Smart Strategies for Therapeutic Agent Delivery into Brain across the Blood–Brain Barrier Using Receptor-Mediated Transcytosis

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Discriminatory drug delivery into target cells is essential to effectively elicit the drug activity and to avoid off-target side effects; however, transporting drugs across the cell membrane is difficult due to factors such as molecular size, hydrophilicity, intercellular adhesiveness, and efflux transporters, particularly, in the brain capillary endothelial cells. Drug delivery into the brain is blocked by the blood–brain barrier (BBB). Thus, developing drugs for the central nervous system (CNS) diseases remains a challenge. The approach based on receptor-mediated transcytosis (RMT) can overcome this impassable problem at the BBB. Well-designed molecules for RMT form conjugates with the ligand and drugs via linkers or nanoparticles. Cell penetrating peptides (CPPs), receptor-targeting peptides, and monoclonal antibodies (mAbs) are often used as ligands. The binding of ligand to the receptor on the endothelial cell surface induces endocytosis. Existing exosomes comprising the conjugates move in the cytoplasm and fuse with the opposite plasma membrane to release them. Subsequently, the transcytosed conjugate-loaded drugs or released drugs from the conjugates elicit activity in the brain. As receptors, transferrin receptor (TfR), low-density lipoprotein receptor (LDLR), and insulin receptor (InsR) have been used to intendedly induce transcytosis. Presently, several clinical trials on CNS drugs for Alzheimer's and Parkinson disease are hindered due to poor drug distribution into the brain. Therefore, this strategy based on RMT is a promising method for CNS drugs to be transported into the brain. In this review, I introduce the practicality and possibility of drug delivery into brain across the BBB using RMT.

Key words receptor-mediated transcytosis; drug delivery system; membrane permeation; blood–brain barrier; ligand–drug conjugate; nanoparticle

1. Introduction

Drug delivery into cells across the membrane is a serious concern in drug discovery and development. In particular, delivering oral or intravenous drugs into brain across the membrane is impeded by the blood–brain barrier (BBB). Thus, developing drugs for diseases of the central nervous system (CNS) remains a challenge; furthermore, this is surprisingly arduous due to the BBB. Several CNS drugs against Alzheimer’s disease (AD) and Parkinson disease (PD) have been rejected during clinical trials because of poor drug absorption and distribution into brain across the BBB. Improving such bioavailability will meet the medical requirement in CNS diseases. Endothelial cells at the BBB are epigenetically subjected to prevent xenobiotics and certain drugs from entering into the brain by physical tight junction and biological efflux transporters such as multiple drug resistance 1 (MDR1) (P-glycoprotein (P-gp)), a representative ATP-binding cassette (ABC) transporter. In contrast, nutrients such as glucose and amino acids required for brain activity are absorbed into the brain by solute carrier (SLC) transporters present on the endothelial cells. Furthermore, some proteins and peptides are transported into the brain via endocytosis and subsequent exocytosis in the endothelial cells at the BBB. Alternatively, metabolites such as soluble amyloid β (Aβ) protein are excreted from the brain to bloodstream across the BBB. Thus, certain substances move in or out across the BBB based on a controlled transportation system.

In general, compounds are divided into three categories, that is, low-molecular compounds (MW < approx. 500), high-molecular compounds (MW > approx. 3000), and middle-molecular compounds (MW approx. 500–approx. 3000). Low-molecular compounds, particularly hydrophilic ones, are absorbed into brain across the BBB by SLC transporters with substrate specificity. Absorption by SLC transporters bypasses the elimination by MDR1 that excretes hydrophobic low-molecular compounds just passing through the lipid bilayer. Glucose is the substrate of glucose transporter 1 (GLUT1). Certain N-containing compounds such as diphenhydramine and morphine are the substrates of the proton-coupled organic cation (H+/OC) antiporter, although its amino acid sequence and its topology have not been identified.3) Thus, drugs designed based on such transporters, that is, transporter-consciously designed drugs, can be transported into the brain using SLC transporters expressed at the BBB.3) In contrast,
high- and middle-molecular compounds are too large to be transported by the transporters. Endocytosis and subsequent exocytosis at the membranes of endothelial cells can function as an alternative transport route. In this process, large molecular compounds (high-molecular compounds and middle-molecular compounds) are endocytosed into the endothelial cells via receptor-mediated endocytosis. Exosomes comprising these large molecular compounds dock to the membrane on the brain side and release them based on fusion. Therefore, a delivery strategy using receptor-mediated transcytosis (RMT) will offer a pharmaceutical avenue to overcome the problem of permeation at the BBB. Several receptors and corresponding ligands have been biologically evaluated for RMT.

