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The highly permeable blood–brain barrier: an evaluation of current opinions about brain uptake capacity

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The blood–brain barrier is often perceived as relatively impermeable, and various cut-off values for zero or limited brain permeability have been suggested. The validity of these values has been evaluated in this review. The barrier appears to have a very high permeability and absorptive capacity: sufficient to absorb compounds with polar surface area >270 Å^2, molecular weight >1000 Da, log D < −3.5 and equilibrium brain-to-blood concentration ratio <0.01 well. Sufficient intestinal uptake indicates good passive brain uptake potential. The uptake is potentially more sensitive to involvement and changes of active transport than in the intestines. A physiologically based *in vitro–in vivo* method for prediction of brain uptake is presented.

In their review, Su and Sinko [1] presented the blood–brain barrier (BBB) as nearly impermeable and according to Pardridge [2–4] <2% of small drug molecules and essentially all large molecules do not cross the BBB. On the basis of evaluations and statements by these authors, it would appear that many drug candidates (CDs) may not achieve sufficiently high CNS exposure to elicit an effect. The recommendation by Pardridge is that brain drug development programs need to be adjusted so that compounds are designed for active brain uptake [4]. An alternative interpretation is that only a small proportion of all drug products may be used for CNS disorders and/or that few compounds that enter the brain produce CNS side effects.

The BBB is often perceived as impermeable to compounds above a certain mass or hydrophilicity (measured as log D or polar surface area (PSA) for example). It is well known that passive drug permeability (P_e) in various endothelial and epithelial cells decrease with increasing molecular weight (MW) and PSA [2,5,6]. According to Kelder *et al.* [6], the PSA for passively absorbed CNS drugs should not exceed ~120 Å^2. Others have proposed that the PSA, log D and MW for BBB penetration should be kept at <70–90 Å^2, 1–4, and <~450 Da [2–4,7]. Kelder *et al.* [6] suggested that CDs can be tailored to good BBB P_e by decreasing the PSA to <60–70 Å^2. Support for such conclusions are given by results showing that CNS-active drugs generally have smaller PSAs and higher log Ds than CNS-inactive drugs [6,8].

Low brain-to-blood or brain-to-plasma total concentration ratios (C_{br}/C_{bl}; C_{br}/C_{pl}) are sometimes used as arguments that a certain compound has low BBB P_e and enter the brain to a limited extent. According to this point of view, it is desirable that CNS-active compounds have large C_{br}/C_{bl}, whereas peripherally acting compounds should have a low C_{br}/C_{bl}.

The P_e is the rate (commonly expressed as 10^{-6} cm/s) at which a molecule passes through endothelial and epithelial cells. The implication is that permeation and organ uptake is avoided only for substances with a P_e of zero.

Following repeated drug administration, and potentially also following single dosing, the concentration of unbound drug in the brain ($C_{u,br}$) and blood ($C_{u,bl}$) will eventually equilibrate or pseudo-equilibrate. The equilibrium $C_{u,br}/C_{u,bl}$ ratio will not necessarily be unity. Deviations occur, or could occur, for actively transported substances [9], and it could also occur for compounds with a slow dissociation rate from binding sites. In a study by Kalvass *et al.* [9], it was demonstrated that those compounds effluxed by P-gp had a tendency to have lower $C_{u,br}/C_{u,pl}$ ratios. The equilibrium C_{br}/C_{bl}

Abbreviations: BBB, blood–brain barrier; BUI, brain uptake index; C_{bl}, blood concentration; C_{br}, brain concentration; CD, candidate drug; C_{pl}, plasma concentration; $C_{u,bl}$, unbound C_{bl}; $C_{u,br}$, unbound C_{br}; F, oral bioavailability; f_a, fraction absorbed; $f_{a,i}$, intestinal f_a; $f_{a,br}$, brain f_a; f_u, unbound fraction; $f_{u,br}$, f_u in brain; $f_{u,pl}$, f_u in plasma; λ, shape factor (of the P_e versus f_a curves); MW, molecular weight; PB-IVIV, physiologically based *in vitro* to *in vivo*; P_e, permeability; P_{e50}, P_e at 50% f_a; PSA, polar surface area; Q_{br}, brain–blood flow rate; S_{br}, BBB surface area; TT, intrinsic transit time; TT_{br}, brain TT; $t_{1/2}$, half-life.

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will also be determined by the binding capacities in brain and blood. Liu *et al.* [10] showed that rapid brain equilibrium requires a combination of high BBB P_e and a low level of brain tissue binding.

