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Prodrug strategy for enhanced therapy of central nervous system disease

Xue Xia, Yang Zhou and Huile Gao *

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Central nervous system (CNS) disease is one of the most notorious arch-criminals of human health across the world. Although considerable efforts have been devoted to promote the development of CNS drugs, ideal therapeutical effects are yet far from enough. The blood–brain barrier remains a major player that impedes the full potential of CNS therapeutical agents as it blocks the entry of CNS drugs into the brain. The past few decades have witnessed the upspring of prodrug strategies as a promising method to accelerate CNS drug development. The prodrug strategy with the ability to overcome the formidable blood–brain barrier enhances the delivery to the brain and hence improves the effects of the CNS therapeutics. In this Feature Article, we summarize the reported barriers and strategies for CNS therapeutics and spotlight prodrug design strategies to improve the efficiency of crossing the blood–brain barrier.

1. Introduction

Analytical statistics from the Global Burden of Diseases, Injuries, and Risk Factors Study (GBD) 2016 showed that the central nervous system (CNS) disease was the second leading cause of deaths worldwide in 2016,[1] giving rise to an enormous socio-economical burden to the whole society. Even though considerable advances have been made in the understanding of the pathophysiological mechanisms of the disease, the development of therapeutics for CNS disorders remains a tall order. Inadequate drug exposure to the brain is a central player which makes the practical use of the therapeutical molecules a failure.[2] The strict requirements for maintaining CNS function spawned evolutions of different barrier systems in the brain. To sum up, there are five typical barriers guarding CNS homeostasis. The most robust and selective barrier is the blood–brain barrier (BBB); other interfaces include the arachnoid barrier, the blood-cerebrospinal fluid (CSF) barrier, the circumventricular organs (CVOs), ependyma and the embryonic cerebrospinal fluid (CSF)-brain barrier. These interfaces altogether make an integrate barrier system for CNS protection.[3] BBB, the most well-studied barrier component, comprised of a structurally continuous endothelial cell layer, is widely accepted as a

Key Laboratory of Drug-Targeting and Drug Delivery System of the Education Ministry, Sichuan Engineering Laboratory for Plant-Sourced Drug and Sichuan Research Center for Drug Precision Industrial Technology, West China School of Pharmacy, Sichuan University, Chengdu, 610064, P. R. China.
E-mail: 1805310867@qq.com, 2946299502@qq.com

Xue Xia

Xue Xia received her BS degree in pharmacy from West China School of Pharmacy in Sichuan University in 2020. Currently, she is pursuing her Master degree under the supervision of Professor Huile Gao. Her current research interests lie in the development of targeting drug delivery system for the therapy of neurodegeneration disease.

Yang Zhou

Yang Zhou received her BS degree in pharmacy from West China School of Pharmacy in Sichuan University in 2018. Currently, she is pursuing her PhD degree under the supervision of Professor Huile Gao. Her current research mainly focuses on the development and biomedical applications of functional nanomaterials for cancer therapy.

gatekeeper to maintain the normal function and homeostasis of the brain. However, it also serves as a formidable hurdle which prevents drugs from entering the brain parenchymal to realize their treatment potential. It has been estimated that almost 98% of small-molecule and nearly all large-molecule therapeutics are excluded by the BBB when they are systematically administered.[4] This urgently calls for the advent of new approaches to circumvent BBB for enhanced CNS drug delivery. The past few decades have witnessed the development of various approaches to improve brain delivery.[5] Generally, these approaches can be divided into two categories, including invasive and non-invasive methods. Invasive methods allow the direct delivery of drugs to the brain; these methods include convection enhanced delivery (CED), intra cerebro ventricular (ICV) and direct intracerebral injection[6] and transient disruption of BBB.[7] Although significantly increased brain drug concentration is achieved using these methods, they are thought to have poor treatment compliance and have strict requirements of techniques and equipment. Therefore, these invasive approaches are accepted as salvage operations for life-threating disease when other therapeutical regimes are exhausted. Less-invasive approaches involving prodrug strategies and various targeted nano-delivery systems,[8,9] such as liposomes, micelles, nanoparticles, *etc.* are attractive due to their relatively higher safety and feasibility. For strategies based on nanoplatforms, low drug loading, potential drug leakage and toxicity of nanomaterials may well confound their application. Among all the non-invasive strategies, prodrugs for brain drug delivery have stood the test of time and some of them have translated from bench to bedside, such as L-DOPA[10] and codeine.[11] Therefore, the idea of implementing a prodrug for brain drug delivery is expected to flourish in the therapy against CNS disease.

In this feature article, we endeavored to systematically elaborate on how prodrug strategies could improve the therapeutical effects of the treatment for CNS diseases. The limiting factors and breakthrough points of CNS therapy mainly provided by BBB along with the rationales of prodrug design are sequentially discussed. We have also reviewed various brain-targeting prodrug strategies, and hope to inspire the medical scientific community to develop CNS prodrugs with higher efficiency.

Huile Gao

Huile Gao is a Professor at Sichuan University. He obtained his PhD in Pharmaceutics from Fudan University in 2013 and then joined West China School of Pharmacy, Sichuan University as a lecturer. In 2017, Gao was promoted as a Professor. His research focuses on the rational design of drug delivery systems for central nervous system diseases and tumors.

2. Understanding BBB: from a static barrier to a dynamic interface

Brain is one of the most well-protected organs in humans, and refined modulation of brain function is vital for normal behaviors. The formation of BBB could be the result of natural selection under evolutionary pressure for tight regulation of the brain homeostasis.[12] Anatomically, BBB is an extensive capillary bed[13] with highly intensive distribution in brain, imparting every neuron access to its own capillary nutrition supply. That is to say, once bypassing the BBB, drugs can easily spread to targeted neurocytes and exert CNS therapeutical effects. However, it remains a hard nut to circumvent BBB due to the existence of tight junctions (TJs), a paucity of fenestrations and pinocytic activity as well as the presence of metabolic enzymes in cerebral vascular cells,[14] which collectively result in an integrated barrier system. The tight junctions (TJs) spanning two adjacent endothelial cells seal the paracellular cleft and herein limit the paracellular permeability of small hydrophilic molecules, which constitutes a physical barrier.[15,16] The abnormal reduced vesicle trafficking featured by the highly selective transporter contributes to a transport barrier.[16] This is exemplified in Chenghua Gu's work,[17] showing that increased vesicular transcytosis through ablation of the Mfsd2a gene in mice could lead to a leaky BBB without significant tight junction deficiency. Besides, the expression of efflux transporters, such as P-gp and MDR1, also contributes to the transport barrier of BBB. What's more, there are also some specific and active enzymes that are identified to enrich the brain vessels,[17] such as carbonic anhydrase IV and γ-glutamyl transpeptidase nucleotidases, monoamine oxidases, cytochrome P450 *etc.*[18] They together generate a metabolic barrier by changing the chemical structure of molecules, altering the solubility, activity and transport ability of the administered drugs.[19] In addition, the negatively charged cerebral ECs can be a natural hurdle for the anionic substances due to electrostatic repulsion. Beyond all the obstacles originating from ECs, it is also believed that various other cell types, *e.g.* pericytes, gliacytes, converge on the modulation of the barrier profiles *via* the secretion pathway or as a substitution when BBB is compromised.[4]

Despite all presented obstacles, accumulating evidence proves that the BBB is not necessarily a static hurdle but a dynamic interface which serves for the energetic and metabolic requirements of the brain.[20] Therefore, understanding how BBB permeable substances can circumvent is conductive to optimize the design of CNS targeted therapeutics (Fig. 1).

Passive diffusion is a pivotal mechanism by which gas and most small lipid molecules enter the brain.[20] It is generally believed that lipid-soluble substances are more prone to penetrate the BBB *via* the free diffusion pathway. However,

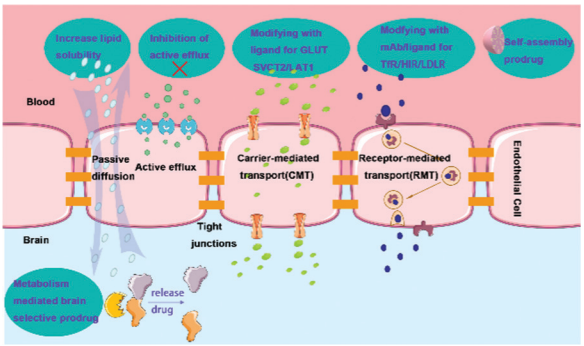

Fig. 1 Well-established mechanisms of BBB transporting and the corresponding brain targeting prodrug strategies.

