European Journal of Pharmaceutics and Biopharmaceutics 172 (2022) 112–122

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Is oral lipid-based delivery for drug targeting to the brain feasible?

Alice Brookes [a], Liuhang Ji [a], Tracey D. Bradshaw [a], Michael Stocks [a], David Gray [b], James Butler [c], Pavel Gershkovich [a,*]

[a] School of Pharmacy, University of Nottingham, Nottingham, Nottinghamshire NG7 2RD, UK
[b] Division of Food, Nutrition and Dietetics, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire LE12 5RD, UK
[c] GlaxoSmithKline Research and Development, Park Road, Ware, Hertfordshire SG12 0DP, UK

ARTICLE INFO

Keywords:
Oral
Lipid
Lipid-based drug delivery
Brain
Blood-brain barrier
Brain targeting

ABSTRACT

This review outlines the feasibility of oral lipid-based targeted delivery of drugs to the brain, including permeation of the central nervous system's (CNS) protective blood–brain barrier (BBB). The structure of the BBB and disruption caused by varying disease states highlights the need for disease-specific approaches to alter permeation. Disruption during disease state, and the effects of certain molecules on the barrier, demonstrate the possibility of exploiting such BBB disruption for drug delivery. Many administration methods can be used to target the brain, but oral administration is considered ideal for chronic, long-term illnesses. Several lipids that have been shown to facilitate drug delivery into the brain after systemic administration, but could also be delivered orally, are discussed, including oleic acid, triolein, alkylglycerol, and conjugates of linoleic and myristic acids. Current data reveal the potential for the use of such lipids as part of oral formulations for delivery to the brain by reaching sufficient plasma levels after administration to increase the permeability of the BBB. However, gaps in the literature remain regarding the concentrations and form of most lipids required to produce the desired effects. The use of lipids via oral delivery for brain targeting has not been investigated thoroughly enough to determine with certainty if similar permeability-enhancing effects would be observed as for parenteral administration. In conclusion, further research to fill research gaps is needed, but the limited evidence suggests that oral lipid-based drug delivery for brain targeting is potentially feasible.

1. Introduction

Delivery of drugs to the brain is necessary for the treatment of a number of diseases. However, the brain is well protected, making this a particularly challenging area of drug targeting, with limited non-invasive options available. Oral lipid-based drug delivery is already established as an approach to improve systemic bioavailability, especially for low solubility drugs [1]. Additionally, some lipids are also known to affect the permeability of the protective blood–brain barrier (BBB), and could, therefore, if delivered in the right concentrations alter the delivery of drugs to the brain. The aim of this review paper is to collate the relevant information and to discuss the feasibility of developing orally administered lipid-based formulations for improved delivery of drugs to the brain.

2. The need for brain targeting

Drugs must reach their pharmacological site of action to be effective. If the target tissue is in the brain, the drug needs to penetrate the blood–brain barrier (BBB). Conditions such as Alzheimer's disease (AD), traumatic brain injury (TBI) and Parkinson's disease, as well as many other illnesses such as brain tumours or human immunodeficiency virus (HIV) reservoirs, are examples where the drug is required to reach the brain tissue for effective treatment.

Treatment for cancers (including brain tumours) usually includes a combination of surgery, radiotherapy and chemotherapy [2]. However, surgery is not always possible, and in many cases, treatment leads to only modest survival improvements (e.g., several months to a year for glioblastoma multiforme (GBM) patients) [2]. Brain surgery to remove the tumour often allows for a one-off direct injection of chemotherapeutics into the surrounding tissue, which is advantageous compared to systemic delivery, as many chemotherapeutic agents struggle to cross

* Corresponding author at: School of Pharmacy, Biodiscovery Institute (BDI), University of Nottingham, University Park, Nottingham NG7 2RD, UK.
E-mail address: pavel.gershkovich@nottingham.ac.uk (P. Gershkovich).

https://doi.org/10.1016/j.ejpb.2022.02.004
Received 18 October 2021; Received in revised form 21 January 2022; Accepted 5 February 2022
Available online 8 February 2022
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the BBB [2]. There has been some development in drug delivery to brain tumours by alternative administration, including biodegradable wafers inserted during surgery for the slow release of chemotherapeutics [2]. Alternative methods of drug delivery to the brain, such as nanocarriers, naturally occurring 'cages' (e.g. apoferritin to transport agents across the BBB) [3], or convection enhanced delivery (CED) of chemotherapeutics across the BBB [4] are also being explored. Continual release by the neurosurgically placed microcatheters allows transfusion of drug through 95% of the tumour, something not possible with systemic administration [3]. However, overall progress of new treatment approaches is slow and the prognosis for brain cancer patients unfortunately remains very poor.

HIV is treated with combination antiretroviral therapy (cART), but a complete cure cannot currently be achieved [5]. Often during treatment, HIV ribonucleic acid (RNA) is not detectable in blood, but once treatment is stopped, viral RNA is detected in the blood again [5]. This is due to reservoirs of HIV-infected cells remaining, even under effective cART treatment. These reservoirs are present in a number of difficult to reach tissues including bone marrow, the lymphatic system, the genital tract, and the brain [5,6]. Since many cART agents do not efficiently penetrate the BBB, it is difficult to achieve effective concentrations of antiretrovirals in the brain reservoir [5,7]. Approaches to improve the delivery of cART agents to the brain include drug delivery systems such as liposomes, micelles, and nanoparticles. However, delivery of cART agents to the brain is only part of the problem. It is also important to ensure cART agents are effective at eradicating HIV from central nervous system (CNS)-residing cells [5]. Asahchop et al compared the EC_{50} (half maximal effective concentration) values of several cART agents including darunavir, etravirine and dolutegravir in human foetal microglia and bone marrow derived macrophages [8]. They found that the EC_{50} was significantly higher for human foetal microglia than bone marrow derived macrophages, indicating a decreased efficacy of the agents against infected microglial cells [8].

Alzheimer's Disease (AD) is an incurable, age-related neurodegenerative disorder. Much research focuses on early diagnosis for the best management of symptoms. The two main hallmarks of AD, β-amyloid plaque deposition and neurofibrillary tangles, are the focus of diagnosis [9,10]. There has also been increased research into multi-target strategies to manage symptoms [10]. However, as most, if not all, AD treatments would need to reach the brain, appropriate drug formulation and administration should be an integral part of the drug development programme.

Traumatic brain injury (TBI) is usually the result of a blow to the head, and varies greatly in how it presents itself, from mild, temporary brain alterations to a comatose state and even death in severe cases [11]. The desired localisation of treatment is sometimes only a small area of torn tissues but can cover the whole brain in extreme cases [12]. Depending on the extent of the damage, treatment can include daily cognitive therapy and surgery as well as preventative measures, such as anti-epileptics [12]. The future of TBI treatment looks to therapeutic neuroprotectors, administered within hours of damage, to minimise the need for invasive surgery, and prevent some of the secondary damage caused by TBI [12]. The effectiveness of such agents would rely on good BBB penetration, as well as drug potency.