CYPs 1A2, 2C9, 2D6 and 3A4 are present in the human brain [11]. There is, however, a limited knowledge and understanding of their role in brain and BBB drug metabolism.

The main objective of this study was to evaluate the correctness of common conclusions and opinions about BBB penetration and brain uptake.

Data selection and model development

BBB P_e data obtained *in vitro* (with brain capillary endothelial cells) and in rats *in vivo* (perfusion, microdialysis and brain uptake index; BUI), animal and human brain physiology data, and CNS side effect-data in humans were used in the analysis. Collection and analysis of data for large and hydrophilic molecules and substances with low C_{br}/C_{bl} was of particular importance.

In vitro BBB P_e and brain physiology data were used to build a physiologically based *in vitro* to *in vivo* (PB-IVIV) model for prediction and visualization of the uptake capacity of the human brain. Intestinal data were added for comparison. Eq. (1) was used for establishing relationships between *in vitro* passive P_e and *in vivo* fraction absorbed (f_a) for each transit through the human intestines ($f_{a,i}$) and brain ($f_{a,br}$):

$$f_a = \frac{P_e^\lambda}{P_{e50}^\lambda + P_e^\lambda} \quad (1)$$

P_{e50} is the P_e corresponding to a f_a of 0.50 and λ is a shape factor. P_e data used in this evaluation were taken from an extensive artificial membrane *in vitro* P_e-data set ($n = 126$; obtained at pH 7.4; [12]). The human *in vivo* $f_{a,i}$ data ($n = 126$) were also taken from [12].

No human BBB P_e and $f_{a,br}$ data are available. Therefore, animal *in vitro* and *in vivo* data were used as surrogates for prediction of the human *in vivo* $f_{a,br}$. It was assumed that humans and animals have similar *in vivo* and *in vitro* BBB P_e (potential involvement of active transport was ignored). Human *in vivo* $f_{a,br}$ was estimated from relationships (equations) between log *in vitro* bovine BBB P_e (taken from [13]) and log *in vivo* rat BBB P_e (taken from [13]), and artifical membrane *in vitro* P_e and *in vitro* bovine BBB P_e. The predicted human *in vivo* BBB P_e was then used together with human brain surface area (S_{br})- and blood flow rate (Q_{br}) data and the well-stirred model (Eq. (2)) to make an approximation of human *in vivo* $f_{a,br}$ for unbound drug molecules:

$$f_{a,br} = \frac{P_e S_{br}}{Q_{br} + P_e S_{br}} \quad (2)$$

Sink conditions and no redistribution back to blood were assumed. The S_{br} and Q_{br} were set to ~20 m^2 and 610 ml/min, respectively [14,15].

The average transit time through the brain (TT_H) and intestines are very different (almost 30,000-fold); ~5 s versus ~40 h, respectively [15–17]. A compound that is absorbed along the whole intestine (because of low P_e) will, therefore, also have ~40 h available for uptake into the brain. In order to visualize the intrinsic uptake capacity of the brain versus the intestines, the TT_H was set to 40 h (assuming sink conditions and no redistribution back to blood) when simulating the P_e relationship versus $f_{a,br}$ relationship.

Rat *in vivo* $f_{a,i}$ ($n = 23$; taken from [18]) and BUI (brain uptake measured 5–15 s after injection of a drug-containing buffer into the carotid artery; $n = 6$; data taken from [13]) were also related to artifical membrane *in vitro* P_e.

Data evaluation

Brain physiology and BBB P_e data

The brain weight, Q_{br} and TT_{br} of the human brain (for a 70 kg person) are reported to be ~1500 g (average from 7 studies), 610 ml/min (average from 18 studies) and ~5 s, respectively [15,17]. The surface area of the BBB (S_{br}) is approximated to 20 m^2 [14], and the total length of the >100 billion capillaries is ~400 miles [4]. Other reported estimates of S_{br} and total capillary length are ~12 m^2 and ~600 km, respectively [19]. The capillary endothelial cells of the BBB, which are believed to control the BBB P_e [4], are very thin (0.3–0.5 μm [1]), and have a cholesterol/phospholipid ratio (0.7) similar to that of the other endothelial cells [5]. This indicates that diffusion across the BBB is comparably rapid. Highly permeable water-selective channels, aquaporins, which are present in the renal tubuli and play an important role in the regulation of water homeostasis, are also present in the BBB [20].