Based on my interests as a medicinal chemist, I have already authored several reviews concerning the drug delivery approaches accomplishing intentional membrane permeability. Their topics include transporter-conscious drug design, the methods of drug delivery into cells using cell-penetrating peptides, and the methods of selective drug delivery into cancer cells across their membrane using receptor-mediated endocytosis. In this review, I describe the means to understand the present situation and possibility of CNS drug delivery system based on RMT across the BBB.

2. Discussion

2.1. The BBB That Faces Pharmaceutical Development

CNS is essential for human beings because it controls the function of the body and mind. Thus, CNS is protected by the biological and physical checking station called the BBB. The BBB does not permit entry of xenobiotics and toxins to the brain. Moreover, large molecular compounds and hydrophilic compounds also have difficulty in being transported into the brain. Brain capillary endothelial cells form a substantive part of the BBB because of the physical tight junction based on adhesion molecules such as claudin and biological efflux transporters such as MDR1 and are linked with astrocytes and pericytes. Furthermore, the transcellular and paracellular pathways of large molecular compounds based on passive diffusion are blocked by the lipid bilayer of endothelial cells and tight junction of endothelial cells at the BBB, respectively. Moreover, large molecular compounds cannot penetrate the transporter pores. Thus, infiltration of these compounds into cells is usually difficult; however, the brain needs certain proteins such as insulin. Large molecular compounds are absorbed into the brain from blood stream by transcytosis. There are several receptors that trigger endocytosis at the BBB. The representative receptors include transferrin receptor (TfR), low-density lipoprotein receptor (LDLR), and insulin receptor (InsR). The binding of the corresponding ligands to the receptors induces transcytosis. The RMT system can enable transport of large molecular compounds into the brain across the BBB.

2.2. Exosomes At present, it has been being clarified that exosomes play a vital role in cell-to-cell communication. Structurally, they are extracellular lipid bilayer vesicles (30–100 nm in diameter) comprising RNAs, DNAs, proteins, and lipids. Exosomes were identified in 1983 and were recognized as garbage cans to discard cellular unwanted components for a long time; however, after observing that they internally comprised mRNAs and microRNA (miRNA), they have gained immense attention as intercellular messengers, apart from cytokines and chemokines, in the field of pharmaceutical and medical sciences. Capsulated RNAs in exosomes are protected against enzymes such as ribonucleases (RNases) and are transferred to the recipient cells along the blood stream. The biosynthesis pathway of exosomes is briefly described here. Early endosomes mature into late endosomes/multivesicular bodies (MVBs), wherein the formation of intraluminal vesicles (ILVs) occurs. Subsequently, the exosomes originating as ILVs are released by exocytosis via MVB fusion with the plasma membrane. Exosomes containing RNAs or other materials are internalized into recipient cells.

Cancer cells expel exosomes to the endothelial cells in the brain as recipient cells before metastasis. Cancer cell exosomes bind to heparan sulfate of proteoglycan on the surface of recipient cells, which is one of the factors for cellular recognition and adhesion, and are internalized. Endocytosed exosomes release miRNA and destroy the tight junction by degradation of claudin, occludin, and actin. As a result, the cancer cells invade the BBB and enter the brain. This reflects the “seed and soil” theory of metastasis by Stephen Paget. The process of exosome formation, secretion, cellular recognition, adhesion, internalization, and trafficking provides various hints for investigation of macromolecular drug delivery into cells across the membrane via the noninvasive route. Viruses have gained immense attention in human research as they partially use the exosome system for their infection and transmission.

