [10] as well as different membrane compositions and characteristics in comparison with ECs [11].

Some of these cell lines have been transfected to express human efflux transporters, such as P-glycoprotein (P-gp) [12] and breast cancer resistance protein (BCRP) [13]. The ratios between basal-to-apical and apical-to-basal permeability coefficients, known as 'efflux ratios', are frequently used to identify cellular efflux pump substrates. In this frame, other elegant models have been developed such as the recently described grasshopper *ex vivo* model, which exhibits a proper P-gp functionality, as seen in the mammalian BBB [14]. This knowledge is of considerable value for avoiding adverse drug interactions at the BBB and is useful for medicinal chemists, because efflux mechanisms can explain, at least in part, a poor CNS distribution. However, high efflux ratios are not necessarily associated with low *in vivo* permeability and some marketed CNS drugs are known to be efflux pump substrates [15]. Consequently, predicting BBB permeation rates with cell lines that overexpress efflux transporters could preclude some potential effective CNS drug candidates from entering subsequent *in vivo* development stages.

To predict the BBB permeation rate, *in vitro* models should possess as many of the relevant *in vivo* properties of the brain endothelium as possible [9]; this is not the case for non-brain-derived cell lines that express an epithelial phenotype.

Primary-culture and low-passage brain ECs have been extracted from various species to design BBB models: bovine [16], porcine [17], rat [18], mouse [19] and also human [20]. They are known to mimic the *in vivo* BBB closely, particularly when co-cultured with glial cells, because glial cells are known to induce and maintain the BBB phenotype *in vitro* [21]. A well-characterized *in vitro* BBB model can also prove useful for studying (i) mechanistic aspects of transport and (ii) biological and disease processes related to the BBB [22]. However, as already pointed out by Andreas Reichel [7], none of the currently available *in vitro* BBB models meets all the pharmaceutical industry requirements for use in early-stage drug discovery: feasibility, rapidity, ease-of-use, reliability and low requirement for human resources.

In the early 1990s, our laboratory developed a co-culture model of the BBB [16] that enabled the investigation of a drug's cellular and molecular effects on the BBB and provided a powerful alternative to the *in vivo* determination of the rate of BBB transport [23]. Because this original model requires a significant amount of expertise and continuous, routine work by dedicated laboratory workers to produce useful and reproducible results, only a few pharmaceutical companies adopted it for in-house use.

To combine the advantages of a well-characterized *in vitro* BBB model with a simpler procedure for permeability screens, we have developed an *in vitro* BBB model that can easily be used in-house in large pharmaceutical companies with major CNS programs and smaller biotech companies with an occasional need for BBB permeability knowledge.

A 24-well miniaturized BBB model

In an initial attempt to design a predictable, higher-capacity, *in vitro* BBB model, we modified the original, well-characterized, co-culture model set up in the 1990s that was based on the co-culture between bovine brain capillary ECs (BBCECs) and rat glial cells [16] (Fig. 1a). The simplification consisted in miniaturizing the format (i.e. using 24-well plates instead of 6-well plates), reducing the use of glial cells and shortening the duration of the cell culture step (4 days instead of 12 days; Fig. 1b). In this model [24] the inductive influence of glial cells and crosstalk between ECs and glial cells is mimicked by the use of 'BBB-inducing medium', containing co-culture-conditioned medium and selected sera. The use of co-culture-conditioned medium enables a reduction in the number of animals necessary for experiments, because it can easily be gathered and frozen for later use. A functional BBB model, with the correct localization of tight junction proteins and the functional expression of P-gp and several other efflux transporters, is obtained just four days after BBCEC seeding. As the original co-culture model, this model presents a low permeability to Lucifer yellow and sucrose, which are used as integrity markers. Furthermore, it shows a good correlation between the measured *in vitro* permeability values and *in vivo* BBB permeability, as assessed by *in situ* brain perfusion [24].