Parkinson's disease is a neurological disorder presenting itself as problems with movement such as tremors and rigidity. Parkinson's disease, like AD, is common in older people, and with an aging population the prevalence of such neurological disorders is increasing [13]. Treatment involves a combination of pharmacological and physical approaches, including exercises and speech therapy [14]. Pharmacological treatment is focused on the best management of symptoms and secondary ailments arising from the disease, rather than preventing or delaying disease progression itself [14]. Improving delivery of therapeutics across the BBB is important in the treatment of symptoms and progression towards a cure [3]. For example, methods such as use of CED, which was initially developed for the treatment of Parkinson's disease, showed promising results, but present other drawbacks, such as infection risk accompanying invasive procedures [15].

As highlighted above, enhancing the delivery of therapeutic agents across the BBB is beneficial for the treatment of many diseases. Sometimes, the need to deliver drugs to tissues within the brain leads to extremely invasive approaches, such as CED, as discussed above, and direct *trans*-cranial injection [2,16]. Another invasive method used is the deposition of an implant, commonly comprised of genetically engineered cells or polymeric matrices containing drug [17]. Implants need to be deposited close to the site of action, as drug diffusion decreases exponentially with distance (generally a 90% reduction in drug concentration at 500 microns distance)[16,17]. This leads to inconsistencies in drug concentrations across the cells, with cells closest to the implant experiencing very high concentrations [17]. This also sometimes leads to the situation in which multiple implants are required at the same time for drug to reach all of the target tissues. Other potential options for administration include intrathecal injection [17] and the exploitation of temporary disruption to the CNS protective BBB [17]. For chronic, long-term illnesses these are not sustainable treatment options, as ideally the patient would be able to administer their own treatments at home [17]. Two main options for non-invasive delivery are oral administration and intranasal spray, both of which are easily self-administered.

Trans-nasal delivery can lead to direct movement of drugs from the submucosal layer in the nasal cavity to the cerebrospinal fluid (CSF), entering predominantly via the olfactory bulb [16,18–20]. For example, *trans*-nasal delivery of progesterone resulted in CSF concentrations of the drug exceeding concentrations in plasma [16,21]. It is unclear if many drugs could be delivered to the brain efficiently using this route due to the delivery method limitations. For instance, large doses cannot be administered this way and the drugs delivered may have very specific physiochemical property requirements. High variability in the amount of delivered drug can also be expected, especially as it is difficult to deliver drug consistently to the olfactory bulb. Induced minor local injury by *trans*-nasal delivery could also occur, meaning some molecules that would not be expected to enter the CSF are eventually able to do so via *trans*-nasal delivery, such as vitamin B12 [16]. However, this often requires delivery of high volumes, which can cause local injury at volumes > 100 μL in adults [16,22].

Intranasal delivery appears to be a convenient option for patients to self-administer treatment at home for long-term illnesses. However, problems arise both in formulation development, and delivery itself. Drugs for intranasal delivery are usually formulated as nanoparticles, rather than as a solution [18]. As mentioned above, there is also a challenge when trying to deliver high doses via the intranasal route, as this could require larger volumes than are feasible, or extensive repeated administration with potential damage to tissues [16,18,22]. There are also limitations due to nasal-mucociliary clearance, a natural defence mechanism that is variable across humans, and in disease states [18,19]. Therefore, whilst on the patient compliance side, intranasal delivery is attractive, only highly potent molecules can be delivered this way at present and there are many challenges that still need to be overcome.

3. The blood brain barrier

The main hurdle to overcome for any approach aimed to deliver the drugs to therapeutic targets within the brain is the BBB. The BBB is a protective barrier, present in all organisms with a developed CNS, preventing the majority of compounds crossing from the blood into the CNS [16,23,24]. A secondary barrier, the blood-cerebrospinal fluid barrier (BCSFB), with larger openings between junctions for fluid transfer, is also able to protect the CNS from some blood-borne substances [23,25].

The BBB protects the brain from potentially neurotoxic substances including metabolites and proteins [23]. Although cells in the CNS in early life are able to repair themselves and replicate during growth, in adults they have lower capacity for replication [23]. The CNS protective barriers deteriorate at a steady rate, as cells and neurons die without

being replaced [23]. This occurs naturally in healthy brains, but the deterioration could be accelerated in disease states [23].

The structure of the BBB, shown in Fig. 1, consists of a monolayer of brain capillary endothelial cells (BCECs) surrounding the blood capillaries [26]. The BCECs create the largest blood–brain interface for molecular exchange, with a total average area of 12–18 m^2 in adult humans [23]. BCECs have small numbers of fenestrations (pores) and much denser content of glycocalyx than other vessels (40% compared to 15% in cardiac and 3% in pulmonary vessels). This structure creates the extensive tight junctions characteristic of the BBB, preventing most large or hydrophilic molecules from interacting with, and therefore penetrating, the BBB [23,24,26,27]. This results in a much lower protein content in the CSF compared to the plasma [23,27]. Small non-ionisable molecules, including O_2 and CO_2, can easily diffuse across the barrier [24]. Transporters and receptor-mediated endocytosis are responsible for the movement of most nutrients, e.g., amino acids, glucose and insulin, into the brain [24,27].

Transcellular permeation is high in peripheral endothelial cells but limited across BCECs. BCECs form a more continuous lining, limiting gaps between the cells. BCECs also have low levels of transcytosis, the main pathway for larger molecules to cross the BBB [23,26]. However, some large molecules are able to cross by receptor-mediated or adsorptive-mediated transcytosis, with a small number of non-specific large molecules being able to cross by non-receptor mediated mechanisms [16,23]. A detailed depiction of the BBB and possible transport pathways across the barrier are shown in Fig. 2.

Surrounding the BCECs are astrocytes and pericytes, forming the basement membrane, which acts as a supportive structure [23,24]. The basement membrane is essential to the key structures allowing movement across the BBB. For example, astrocytes have been shown to tighten the endothelium in leaky vessels and are suggested to be responsible for inducing BBB properties in endothelial cells [24,28].

Tight junctions, shown in Fig. 2 (A), are one of the first BBB features to develop, forming in the cell adhesions, creating tight gaps for very small, water-soluble molecules to move through via paracellular transport [23]. Tight junctions are unique in their structure and molecular composition (proteins present in the gaps link the two cells together), creating a high-resistance electrical barrier [23,26]. Tight junction proteins consist mainly of claudin, occludin and junction adhesion molecules, all of which are variable in number across the junctions [24]. Claudins are essential to the barrier function as they maintain the seal, or 'tightness', of the junction [24]. Passive diffusion across BCECs of the BBB is also possible for nonpolar molecules.