2.3. Endocytosis/Transcytosis Cells absorb large molecular compounds such as proteins via endocytosis to maintain homeostasis. The membrane invaginates to form exosomes that comprise the absorbed compounds. Subsequently, endosomes follow the degradation or the secretory pathway. Endothelial cells at the BBB are destined to transfer the nutrients from blood stream to brain, and inversely, the waste materials from brain to blood stream. Thus, endothelial cells at the BBB have relatively evolved RMT machinery system. Through fusion of exosomes with the opposite plasma membrane, large molecular compounds are released to the brain, based on the secretory pathway. Therefore, this RMT machinery system acts as a promising approach to deliver large molecules. In the degradation pathway, pH in endosomes gradually reduces from the early endosome (pH approx. 6.5) to late endosome (pH approx. 5.5) and then lysosome (pH approx. 4.5) by vacuolar H⁺-ATPase proton pumps. Eventually, large molecules in the lysosome are degraded into pieces under acidic conditions. To avoid such degradation, it is essential to select receptors that are subjected to trigger RMT via the secretory pathway at the BBB. Nevertheless, cargos that escape from the endosome under acidic conditions can inefficiently reach the brain by passive diffusion over the membrane on the brain side.

In practice, the precise mechanism of endocytosis remains unclear. Furthermore, various types of endocytosis complicate the issue. These include (i) macropinocytic, (ii) clathrin-dependent, (iii) caveolae-dependent, or (iv) clathrin- and caveolae-independent considering the plasma membrane invagination and (i) receptor-initiated, (ii) nonreceptor-initiated, or (iii) caveolae-initiated considering the trigger. Most types are energy-dependent and presumed to depend on the clathrin pathway. Even molecules with same ligands inducing endocytosis are internalized via several endocytosis modes.
simultaneously in certain cases depending on the conditions. The endocytosis mechanisms that designed molecules follow are determined via experiments using inhibitors of the type of endocytosis. Unknown trafficking trajectories of arbitrary compounds can be inferred by incorporating optical active dyes or radioactive elements into them.

To unveil the unclear transportation mechanism and entire trajectory, imaging components such as dyes have often been incorporated with the transported molecules through a suitable covalent linker. However, even such slight modification can presumably change the transportation mechanism and entire trajectory between the original label-free molecules and the corresponding labeled molecules due to the coexistence of several trafficking machineries in the cells. In fact, it was reported that incorporation of the dye\(^{16}\) or the linker chain\(^{17}\) resulted in changing the type of endocytosis. Researchers should be aware of such possibility.\(^{18}\) That reminds me of the uncertainty principle, by the German physicist Werner Heisenberg, in quantum mechanics.

### 2.4. Ras-Associated Binding (Rab) Proteins in Trafficking

In cells, vesicular trafficking is strictly controlled by Rab proteins\(^{19}\) that form the largest family of the Ras superfamily of small guanosine triphosphatases (GTPases). Their conformational switch between the inactive and the active GTP-bound forms regulates the interaction with effector proteins. Effector proteins bind only to the active GTP-bound form. Particularly, in the brain capillary endothelial cells, certain endosomes are fused with apical membrane on the blood stream side. In this process, the endocytosed receptors in some endosomes are recycled to the apical membrane, and some endosomes are fused with the basolateral membrane on the brain side. As a result, the substances are then released to the brain. Other endosomes are maturated to become lysosomes and are degraded. All these exosome pathways are regulated by Rab proteins and related effector proteins via multiple sequential and interrelated steps. Exosome pathway of transcytosis is briefly described here. Endosomes derived from the clathrin-dependent receptor-mediated membrane invagination possess Rab proteins on the surface, ligand-receptor complexes, and transmembrane vesicle soluble NSF attachment protein receptor (v-SNARE). Membrane components and inclusion composition are scrutinized by the Rab proteins. Next, the binding of the effector protein to Rab5 is involved in uncoating of the clathrin-coated pits. Moreover, the endosomes are subjected to the basolateral membrane along the cell cytoskeleton by motor proteins as the effector binds to Rab proteins. Subsequently, the Rab proteins are bound to the tether protein on the membrane. The complex formation of v-SNARE on tethered endosomes and t-SNARE on the membrane mediate membrane fusion to release the ligand to the brain.\(^{20}\) The Rab protein cascade plays a pivotal role in intracellular vesicular transport. Sophisticatedly, handling intracellular trajectory is a key strategy for success of drug delivery across the BBB based on RMT. Accordingly, Rab protein control by well-designed functional units contained in endocytosed compounds is the next challenge to enhance transcytosis.

### 2.5. Ligands for the Receptors That Induce Transcytosis

Molecules design appropriate for drug delivery crossing the BBB based on RMT conjugates with the ligand as the vector and drug as cargo, connected through the linker or nanoparticle as carriers (Fig. 1). The ligand and drug are covalently tethered to a suitable linker. Cell penetrating peptides (CPPs), monoclonal antibodies (mAbs), and receptor-targeting peptides as endogenous substance mimics are used as ligands.