A ready-to-use BBB model

Trypsinization of BBCECs (Fig. 1b) requires technical skill and adequate training, and

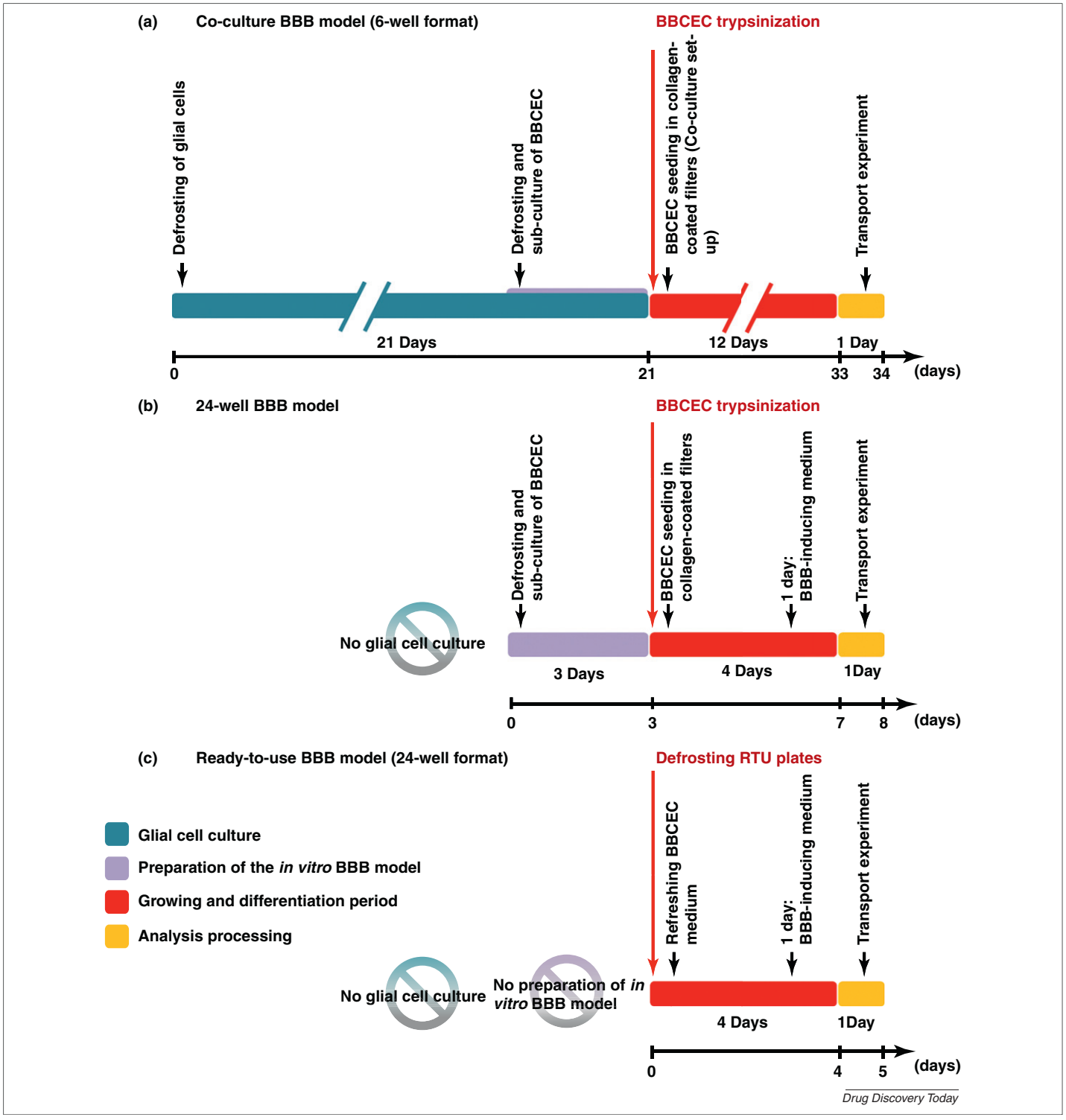

FIGURE 1

The adaptation of *in vitro* blood–brain barrier (BBB) models for use in early-stage drug discovery. **(a)** The original co-culture model developed by Dehouck *et al.* [16] consists of bovine brain capillary ECs (BBCECs) cultured on a filter and rat glial cells cultured at the bottom of the well (the 6-well format). Setting up this system is time-consuming because glial cells require three weeks of culture and the subsequent co-culture of BBCECs and glial cells lasts for 12 days. The procedure features several delicate steps (i.e. BBCEC trypsinization and filter coating) but enables the reliable production of an *in vitro* BBB model with *in vivo* BBB properties. The 6-well model is of value for studying the BBB under physiological and pathological conditions and takes account of EC and glial cell interactions. **(b)** A 24-well model was designed by simplifying the co-culture model for use in automated, high-throughput screening. The format was miniaturized (i.e. 24 wells instead of six), the glial cells were replaced by BBB-inducing medium and the duration of cell culture was shortened (i.e. 4 days instead of 12 days) [24]. The procedure still includes a trypsinization step and requires three days to prepare the model. **(c)** The ready-to-use (RTU) model was designed to be even more user-friendly because the crucial trypsinization step is avoided, therefore the final user does not need any specific technical training. The RTU *in vitro* BBB model is supplied in a frozen format and is ready for experimental use just four days after thawing and with only two medium changes.

represents the most crucial step for establishing the 24-well model in the screening laboratory because BBCECs are highly sensitive to this manipulation. Hence, to create an even more user-friendly *in vitro* BBB model and to guarantee a reproducible low permeability and phenotype, BBCECs were seeded into 24-well filter