Transporters can both aid molecules entering the brain, as well as remove substances from endothelial cells back into the blood. Solute transporters carry substrates such as glucose, amino acids, and fatty acids down the concentration gradients [23,26,27]. Efflux, or ATP-binding cassette (ABC) transporters, consume ATP to remove small molecules from the brain into the blood [23,26]. Of particular note is drug efflux pump P-glycoprotein (P-gp; ABCB1; multidrug resistance protein-1), where enhanced expression is induced by chemotherapeutic agents, and it is therefore largely responsible for multi-drug resistance [29]. The presence of P-gp on BCEC exports and blocks the retention of many potentially efficacious drugs.

Fig. 2 (D) and (E) describe the limited transcytosis across the BBB. Receptor-mediated transcytosis transports specific peptides and proteins, such as insulin, across the BBB. Reduced intracellular degradation, allowing the transport of specific molecules through the cells is thought to be a functional feature of BCECs. This feature is an important homeostatic requirement [30]. Absorptive-mediated transcytosis describes the uptake of cationic molecules into the brain, such as albumin.

Low-density lipoprotein receptors (LDLR) are another important aspect of the BBB, allowing transcytosis across the BBB [31]. Such receptors have led to the approach of administering statins with a LDLR targeting encapsulated poorly BBB-permeable drug, in order to increase permeability via LDLRs [32]. Low-density lipoprotein receptor-related protein 1 (LRP-1) is of particular interest, playing an important role in physiological and pathological conditions. LRP-1 is reported to regulate certain tight junction proteins, and is responsible for inducing BBB opening after ischemic attack [33].

There are some empirical rules describing which molecules are likely to permeate the BBB, similar in principle to Lipinski's rule of five for intestinal absorption [16]. A molecule should be nonpolar, or have a low polar surface area (<90 $Å^2$) with a maximum of 5 hydrogen bond donors and 10 hydrogen bond acceptors (reducing the free energy requirement of moving from aqueous to lipid environments) [16,23]. The molecule should also be<500 Da and should not have many rotatable bonds (<5 as the presence of rotatable bonds appears to limit permeability across the BBB) [16,17,23,34]. In addition, it has been demonstrated that weak bases penetrate the BBB more readily than neutral molecules and

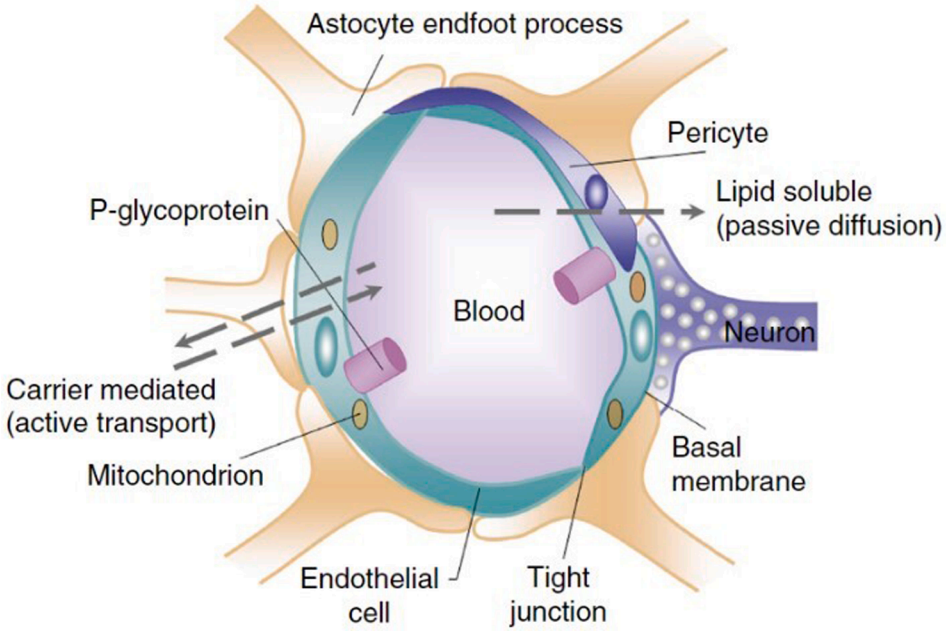


Fig. 1. Schematic diagram of the neurovascular unit/cell association forming the BBB. Reproduced from [30], with permission from Elsevier.