In comparison to the liver and intestines, the human brain has a short transit time (TT) and small surface area. TT and surface area estimates for the liver and intestines are ~30 s and ~40 h, and ~180 and ~70 m^2 (effective *S*), respectively [12,21,22]. The hepatocyte sinusoidal surface area was estimated based on a rat estimate (0.1×10^{12} μm^2/g liver) and a human liver weight of 1800 g [22]. Brain capillary endothelial cells are considerably thinner than enterocytes (0.3–0.5 μm versus 17–30 μm) [1,23]. The human brain is also comparably large (2.2, 0.75 and 0.39% of body weight in humans, rats and rabbits, respectively) [24] and has longer TT_{br} and greater S_{br} than in smaller species.

Species similarities in cell membrane composition, and passive P_e and uptake have been demonstrated [21,25,26]. *In vitro* P_e data for 19 radiolabelled substances of various characteristics and with a large range of P_e across human primary brain endothelial cells were comparable (in some cases the P_e was significantly larger or smaller) to those obtained with bovine and rat capillary endothelial cells [27]. On this basis, it is anticipated that humans and animals have similar passive drug BBB P_e. This, together with the comparably large human brain, indicates that humans might potentially have a greater capacity than laboratory animals for absorbing compounds into the brain.

The *in vitro* P_e of the BBB is reported to be lower than of hepatocytes (for mannitol and urea [28]), but higher than in intestinal Caco-2 cells [13]. The *in vitro* study by Lundquist *et al.* [13] shows, on average, an 11-fold and maximally a 34-fold higher P_e across BBB (highly differentiated model with well-developed tight junctions) than across Caco-2 cell monolayers ($n = 15$). *In vitro* P_e data across isolated rat hepatocytes ($n = 2$), bovine BBB cell line ($n = 16$), rat small intestinal cell line (2/4/A1) ($n = 5$), human colonic cell line (Caco-2) ($n = 5$–15) and dog distal renal tubular cell line (MDCK) ($n = 8$) obtained at different laboratories and by different techniques are shown in Fig. 1. Data were taken from [13,29–31]. The hepatic P_e was estimated from *in vivo* P_eS and total hepatocyte *S* data (~3 m^2 in the rat [22]). The figure demonstrates the comparably high *in vitro* P_e across the BBB (comparable to the

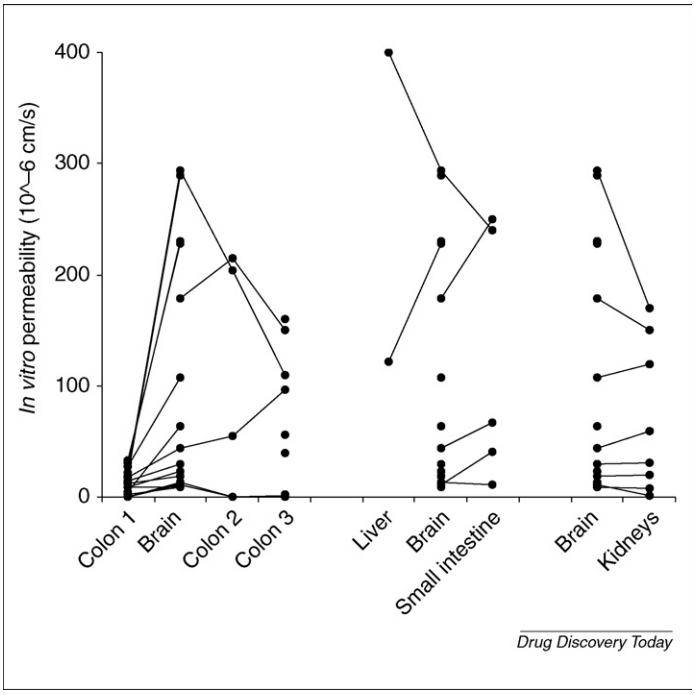

FIGURE 1

The *in vitro* permeability (P_e) across cells or cell lines of various organs. Data have been obtained at different laboratories and by using different techniques. Data for isolated rat hepatocytes ($n = 2$), bovine BBB cell line ($n = 16$), rat small intestinal cell line (2/4/A1) ($n = 5$), human colonic cell line (Caco-2) ($n = 5$–15) and dog distal renal tubular cell line (MDCK) ($n = 8$) were taken from [29] (liver), [30] (colon 3 and kidneys), [13] (brain and colon 1), and [31] (colon 2). The compounds are acetylsalicylic acid, antipyrine, caffeine, dexamethazone, diazepam, dopamine, hydrocortisone, mannitol, nicotine, phenytoin, pindolol, pirenzepine, propranolol, sucrose, terbutaline and urea. For visualization the brain data are presented three times.

highly permeable hepatocytes), but also the significant P_e differences between laboratories for Caco-2. The *in vivo* BBB/liver P_eS ratio for diazepam and propranolol (based on data shown in [13,29]) were estimated to be 0.2 and 0.04, respectively. The low values could partly be explained by the smaller surface area (~1/10) of the BBB. The Caco-2 cell line has been shown to be comparably less permeable for low P_e compounds [32], and this appears also to be the case for the BBB.