plates at a density of 30 000 cells/cm^2, immediately stored at 0 °C for 1 h upon seeding in the inserts and finally transferred to −80 °C to freeze the entire system, for future shipment and use in another laboratory. Because of the freezing step, the final user does not need specific knowledge of brain EC culture and subculture. The time necessary for the whole experiment is substantially reduced because no preparation of BBCEC culture is needed: the new model is ready for use 4 days after thawing (Fig. 1c). Immunocytochemical assays show that the BBCECs form a regular monolayer of tightly packed, non-overlapping cells with correctly localized tight junction proteins (Fig. 2a), suggestive of a restrictive paracellular pathway. Permeability to the fluorescent BBB integrity marker Lucifer yellow remained low for cultures thawed after up to eight weeks of storage at −80 °C (Fig. 2b). The permeability measured in the ready-to-use (RTU) model was as low as that obtained in the original 24-well model (Fig. 2c) and attests to the reproducible tightness of the cell barrier (n = 147).

Expression of P-gp – an important BBB feature – in the RTU model was demonstrated by immunocytochemistry (Fig. 2a). Similarly, the presence of efflux pumps was assessed by RT-PCR (reverse transcriptase polymerase chain reaction), revealing the transcriptional expression of other efflux transporters – P-gp, multidrug resistance protein (MRP)1, MRP4 and MRP5 – as described in the original 24-well model [24] and co-culture model [25]. The functionality of efflux pumps was evidenced by greater uptake of rhodamine^{123} (^{123}R; 20 μM) by the BBCECs (Fig. 3a) and greater permeability of the BBB to quinidine (10 μM; Fig. 3b) in the presence of verapamil (25 μM). Furthermore, we found a strong correlation (R^2 = 0.87) between the permeability values measured for 33 marketed drugs (comprising CNS and peripheral acting drugs; P-gp and non-P-gp substrates exhibiting different molecular weights and lipophilicity) using the RTU model and the 24-well miniaturized model, respectively (Fig. 3c).