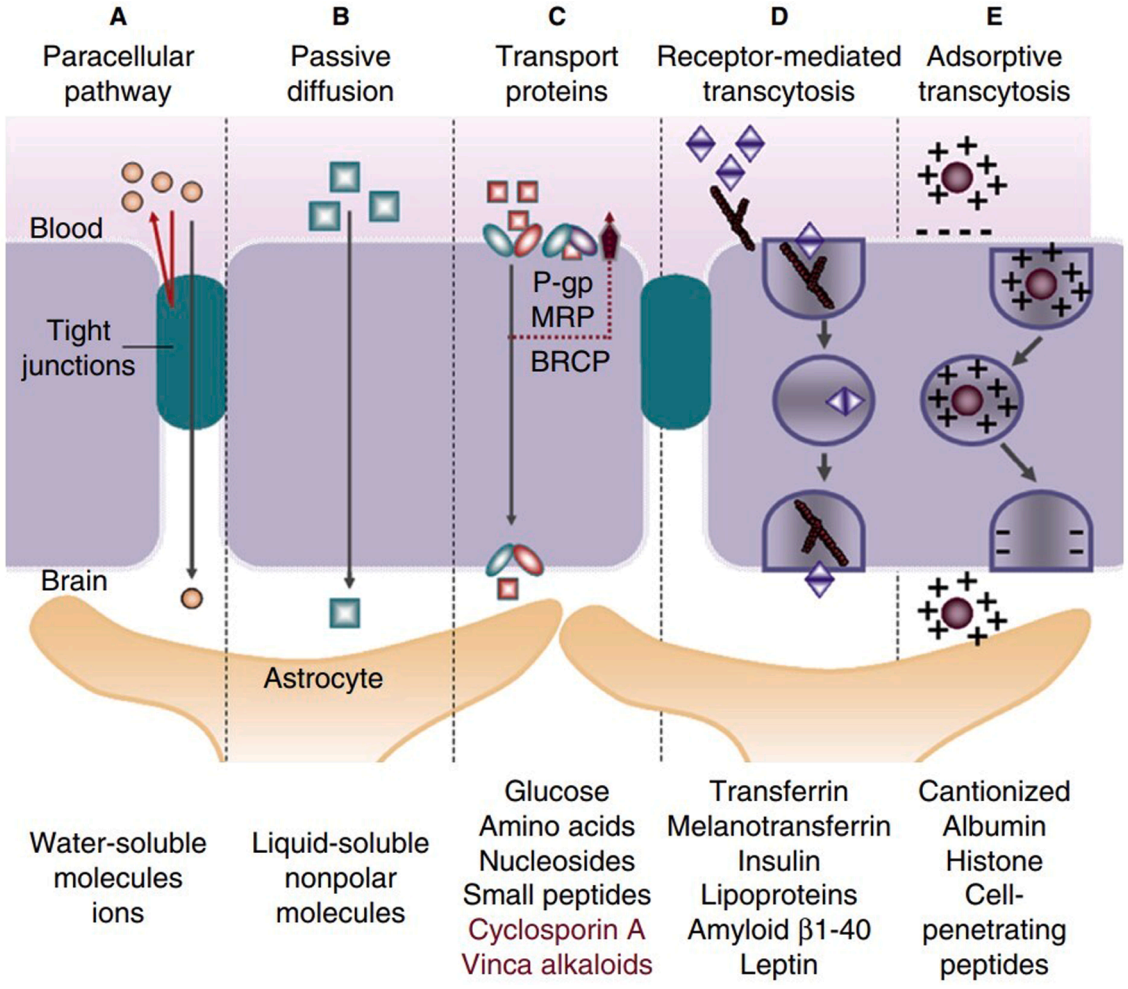


Fig. 2. Routes for transport across the BBB. Reproduced from [30], with permission from Elsevier.

zwitterions, with acidic molecules having the lowest CNS penetration [23,35]. This is partly due to the interaction with the negatively charged glycocalyx as well as other factors, such as P-gp efflux [23,35,36]. These guidelines quite successfully describe the limitation of BBB permeation for almost 98% of small molecules (and almost all large molecule) drugs [37].

As mentioned above, certain diseases, such as AD and multiple sclerosis, can alter the structure and integrity of the BBB [23,38,39]. BBB disruption from brain tumours is highly variable and is not well characterised in many disease states [38]. Disruption of the BBB is central to pathologies such as multiple sclerosis and epilepsy, but is often a secondary effect of disease, such as in brain cancers [26]. Therefore, barrier disruption is frequently disease-dependent. For example, multiple sclerosis has been reported to lead to BBB breakdown by tight junction abnormalities, loss of claudin-3 and downregulation of laminin. On the other hand, HIV is reported to cause tight junction disruption as a result of leukocyte migration into the brain [23,24]. In most cases of BBB disruption in disease states, there are changes to the tight junctions, causing their opening and allowing the permeation of larger molecules across the barrier [23]. As expected, a number of abnormalities can occur when the BBB is disrupted, leading to a range of effects [26]. For instance, disruption of glucose transporter GLUT1 leads to seizures, whereas disruption in LAT1 amino acid transporter can lead to autism spectrum-type effects [26].

BBB disruption in AD is an ongoing area of research, including as a potential early biomarker and target for treatment [26,39]. Certain disruptions in AD patients, such as synaptic dysfunction, are shown to be the result of pathway activation by β-amyloid [24]. Other disruptions, such as alterations in white matter lesions, have been observed but have no known definitive cause yet [25]. However, research is still ongoing into what stage in the disease progression BBB dysfunction occurs, and a full understanding of the barrier properties affected [26].

In patients with glioblastoma multiforme (GBM), the most aggressive brain cancer, loss of claudin-1 and claudin-3 from tight junctions leads to reduced BBB integrity and some functional loss of the barrier [2,23,24]. Exploring the possibility of exploitation of BBB disruption in cancer patients has been ongoing to selectively target chemotherapeutics to tumours [17]. For example, administration of bradykinin further increases the permeability of BBB capillaries in patients with brain tumours, with the greatest effect seen close to the tumour site, but not in healthy brains [40,41]. This process is thought to occur by activation of B2 receptors on endothelial cells to open ATP-sensitive potassium channels [41,42].

During stroke, BBB disruption follows a biphasic time course, visible within hours before decreasing, and increasing again the following day [43,44]. Stroke causes structural changes to tight junctions via loss of claudin-1 and increased transcytosis [24,45]. The second phase of disruption is a result of increased cell death that occurs in the days following the stroke [26]. Approaches to reduce cell death during the second phase include poly(ADP-ribose) polymerase (PARP) inhibition (where over-activation leads to DNA damage and ATP-depletion), or inducing therapeutic hypothermia [46–48]. However, there are still many unknowns in the mechanism of BBB disruption during stroke, including the differences in disruption between the first and second

wave.

It is important to recognise that in disease states the BBB is often disrupted, and often in different and sometimes unpredictable ways by the same disease [26]. Although some research is underway to exploit BBB disruption in disease states in order to enhance drug permeability, this is not a straight-forward option and would likely require patient- and disease stage-specific approaches [16]. Inducing reversible BBB disruption as a treatment enhancer, for example by mannitol, is also under investigation [17]. The BBB is complex, and even more so when disrupted, leaving many unanswered questions. Transport across the BBB is an important area of research, where the understanding of a molecule's interaction with the barrier is key [26].

4. Oral lipid-based drug delivery

Oral delivery of drugs is considered the easiest administration for patients, allowing convenient at home treatment, and therefore high compliance [49]. There are several other advantages associated with oral drug delivery, such as reduced costs to healthcare systems, pre-defined doses and, dosing times and the non-invasive nature of this delivery method [50]. Therefore, as highlighted above, oral administration is ideal for chronic illnesses. However, successful oral administration presents many hurdles. Firstly, as with all administrations, the delivery system needs to be biocompatible and not alter the drug's activity [49]. Factors such as low water solubility, molecular size (i.e., oral delivery of peptides and proteins is more challenging) high first-pass metabolic loss and low membrane permeability can all limit a molecule's oral bioavailability [1,50]. Secondly, for good patient compliance, it is important that oral formulations do not have unpleasant taste. Often, taste-masking agents are used for this purpose [51].

Oral lipid-based delivery systems are a well-studied formulation approach first reported in the 1970 s, with the use of lipids to enhance drug absorption [1,52]. It is important to understand the many mechanisms leading to enhanced bioavailability of drugs administered with lipid-based delivery systems. These include: increased absorption from the gastrointestinal tract (by a combination of prolonged exposure and increased wall permeability), altered enterocyte-based transport, avoiding first-pass metabolism and enhanced transport to the systemic circulation by the intestinal lymphatic system [53,54].