#### 2.5.1. CPPs as Ligands

Presently, substance delivery across the membrane into cells using CPPs has been investigated. CPPs are oligopeptides 5–30 residues in length and are internalized into cells across the membrane. Thus, CPP-cargo conjugates through covalent bonds or noncovalent electronic interactions are often utilized for substance delivery into cells. The internalization mechanism of CPPs is still steeped in mystery. Direct translocation and endocytosis are universally recognized as the actual internalization pathway. These two mechanisms may occur simultaneously depending on conditions such as the sort and concentration of CPPs. Trans-activator of transcription (TAT) protein (YGRKKRRQRRR), R9 (RRRRRRRR), and penetratin (RQIKIWFQNRRMKWKK) are well-known CPPs; however, they possess trade-off problems between the simplicity of preparation and cell selectivity. Therefore, CPPs with cargos may present off-target side effects due to unintended wrong distribution.

#### 2.5.2. mAbs as Ligands

Molecular Trojan horse strategy\(^{21}\) is the pioneer transport system to deliver substances into the brain across the BBB based on RMT. In this strategy, the delivered substances are linked with mAb against the receptor expressed on the surface of endothelial cells. The binding of mAbs to the receptor induces receptor-mediated transport. mAbs are immunoglobulins against a specific epitope on a single antigen. Thus, mAbs present high specificity as ligands; as a result, their off-target side effects can be minimized.

#### 2.5.3. Receptor-Targeting Peptides as Ligands

Certain receptors on the cell surface are internalized via endocytosis. The endogenous ligand and artificial receptor-targeting peptide ligands trigger this process by binding to the receptors, although the binding sites might differ in few cases. As for mAbs, their own epitopes are often different sites each other even on the same receptors. The rank order of ligand specificity is mAbs > receptor-targeting peptides > CPPs. Thus, the usage of receptor-targeting peptides is technologically preferred and is cost-effective in developing materials that can cross the BBB.

### 2.6. Receptor-Mediated Transcytosis

Several receptors are expressed on the surface of endothelial cells at the BBB.
Table 1. Receptors That Are Relevant to RMT and Their Endocytosis Mechanism

| Receptors | Plausible endocytosis mechanism | References |
|-----------|---------------------------------|------------|
| TIR       | Clathrin-mediated                | 22, 24–28, 44) |
| LDLR      | Clathrin-mediated                | 31)        |
| InsR      | Clathrin-mediated                | 33, 34, 35) |
| LRP1      | Clathrin/caveolae-mediated       | 36, 37)    |
| CD98hc    | Uncertain                       | 40)        |
| LepR      | Uncertain                       | 41)        |
| HSPG      | Uncertain                       | 47)        |

Some of these are engaged in transcytosis including TIR, LDLR, InsR, and low-density lipoprotein receptor-related protein 1 (LRP1) (Table 1); however, when ligand avidity for the receptor is extreme, the ligand continues to bind to the receptor. Notably, loaded drugs coexisting with the ligand are not released in the brain via transcytosis. Thus, the ligand–receptor avidity must be moderate for dissociation.

2.6.1. TIR

TfR (transferrin receptor) is an endogenous macromolecule to be transferrin system. After internalization, transferrin (Tf) is released into the cytoplasm, where iron is extracted, and Tf is recycled back to the plasma membrane. This process can be modulated by binding ligands to TfR.

**Fig. 2. Structures of Bispecific RmAb158-scFv8D3 and Nanoparticle PELGA-gH/CRT**

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Tf and then induces iron-bound holo-Tf conformation recognized by TR. Thus, PELGA-gH/CRT-33/66 at the ratio 33% gH-66% CRT crosses the BBB endothelium in vitro assays using the flow chamber. Thus, PELGA-gH/CRT-33/66-loaded drug can be transported into brain across the BBB. Yet, the mechanism of crossing the BBB is not clear, although RMT might be involved.

Recently, systematic methods of substance delivery across the BBB have been developed. J-Brain Cargo is the system to deliver drugs into the brain using TR-mediated transcytosis. Drugs for lysosome storage diseases have been investigated using this system. In addition, several pharmaceutical companies have applied it to CNS drug research. Accordingly, innovative drugs will be produced via this technology.