The BBB is reported to lack fenestrations and have nearly impermeable tight junctions [1]. Paracellular absorption (via the tight junctions) in the human intestine has previously been shown or suggested to lack importance for molecules with a MW > 200 Da [33,34]. On this basis, it is anticipated that drugs are absorbed across the BBB mainly transcellularly. CNS uptake could occur at the comparably permeable choroid plexus, but extensive absorption via this route is also significantly limited by a small surface area (~1/5000 of the S_{br}) [35,36].

Many compounds utilize active uptake and efflux across the BBB, and this could be of great importance [35,37,38].

Brain uptake of large and hydrophilic molecules

Several compounds with PSA > 70–120 Å^2, MW > 450 Da and log $D < 1$, and with apparent CNS uptake and activity were found. For example, there are CNS-penetrating compounds (in rats and mice) with PSA values between 127 and 279 Å^2 (sulpiride (PSA = 142 Å^2), vinblastin (PSA = 154 Å^2; MW = 909 Da), vincristin (PSA = 171 Å^2; MW = 923 Da), cyclosporin A (PSA = 279 Å^2; MW = 1203 Da; log $D = 5.0$) [7,13,19,38–42]. Borg and Ståhle [43] demonstrated significant rat brain absorption of two hydrophilic compounds, zalcitabine (log $D = -1.2$) and BEA005 (log $D = -1.5$); in addition, caffeine (log $D = -0.1$) is rapidly and extensively absorbed across the rat BBB [13]. Cyclosporin A, which also has good intestinal uptake for such a comparably large molecule, appears to diffuse into rat BBB endothelia cells *in vivo* and, thereby, inhibit P-pg transport of verapamil [38]. Octa- and heptapeptides with MW > 1000 Da enter the CNS in rats and mice [36,44], and inulin (MW ~ 5000 Da; log $D = -3.0$) is taken up by the rat BBB [45]. The fact that other low P_e molecules such as sucrose (PSA = 190 Å^2; MW = 342 Da; log $D = -3.7$), vincristin and cyclosporin A have higher BBB P_e than inulin [27,42,46] strongly indicates that these actually permeate the BBB and that no threshold for zero brain uptake, other than the anticipated zero P_e, has been established. It has been reported that some plasma proteins are excluded from the CNS [47]. According to several other reports (presented in [48]), however, plasma protein-bound drugs appear to cross the BBB. Albumin has a diameter of ~3–4 nm [49].

Solid nanoparticles (~50 to ~200 nm) have been demonstrated to cross the rat BBB (possibly by endocytosis or transcytosis) without damaging the endothelium [50,51]. Viruses (e.g. HIV, herpes simplex, West Nile and encephalitis) and bacteria (e.g. *Streptococcus*) may also penetrate the BBB and enter the brain [52–54]. The exact mechanism(s) for viral and bacterial CNS uptake are not known, but it might be as a result of damaging or P_e-enhancing effects of the infectious species on the BBB [50,52].

CNS effects of large and hydrophilic molecules, and substances with low C_{br}/C_{bl}

Cyclosporin A and metotrexate (PSA = 212 Å^2), which have a BBB P_e about one order of magnitude greater than sucrose and mannitol [35], may give CNS side effects in humans (see http://www.fass.se).

There are CNS-active compounds with C_{br}/C_{bl} ratios in the range of 0.007–0.01 [6,48,55]. Therefore, relatively low brain levels of a compound should not necessarily be interpreted as a low BBB P_e. *N.B.: total brain homogenate may contain a small fraction (in the order of a few percent) of cerebral blood, and this could produce a significant artifact C_{br}/C_{bl} ratios for compounds with low estimates* [19]. CNS binding- and effect-potentials are also a matter of binding capacity of other tissues/compartments, dose, potency (low amounts and concentrations could be sufficient to produce significant CNS effects) and the systemic half-life ($t_{1/2}$). Acidic compounds, which generally have a low capacity for binding to tissues and comparably high binding capacity to blood components (especially to albumin) often show lower C_{br}/C_{bl} ratios than neutral compounds and bases [56].