Most recently newly discovered drugs are classified into biopharmaceutics classification system (BCS) class II, i.e., poor aqueous solubility and high membrane permeability [53]. In order to improve the poor solubility of a drug, a lipid-based formulation may be used. In 2000, Pouton reported a lipid formulation classification system (LFCS) to group the different lipid-based systems used for drug delivery [55]. Formulations were divided into categories with increasing hydrophilic content from type I to type IV (type IV being added in 2006 to describe formulations not containing oils), summarised in Table 1 [55,56]. Briefly, Type I formulations use 100% oils, triglycerides, or mixed mono- and di-glycerides. Type II use a combination of oils and water-insoluble surfactants. Type IIIA use predominantly oils and a mixture of water-soluble surfactants and hydrophilic co-solvents, i.e., polyethylene glycol (PEG) or propylene glycol. Type IIIB contains the same excipients as type IIIA but lower oil content (<20%). Finally, type IV contains both water-soluble and water-insoluble surfactants and hydrophilic co-solvents.

Table 1
The lipid formulation classification system (LFCS) as described by Pouton in 2000 and 2006 [55,56].

Excipients in formulation	Content of formulation (%, w/w)				
	Type I	Type II	Type IIIA	Type IIIB	Type IV
Oils	100	40–80	40–80	<20	–
Water-insoluble surfactants	–	20–60	–	–	0–20
Water-soluble surfactants	–	–	20–40	20–50	30–80
Hydrophilic cosolvents	–	–	0–40	20–50	0–50

Lipid-based drug delivery systems can be advantageous not only to increase the solubility of poorly soluble molecules, but also to increase permeability, avoid fist-pass metabolism and decrease food effects [1,51–53]. As the formulation already contains lipids, the further effects of lipids in the diet are usually limited. However, this does vary depending on the lipid formulation and drug solubility [52–54]. Birnbaum et al showed this with oral cannabidiol (CBD) delivery, showing 14 times higher C_{max} and 4 times higher area under the curve (AUC) in the fed state compared to control [57]. Another advantage of oral lipid-based delivery is the relative ease in the scaling-up of the product. Usually, the same, or a very similar, liquid formulation that is given to animals in early pre-clinical studies can be scaled up for clinical trials [1].

Surfactants are often added excipients in lipid-based formulations, which can lead to the creation of micelles (with both hydrophobic and hydrophilic regions) in aqueous environments [51]. Surfactants are usually a secondary addition, when the use of water-insoluble oils only (such as peanut and soybean oil) does not improve bioavailability [51]. For example, the addition of d-α-tocopheryl polyethylene glycol 1000 succinate (vitamin E-TPGS) to poorly water-soluble antiretroviral drug amprenavir created micelles to stabilise the molecule, which enhanced the solubility and permeability of the drug [58]. Commonly used surfactants include Cremophor EL (polyoxyl 35 castor oil), Cremophor RH 40 (polyoxyl 40 hydrogenated castor oil), Tween 20 and 80 (polysorbate 20 and 80), TPGS, Solutol HS-15 and a number of polyglycolysed glycerides [51]. Polyglycolysed glycerides include fatty acids such as oleic acid, linoleic glycerides and mono-, di- and triglycerides [51].

Lipid nanocarriers have gained much interest in recent years as a delivery method to overcome the poor bioavailability of certain drug molecules. Some examples include the development of nanocarriers for antihyperlipidaemic drug simvastatin, which led to a 4-fold increase in oral bioavailability compared to a simvastatin suspension. Another example is polymer-lipid nanoparticles of enoxaparin, which showed a 4–5-fold increase in oral bioavailability, compared to an enoxaparin solution [59,60]. Lipid liposomes have also been shown to aid in the delivery of peptides by oral administration. For example, lipid glycerylcaldityltetraether (GCTE) liposomes of antibiotic vancomycin protected the peptide structure and integrity *in vivo* after oral delivery [61].

4.1. Lipid digestion and transportation

Oral lipid-based drug delivery systems utilise lipid digestion and transportation processes. Therefore, a brief description of these processes is provided here for background (Fig.3).

Most dietary lipids are in the form of triglycerides, and after a series of digestive processes, the triglycerides are broken down into fatty acids and monoglycerides, which are then absorbed by enterocytes [62]. Subsequently, the triglyceride chain length determines the intracellular processing. In general, short- and medium-chain fatty acids (carbon chain length < 12) diffuse through the enterocyte and then enter the blood circulation through the portal vein and liver. Longer chain fatty acids (carbon chain length > 12) are re-acylated into triglycerides and assembled into chylomicrons (CMs), which are secreted into the mesenteric lymph and enter the blood circulation via the thoracic duct. CMs entering blood capillaries of certain tissues will activate the lipoprotein lipase on the surface of BCECs to generate fatty acids. These fatty acids then enter muscles and adipose tissues for storage or use. The CM remnants (mainly containing cholesteryl esters) will be recognised by the apoE receptor on the liver cell membrane and enter the liver cells.

Due to the hydrophobic nature of lipids, their transportation in the body requires lipoprotein vehicles. Lipoproteins circulate in the blood until their triglycerides are consumed by the surrounding tissues, or the lipoproteins themselves are eliminated by the liver. CMs are the largest

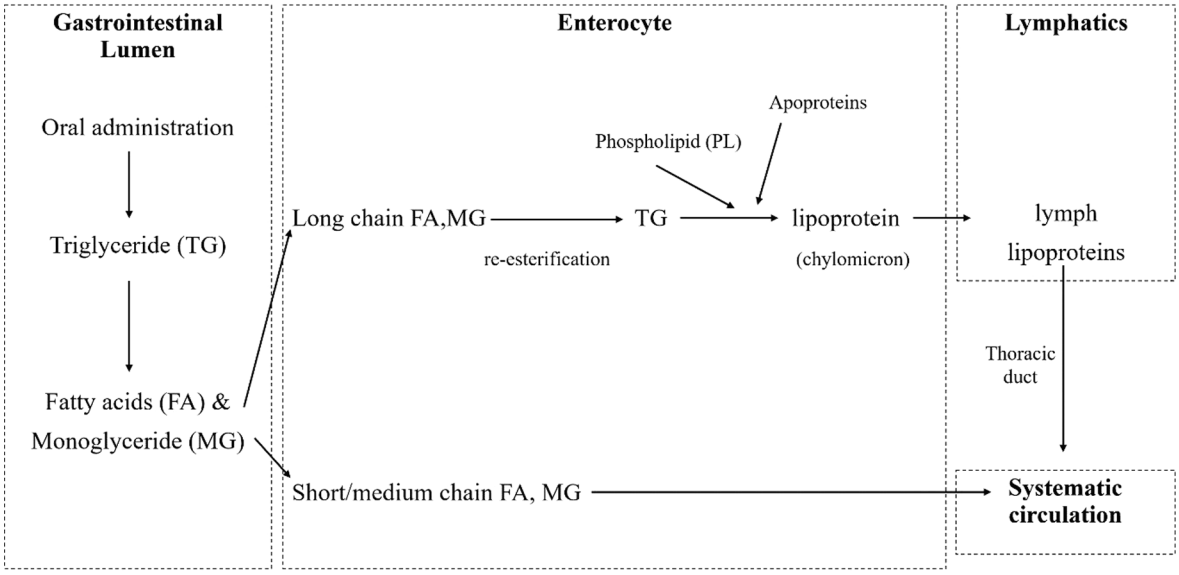


Fig. 3. Schematic diagram describing the sequential steps in the digestion of lipids and subsequent absorption via the portal blood and intestinal lymphatics. Reproduced from [95], with permission from Elsevier.