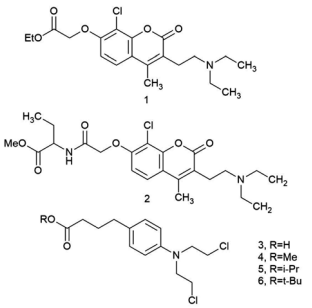

Fig. 2 Chemical structures of AD6 (**1**), CLOR-C4 (**2**), chlorambucil (**3**), chlorambucil-methyl ester (**4**), chlorambucil-isopropyl ester (**5**), and chlorambucil-tertiary butyl ester (**6**).

extremely high lipo-solubility also compromises brain permeability. It not only accelerates plasma protein-binding mediated systematic clearance but also increases drug retention in the periphery and ECs,[20,21] leaving only a small proportion available to brain parenchyma. Therefore, lipid solubility indicated by the lipid/water partition coeffective is one of the crucial factors that affects passive diffusion. Other contributors include molecular weight (MW), the number of H-bond donors and acceptors as acclaimed by Lipinski's "rule of five".[22–25]

Additionally, various transport systems within endothelial cells supplement the way for non-lipophilic molecules to enter the brain, namely the carrier-mediated transport (CMT), receptor-mediated transport (RMT) and active efflux transport.[26] The CMT systems are composed of solute carrier (SLC) superfamilies and are involved in transport of small solutes across the BBB, such as hexoses, monocarboxylic acids, neutral amino acids, basic amino acids, quaternary ammonium molecules, purine nucleosides and purine bases.[26] In view of the strict size and structural requirements brought up by the CMT systems, chemical modification to yield high affinity for a transporter has never been an easy thing. Therefore, it is essential to precisely adjust the compound's structure so that it can penetrate the BBB as easily as nutrients do. For the large molecules, they tend to be ferried into brain parenchyma *via* the RMT approach. Multiple studies have found that RMT is a reliable method for shuttling large molecules with a wide range of sizes, from transferrin (~80 kDa) to lipoprotein (up to 80 nm in diameter).[27] Transferrin receptor (TfR), insulin receptor (IR) and low density lipoprotein receptor family receptor (LDLRf receptor) are the most well-studied targets,[28,29] whose ligands have been widely exploited to enhance the CNS delivery of major molecules. For these polycationic molecules, their entry into the BBB seems to be initiated by their interactions with negatively charged EC membrane or proteoglycan on the plasma membrane, which is known as absorptive-mediated transcytosis (AMT). However, it is frustrating that this method is not restricted to BBB but all the membrane structure throughout the whole body, implying the potential widespread absorption and the resultant limited application value.

Generally, the gist of representative barrier properties and BBB transporting mechanisms has provided some basic guidelines for designing a brain-targeting system.

3. Considering prodrug as an optimal delivery strategy

The complex structural and functional characteristics of BBB confounded the endeavors for enhanced CNS therapy; however, attempts have never stopped in order to enhance the therapeutical effects of CNS drugs. The concept of prodrug was first introduced by Albert in 1958,[30] but its roots can be dated back to several century ago, which was supported by methenamine, phenacetin and prontosil.[31] To be concise, prodrug is a masked drug with more desirable physiochemical profiles. It is initially inactive and undergoes bioconversion at specific sites to yield active products *via* chemical or enzymatic reactions or their combinations. For improved pharmacokinetic and biopharmaceutical performances, prodrug strategies have been utilized to alter the drug structure and therefore the inherent physiochemical properties of the molecules.[31] Basically, prodrug consists of three key components: parent drug, pro-moiety and the linker that conjugates them.

Parent drug is the core part which is responsible for the therapeutical effects. For modifications to generate a successful prodrug, a parent drug should better bear a "synthetical handle" that bestows modifiable sites. The most common functional groups reported are hydroxyl, carboxyl or amine groups, phosph(on)ate groups and amidine or guanine groups.[31] For the parent drugs without a "synthetical handle", the first consideration is to generate suitable functional groups so that they can be further decorated with other moieties to construct successful prodrugs. For example, the first hinderance of prodrug design for thyrotropin-releasing hormone (TRH, pGlu-His-Pro-NH2) was overcome *via* substituting pGlu with Gln to provide an amine group for further connection, which can be efficiently converted to pGlu by glutaminyl cyclase (QC) after bioconversion at specific sites.[32] Moreover, studies also showed that the structure of the parent drug might influence or even change the final results even when other

components of prodrug are fixed.[33] This reminded us of the importance of screening the parent drug structure in order to achieve optimal therapeutical effects.

As for pro-moiety, it dictates the basic chemical identity of the prodrug[34] and it seems the armory where optimized pharmacokinetic and pharmacodynamic properties come from. In most prodrug cases, pro-moieties were carefully selected, edited, adapted and tailored to meet different requirements for drug delivery. For example, a lipophilic pro-moiety was introduced to increase its lipid solubility for membrane permeability[35] or a specific targeting ligand pro-moiety was added to ensure selective targeting.[36] In a nutshell, it is reasonable to predict that utilizing multi-pro-moieties that cater to different requirements of a specific site can give full play to a prodrug delivery system.

Last but not least, efficient bioconversion is a crucial part for a successful prodrug design, which is universally accepted to be determined by a linker with build-in-liability. A great proportion of prodrugs use an ester or amide as their linkers. Ester bonds can be easily hydrolyzed by water or various esterases, while amide linkages are more stubborn and require the activity of a specific peptidase/protease.[37] Some linkages can be detached in a specific microenvironment amid targeting sites, such as acid-sensitive and reduction-sensitive linkers. In addition, more and more dimensions of a linker that affect the characteristics of prodrug have gradually been unveiled. For example, a study showed that an addition of a methylene linker between the parent drug and pro-moiety almost doubled the unbound fraction of the prodrug in brain and plasma and substitution of an amide linker with an ester-bond can even alter the cell uptake mechanism of the prodrug.[38]

In general, to generate a successful prodrug design targeted for CNS therapy, there are two key steps: first, the prodrug should meet the goal of improving BBB permeability; second, it should be able to release its active form in brain parenchyma to take effect. In order to accomplish these two steps smoothly, not a single detail should be neglected in the process of prodrug design. As mentioned above, any subtle alteration in the prodrug structure resulting from parent drug selection, pro-moieties, linkers or combined, can have an apparent influence on brain endothelial cell uptake efficacy. Besides, stability profiles of prodrug should be underlined and the structure of prodrug should also be well tuned to serve for the various requirements on stability in different medication treatment. It is extensively believed that the prodrug should undergo a rapid transformation into its active form at the targeted site in order to achieve adequate concentration.[39] However, when sustained effects are desired, the releasing rate should be slowed down. SER-214, rotigotine-polyoxazoline (POZ) polymer conjugation, is a typical prodrug representative with long-term function.[40] In some cases, prodrugs were designed to harbour rapid metabolic elimination profile to prevent accumulated toxicity. All together, when designing a CNS prodrug, concentration should not just be limited to every component of the prodrug *per se*, other ingredient such as varied medication purposes (for immediate action or long term use) should also be taken into consideration. Table 1 provides an outline of the current prodrug strategies that are being used to improve brain therapy.

4. Invoking prodrug strategies for enhanced CNS therapy

The attempts to identify more therapeutical molecules and to increase their brain exposure should go hand in hand to enhance CNS therapy. Various approaches to improve the influx of medication into the brain have been extensively studied, among which the prodrug strategy is one of eye-catching directions. The combined knowledge of BBB physiology and transport profiles as well as rationale of prodrug fuels the development of prodrug therapy for CNS diseases.