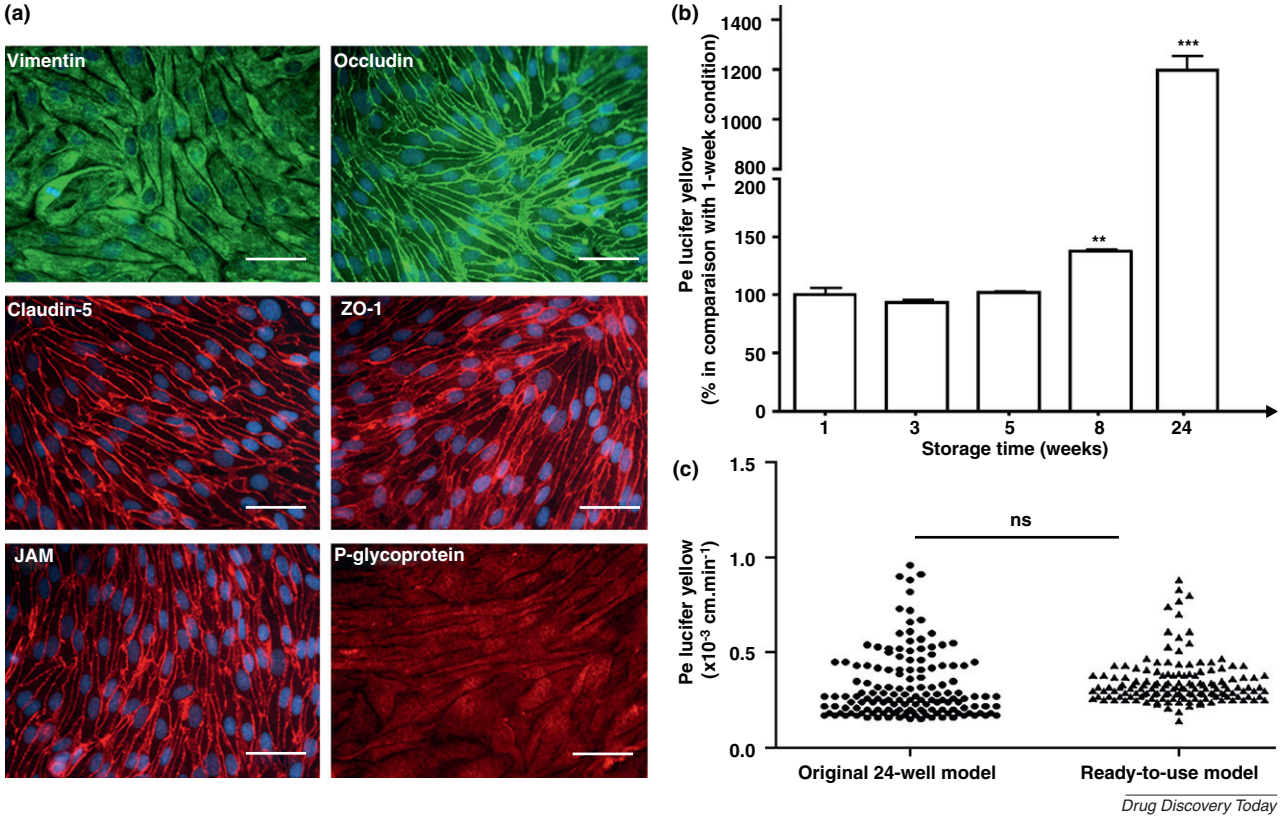

FIGURE 2

(a) Immunocytochemical characterization of the ready-to-use (RTU) blood–brain barrier (BBB) model. **(b)** The paracellular permeability of the endothelial monolayer to Lucifer yellow as a function of storage time at −80 °C. **(c)** A comparison with the original 24-well model. **(a)** The cell monolayer was immunostained to reveal an endothelial morphology (vimentin), the expression of tight junction proteins [occludin, claudin-5, zonula occludens-1 (ZO-1) and junctional adhesion molecule (JAM)] and the expression of P-glycoprotein. Scale bars: 100 μm. **(b)** Effect of storage time at −80 °C and delivery (48 h in dry ice) on BBB integrity, as assessed by measuring BBB permeability (Pe) to the small, hydrophilic dye Lucifer yellow (molecular mass: 457 g/mol). Results are presented as the mean ± SEM, n = 12 monolayers for all tests (except for the 8- and 24-week conditions, where n = 3). Unpaired t-test: $^{**}P = 0.082$, $^{***}P < 0.001$. **(c)** Monolayer paracellular permeability (Pe, in cm/min) was assessed by measuring the transport of Lucifer yellow in the original 24-well model ($0.32 \pm 0.18 \times 10^{-3}$ cm/min) and in the RTU model ($0.35 \pm 0.13 \times 10^{-3}$ cm/min), as already described [24]. Results are presented as a scatter plot vertical representation for n = 147 monolayers. Paired t-test: ns (non-significant), $P = 0.19$.