lipoproteins found in the body, synthesised in the intestinal wall. Other lipoproteins are characterised as either very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL) or high-density lipoproteins (HDL) [63]. VLDL are predominantly synthesised in the liver. VLDL's surface Apo C-II activates lipoprotein lipase, which, as described above, hydrolyses triglycerides into free fatty acids and glycerol to be absorbed by cells. LDL are rich in cholesterol and are mainly generated following VLDL metabolism in the plasma. A small proportion of LDL is synthesised by the liver and secreted directly into the blood. LDL function is mainly to transport cholesterol to peripheral tissue cells. Between 40 and 60% of LDL can be cleared by the liver (mediated by apo B and hepatic LDL receptors), and the rest is absorbed through hepatic LDL or non-hepatic non-LDL receptors. HDL is synthesised by the liver and enterocytes, and newly generated HDL does not contain cholesterol. HDL function is to obtain cholesterol from peripheral tissues and lipoproteins in order to transport it to tissues where cholesterol is needed. Additionally, long-chain fatty acids tend to exist in the form of triglyceride in lipoproteins, whereas short- or medium-chain lipids remain in the fatty acid form in the blood [64].

5. Research indirectly indicating that oral lipid-based drug delivery for brain targeting is potentially possible

To the best of our knowledge, there is no published literature discussing or reporting oral lipid-based drug delivery systems for the enhanced uptake of drugs into the brain to date. However, there is substantial evidence of systemically administered lipids reversibly affecting BBB permeability. There are also reports that some of these lipids can be administered orally, and made available in the systemic circulation after administration. The different aspects of these reports, as well as currently existing gaps in the available information are discussed below.

5.1. Lipids affecting BBB permeability and suitable for oral administration

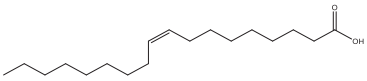
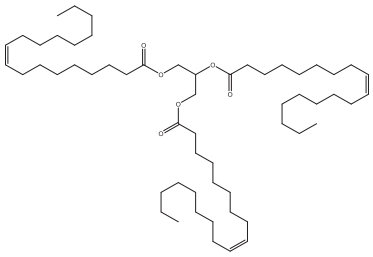
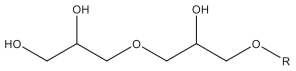
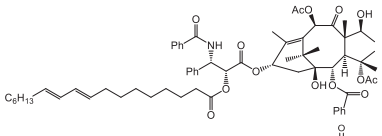
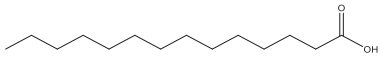
Due to the physiological characteristics of the BBB, lipophilic substances can enter the brain relatively easily. This suggests that lipids could potentially be used to facilitate the delivery of drugs to the brain. However, as the chain length of lipids, and their metabolic pathways, differ, not all lipids are suitable for oral administration for these purposes. The lipids that can affect BBB permeability following systemic injection and are also suitable for oral administration to then become available in the systemic circulation are summarised in Table 2.

5.2. Long-chain lipids

Oleic acid (C18:1) is abundant in human adipose tissue and is also present in animal and vegetable fats. Oleic acid circulating in the systemic blood in the free form has been shown to increase BBB permeability (the suggested mechanism of this is via albumin binding) [65,66]. Sztrlha and Betz examined the effects of oleic acid infusion on BBB permeability in 1991. A sodium oleate solution was injected into the right internal carotid artery of adult rats (6 mL/min for 30 s) and uptake of α-aminoisobutyric acid into the brain was studied [65]. Uptake was found to be facilitated by a 1×10^{-5} M oleic acid formulation. Interestingly, and encouragingly, the effect was reversible. At 80–90 min post injection, BBB permeability had returned to normal. Kim et al observed a similar phenomenon when administering an oleic acid emulsion (0.05 mL oleic acid and 20 mL saline) into the carotid artery of cats (4 mL/min for 5 min) [67]. The oleic acid emulsion was found to reversibly increase BBB permeability. The effects of oleic acid are proposed to be the result of interaction of lipids with BCEC membranes. Oleic acid allows the incorporation of fatty acids into the lipid membrane, affecting the properties and functions of the cell membrane [65]. However, there is no sufficient evidence to prove this hypothesis at this stage and further research is necessary.

In order to consider oleic acid as a vehicle for oral lipid-based delivery to the brain, it is important to take into consideration the digestion of orally administered oil. The saturation of fatty acids plays a role in their absorption, with mono-saturated oleic acid resulting in good digestion, regardless of the oil form administered. Oleic acid is transported via the lymphatic system, and as discussed above, stored as triglycerides in tissues until use [68]. Studies examining the biodistribution of oleic acid have traced radiolabelled molecules to a wide range of tissues including the heart, liver, lung, spleen, kidney, and brain [68,69]. Several studies have already utilised this pathway to design oral formulations for lipophilic compounds. For instance, Caliph et al observed a significantly higher systemic exposure of highly lipophilic substance Org 49,209 using oleic acid-based formulations compared with a lipid-free vehicle in rats [70]. This substance has also been detected in the brains of rats that were orally administered with

Table 2
Lipids that can affect BBB permeability and are suitable for oral administration.

Name and Dosing Pathway	Structure	Species	Minimal Dose that can affect BBB	Proposed Mechanism
Oleic acid (intracarotid administration)		Rats: 0.35–0.4 Kg	Rats: 3 mL of 10–5 M sodium oleate solution (dissolved in saline) for each animal Cats: 20 mL of 0.25 mL/mL oleic acid emulsion (with saline) for each animal	Interaction with cell membranes
Triolein (intracarotid administration)		Cats (weight not reported) Rabbits: 2.5–3.0 Kg Rats: 0.29–0.31 Kg Cats: 2.8–3.6 Kg	Rabbits: 10 mL of 10 μL/mL triolein emulsion (with saline) for each animal Rats: 0.67 mL/Kg of 20 μL/mL triolein emulsion (with saline) Cats: 3 mL/Kg of 5 μL/mL triolein emulsion (with saline)	Tight junction breakdown ; Endocytosis efficiency increase
Short-chain Alkylglycerols (intracarotid administration)	 (rac)-1-O-alkyldiglycerol R: pentyl or hexyl	Rats (weight not reported)	36 mg/Kg for 1-O-hexyldiglycerol (lipid mass per Kg body weight)	Tight junction breakdown
Conjugated linoleic acid with Paclitaxel (intravenous injection)		Rats: 0.18–0.22 Kg	Equivalent 5 mg/Kg dose of PTX	Possibly increased lipophilicity
Myristic acid conjugates (intravenous injection)		Mice: 20–25 g	–	Possibly structure of myristic acid, especially the chain length

oleic acid-based formulations, although it is not yet clear whether the presence of oleic acid increases the brain's uptake of Org 49,209 [70].