2.6.2. LDLR

LDLR is a single transmembrane glycoprotein. LDL binds to the cell-surface domain of LDLR via apoprotein B100 (apoB-100) as the protein component of LDL particles or apolipoprotein E (apoE) and is followed by endocytosis. About 25% or more of the total cholesterol in human body is located in the brain to maintain neuronal physiology; therefore, nanoparticles as carrier also are efficient for delivering drugs into the brain using LDLR-mediated transcytosis. Thus, nanoparticles as carrier also are efficient for delivering drugs into the brain based on RMT; however, the entry and exclusion of substances across the BBB is difficult. Thus, metal nanoparticles might stay in the brain for a long time.

2.6.3. InsR

InsR expressed on the endothelial cells transports insulin from blood stream to brain based on RMT. Recently, it was suggested that insulin uptake to brain was not affected by the presence of InsR antagonists such as S961 and by the absence of InsR due to alternative transportation routes; however, InsR has been utilized for CNS drug delivery to brain by RMT. Peptide radiopharmaceuticals are useful for earlier quantitation diagnosis of brain diseases such as AD. Aβ peptides possess the high affinity to preexisting Aβ. The monobiotinylated(bio) peptide radiopharmaceutical, that is 125I-bio-Aβ1-40, was conjugated with 83-14 HIR mAb against InsR via linked streptavidin (SA) with a strong affinity to biotin, in particular, 125I-bio-Aβ1-40/8314-SA (Fig. 3). 125I-bio-Aβ1-40/8314-SA exhibited higher uptake in the brain than 125I-bio-Aβ1-40 in vivo assay after intravenous injection to rhesus monkeys. At present, this strategy is presumed to be simpler than using 125I-PET.

Mutations of the gene encoding lysosomal enzyme α-L-iduronidase (IDUA) cause mucopolysaccharidosis type I (MPSI), a type of lysosome storage diseases. The fusion protein conjugate of mAb against human InsR and IDUA, HIRMAb-IDUA (valanafusp alpha), was evaluated in phase I–II clinical trials (NCT03053089, NCT03071341) (Fig. 4). HIRMAb-IDUA was subjected to RMT through InsR and then was transferred into the brain across the BBB. HIRMAb-IDUA demonstrated cognitive stabilization in MPSI based on RMT through InsR.

Administration of insulin for AD patients improved their memory. Insulin-coated gold nanoparticles, that is, INS-GNPs (20 nm in diameter) crossed the BBB in brain and exhibited greater permeation compared to the corresponding INS-GNPs (50 and 70 nm in diameter, respectively), in the in vivo assay where mice were used after injecting their tails with these particles, and the results were analyzed via computed tomography (CT). This uptake was attributed to RMT via InsR. Thus, nanoparticles as carrier also are efficient for delivering drugs into the brain based on RMT; however, the entry and exclusion of substances across the BBB is difficult. Thus, metal nanoparticles might stay in the brain for a long time.

2.6.4. LRP1

LRP1 belonging to the LDLR family has various ligands such as apoE, amyloid precursor protein (APP), and Aβ, and is involved in RMT. Angiopep-2 (TFFYGGSRGKRFKKTEEEY) is well-known LRP1 peptide ligand derived from human Kunitz domain. LRP1 is highly expressed in the endothelial and glioma cells. Thus, the drug against glioma can be effectively delivered to the target diseased part across the BBB using LRP1-mediated transcytosis.

Fig. 3. Structures of VH4127-Fc and 125I-bio-Aβ1-40/8314-SA

Fig. 4. Structures of HIRMAb-IDUA and Biotin-Labelled ANG-TAT
Biotin-labelled ANG-TAT, the conjugate of Angiopep-2 and TAT (Fig. 4), exhibited 1.8-fold higher brain uptake than biotin ANG in in situ brain perfusion using xenograft mice, intracerebrally implanted with U87 glioblastoma cells. ANG-TAT-PIX, the conjugate of Angiopep-2, TAT, and paclitaxel, increased the survival rate greatly than ANG-PTX or PTX alone in the in vivo assay using similarly xenografted mice.35) TAT assisted the delivery of glioma in brain. Furthermore, ANG1005, the conjugate of Angiopep-2 and paclitaxel, completed phase II clinical trial for the treatment of brain cancer (NCT01967810) and recurrent brain metastases from breast cancer (NCT02048059) and is being prepared for phase III trial.