There are also examples of CNS-active drugs with a very low unbound fraction in the brain ($f_{u,br}$) and a low brain/plasma f_u ratio ($f_{u,br}/f_{u,pl}$). The $f_{u,br}$ of the highly permeable fluoxetine ($P_eS \sim 2 \times Q_{br}$ in the rat) in rats and mice is only 0.00094 and 0.0023 [10,19], and its $f_{u,br}/f_{u,pl}$ in mice is reported to be 0.074 [19]. The binding of this compound in the mouse brain is greater than to plasma ($C_{br}/C_{pl} = 12$) [19].

The role of BBB P_e for the extent and rate of brain uptake

BUI data obtained in the rat demonstrate rapid and extensive brain uptake of high P_e compounds and some absorption of low P_e substances (Fig. 2). For example, antipyrine, caffeine, nicotine and propranolol were absorbed to ~70–100% within 5–15 s, and the uptake of hydrocortisone and sucrose was 1.4% [13]. Although these results are, or might have been, influenced by some redistribution back to blood and distribution to other compartments, they give an indication of the high absorptive capability of the rat brain. Smith *et al.* (2001) [57] also concluded that the CNS is more permeable than imagined. They found that poorly permeable compounds do not take longer time to reach equilibrium with CNS tissue than more lipophilic compounds.

Many highly permeable substances, including anaesthetics and nicotine, have a very rapid onset of CNS effects (in the order of seconds) following injection or inhalation, and compounds with a brain $P_eS > Q_{br}$ have been found in the rat [10,58]. An example of a CNS-active drug with low P_e is theobromine, which has a P_eS in the rat that is only 7% of the Q_{br} [10].

The BBB passage time for different molecules can be estimated by dividing the thickness of the capillary endothelial cells by the BBB P_e. Calculations based on data from [13] show that it takes between ~0.1 and ~4 s for propranolol (high P_e) and sucrose (low P_e) to diffuse through the *in vitro* BBB. Corresponding estimates based on rat *in vivo* BBB P_e data are ~0.3 s and ~12 min, respectively. These results show the non-linear *in vitro*/*in vivo* BBB P_e relationship, and also a quite short time for low P_e molecules to be transported across the BBB. The *in vivo* BBB passage time for caffeine, morphine and inulin (MW ~ 5000 Da) molecules was approximated to 0.2 s, 7 s and 50 min, respectively. *N.B.: These BBB passage time-estimates should not be mixed with the time to equilibrium.*

For an unbound compound with an *in vitro* artifical membrane P_e of 1×10^{-6} cm/s (similar to the passive *in vitro* P_e of digoxin), the brain and intestinal f_a (for each organ passage; assuming no redistribution of absorbed drug back to the blood circulation) are approximated to 3 and 66%, respectively. The corresponding average percent f_a per minute per absorptive surface area is 1.8 and 0.002, respectively. Thus, the brain has higher absorptive capacity per minute per surface area. The hepatic f_a for free acetylsalicylic acid in rats, dogs and sheep has been estimated to 60–80% [25]. The rat *in vivo* BUI for this compound is 1.8% [13]. This demonstrates the high capacity of the liver to absorb this compound.

The relationships between artificial membrane *in vitro* passive P_e and *in vivo* $f_{a,i}$ (observed; fitted line) and $f_{a,br}$ (predicted) is presented in Fig. 3. The P_{e50} and λ for the intestinal data set are approximated to 0.66×10^{-6} cm/s and 1.6, respectively. For brain