4.1 Enhanced passive diffusion

Supposing that the BBB behaves like a lipid membrane and allows the entry of small lipid molecules *via* the free diffusion method, the initial consideration for enhanced brain delivery would be to increase the lipid solubility of the molecule. One prominent example is that either *O*-methylation or *O*-acetylation of morphine, producing codeine and heroin,

Table 1 Current prodrug strategies for enhanced BBB transport

Transport mechanisms	Examples	Methods	Ref.
Passive diffusion	CLOR-C4	Increased lipid solubility by alkylamino acid modification	41
Carrier-mediated transport	Glu-DAPPD, V-TDS-G, L-4-chlorokynurenine, L-Phe-SA, and FU-D	β-D-Glucoside modification, L-AT1 mediated transport by L-ascorbic acid modification, Pyrilamine-sensitive H+/OC antiporter mediated transport by *N*,*N*-dimethylethylenediamine modification	33, 48, 50, 60 and 63
Receptor mediated transport	OX-26-MTX, β-galactosidase-8D3, IDUA-HIR mAb, and ANG-1005	TfR/HIR mAb conjugation, LDLR mediated transport by Angiopep-2 conjugation	71, 72, 76 and 78
Inhibition of active efflux transporter	Pal-8SSMe	Inhibition of P-gp and ABCG2	80
Metabolism-based pro-drug for CNS-selective therapy	C12–C12-Pro-Pro-Gln-His-Pro-NH2, sob-AM1 and sob-AM2	Post-proline cleaving enzyme (POP)/fatty acid amide hydrolase (FAAH) related selective brain activation	32 and 85
Self-assembly prodrug for enhanced CNS therapy	C18-SS-EM1 NPs, CPD@IR780	Combination of prodrug and nano-technology	98 and 99

respectively, largely elevates the proportion of brain drug uptake by tenfold and more than thirtyfold compared with that of intact morphine.[11] It was observed that these alkyl-modified prodrugs could undergo rapid BBB transport within 15 seconds, which accounts for the large retained percentage in brain. After reaching the brain, codeine and heroin could be hydrolyzed to morphine, which could interact with opioid receptors and exert enhanced pharmacological effects. The significant increase of drug level in the brain was not only due to elevated BBB transport *via* lipidation, but also because the transformations to lower BBB-permeable morphine within the brain retarded the redistribution of active formation from brain to blood, which is known as the ''lock in'' phenomenon.

Another example was reported by R. Pignatello *et al.*[41] They found that intraperitoneal administration of a lipophilic alkylamino acid (LAA) modified cloricromene (AD6, **1**), namely CLOR-C4 (**2**), resulted in significantly higher active metabolic exposure to brain compared with its parent drug administered in the same way (Fig. 2). This alkylamino acid (4 carbon atoms) pro-moiety rendered facilitated BBB passage, as it can increase the lipophilicity of the prodrug without compromising its solubility, which indicated the importance of striking an overall balance between lipophilicity and hydrophilicity in order to favor BBB permeability without damaging its edge.

In the case of chlorambucil,[42] a water-soluble alkylating anti-tumor agent, several lipophilic derivatives (chlorambucil, **3**, chlorambucil-methyl ester, -isopropyl ester, -tertiary butyl ester, **4–6**), were synthesized and then administered intravenously to rats. The endowment of lipophilicity was supposed to increase brain uptake, thereby upgrading its activity against CNS tumors. However, ubiquitous unspecific enzymes resulted in early activation of these esterized prodrugs, thereby impeding brain crossing. *In vitro* hydrolysis results showed that chlorambucil-methyl and -isopropyl esters underwent quick hydrolysis within 30 s in blood and liver, while extended half-life was observed in chlorambucil-tertiary butyl ester. Considering that the anti-tumor efficacy of chlorambucil arose from the its nitrogen mustard moiety, its anti-tumor activity could also be seen even with a low bio-conversion rate with brain. For such types of agents, the esterification to prolong their circulation time and promote brain drug exposure is pivotal and may also achieve elevated therapeutical effects even when bioconversion efficacy is to some extent sacrificed.

4.2 Carrier-medicated transport

Despite that rapid BBB permeability was achieved by the lipidation of parent drug, it is likely to increase plasma protein binding and accumulation in other peripheral organs, which to a large extent compromises its practical application in CNS therapy. In view of the fact that the specific carrier systems are crucial to brain nutrients supply, it is believed that leveraging the transporting capacity of these specific carriers hold a great promise for brain drug delivery. To date, transporters have be identified to facilitate the transport of six classes of nutrients into CNS,[43–45] namely the glucose transporter type 1 (GLUT1) for hexose, sodium-dependent vitamin C transporter 2 (SVCT2) for ascorbic acids, L-type amino acid transporter 1 (LAT1) for neutral amino acids, monocarboxylic acid transport type 1 (MCT 1) for monocarboxylic acids, cationic amino acid transport type 1 (CAT1) for cationic amino acids, and concentrative nucleoside transporter type 2 (CNT2) for nucleosides. Since the first three aforementioned carriers are extensively implicated in brain-targeting prodrug design, we will focus our attention on their applications.

4.2.1 GLUT 1 mediated transport. GLUT1 tops the list of high-efficient transporters *via* BBB and it is responsible for the transport of various cargos, such as D-glucose, 2-deoxyglucose, 3-*O*-methylglucose, but not L-glucose. Coupling glycosyl to an active molecule provides a strategy to ferry cargos into the brain.[46] A case in point is the glycosylation of enkephalins. Enkephalin is potent analgesic substance, whose function is largely compromised due to its inability to overcome the BBB. It was demonstrated that L-serinyl β-D-glucoside modified enkephalin analogues (3-0-serinyl 3-D-glucosides) produce a significant and longer-lasting analgesia in both classic analgesic experiments, the warm-water tail-flick test and hot-plate test, compared with their unmodified parent drug.[47] The inserted glucose moieties reduced the lipophilicity of the compounds but did not reduce BBB permeability as previously thought, suggesting that GLUT1 played a key role in their BBB transport. In another report,[48] β-D-glucoside was linked to an anti-neuroinflammatory agent, *N*,*N*′-diacetyl-*p*-phenylenediamine (DAPPD) to produce Glu-DAPPD, and it was demonstrated that GLUT1 can facilitate BBB crossing of Glu-DAPPD and after entering the brain parenchyma, active DAPPD can be released from the prodrug through the actions of enzymes, such as glucosidases and *N*-acetyltransferase (Fig. 3a). *In vivo* pharmacodynamics evaluation results elucidated that glucose-modified DAPPD could decrease Aβ aggregation and improve cognition function in APP/PS1 mice (Fig. 3c–e).

Guided by similar rationales, Zhang' s group synthesized a series of glycosyl derivatives based on ibuprofen, with D-glucose as the brain targeting agent. In these new derivatives, ibuprofen was linked directly to the C-2, C-3, C-4, and C-6 positions of glucose to generate prodrugs I, II, III, and IV (**7–10**) *via* ester bonds, hoping to overcome its poor brain delivery.[49] It was observed that the mean retention times in plasma (MRT) of ibuprofen, I, II, III, and IV were 26.21 ± 0.75, 58.65 ± 1.31, 61.27 ± 1.12, 47.84 ± 1.32, and 43.92 ± 0.06 min (n = 3), respectively. What's more, elevated brain concentrations were also achieved by these glycosyl derivatives and GLUT1 fueled their transport across the BBB. The understanding of how different alterations in the chemical structure affect these interactions prompts the development of promising prodrugs.

As has been shown in examples above, glucosylation has verified its potential to enhance brain targeting efficacy of drugs, and likewise, glucose-modified venlafaxine was proposed to increase its accumulation in the CNS. However, GLUT1 acts as a bidirectional transporter which can not only transport glucose from blood to brain but also from brain to blood, which might threaten its accumulation in brain parenchyma. In light of this, Wu's group[50] came up with an idea to insert a ''lock-in'' thiamine disulfide (TDS) into the prodrug