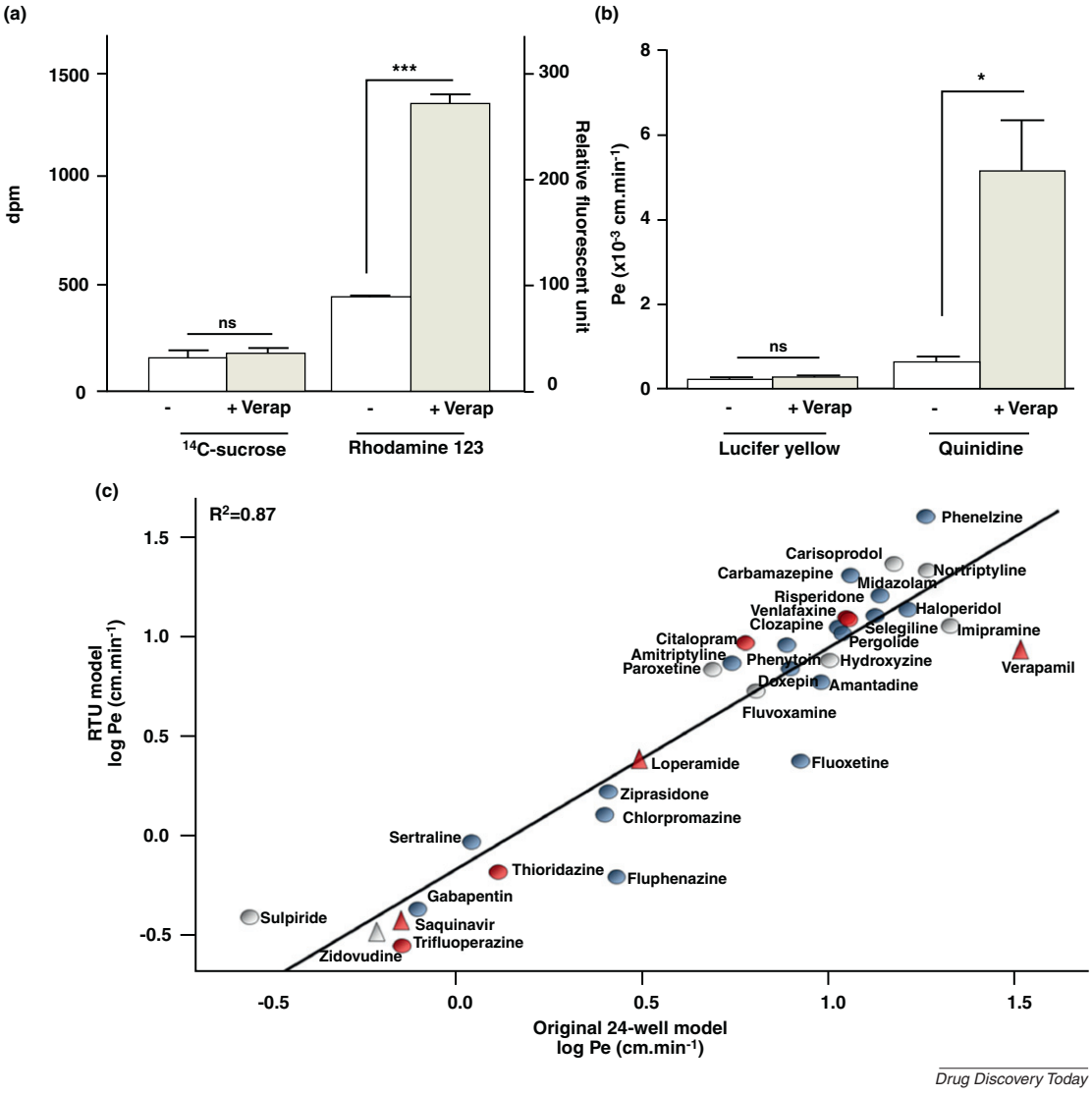

FIGURE 3

Assessment of efflux pump activity in the ready-to-use (RTU) blood–brain barrier (BBB) model. **(a)** Accumulation of rhodamine^{123} (^{123}R) in the presence and absence of verapamil. **(b)** Transport of quinidine in the presence and absence of verapamil. **(c)** The correlation between the permeability values of 33 compounds measured with the original 24-well model and the RTU model, respectively. **(a)** Efflux pump functionality was assessed by studying the accumulation of rhodamine^{123} in endothelial cells (ECs) in the presence and absence of verapamil (verap; 25 μM). Verapamil increased ^{123}R endothelial uptake <3-fold (273.2 ± 20.8 relative fluorescent units or RFUs vs 89.22 ± 6.4 RFUs for untreated cells). ^{14}C-sucrose was co-incubated with ^{123}R to confirm the absence of alterations in EC monolayer integrity and the absence of accumulation in the cells [164 ± 42.15 disintegrations per minute (dpm) without verapamil vs 185.8 ± 29.59 dpm with verapamil]. Results are expressed as the mean ± SEM, $n = 3$ monolayers for the sucrose assay and $n = 6$ monolayers for the ^{123}R assay. Unpaired *t*-test: ns (non-significant), $P = 0.59$, ***$P < 0.001$. **(b)** Efflux pump functionality was also assessed by studying the transport of quinidine (10 μM) across the BBB in the presence and absence of verapamil (verap; 25 μM). Verapamil increased quinidine transport 8-fold [endothelial permeability (Pe) of 5.16 ± 1.24 × 10^{-3} cm/min vs 0.66 ± 0.09 × 10^{-3} cm/min for untreated cells]. Lucifer yellow was co-incubated with quinidine to confirm the absence of alterations in EC monolayer integrity as a result of verapamil (Pe of 0.24 ± 0.02 × 10^{-3} cm/min vs 0.29 ± 0.04 × 10^{-3} cm/min for treated cells). Results are expressed as the mean ± SEM, $n = 3$ monolayers for the sucrose and quinidine assays. Unpaired *t*-test: ns (non-significant), $P = 0.26$, *$P < 0.05$. **(c)** Plot of permeability (Pe) values for 33 compounds measured in the original 24-well BBB model [24] and the RTU BBB model, $n = 3$ monolayers for each compound. CNS compounds