LRP1 interacts on its domains such as cluster 2 (Arg786-Leu1165) and cluster 4 (Ser3332-Arg3779) with its endogenous ligands. Through peptide library screening against human Fc-fused LRP1 (Arg786-Leu1165) or human Fc-fused LRP1 (Ser3332-Arg3779) based on phage display technology, novel LRP1 ligand L57 (TWPKHFDKHTYSILKGLKH) was found and possessed avidity for cluster 4. Incidentally, Angiopep-2 had the avidity to cluster 2. L57 demonstrated higher BBB permeability than Angiopep-7 in in situ brain perfusion assay using mice.27) Angiopep-7, which involves Arg substitutions from Lys at position 10 and 15 of Angiopep-2, revealed slightly higher BBB permeability than Angiopep-2 in the same in situ brain perfusion assay. Therefore, L57 is potent ligand candidate for LRP1 to deliver the CNS drugs based on RMT, although the internalization mechanism of L57-mediated transport needs to be elucidated.

The local flow in blood stream has an effect on crossing the BBB. Blood stream produces fluid shear stress (FSS) on the blood vessel wall. Flowing ligands in the blood are dragged along the vessel wall by frictional force and thereby increase the number of collisions against receptors on the endothelial cells. The average wall FSS is approx. 4 dyne/cm² in normal mice.38) Angiopep-2 coupled liposomes, that is, Ang2-liposomes (80–95 nm in diameter) (Fig. 5) were transported 2.7-fold at 1 and 3.5-fold at 6 dyne/cm² of FSS greater than with static incubation using the model microfluidic BBB. Alternatively, Ang2-liposomes bound and then were internalized at 1 dyne/cm² of FSS or after static incubation greater than at 6 dyne/cm² using the brain endothelial cells. Accordingly, Ang2-liposomes were likely to be transported at 6 dyne/cm² by paracellular route based on the leakage through cellular junctions, in addition to the transcellular route based on RMT.39) Therefore, the appropriate enhance of avidity based on multivalent effect is important at high FSS.

2.6.5. Other Receptors

Various proteins are expressed on the surface of endothelial cells at the BBB. Among these, CD98hc is amino acid transporter (SLC3A2) and has a large population of a single-pass transmembrane protein in the endothelial cells. Bispecific mAb against CD98hc and BACE1 (Fig. 5) exhibited reduction of brain Aβ level by 30–45% based on RMT compared to IgG alone as control in in vivo assay using mice after a single 50 mg/kg intravenous administration of antibody.40) In this case, mAb against CD98hc functioned as a ligand, whereas mAb against BACE1 worked as a cargo. The strategy of substance delivery crossing the BBB using bispecific mAb through RMT is an effective method due to high avidity both for the receptor and the target brain molecule.

Leptin is a hormone secreted from adipose cells and is transferred into the brain based on RMT via leptin receptor (LepR) to repress the eating behavior as a feedback adaptation to satiety. In fact, 125I-leptin uptake was considerably reduced in mice lacking LepR on endothelial cells compared to the normal mice in in situ brain perfusion assay.41) Thus, LepR can be utilized for drug delivery into brain across the BBB; however, the expression level of LepR changed, depending on the conditions. Fatty diets induced LepR upregulation, which might influence the extent of drug uptake based on RMT through LepR.42)

CPPs tethered with the cargo via covalent linker crossed the BBB. It is known that THR (THRPMWSPVWP) binds to TFR43), however, CPPs are enzymatically degraded in serum. CPPs acquired resistance to hydrolysis by conversion into D-form or cyclization. Thus, the retro-enantio version of THR, that is, THRpe (pwypsnmprpt), became enzymatically stable. Moreover, bivalent branched fashion showed more potent avidity than the monovalent linear fashion due to multivalent effect. Accordingly, branched THRre peptides were designed and developed. Bivalent green fluorescent protein (GFP)-THRre_2m possessing 6-maleimidohexanoic acid as linker exhibited approx. 2.6-fold higher membrane permeability than the monovalent GFP-THRre_1m or GFP alone, respectively, in the in vitro assay using bEnd.3 cells as the BBB model (Fig. 6). This suggested that the internalization included the receptors, although it was uncertain that THRre bound to TFR. It was revealed that THRre_2f possessing carboxyfluorescein moiety as fluorophore was engaged in transcytosis.45) Therefore, branched THRre approach can be applied for CNS drug delivery based on RMT.