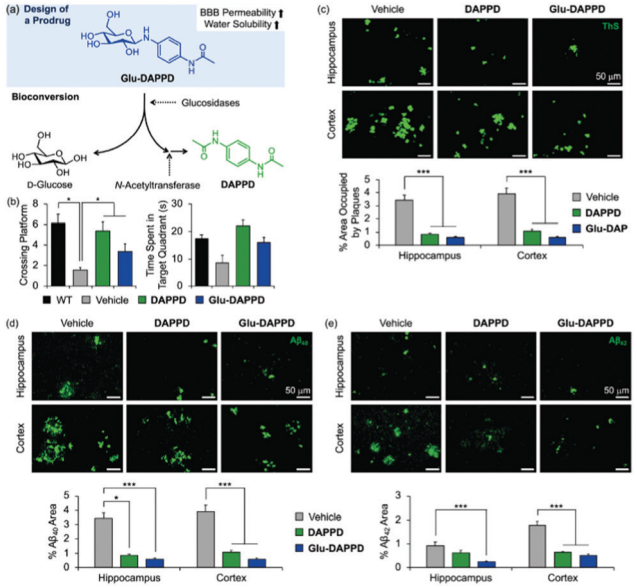

Fig. 3 (a) Bioconversion of Glu-DAPPD. (b) Measurements of platform crossing frequency and time spent in target quadrant during the probe test. Analyses of (c) Aβ plaques, (d) Aβ40 aggregates, and (e) Aβ42 aggregates detected using ThS, anti-Aβ40 antibody (G30), and anti-Aβ42 antibody (20G10), respectively, after the daily treatments of each molecule for 2 months (2 mg kg^{-1} day^{-1}; i.p.) in APP/PS1 mice starting at 8 months of age. Scale bars = 50 μM. Animal number: (b) n = 7 for WT mice and vehicle- or Glu-DAPPD-treated APP/PS1 mice; n = 5 for DAPPD-treated APP/PS1 mice; (c–e) n = 3 for vehicle-, Glu-DAPPD-, or DAPPD-administrated APP/PS1 mice. $^{*}P < 0.05$; $^{***}P < 0.001$ by Student's t test or repeated-measures ANOVA, Tukey's *post hoc* test. All error bars indicate SEM.

structure so that the lipophilic TDS part subsequently forms a hydrophilic thiazolium quaternary salt after being reduced by disulfide reductase in the brain, prohibiting its reentry into peripheral circulation (Fig. 4b). It was shown that the relative

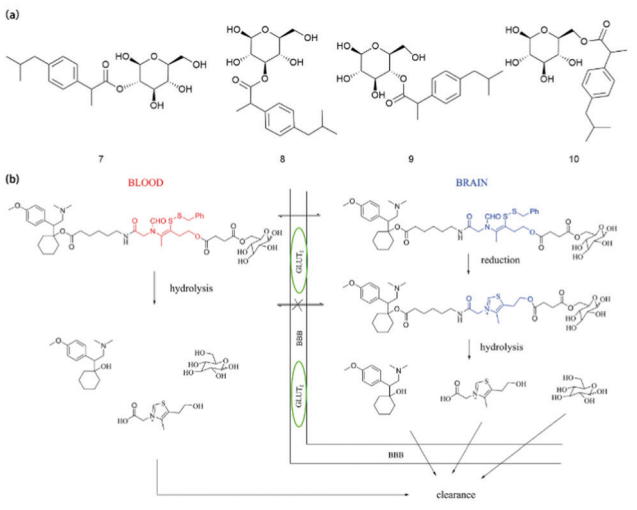

Fig. 4 (a) Chemical structures of the four glucose-modified ibuprofen derivatives (**7–10**), and (b) a scheme of the brain "lock-in" mechanism of glucose-modified, TDS inserted venlafaxine prodrug.

uptake efficiency (RE) and concentration efficiency (CE) were increased by 5.69 and 5.70 times compared with that of naked venlafaxine through this strategy, respectively, suggesting its enormous potential to promote CNS delivery.

4.2.2 SVCT2-mediated transport. Vitamin C, also known as L-ascorbic acid (AA), is an essential substance for maintaining normal functions of the brain, whose concentration is evidently higher (generally tenfold higher) in the CNS than in its peripheral counterparts.[51] Two distinctive ways have been reported to mediate the transport of L-ascorbic acid into the brain: the oxidized form of AA, dehydro-ascorbic acid (DHAA), is ferried by GLUT1and is then reduced into AA in the brain;[52] while the Na^{+}-dependent Vitamin C transporter SVCT2 transports AA directly to the brain.[53] In an investigation carried out by Alhawi Mohammad,[54] a sodium-dependent vitamin-C transporter was used for the brain-specific transport of naproxen. They synthesized vitamin C-based naproxen prodrug, either by the direct coupling vitamin C and naproxen to obtain derivative **11**, or *via* conjugation through a glycolic acid spacer to give derivative **12**. It was demonstrated that AA conjugation enabled increased drug exposure to brain. Furthermore, *in vitro* statics showed that the inserted glycolic acid spacer endowed a faster hydrolysis rate both in plasm and brain, which indicated its fast clearance and efficient bioconversion in the brain. This further guaranteed reduced systemic toxicity, which is of great importance for the application of NSAIDs in CNS disease treatment. Collectively, coupling AA with a parent drug and a hydrolysable linker is a promising strategy for enhanced therapeutical effect with no overt systemic toxicity.

Similar to GLUT1, SVCT2 was also shown to be a bidirectional transporter that located in both the luminal and abluminal sides of BBB. Therefore, it is likely that AA–ibuprofen conjugation can be pumped back into the blood even after its successful entry into the brain. In order to solve this problem, exactly the same strategy of the above-mentioned GLUT1-mediated transport of venlafaxine prodrug was applied to enhance SCVT2-mediated transport of ibuprofen prodrug[55] (Fig. 5b). They prepared TDS modified AA conjugated prodrug, and both *in vitro* and *in vivo* results showed that this lock-in strategy contributed to significant increase in the relative uptake efficiencies (REs) and concentration efficiencies (CEs) for brain compared with those of the direct AA-conjugated ibuprofen prodrug.

4.2.3 LAT1 mediated transport. LAT1 is a well-established transporter that can mediate Na^{+} and pH independent exchange of essential amino acids.[56] There are two general methods for prodrug design that use LAT1 as their targets, where either the whole prodrug acts as a pseudo-nutrient to bind LAT1 or neutral amino acids are utilized as a pro-moiety to connect with the active drugs to produce prodrugs for the LAT1 system.

A typical example of fabricating nutrient-resembling prodrug for the LAT1 system is L-DOPA, which is known to be the mainstay for Parkinson's disease (PD) treatment.[57] L-DOPA can cross the BBB *via* LAT1 mediated transcytosis. After getting into the brain, it can be decarboxylated to DA as a supplement for

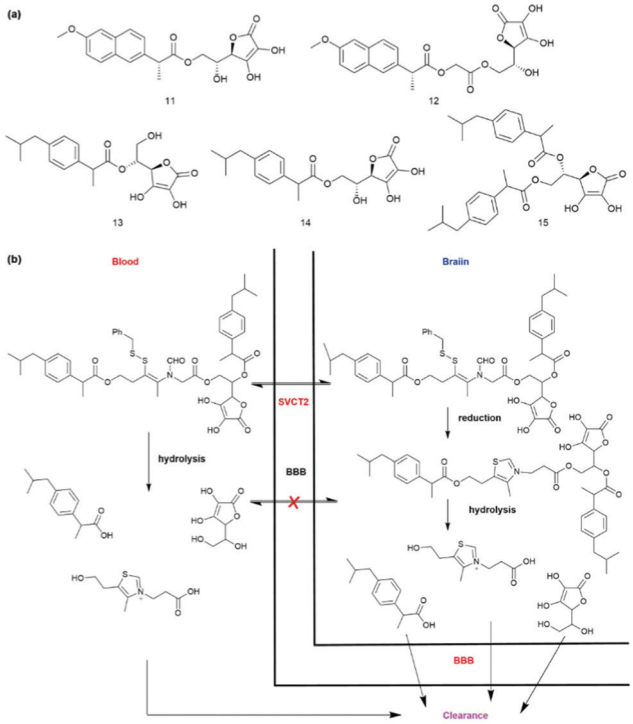

Fig. 5 (a) Vitamin C based naproxen prodrug (**11** and **12**) and vitamin C based ibuprofen prodrug (**13–15**). (b) A scheme of the brain "lock-in" mechanism of AA-modified, TDS inserted ibuprofen prodrug.

DA deficiency in PD therapy. Another good example is the prodrug design of 7-chlorokynurenic acid.[58] It is a competitive antagonist of *N*-methyl-D-aspartate (NMDA) receptor, which exerts neuro-protective effects. However, its poor CNS penetration limit its application in neuro-degeneration disease therapy. This shortcoming can be overcome by its prodrug, L-4-chlorokynurenine, which can undergo bioconversion to produce 7-chlorokynurenic acid through the action of kynurenine aminotransferase after entering into the brain. In these two classic examples, molecules mimic essential nutrients to trick the carriers so that they can circumvent the BBB.