are represented with circles and peripheral acting drugs with triangles. Known P-gp substrates are represented in red and compounds that are not P-gp substrates are in blue; the molecules for which this information is lacking or controversial are in grey. All compounds were tested at 5 μM, and their concentrations were determined by chromatography coupled with mass spectrometry. The correlation coefficient was $R^2 = 0.87$ and the slope differed significantly from zero, $P < 0.0001$.

Conclusions and future opportunities

The RTU system described herein appears to be more suitable than previously characterized models for use in higher-throughput BBB permeability screens. It reproduces the *in vivo* BBB phenotype well, with reproducible, low, paracellular permeability and functional efflux pumps. The RTU system can be prepared rapidly for use and is easy to handle; as such, it offers pharmaceutical and smaller biotech companies an opportunity to generate useful BBB permeation data in-house. This enables information on drug optimization to be fed back rapidly to the medicinal chemists.

Determining the rate of BBB transport (i.e. permeability) at an early stage in the drug discovery process helps to predict whether drug candidates are likely to achieve high enough

CNS exposure to elicit the desired pharmacological effect – a parameter that has to be confirmed *in vivo* later in the development process. To facilitate compound selection, it might be necessary to relate potency to the estimated unbound brain concentration. Recently, high-throughput *in vitro* methods for rapid determination of brain tissue binding have been developed [26] and constitute a relevant means of predicting the extent of brain penetration based on *in vitro* and *in silico* models. In the future, we intend to extend the use of our *in vitro* BBB models for the assessment of blood–brain partitioning. In fact, by combining the latter data with knowledge of the unbound brain fraction, it should be possible to predict the unbound concentrations of a therapeutic agent reaching the CNS at an early stage in the drug discovery process.