Cationic CPPs such as TAT and R8 as ligand bound to negatively charged heparan sulfate chains branching from proteoglycan (HSPG) on the cell surface, and then induced endocytosis.46) Thus, the strategy based on RMT using CPPs can be applied for drug delivery into brain across the BBB. CPPs connected with drugs as cargo are usually used for delivery based on cell membrane permeation. Interestingly, insulin and...
noncovalent D-R8 after intravenous coadministration were
delivered into the brain abundantly than insulin alone in the in vivo assay using rat.\textsuperscript{47} Insulin and D-R8 were electrostatically interacted. This uptake was conducted in energy-independent and R8 dose-dependent manner. Thus, it was suggested that insulin was internalized with R8 via a type of receptor-mediated endocytosis that differed from energy-dependent endocytosis. It was assumed to be difficult for insulin to internalize with D-R8 into cells based on direct translocation due to the protein size and hydrophilicity. Recently, insulin is known to ameliorate dementia derived from AD. Noncovalent CPP method for insulin delivery is so low-cost that it is attractive therapeutic treatment; however, CPPs do not have enough specificity to target cells or organs. In particular, insulin was distributed to the liver and kidneys after intravenous coadministration with D-R8. These issues must be addressed before application.

2.7. Nanoparticles as Scaffold for Ligands Investigation of substance delivery system using nanoparticles as carriers such as liposomes, micelles, and polymers has been increasingly carried out.\textsuperscript{48,49} Nanoparticles are superior in terms of stability and carrier capacity, compared to the ligand-cargo conjugates with linker. The feature of nanoparticles is determined by the factors such as size, shape, component material, ligand, cargo, and additive.

Nanoparticles comprise natural component material such as chitosan or synthetic component material such as polymeric organic substances and inorganic substances. Worldwide, an abundance of undegradable plastics in ocean and soil have been a crucial issue due to the environment pollution and ecological hazard. In a similar way, the undegradable nanoparticle accumulation in living body for a long time might cause chronic toxicity, still more in CNS. Inorganic nanoparticles stay in the brain without being degraded and might present toxicity there. Thus, the biologically degradable organic nanoparticles should be used. Degradable polymer-based nanoparticles were prepared from poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), poly(glycolic acid) (PGA), or polycaprolactone (PCL) (Fig. 7). The U.S. Food and Drug Administration (FDA) has permitted the use of PLA, PLGA, PCL, and PEG for drug products.

Surface modification of nanoparticles determines the behavioral characterization in the trajectory at the BBB. Ligands against Tfr, LDLR, InsR, and LRP1 are often chosen to let the nanoparticles cross the BBB. PEGylation elongates the half-life period of nanoparticles due to increased enzymatic stability and due to decreased macrophage clearance. Longer circulating of PEGylated nanoparticles in blood stream augmented the amount distributed to brain.

2.8. Ligand-Receptor Clustering Endocytosis is enhanced by the cluster formation based on cross-linking of ligands and the corresponding receptors on the cell surface. Intriguingly, although intercellular adhesion molecule 1 (ICAM-I) alone did not induce endocytosis, nanoparticles covered with ICAM-I were internalized through endocytosis due to cross-linking.\textsuperscript{49} Moreover, bispecific humanized antibody with a particular affinity for EGFR and PEG (i.e., PEG engage\textsuperscript{\textsc{EGFR}}) formed the cross-linking between PEGylated liposome containing doxorubicin (i.e., Doxosome) and EGFRs (Fig. 8). As a result, such cluster based on EGFR cross-linking induced endocytosis.\textsuperscript{50} As a nonselective ligand, TAT electrostatically bound to heparan sulfate proteoglycans to form a cross-linked formation. The clustering activated rac-1, which is one of the Rho GTPase family, and triggered actin network remodeling to induce endocytosis.\textsuperscript{51,52} The clusters formed by even nonselective ligands such as TAT presented endocytosis. Therefore, it was suggested that the clustering of ligands and receptors played a pivotal role in endocytosis trigger.

In general, the receptors involving receptor-mediated transcytosis formed the cluster and subsequently were endocytosed. Local clustering of Tfr induced clathrin-mediated endocytosis.\textsuperscript{53,54} LDLR clustering interacted with the clathrin terminal domain in coated pits during clathrin-mediated endocytosis process.\textsuperscript{55} Moreover, EGFR oligomerization through the ligand binding enhanced internalization presumably by interacting with the clathrin-coated pits.\textsuperscript{56} Polymeric immunoglobulin receptor clustering also enhanced transcytosis.\textsuperscript{57} Accordingly, receptor clustering is essential for receptor-mediated transcytosis triggering. Molecular design using nanoparticles to accomplish receptor clustering is important for effective internalization; however, the mechanism of ligand-receptor clustering-induced internalization remains still unknown.