In most cases, the parent drugs were conjugated to large neutral amino acids in a bio-reversible manner to allow efficient LAT1 binding. Ketoprofen (KPF) is an example of a COX inhibitor and bears anti-inflammation profile, and may be a potent drug for neurodegenerative diseases if its BBB permeability is elevated. When coupled with L-tyrosine, a LAT1 substrate, KPF can change into a substrate for LAT1 and cross the BBB *via* this pathway.[59] Considering that the full potential of KPF can only be achieved when it is taken up by neuron cells at the lesion sites, LAT1 is the best consideration for its prodrug design, as these transporters are shown to express not only at the BBB but also on various brain cells.

Inspired by the aforementioned rationales, it is reasonable to believed that for the drugs whose therapeutical effects can be exerted only when they are taken up by cells, LAT1 utilized prodrug design has a great edge over other designs. Therefore, in an investigation, conjugations of L-phenylalanine and a series of non-steroid anti-inflammatory drugs, such as flurbiprofen (FLB, L-Phe-FLB), salicylic acid (SA, L-Phe-SA), ibuprofen (IBU, L-Phe-IBU), and naproxen (NAP, L-Phe-NAP) were prepared and then their *in vitro* cell uptake efficacy and *in vivo* pharmacokinetics were evaluated. Although all prodrugs showed improved cell uptake efficacy compared with their parent drugs, their *in vivo* performances were not well aligned with the *in vitro* results. Results showed that only L-Phe-SA could increase the amount of released SA by nearly 5 times, while others could not,[33] which might be contributed to instability in plasma, unspecific binding with protein and poor bioconversion. Therefore, what is worth repeatedly noting is that many factors other than brain delivery efficacy should be well-tuned to generate a promising candidate, when considering a LAT1-mediated prodrug design.

4.2.4 Newly-identified carrier-mediated transport. Except the most well-established carriers expressed on BBB, a recently identified carrier, pyrilamine-sensitive H^+/OC antiporter, is also expected to facilitate the development of some novel prodrug candidates despite the relatively less knowledge of this carrier in comparison to that of others. It was first reported by Yamazaki's group in 1994,[60] with preferential entry for drugs bearing secondary or tertiary amine moieties, including pyrilamine, diphenhydramine, oxycodone, and clonidine.[61] In parallel to this exploring journey, Zhang's group has done considerable work on brain-targeting prodrug design based on alkali amine pro-moieties, whose preferential brain accumulation was later proven to be mediated by this newly identified carrier.

In their early works, they applied *N*,*N*-dimethylethanolamine-related structures to different model drugs, such as dexibuprofen,[62] flurbiprofen (FLU),[63] 5-fluorouracil (5-FU)[64] and dopamine (DOPA).[65] In the study based on dexibuprofen, they synthesized a variety of dexibuprofen prodrugs, with small alterations in their *N*,*N*-dimethylethanolamine-related pro-moieties. Increased brain-targeting efficacy was seen in all groups; however, they identified the best candidate was the one that utilized *N*,*N*-dimethylaminoethanol as its targeting moiety. Therefore, this specific structure was introduced into their follow-up works. Flurbiprofen (FLU) was then used as a model drug to reaffirm the excellent brain-targeting potential achieved by this pro-moiety and also to study the effect of different linkers on bioconversion efficacy of prodrugs, where FLU was linked *via* amino and ester bonds, respectively. Statistics demonstrated that the linker type did not significantly change the targeting profile but did influence the rate of bioconversion in the brain, with amino bond showing poor ability in releasing its parent drug. Therefore, it is highly likely to compromise the therapeutical effect with an amino linker even when increased prodrug brain exposure is obtained. However, it was not an immortal rule. They linked 5-FU, a common anti-tumor agent with *N*,*N*-dimethylethylenediamine (named after FU-D) *via* amino bond. Although FU-D barely released 5-FU in the brain due to the existence of the robust amino bond, it could still retain excellent anti-tumor effect,

which seemed to be the result of almost no impact of the modification on the pharmacophore of 5-FU. Therefore, it seems that efforts to elevate bioconversion are no longer urgent in this kind of situation where modified molecules can exert the therapeutical effects as a whole.

In another example,[65] the same pro-moiety was applied to dopamine (DDP) but did not provide the desired brain-targeting effect. Careful examination showed that the inadequate lipophilicity of parent drug, dopamine, was a key player. Therefore, in order to increase the overall lipophilicity of the conjugate, dipivaloyloxy functional group was introduced into DDP *via* an ester linker to produce *N*-3,4-bis(pivaloyloxy)-dopamine-3-(dimethylamino)propenamide (PDDP). Overall, this prodrug presented elevated brain accumulation and its preliminary hydrolysis product, DDP, in the brain as a whole exerted effects similar to dopamine. Aside from the elevated therapeutical effect, this design can also decrease the side effects of dopamine-therapy. On the basis of all these merits, it is safe to conclude that this strategy is a promising direction for CNS targeting prodrug design.

4.2.5 Dual-targeting moiety mediated transport. Strategies of single modification with one targeting moiety have been widely investigated as above-mentioned. However, research studies on integrating a hybrid of more than one BBB targeting moieties into a parent drug are rare to see. Here, Wu's group designed a dual-targeting prodrug conjugated with glucose and ascorbic acid based on parent drug naproxen, named G–V–Nap.[66] Based on their previous research studies, they identified glucose and ascorbic as two promising pro-moieties, both of which presented excellent performances in facilitating BBB transport. Therefore, they separately examined brain targeting efficacy of naked naproxen, single target modified naproxen prodrug, glucose-modified naproxen, named G–Nap, ascorbic acid-modified naproxen, named V–Nap and dual target modified naproxen, named G–V–Nap. Results demonstrated that G–V–Nap showed the highest brain uptake percentage among all the groups. The best therapeutical effect was also achieved by the G–V–Nap group. This might provide us with an idea of combining different transporter substances to obtain a multi-targeting moiety when designing a brain-targeting prodrug. Likewise, a similar strategy was utilized to generate an ibuprofen prodrug co-modified by organic amine and L-ascorbic acid for CNS delivery.[67] As expected, the dual-modified prodrug achieved elevated brain uptake efficiency and concentration efficiency, which were 3.16 and 4.92 times higher than those of naked ibuprofen, respectively. However, more efforts need to be devoted to elucidate the specific mechanisms that mediate BBB transport, whether the improved CNS penetration is due to the increase in the number of moiety or it is attributable to the combination of different targeting moiety. It would give us more insights into prodrug design if all these problems are solved.

4.3 Receptor-mediated transport

RMT system is employed to ferry large molecules across the BBB. As for drug delivery, understanding of how substance can be routed from the luminal (blood) to abluminal (brain) side *via* RMT is conductive to a CNS drug delivery design. Generally, this whole process can be divided into four parts: first, a ligand binds to an endogenous receptor expressed on the luminal plasma membrane; second, endocytosis takes place and an intracellular vesicle that contains receptor–ligand complexes is formed; third, the intracellular vesicle can either bind to cellular vesicles or lysosomal vesicles for further transcytosis or degeneration; and fourth, in the case of transcytosis, the vesicle is shuttled to the abluminal plasma membrane and then exocytosis happens, releasing the vesicle's contents into the brain parenchyma.[27] In light of its edge on the transport of endogenous large molecular substances, RMT has gained increasing attention as a target for CNS drug delivery system. Two main approaches are exploited to formulate RMT-targeting system including direct conjugation of ligand for RMT to therapeutics of interest and construction of a nanoplatform modified with RMT-targeting moiety. The latter approach has been widely discussed else where,[68–70] so in this section, we will discuss works in which drugs of interest are directly tethered to RMT ligands *via* different linkages such as chemical linkers, streptavidin (SA)/biotin linkage, or construction of a fusion protein. RMT can facilitate transport of different kinds of cargos,[27] *e.g.* small molecules, mAbs, recombinant proteins, nucleic acid and nanomedicines, especially those with large molecules. One good explanation is that RMT is mediated by vesicle transport with barely size selectivity, thereby bearing an edge over CMT in terms of ferrying large molecules. Here, we focus on RMT-mediated drug delivery based on the well-studied RMT targets.