The BBB restricts the access of many conventional, low-molecular-weight compounds to their CNS targets. Therefore, the delivery of biotherapeutics to the brain represents an even greater challenge and will require a better understanding of the active and passive processes governing transport across the BBB. Accordingly, identifying a brain delivery strategy for biopharmaceuticals will become essential. Several peptides, proteins, cytokines and neurotrophins are transported across the BBB by endogenous mechanisms, some of which could be exploited for brain delivery. The use of antibodies for receptor-mediated transport across the BBB might be one way of interacting with targets currently beyond the scope of small-molecule drugs [27]. To this end, the use of *in vitro* models derived from human cells could be crucial for the successful evaluation and development of large-molecule therapeutics. To date, several reasons prevent primary human brain ECs and human brain EC lines from being used in permeability screens. Indeed, primary human brain ECs cannot be provided on a regular basis and the yield of ECs from human surgical samples is low. As a consequence, they cannot be used in permeability screens within the pharmaceutical industry, at least in early-stage drug discovery, where high-throughput screening is needed. However, their use might help in later stages (e.g. in the development process) for confirming and refining the predictions made earlier, as soon as optimized molecules have been generated and selected. The currently available human EC lines do not provide high paracellular restriction enough to be useful in permeability screens, even if their high throughput and ease of culture are of advantage in this case. Another possibility would be to use human pluripotent stem cells, because it might constitute a new way of obtaining a reliable human *in vitro* BBB model if they can be made to differentiate into ECs [28,29] displaying the BBB phenotype. Their great self-renewing potency would enable a high yield and, taking advantage of intercellular communication of the neurovascular unit-forming cells in co-culture conditions, could enable these ECs to acquire the BBB phenotype *in vitro*. With an efficient differentiation and maintenance of BBB features in culture, scientists could successfully design human stem-cell-based models, on the basis of other successful applications of this strategy in other fields [30].