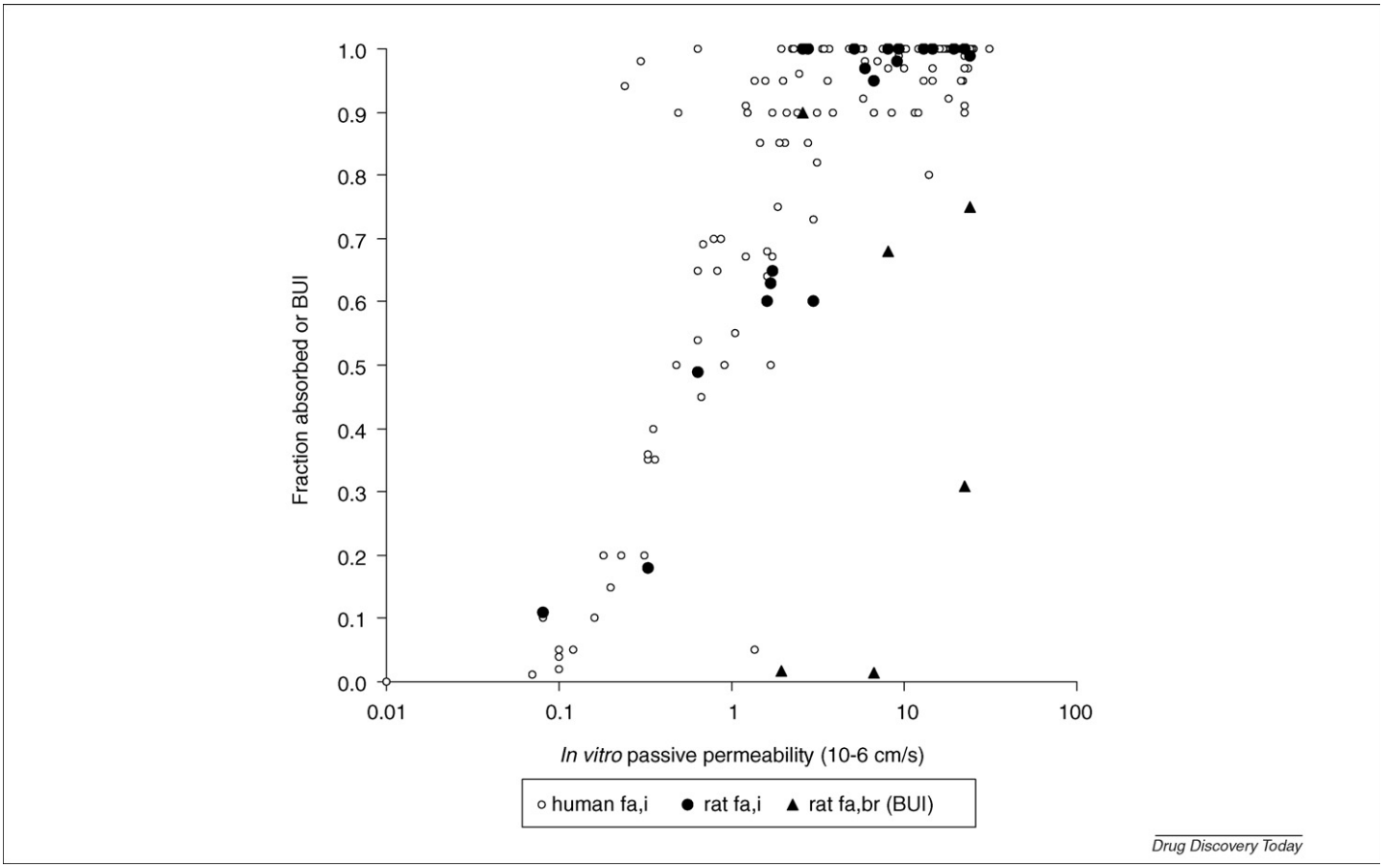

FIGURE 2

The *in vitro* passive permeability (P_e) versus *in vivo* intestinal fraction absorbed ($f_{a,i}$) and brain uptake index (BUI) during transit through the rat and human intestines (one transit) and rat brain (after 5–15 s), respectively. Rat (n = 23) and human (n = 126) $f_{a,i}$ data were taken from [18] and [12], respectively. BUI data (n = 6) were taken from [13].

uptake, a better fit was obtained when using different relationships for compounds with low ($<10 \times 10^{-6}$ cm/s) and high P_e ($>10 \times 10^{-6}$ cm/s). It should be noted that this is a rough approximation based on P_e data with comparably high variability. Hansen *et al.* [59] also found a similar relationship between log *in vitro* BBB P_e and *in vivo* brain uptake clearance in the rat (assessed by microdialysis sampling). The P_{e50} and λ for the low and high P_e compounds are estimated to be 150 and 24×10^{-6} cm/s, and 0.7 and 2, respectively. Predicted estimates for humans agree quite well with BUI data obtained in the rat *in vivo* (Fig. 2), which gives further validation to the predictions. The λ for the well-stirred model, and also the parallel-tube model at low f_a, is 1. Possible reasons for the deviation (0.7 and 2 versus 1) and biphasic character of the P_e versus $f_{a,br}$ curve include active transport, *in vitro–in vivo* differences and uncertainties in the data.