The most well-known RMT targets are transferrin receptor (TfR), insulin receptor and low-density lipoprotein receptor. Their corresponding ligands, such as endogenous RMT ligands, and anti-receptor antibodies have been widely investigated to support RMT-mediated brain drug delivery. However, endogenous RMT ligands were not good candidates due to their high natural concentrations *in vivo* and might even present as competitors for receptor-binding. Therefore, certain peptidomimetic monoclonal antibodies (mAb) against their corresponding receptor were used to promote BBB message, referring as molecular Trojan horses. An early study in 1991[71] connected an anti-TfR antibody murine OX-26 with methotrexate (MTX) *via* a hydrazone-link and yielded OX-26-MTX conjugates. These conjugations could bind to receptors present on the luminal surface of capillary endothelial cells and facilitate selective distribution in brain vascular cells in a dose- and time-dependent manner. In another study,[72] the rat 8D3 mAb was conjugated to β-galactosidase (116 kDa) *via* a SA–biotin linkage. The high affinity between SA and biotin allowed for the formation of conjugation immediately after mixing of the mono-biotinylated β-galactosidase and the TfR mAb-SA. The acquired results revealed that this β-galactosidase-8D3 mAb conjugation significantly increased the uptake of β-galactosidase by 10-fold. In multiple research studies, genetic engineering technology has been used to construct a fusion protein of a mouse/rat chimeric mAb against TfR and a

therapeutic single chain Fv (ScFv) antibody.[73,74] In examples of PD treatment,[53] glial-derived neurotrophic factor (GDNF) and erythropoietin (EPO), were fused to the carboxyl terminus of the heavy chain of a chimeric TfR mAb, termed as cTfR mAb, to gain cTfR mAb-GDNF and cTfR mAb-EPO fusion proteins. It was demonstrated that administration of these two fusion proteins resulted in the recovery of both motor activity and striatal tyrosine hydroxylase (TH) enzyme activity, thus suggesting their therapeutical effect. Human insulin receptor (HIR) is another extensively-applied target for brain drug delivery. As with TfR targeting, a brain-targeting siRNA delivery system based on HIR mAb was brought up by William M. Pardridge.[75] This system was composed of mono-biotinylated siRNA (with mono-biotinylated modification on the 3′-terminus of the sense strand) and a HIR mAb coupled with SA. The siRNA–HIR mAb conjugation was formed by high affinity SA–biotin linkage and the gene silencing efficacy obtained by siRNA-HIR Mab was comparable to that achieved by cationic liposome, such as oligofectamine. Based on the same HIR target, William M. Pardridge[76] prepared a BBB-penetrating iduronidase (IDUA) by fusing the IDUA enzyme to the heavy chain of HIR. In order to assess the biodistribution of the conjugation, the naked IDUA and IDUA-HIR mAb were radioiodinated and then were injected intravenously in adult rhesus monkey. Biodistribution results demonstrated that brain uptake of IDUA-HIR mAb (1.2% injected dose per brain) was higher than that of IDUA dosed alone (0.04–0.07% injected dose per brain). Apart from TfR and HIR, another eye-fixing target is the low density lipoprotein family (LDLRf) receptors, which involve the low density lipoprotein receptor (LDLR) and low density lipoprotein receptor-related proteins 1 (LRP1) and 2 (LRP2). They are abundantly expressed on the BBB and are acclaimed to facilitate the transport of lipoproteins and various other ligands cross the BBB *via* RMT.[53] Unlike targeting strategies for TfR and HIR, where corresponding mAbs were extensively applied, reports that have explored the usage of mAbs against LDLRf receptors for BBB transport are rare. However, numerous investigations used peptide ligand mimics for LDLRf receptors as vectors for brain drug delivery. As an example, angiopep-2, a 19-amino-acid peptide, was discovered as a ligand for LDLR to mediate transcytosis across the BBB.[77] Under the guidance of this discovery, J–P Castaigne[78] synthesized paclitaxel-Angiopep-2 compound, named ANG1005, with each molecule comprising three paclitaxel molecules attached to one angiopep-2 by cleavable ester linkages. Both *in vitro* and *in vivo* experiments indicated that ANG1005 presented increased brain uptake (12–15 times higher) compared with paclitaxel *in situ* brain perfusion in mice. Besides, the potential of bypassing P-gp endowed by ANG1005 made this strategy even more enticing, which could not only further promote brain drug accumulation by eliminating the efflux function but could also alleviate drug resistance. In another study,[79] angiopep-2 was fused with the C-terminal domain of single-chain antigen binding fragment of anti-VEGF antibody using recombinant protein technology, yielding recombinant scFab-ANG protein and this also achieved good results. To sum up, collaborative evidences suggest the great potency of RMT in CNS drug delivery system.

4.4 Inhibition of active efflux transporter

Ubiquitous existence of active efflux transporter has always remained a hinderance for CNS drugs to elicit therapeutical effect and paliperidone is a case in point. As a long-term prescribed drug for the treatment of schizophrenia, its limited BBB permeability puzzled the scientists because it acted as an overlap substance of active efflux transporters, P-gp and ABCG2.[80] Previous reports demonstrated the potential of homodimerization of substance for transporters to generate a functional transport inhibitor.[81] Based on above mentioned experience, Kelsey Bohn *et al.*[80] synthesized a series of dimer of paliperidone (Pal) tethered by ester linkages. They identified a top-performing dual inhibitor containing an internal disulfide bond in the tether (Pal-8SS) and then optimized this compound *via* adding two hindering methyl groups alpha to the carbonyl of the ester moiety within the tether. The final obtained prodrug (Pal-8SSMe) showed increased ester enzyme stability in bloodstream and could be cleaved in a cellular reductive environment to generate the monomeric therapeutical form (Fig. 6). Data at the cell level indicated that Pal-8SSMe could effectively inhibit the activity of both two active efflux transporters and resulted in a great deal of monomeric paliperidone accumulation in BBB cells at 24 h.

4.5 Other prodrug strategies for CNS therapy

4.5.1 Metabolism-based prodrug for CNS-selective therapy. The tactics of harnessing abundantly expressed enzymes or robust enzyme activity at specific sites are more than useful during therapeutical design.[82–84] In virtue of the highest activity and selective distribution of prolyl oligopeptidase (POP, a.k.a. post-proline cleaving enzyme) in the brain, integrating a POP-sensitive structure into prodrugs seems to be a feasible approach. Therefore, Katalin[32] came up with an idea of introducing a POP-active linker that can covalently connect thyrotropin-releasing hormone (TRH, 16) with a lipoamino acid to produce a brain-targeting prodrug (C12-C12-Pro-Pro-Gln-His-Pro-NH2, TRH, 17). They found that excellent membrane affinity and metabolic stability in peripheral circulation were achieved by this prodrug. They also confirmed the crucial role of POP in the design using a POP inhibitor, KYP-2047, whose

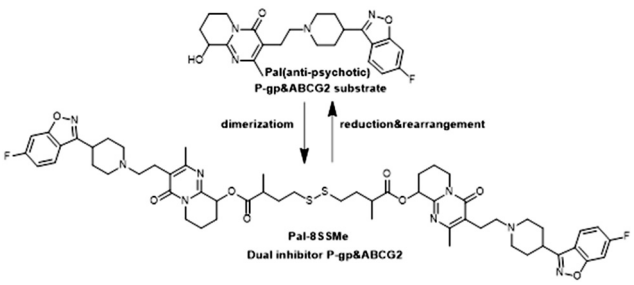

Fig. 6 The scheme of transformation in anti-psychotic and the dual-inhibitor effect of Pal and Pal-8SSMe.

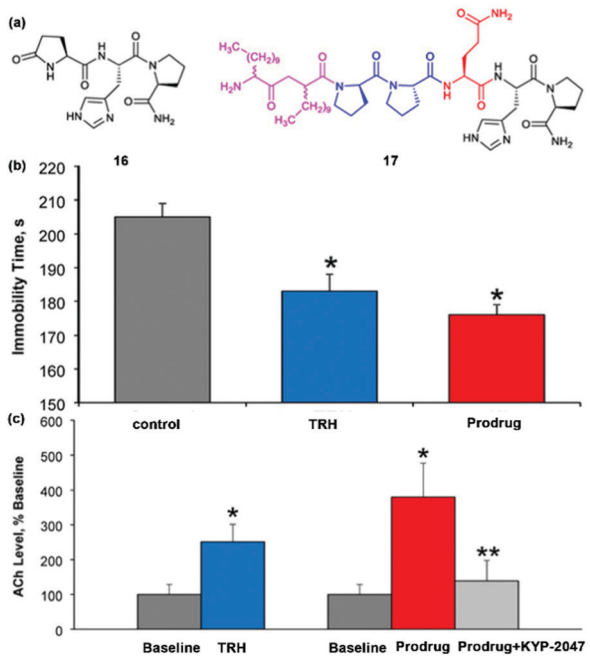

Fig. 7 (a) Chemical structure of TRH (**16**) and TRH prodrug (**17**), and (b) antidepressant-like effects and (c), acetylcholine (ACh) release effects.