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References

1 Jeffrey, P. and Summerfield, S. (2010) Assessment of the blood-brain barrier in CNS drug discovery. *Neurobiol. Dis.* 37, 33–37
2 Di, L. *et al.* (2008) Strategies to assess bloodbrain barrier penetration. *Expert Opin. Drug Discov.* 3, 677–687
3 Aasmundstad, T.A. *et al.* (1995) Distribution of morphine 6-glucuronide and morphine across the blood-brain barrier in awake, freely moving rats investigated by in vivo microdialysis sampling. *J. Pharmacol. Exp. Ther.* 275, 435–441
4 Smith, D.A. *et al.* (2010) The effect of plasma protein binding on in vivo efficacy: misconceptions in drug discovery. *Nat. Rev. Drug Discov.* 9, 929–939
5 Hammarlund-Udenaes, M. *et al.* (2008) On the rate and extent of drug delivery to the brain. *Pharm. Res.* 25, 1737–1750
6 Takasato, Y. *et al.* (1984) An in situ brain perfusion technique to study cerebrovascular transport in the rat. *Am. J. Physiol.* 247, 484–493
7 Reichel, A. (2006) The role of blood-brain barrier studies in the pharmaceutical industry. *Curr. Drug Metab.* 7, 183–203
8 Toth, A. *et al.* (2011) Patented in vitro blood-brain barrier models in CNS drug discovery. *Recent Pat. CNS Drug Discov.* 6, 107–118
9 Gumbleton, M. and Audus, K.L. (2001) Progress and limitations in the use of in vitro cell cultures to serve as a permeability screen for the blood-brain barrier. *J. Pharm. Sci.* 90, 1681–1698
10 Lacombe, O. *et al.* (2011) In vitro primary human and animal cell-based blood-brain barrier models as a screening tool in drug discovery. *Mol. Pharm.* 8, 651–663
11 Di, L. *et al.* (2009) Comparison of blood-brain barrier permeability assays: in situ brain perfusion, MDR1-MDCKII and PAMPA-BBB. *J. Pharm. Sci.* 98, 1980–1991
12 Pastan, I. *et al.* (1988) A retrovirus carrying an MDR1 cDNA confers multidrug resistance and polarized expression of P-glycoprotein in MDCK cells. *Proc. Natl. Acad. Sci. U.S.A.* 85, 4486–4490
13 Xiao, Y. *et al.* (2006) A 96-well efflux assay to identify ABCG2 substrates using a stably transfected MDCK II cell line. *Mol. Pharm.* 3, 45–54
14 Nielsen, P.A. *et al.* (2011) Models for predicting blood-brain barrier permeation. *Drug Discov. Today* 16, 472–475
15 Summerfield, S.G. *et al.* (2007) Central nervous system drug disposition: the relationship between in situ brain permeability and brain free fraction. *J. Pharmacol. Exp. Ther.* 322, 205–213
16 Dehouck, M.P. *et al.* (1990) An easier, reproducible, and mass-production method to study the blood-brain barrier in vitro. *J. Neurochem.* 54, 1798–1801
17 Zhang, Y. *et al.* (2006) Porcine brain microvessel endothelial cells as an in vitro model to predict in vivo blood-brain barrier permeability. *Drug Metab. Dispos.* 34, 1935–1943
18 Nakagawa, S. *et al.* (2009) A new blood-brain barrier model using primary rat brain endothelial cells, pericytes and astrocytes. *Neurochem. Int.* 54, 253–263
19 Coisne, C. *et al.* (2005) Mouse syngenic in vitro blood-brain barrier model: a new tool to examine inflammatory events in cerebral endothelium. *Lab. Invest.* 85, 734–746
20 Josserand, V. *et al.* (2006) Evaluation of drug penetration into the brain: a double study by in vivo imaging with positron emission tomography and using an in vitro model of the human blood-brain barrier. *J. Pharmacol. Exp. Ther.* 316, 79–86
21 Hamm, S. *et al.* (2004) Astrocyte mediated modulation of blood-brain barrier permeability does not correlate with a loss of tight junction proteins from the cellular contacts. *Cell Tissue Res.* 315, 157–166
22 Mysiorek, C. *et al.* (2009) Peroxisome-proliferator-activated receptor-alpha activation protects brain capillary endothelial cells from oxygen-glucose deprivation-induced hyperpermeability in the blood-brain barrier. *Curr. Neurovasc. Res.* 6, 181–193
23 Lundquist, S. *et al.* (2002) Prediction of drug transport through the blood-brain barrier in vivo: a comparison between two in vitro cell models. *Pharm. Res.* 19, 976–981
24 Culot, M. *et al.* (2008) An in vitro blood-brain barrier model for high throughput (HTS) toxicological screening. *Toxicol. In Vitro* 22, 799–811
25 Berezowski, V. *et al.* (2004) Contribution of glial cells and pericytes to the mRNA profiles of P-glycoprotein and multidrug resistance-associated proteins in an in vitro model of the blood-brain barrier. *Brain Res.* 1018, 1–9
26 Wan, H. *et al.* (2007) High-throughput screening of drug-brain tissue binding and in silico prediction for assessment of central nervous system drug delivery. *J. Med. Chem.* 50, 4606–4615
27 Yu, Y.J. *et al.* (2011) Boosting brain uptake of a therapeutic antibody by reducing its affinity for a transcytosis target. *Sci. Transl. Med.* 3, 84ra44
28 Levenberg, S. *et al.* (2010) Isolation, differentiation and characterization of vascular cells derived from human embryonic stem cells. *Nat. Protoc.* 5, 1115–1126
29 James, D. *et al.* (2010) Expansion and maintenance of human embryonic stem cell-derived endothelial cells by TGFβ inhibition is Id1 dependent. *Nat. Biotechnol.* 28, 161–166
30 Asai, Y. *et al.* (2010) Combination of functional cardiomyocytes derived from human stem cells and a highly-efficient microelectrode array system: an ideal hybrid model assay for drug development. *Curr. Stem Cell Res. Ther.* 5, 227–232

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