The unbound fraction for highly permeably molecules may reach ~50% brain uptake during a single transit through the brain, and complete brain uptake is often demonstrated in rats *in vivo* (within 5–15 s; comparably long time; rats have shorter TT_{br} than humans) [13]. The intrinsic $f_{a,br}$ for the unbound fraction of a compound with an $f_{a,i}$ of ≥20% (generally required for assuring a sufficiently good oral bioavailability (F)), and negligible BBB efflux is ≥~1% per each 5 s transit (the P_e corresponds to a maximum BBB passage time of ~15 s). The implication of this approximation is that intestinal permeation (at least if not active uptake) could be the rate-limiting step for the absorption rate into the brain and that mainly passively absorbed substances with sufficiently high $f_{a,i}$ and F will be readily absorbed into the CNS. This is demonstrated in Fig. 3. When assuming similar time for brain and intestinal absorption (40 h), sink conditions and no redistribution, the BBB has considerably higher $f_{a,br}$ than $f_{a,i}$ for compounds with similar P_e (about three magnitudes; except for those with very high P_e and complete uptake).

In order to limit (or avoid) transport into the brain, drugs probably need efficient BBB efflux (P-gp substrate probability can be predicted reasonably well using *in silico* approaches [60]), short systemic $t_{1/2}$ and extensive/restrictive binding to blood components.

The impact/importance of active transport across the human BBB is difficult to predict. Generally the reasons underpinning this