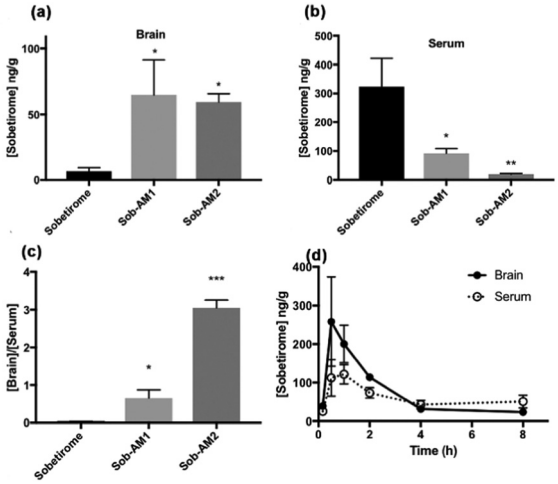

Fig. 8 Sobetirome levels in the (a) brain and (b) serum 1 h after administration, (c) brain-to-serum concentration ratios at this 1 h time point, and (d) sobetirome in the Sob-AM2 groups was measured from mouse cohorts treated at t = 0 (iv, 9.15 μmol kg^{-1}) and measured over 8 h post dose ($^{*}P \leq 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

treatment could significantly increase prodrug stability in brain homogenate ($t_{1/2} > 24$ h, in contrast to $t_{1/2}$ of 47 ± 6 min without KYP-2047). Antidepressant-like and acetylcholine (ACh) release effects elicited by TRH were evaluated *in vivo* to assess the neurotherapeutic potential of this prodrug approach. The immobility time was a typic index for depression evaluation. As shown in Fig. 7b, a significant decrease could be seen in the prodrug treated group compared with the control group. Although there was no obvious improvement on the basis of TRH treated alone, it was still reasonable to believe that this prodrug strategy is promising because it indeed enhanced the peripheral stability and it left room for further alterations to obtain optimized CNS therapeutical effects. In ACh release evaluation, the prodrug group resulted in a 4 times higher ACh level than baseline, with positive control group (treated with TRH) producing a 2.5-fold increase (Fig. 7c). All these accumulated results verified the possibility of utilizing this method for enhanced brain delivery.

Similar rationales of CNS prodrug design were applied by J. Matthew Meinig,[85] who took advantage of the abundant fatty acid amide hydrolase (FAAH) in brain to synthesize a series of amide prodrugs of sobetirome. These prodrugs, sob-AM1 and sob-AM2, shared structural similarity with substrates of FAAH, such as anandamide (AEA), and could liberate sobetirome in CNS by FAAH, guaranteeing their minimized peripheral exposure. Sobetirome concentration 1h after injection in brain and plasma were followed when sob-AM1 and sob-AM2 were administered peripherally and the brain/blood ratio (K_p) was calculated. Sob-AM1 and sob-AM2 showed ~20-fold and ~100-fold increase respectively, in contract to their parent drug (Fig. 8). These enticing improvements in sobetirome's CNS distribution were greatly explained by selective FAAH-activation in brain and were believed to elicit the desired CNS therapeutical effects.

Apart from above-mentioned enzymes, there are still a bulk of brain-specific metabolic systems, such as adenosine deaminase,[86] xanthine oxidase,[87] and monoamine oxidase.[88] In addition, cell-directed enzyme prodrug therapy has been widely leveraged to improve site-selective bioconversion, especially in tumor treatment, which is also believed to make a difference in brain tumor treatment.[89,90]

It is well known that nicotinamide adenine dinucleotide (NADH) and its phosphorylated form (NADPH) are fundamental for CNS metabolic activities.[91] Various examples proved the efficacy of utilizing dihydropyridinium and its bio-oxidate quaternary pyridinium salt as an analogous of NADH/NADPH oxidation–reduction system to improve the access of therapeutic agents to brain. These works has been reviewed by Laszlo Prokai elsewhere in the early twenty first century.[92] Despite long-time attention paid on this strategy, interest in this aspect has never faded away. In a recent study carried by Vincent

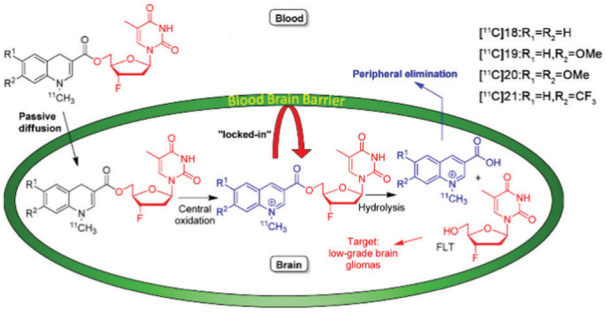

Fig. 9 Targeting FTL to brain by means of [^{11}C]-1,4-dihydroquinoline.

Levacher,[93] they prepared a set of 1,4-dihydroquinolines-based prodrugs 18–21 and assessed their ability to deliver [18F]FLT (3′-deoxy-3′-18F-fluoro-L-thymidine, a valuable PET contrast agent) into the brain (Fig. 9). They finally identified prodrug 18 as the most promising candidate for FLT delivery and further explained the distinct delivery efficacy occurred mainly because the different substitution groups on the quinoline moiety affected both lipophilicity and redox properties of these prodrugs. Their discoveries underlined the importance of fine-tuning the structure of pro-moiety to acquire a candidate with high potential. Same approach was again adopted by Vincent Levacher[94] to deliver MIBG [123I/131I], a widely used tracer for neuroendocrine tumors, into the brain. However, the most promising candidate produced with the highest brain penetration had a different structure with the aforementioned best FLT prodrug candidate, implying that the core value of prodrug strategy lied more in providing a guideline for prodrug design than in providing a fixed prodrug structure with a fixed pro-moiety.

Vincent Levacher also developed a new central selective AChE inhibitor prodrug devoid of peripheral side effects on the basis of the dihydropyridinium structure.[95] The 1,4-dihydroquinoline carbamate derivative, which is a cyclic analogue of rivastigmine, appeared to be a relevant ''bio-oxidizable prodrug'' candidate. The mechanisms of how this strategy can be effective are shown in Fig. 10. The lipophilic prodrug 22 could cross the BBB *via* passive diffusion. Once in the brain, it was bio-oxidized into its active form, parent drug 23, and the following decarbamylated metabolite 24 was routed to peripheral elimination phase. Gathering information from the whole process provided a clear picture of all the merits of this prodrug system: first, in terms of improved CNS delivery, the protonated prodrug at physiological pH in peripheral circulation allowed its preferential BBB transport. After undergoing bio-conversion into its oxidized, ionic parent drug, the unfavorable membrane penetrating structure could exert locked-in effect in the presence of the BBB. Then, for its potential in reducing adverse reaction, the peripheral bio-oxidation prevented the side effects caused by peripheral cholinergic activation, which commonly occurred in AChE inhibitors, as it could undergo rapid systematic clearance. Moreover, they also proved that the metabolite 3 in CNS was a substrate of P-gp and could be pumped back to blood for further elimination, which mitigated underlining risks arising from accumulation of this metabolic byproduct. Furthermore, Laszlo Prokai *et al.*[96] dedicated to reduce the toxicity of metabolic byproduct by ingenious original design. In their scheme, pro-moiety from which the ''lock in'' effect come was omitted, but was ''added'' through a transit chemical alteration within the prodrug molecule (DHED) itself. Therefore, the potential risks brought by 1,4-dihydroquinoline pro-moiety after conversion into pharmacologically active metabolites were largely removed. What's more, bioanalytical results confirmed its preferential activation to E2 in the brain compared with estrogen-sensitive peripheral tissues. Herein, efficacy of the prodrug was guaranteed together with alleviated side effects.