Triolein emulsion (a symmetrical triglyceride containing 3 units of oleic acid) is another lipid that has been shown to increase the permeability of the BBB when administered directly into the blood circulation. Kim et al demonstrated that a doxorubicin-loaded triolein emulsion (0.10–0.20 mL triolein with 10 mL saline) injected into the right carotid artery in rabbits increased BBB permeability [71]. At 2 h following administration, doxorubicin concentration in ipsilateral hemispheres increased several fold, compared to the control of administration without triolein emulsion. Drug concentration changes in the ipsilateral brain over time were also investigated in cisplatin-treated rats after injection into the right carotid artery [71]. The highest cisplatin concentration in the ipsilateral brain was achieved 6 h post injection of 20 μL/mL triolein emulsion (0.67 mL/Kg dose). Additionally, Ryu et al determined that the lowest dose of triolein emulsion that can increase the BBB permeability by intra-arterial injection in cats is 3 mL/Kg (5 μL/mL triolein emulsion in saline)[72]. Choi et al repeated the study to confirm the minimal dose to increase BBB permeability is 3 mL/Kg (5 μL/mL triolein emulsion in saline)[73].

Based on nutrition literature [74–76], it appears feasible to achieve appropriate triolein levels to affect BBB permeability in the systemic blood circulation by oral administration. Ryu et al calculated that the lowest dose required to increase BBB permeability in cats by intracarotid injection is ~ 13.73 mg/Kg [72]. BBB permeability is shown to still be altered 2 h post intracarotid injection of triolein [71–73]. This could indicate that the initial high plasma concentration resulted in a prolonged increase in BBB permeability, or alternatively, that a lower concentration than the C_{max} is effective in altering the BBB permeability. Triolein is well absorbed in humans [77,78], therefore converting this dose to humans based on body weight and body surface area (Human Equivalent Dose), the lowest dose required to increase the BBB permeability in an average adult by intra-arterial injection would be ~ 5.47 mg/Kg [79,80]. For an adult weighing 60 Kg, as little as 330 mg triolein might be sufficient to effect BBB permeability. However, as the plasma C_{max} after oral administration is likely to be substantially lower, and some triolein would be broken down and re-assembled into different triglycerides post absorption, it's sensible to assume a higher dose of triolein would be administered. This dose is achievable in a lipid formulation and suitable for oral administration.

It should be noted that these calculations are preliminary, as additional data will be needed to prove the theoretical calculations made here. The behaviour of triolein in plasma after intracarotid vs. intravenous injection is thought to differ, and the rate of change of triolein concentration in plasma after intracarotid injection is still unclear. In addition, triolein levels in the blood following oral administration are not conclusive from reported literature. Also note-worthy is that all calculations reported here use the lowest triolein levels reported in the literature, so as to prevent any underestimation.

The mechanism by which triolein increases BBB permeability is not yet completely clear. To investigate the phenomenon, Sol et al infused triolein emulsion (3 mL of 5 μL/mL triolein with saline) into the carotid artery of rats [81]. They report that 2 h after injection, transcellular vesicles in the BBB endothelium increased in number ~ 5 fold, compared to the control group of infusion without triolein emulsion. Paracellular transport via tight junctions, which did not occur in the control group, was also observed after infusion with triolein. Based on this, Lee et al speculated that the increase in BBB permeability caused by triolein

emulsion is at least partially explained by enhancement of paracellular and transcellular pathways [82].

5.3. Short-chain lipids

There are not as many studies investigating the effects of short-chain lipids on BBB, although short-chain alkylglycerols (an ether lipid abundant in shark liver oil) have been shown to increase BBB permeability after systemic administration. Erdlenbruch et al co-injected alkylglycerol (0.01–0.3 M) with cisplatin or methotrexate into the carotid artery in rats and measured drug levels in the ipsilateral brain. They found that drug concentrations increased in an alkylglycerol concentration-dependent manner (cisplatin concentration range was from 2 to 230 fold higher) [83]. Importantly, the effect was reversible, and the BBB returned to the original state within several minutes. Further research using methotrexate as the marker to study the effects of alkylglycerol chain structures on the BBB was conducted. The highest BBB penetration of methotrexate was found with 100 mM 1-O-hexyldiglycerol (more than a 1400 fold increase compared to the control group).

Erdlenbruch et al also demonstrated that different alkylglycerol chain structures affected BBB permeability in different manners after systemic administration. The increased permeability induced by 100 mM 1-O-pentylglycerol in rats returned to normal within 3 min, while the permeability increase caused by 50 mM 1-O-hexylglycerol remained 1 h post injection. In addition, the increased BBB permeability caused by alkylglycerols is effective for both small molecules (e.g. fluorescein and sodium) and large molecules (e.g. lissamine-rhodamine B200–albumin) [84]. Tight junction changes were observed through confocal microscopy and it was found that at least part of the enhanced BBB permeability is the result of increased paracellular transport [84].

5.4. Drug conjugates with fatty acids

Chemical conjugation of drugs with lipid molecules is another approach that has been reported to lead to increased BBB permeability after systemic administration. Ke et al covalently linked linoleic acid (C18:2) with paclitaxel and injected the conjugate intravenously in rats [85]. The half-life and area under the curve (AUC) of the conjugate in plasma were significantly increased compared to the control group (unconjugated paclitaxel). The conjugate was quickly distributed to the brain 30 min after injection and was still detectable at high levels after 360 h. The highest conjugate concentration detected in the brain was 1 μmol/Kg, whereas in the control group no paclitaxel was found in the brain. Furthermore, the ability of the conjugate to reduce tumour weight was studied in C6 glioma tumour-bearing rats after cell implantation. While in the control group, unconjugated paclitaxel did not reduce tumour weight, conjugated paclitaxel significantly reduced the tumour weight. Linoleic acid itself can cross the BBB, and the lipophilicity of the conjugate is higher compared to paclitaxel alone, therefore, it is suggested that the conjugate is able to penetrate the BBB more easily than the parent drug.

Shen et al reported the effects of myristic acid (C14:0) conjugates on BBB permeability after intravenous injection [86]. Transfection reagent polyethylenimine was covalently linked with myristic acid and administered intravenously in mice. The transfection effect was further elevated by plasmid-enhanced green fluorescent protein (pEGFP). GFP was successfully synthesised in the brain only in mice treated with the conjugate. The hydrophobicity of myristic acid is suggested to play a key role in the observed effects. The C14:0 chain length provides sufficient hydrophobic interaction to cross the BBB, compared to octanoic acid (C8:0) or lauric acid (C12:0). Unlike palmitic acid (C16:0) or stearic acid (C18:0), the C14:0 length prevents strong binding to the BBB membrane itself.