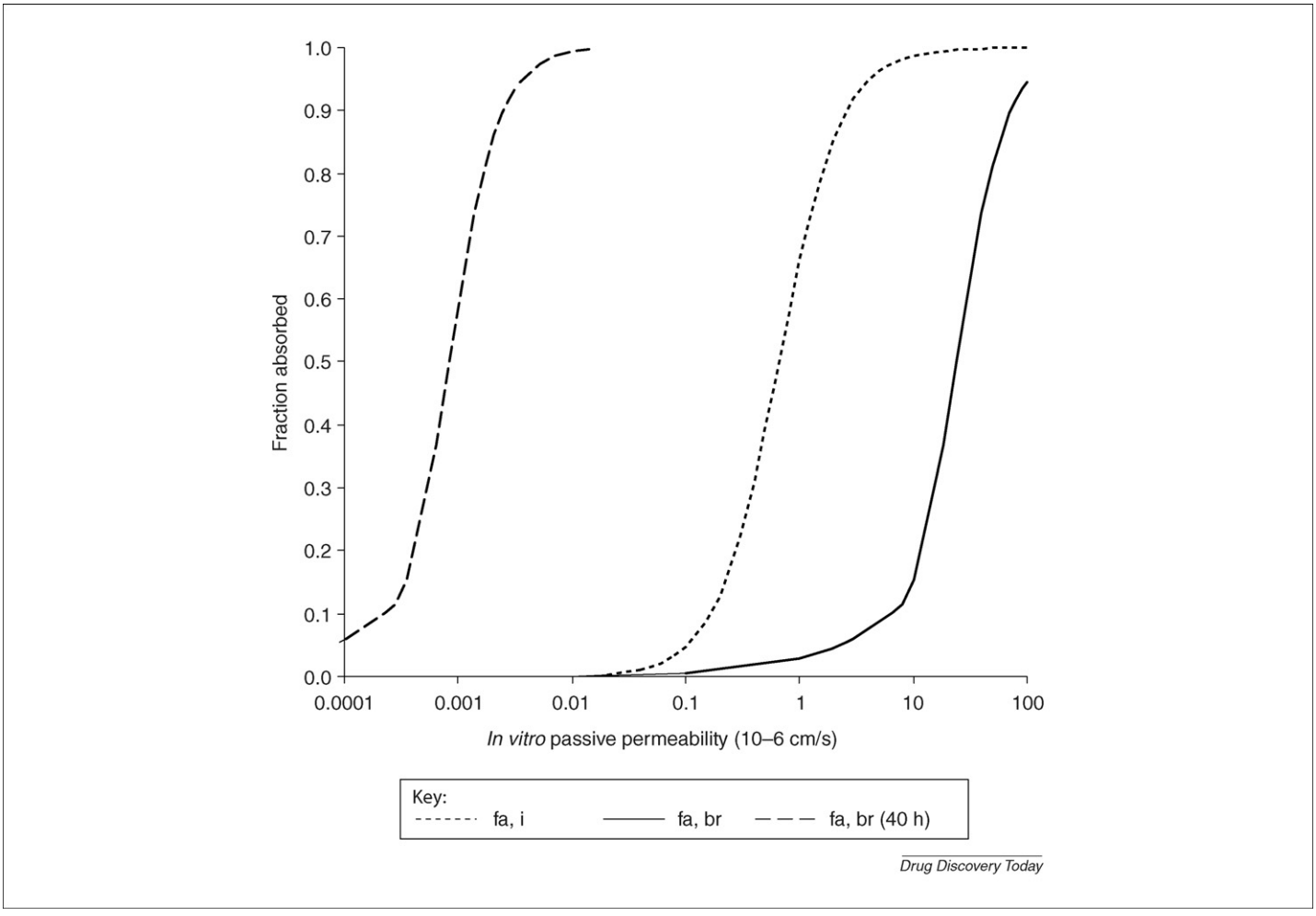

FIGURE 3

The *in vitro* passive permeability (P_e) versus *in vivo* fraction absorbed (f_a) during one transit through the human intestines ($f_{a,i}$; fitted line to observed data) and brain ($f_{a,br}$; predicted average). *In vitro* P_e and *in vivo* $f_{a,i}$ data (n = 126) were taken from [12]. *In vivo* brain f_a data (for the unbound fraction) were predicted using bovine *in vitro* BBB P_e (data taken from [13]), rat *in vivo* BBB P_e (data taken from [13]) and human brain physiology data, and the well-stirred model. Sink conditions and no redistribution were assumed. The brain uptake was also predicted for a 40 h residence time in the brain (similar to the average intestinal transit time). Drugs generally have a passive P_e between 0.3 and 30×10^{-6} cm/s.

are the steepness of the P_e versus f_a relationship, potential differences in active transport capacity between species and between *in vitro* and *in vivo* conditions. A challenging question is how well (or poorly) animal *in vivo* and *in vitro* BBB P_e data predict the *in vivo* BBB P_e for actively transported compounds in man.

The endothelial/epithelial transport of compounds with a passive P_e at, or close to, the slope (of the P_e versus f_a relationships) is potentially more sensitive to influences and changes (e.g. saturation, induction and inhibition) of active transport than for compounds with high passive P_e and complete organ uptake. Many high passive P_e compounds are completely taken up by the intestines and are less, or not, likely to be influenced by active transport (such as for verapamil [61]) (Fig. 3). These will, however, not be completely absorbed by the brain (because of the short TT_{br}), which makes their brain uptake potentially more sensitive to involvement and changes of active transport (Figs 2 and 3). The brain uptake of verapamil in rats *in vivo* during infusion is significantly enhanced in the presence of the P-gp inhibiting cyclosporin A [38]. Animal *in vivo* studies have demonstrated significantly enhanced brain/plasma ratios for highly permeable substances in animals lacking P-pg [39], and the P-gp inhibitor quinidine was able to increase the brain uptake of the highly permeable P-pg substrate loperamide in humans, and thereby, causing respiratory depression [62].

Conclusions

This evaluation challenges common opinions about BBB permeation and brain uptake capacity, the requirement for active BBB uptake, and the usefulness or requirement of *in vitro* and animal *in vivo* BBB P_e studies.

The BBB does not appear to be as impermeable as often perceived, and previously suggested MW-, PSA-, log *D*- and C_{br}/C_{bl} thresholds for no/limited brain uptake do not seem accurate. The BBB has an absorptive capacity similar to that of the highly permeable hepatocytes, and is able to absorb compounds of considerably greater size and lower lipophilicity than previously suggested. The BBB passage time for drug molecules, in general, is approximated to <1 s to ~1 min. Low C_{br}/C_{bl}- and/or $f_{u,br}$ estimates should not be interpreted as the compounds stay outside the brain and cannot produce CNS effects. The suggestion that only a few drugs enter the brain appears to have been based on studies including a large portion of CNS-inactive drugs with good BBB P_e. The proposed requirement to design CNS compounds for active BBB uptake appears exaggerated.

Data show that compounds with sufficiently good intestinal uptake and no, or minor, BBB efflux and metabolism, should have no problems to readily enter the brain. The intestinal uptake could be rate-limiting for the absorption rate into the CNS. Intestinal uptake data could, therefore, potentially replace BBB data for compounds with no or negligible active transport.

Unbound compounds are not (or are not expected to be) completely absorbed during a transit through the brain because of the short TT_{br} (~5 s). For high P_e compounds, the uptake of unbound fraction during each transit may reach approximately 50%. This makes brain uptake comparably (e.g. versus intestinal and hepatic uptake) sensitive to involvement and changes of active transport for compounds with high passive P_e. This has been demonstrated in animals and humans *in vivo*, and in simulations with a newly developed human brain uptake PB-IVIV prediction method.

Conflicts of interest

This work has not been funded, and the author has no conflicts of interest that are directly relevant to the content of this study.

This paper includes personal opinions of the author, which do not necessarily represent the views or policies of AstraZeneca.

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