4.5.2 Self-assembly prodrug for enhanced CNS therapy. Conventional prodrug strategies are mostly based on chemical structure modifications barely with any influence on the state of assembly. Recently, self-assembling prodrug (SAPD) where active therapeutics is rationally modified and can be self-assembled into a well-defined nanostructure has gained considerable attention. Merits of SAPDs are easy to see: firstly, improved metabolic stability of the active parent drug due to protection provided by this nano-structure; secondly, the slowing down renal clearance because of its relatively large size; thirdly, the controllable release of parent drug upon exposure to some stimulus; and fourthly, improved targeting ability arising from its surface decorations.[34] Besides all the merits

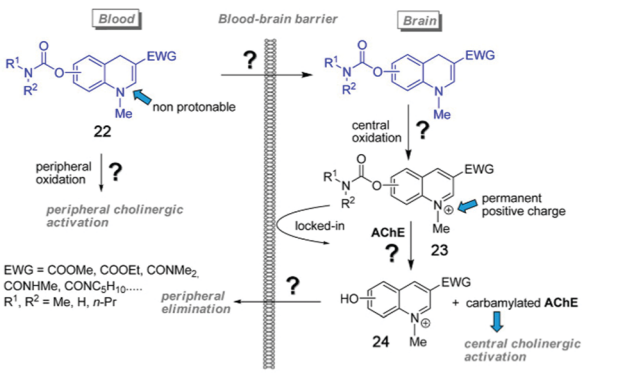

Fig. 10 Rational design of central selective AChE by means of a bio-oxidizable prodrug approach.

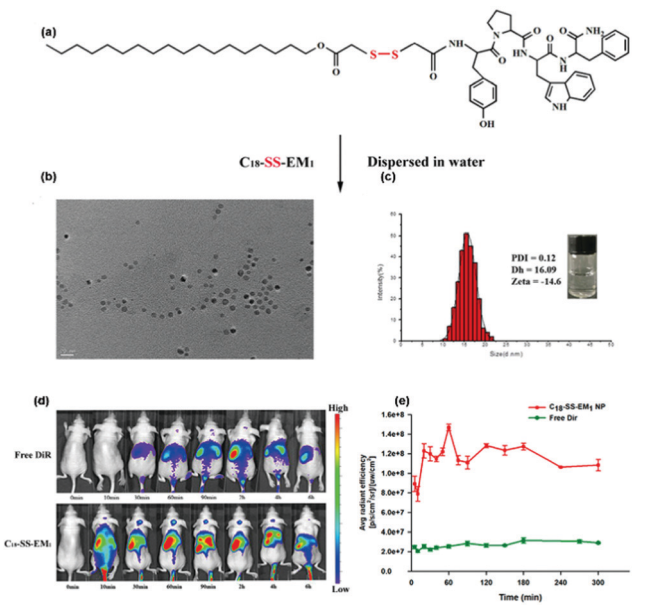

Fig. 11 (a) Chemical structure of C18-SS-EM1, (b) TEM image of C18-SS-EM1 nanoparticles in water. Scale bar: 20 nm, (c) DLS curve of C18-SS-EM1 nanoparticles, (d) *in vivo* imaging of biodistribution of DiR-load C18-SS-EM1 NPs and free DiR after i.v. administration, and (e) time course of fluorescence intensity (p s^{-1} cm^{-2} sr^{-1}) after i.v. dose.

inherited from the nano system, SAPD also showed an enticing advantage in terms of drug loading efficacy, circulation stability and carrier-related issues.[97]

For example, Hui Liu[98] used endomorphin-1 (Tyr-Pro-Trp-Phe-NH2, EM-1), an endogenous short peptide with excellent antinociceptive activity, as a model drug to synthesize EM1 derivative C18-SS-EM1(Fig. 11a). This amphiphilic derivative was obtained by covalently connecting a stearyl moiety with EM1 *via* a disulfide and presented self-assembly profile in aqueous media. Statistics also showed that the self-assembly C18-SS-EM1 NPs could increase the peripheral metabolic stability of EM1 to a large extent.

The real-time *in vivo* imaging of biodistribution also proved that C18-SS-EM1 NPs can achieve satisfying brain-targeting efficacy (Fig. 11d and e). In conclusion, the resulting C18-SS-EM1 nanoparticles largely fulfilled the potential of lipidation and nano-carrier to improve brain targeting, as they protected EM1 against enzymatic hydrolysis in blood. This shed light on the glamor of self-assembled prodrug NPs in brain drug delivery. Another advanced example was proposed by Kaiyong Cai;[99] they constructed iRGD-modified redox-responsive camptothecin (CPT) prodrug self-assembly micelles and then encapsulated the photosensitizer IR780 into micelles (Fig. 12a). The resultant prodrug micelles, named CPD@IR780 showed great potential for glioma treatment with enhanced anti-glioma efficacy (Fig. 12d) as well as reduced side effects. Experiments showed CPD@IR780 presented the highest signal accumulation at both cell and *in vivo* level compared with that of non-iRGD modified self-assembly prodrug nanoparticle (CPC@IR780) and the naked IR780 (Fig. 12b and c). Besides, the formed micelles with CPT conjugated covalently rather than simple encapsulation was confirmed to mitigate side effects because they reduced potential peripheral drug leakage. Therefore, the self-assembly prodrug strategy paves a new way for brain disease treatment with the combination of prodrug and nanotechnology.

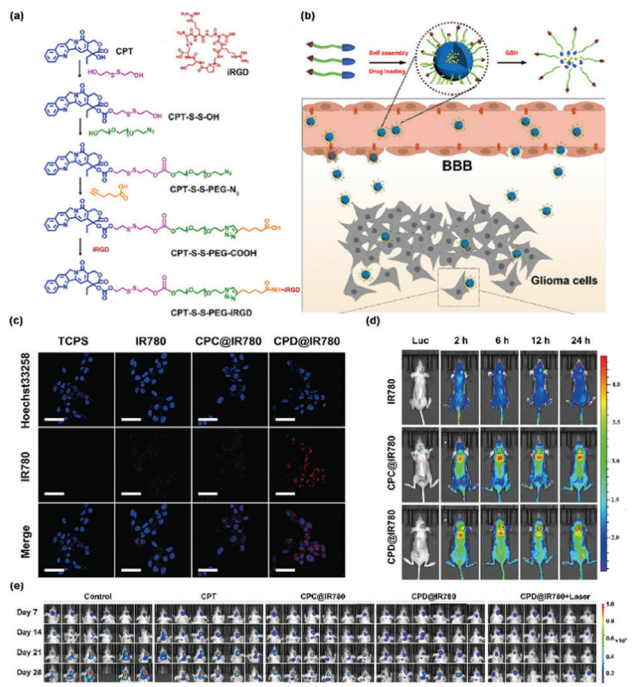

Fig. 12 (a) Synthesis of the CPT-S-S-PEG-iRGD polymer, (b) preparation of self-assembled micelles, and (c) cellular uptake efficacy of micelles. Scale bar: 50 μm. (d) *In vivo* biodistribution of IR780-loaded micelles in U87 orthotopic glioma bearing mice, and (e) anti-glioma efficacy on the U87 orthotopic glioma bearing mice model.

5. Conclusions

In this feature article, we have sketched out the development of prodrug strategies for enhanced brain therapy on the basis of understanding the complex BBB physiology and prodrug design. Gaining inspirations from how some endogenous and exogenous essential substances get through the formidable BBB, approaches have been adopted to utilize the corresponding transporting mechanisms to overcome the corresponding barriers. Lipidation of an original hydrophilic molecule upgrades the possibility of brain accumulation *via* passive diffusion; different ligands or substrate mimics of specific transporters are utilized to bypass transporting barrier; the unique metabolic properties of brain have also been leveraged to realize selective brain delivery; what's more, the expansion of prodrug design into self-assembly strategy is gradually stepping into public's sight due to its excellent performance in enhanced CNS therapy. Since its unrivalled edges in terms of enhanced pharmaceutical, pharmacokinetic and pharmacodynamic performance, prodrug has a flourishing prospect for clinical application,[100] especially in CNS diseases.

Although efficient BBB penetration could be obtained by various prodrug approaches, the practical potency of these methods still remained to be discussed. This is mainly because there are no golden standards for the measurement of BBB transport. For example, William M. Pardridge[101] pointed out that the previously believed concept "CSF drug distribution is a measurement of BBB transport" seemed to be misunderstood. The different applied evaluating approaches may affect the obtained results, which calls for some more authoritative evaluation of prodrug delivery system. What's more, BBB is not a static barrier whose properties and functions remain all the same, rather, a dynamic one that can actively respond to different subtle changes in the CNS micro-environment. Therefore, incremental discoveries to uncover different alterations in different pathological situations are highly likely to provide us with new perspectives in CNS disease treatment.

Conflicts of interest

There are no conflicts to declare.

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