To test this hypothesis a number of polyethylenimine conjugates, using polyethylenimine molecular weights ranging from 1.8 kDa to 25 kDa, were studied. Similar brain distribution profiles were observed, suggesting that myristic acid plays a key role in delivery to the brain. It is important to note that intravenous administration was used in this study, and the use of lipid conjugated drugs for delivery to the brain by oral administration has not yet been investigated. The stability of the conjugates in the intestinal lumen and the extent and rate of absorption of these conjugated molecules would also be important factors to consider.

Taken together, current studies show that certain lipids injected into systemic blood circulation can increase drug permeability across the BBB. Considering the processing and digestion of lipids in the gastrointestinal tract, some, but not all, of these lipids would likely be suitable for oral administration.

6. Gaps between current research and the potential feasibility of oral lipid-based drug delivery to the brain

The delivery of drugs to the brain using oral lipid-based formulations offers a tantalising opportunity, if feasible. The studies highlighted here show that some lipids can indeed affect the BBB after systemic administration, resulting in enhanced drug uptake into the brain. To note, systemic administration of these lipids is of course a limitation for translation of this into potential oral lipid-based brain targeting. The gap between the findings discussed here and clinical practicality of oral administration of these lipids to achieve the goal of enhanced drug delivery to the brain is still quite substantial.

Firstly, the data collected from existing research are insufficient to directly translate into the design of oral lipid-based drug delivery systems for brain targeting. For example, most of the literature mentions the specification of the formulation administered, rather than the level and form of lipid required in the blood to produce the desired effects. This leads to a lack of a credible standard (such as AUC) to assess the lipid levels in the blood sufficient to enhance drug permeation into the brain. In addition, in-depth quantitative assessment suggests that the current knowledge on the relationship between the oral dose of a lipid and following blood levels is limited. The understanding of this relationship is currently insufficient to translate into the design of oral lipid-based formulations. For instance, it can be speculated from nutrition literature that the triolein levels required to affect the BBB can be achieved by oral intake. However, there is a need for more data to support or reject this assumption [74,75].

Secondly, existing research utilises intra-arterial or intravenous injection, resulting in different physiological metabolic processing to oral administration. Oral lipids are first affected by the digestive system, before entering the circulation, for example as substrates for various lipases involved in lipolysis in the gastrointestinal tract. The levels of these enzymes, as well as the pH and bile secretion, are dependent on a number of factors including age and gastrointestinal disease, which can greatly affect drug therapy [87]. The effects of lipid digestion should be studied and evaluated when oral lipid-based formulations for BBB enhancement are designed. In addition, the form of lipid affecting the BBB is also important. Long-chain lipids following oral administration are more likely to exist in the blood in the form of triglycerides. On the other hand, short-chain lipids would mainly circulate the blood in the unesterified form (free state or protein-bound) [83,84]. As a result, long-chain fatty acids are unlikely to appear in the blood in their free state at high levels after oral administration. In existing studies, triolein is often formulated as an emulsion and administered via injection [71–73]. The metabolism of triolein emulsions in the blood is similar to that of CMs produced following oral administration of lipids. Therefore, triolein may be feasible for oral administration as part of a formulation facilitating brain targeting [8].

Thirdly, research into the mechanisms of how lipids affect the BBB is still incomplete, and many assumptions have been made. Sol et al report that intracarotid injection of triolein emulsion into rats resulted in increased frequency of BCECs transcellular vesicles (~5 fold compared

with groups without triolein administration) [81]. They also observed that some substances that usually do not penetrate normal BCECs, were able to pass through tight junctions [81]. However, it is still unclear whether the chemical nature of triolein emulsion, the interaction with enzymes or the effect of cells' physiological response plays the major role. Moreover, are there only these specific lipids that could affect the BBB, or could a wide range of lipids influence BBB transport? What is the physiological significance of this phenomenon? These questions should be addressed for mechanistic design of oral lipid-based formulations for brain targeting.

Finally, the potential adverse health effects of excess lipids should also be considered. Although studies have shown that substances such as 1-O-pentylglycerol, 2-O-hexyldiglycerol and other common edible lipids (oleic and linoleic acid) are harmless to laboratory animals, some potential risks caused by lipids require caution [88]. Additionally, long-term high-fat diets can cause abnormal metabolism and cognitive impairment in mice [89,90]. Studies also found that long-term high-fat diets in rats can actually damage the integrity of the BBB and cause cognitive dysfunction [91–93]. This could be interpreted that potentially oral lipid-based formulations with high lipid contents should not be used long-term, which poses a potential issue for the treatment of chronic conditions. However, researchers have not yet reached a consensus on the impact of high-fat diets. Shirin et al found that a high-fat diets can improve the cognitive function of transgenic AD mice [94]. Furthermore, some lipids facilitate entrance of drugs into the brain by causing varying degrees of damage to the BBB (such as tight junction disruption), indicating that increased permeability might not be selective and could cause brain inflammation or oedema [72,73,91–93]. Thus, the use and dose of lipids to affect drug delivery to the brain should be considered with caution until these concerns are better understood.

7. Conclusion

The potential of oral lipid-based formulations to enhance delivery to the brain has not been widely explored to date. There is a clear unmet clinical need for brain targeting as exemplified by the various diseases involving the brain that are currently poorly treated. Oral lipid-based drug delivery for chronic, long-term illnesses may be ideal due to the ease of oral administration. Several lipids have been shown to affect the BBB and facilitate drug delivery into the brain after systemic circulation: oleic acid, triolein, alkylglycerols, and conjugates of linoleic and myristic acid. The main limitation of translating the results from these studies into the potential feasibility of oral lipid-based drug administration is that the lipids were administered systemically in these studies. Preliminary calculations suggest that triolein could reach the required plasma levels to affect the BBB permeability via oral administration. These examples highlight that certain lipids could be investigated for oral administration to facilitate drug delivery to the brain. However, the gaps between the current research based on systemic administration of lipids and envisioned potential oral lipid-based drug delivery systems are still substantial. The primary concern is that the data collected from existing parenteral delivery research are insufficient for direct translation into the design of potential oral lipid-based drug delivery systems for brain targeting. Future studies should involve oral rather than systemic administration of lipids. Although there are still many questions to be answered, there are potential opportunities for oral lipid-based drug delivery for brain targeting.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to acknowledge Professor Morgan Alexander and Dr David Scurr from the University of Nottingham for their support in this work.

Funding

This research was supported by the Engineering and Physical Sciences Research Council [grant number EP/L01646X/1]; the University of Nottingham CDT in Advanced Therapeutics and Nanomedicine.

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