2.9. Promising Molecular Design of Nanoparticles That Specially Cross the BBB Tfr, LDLR, LRP1, InsR, and LepR have been often used for drug delivery across the BBB based on transcytosis; however, each receptor expresses widely in several tissues in addition to the endothelial cells at the BBB. As the protein level, (i) Tfr is expressed highly in bone marrow, immune system, and female tissues, moderately in brain, endocrine tissues, lung, gastrointestinal tract, kidney, urinary bladder, male tissues, and skin, and low in muscle tissues, liver, gallbladder, adipose, and soft tissue, aside from serum. (ii) LDLR is expressed highly in male tissues, moderately in endocrine tissues, lung, liver, gallbladder, gastrointestinal tract, kidney, and urinary bladder, and low in brain, muscle tissue, and female tissues. (iii) LRP1 is expressed
moderately in brain, lung, liver, gallbladder, male tissue, and female tissue, and low in adipose, soft tissue, and scarcely in skin. (iv) InsR is expressed moderately in the endocrine tissues, bone marrow, immune system, muscle tissues, lung, liver, gallbladder, pancreas, gastrointestinal tract, kidney, urinary bladder, male tissue, female tissues, and skin and low in brain, adipose, and soft tissue. (v) LepR is expressed highly in the brain.28)

Thus, TTR and LRPI are expressed moderately in the brain, whereas InsR is less expressed in the brain. All of TTR, LDLR, and InsR are suggested to be internalized by clathrin-mediated endocytosis. LRPI is suggested to be internalized by clathrin-mediated endocytosis and caveolae-mediated endocytosis.29) In contrast, LepR is expressed highly in the brain, but the internalization mechanism of leptin receptor remains uncertain. Multiple ligands on the nanoparticle multivalently interact with the corresponding receptors on the cell surface.30) The receptors at the BBB used for RMT are expressed not only at the BBB but also in other tissues. Thus, tissue-selective delivery into the brain using RMT is not completely accomplished yet. To avoid the off-target distributions, multifunctionalized nanoparticles covered with receptors highly expressed at the BBB and possessing the same endocytosis mechanism might stochastically facilitate convergent selective binding to the surface of the endothelial cells there and subsequent receptor clustering. Therefore, as molecular design, dual-functionalized nanoparticles covered simultaneously with both TTR ligands and LRPI ligands, possessing moderate ligand-receptor avidity that allows mutual dissociation probably influenced by acidification in endosomes, are expected to be promising agents that can be selectively and efficiently delivered into the brain across the BBB due to clathrin-mediated endocytosis based on receptor clustering. Nonetheless, design using LepR ligands alone on nanoparticles is also an attractive strategy.

Table 2. Summary of RMT Technologies Described in This Review

| #  | Technologies                                      | Designed and tested compounds | Target receptors | Ligands/ vectors                                      | Carriers/scaffolds | Cargos/payloads | Additives                      | Current status of development stage | References |
|----|---------------------------------------------------|--------------------------------|------------------|-----------------------------------------------------|--------------------|-----------------|-------------------------------|-----------------------------------|------------|
| (i) | mAb                                               | Bispecific                    | TTR              | mAb, scFv8D3                                        | Linker             | mAb, 158 against soluble β-protofibrils | Basic research                     | 22)       |
| (ii) | mAb                                               | Bispecific                    | TTR              | mAb, Anti-TfR1                                       | Linker             | mAb, Anti-BACE1 against BACE1    | Basic research                     | 26)       |
| (iii)| Receptor-targeting peptide J-Brain Cargo8        | PELGA-gH/ CRT                 | TIR              | Tf-CRT complex mAb                                   | Nanoparticle none  | PEG              | Basic research                  | 28)       |
| (iv) | mAb                                               | VH4127-Fc                     | LDLR             | VH4127                                              | Linker             | arbitrary substances           | Preclinical, phase I-II (JR-141) | 29)       |
| (v)  | mAb                                               | 125I-bio-Ap1-40/8314-SA       | InsR             | mAb, 83-14 HIR                                       | Linker             | 125I-bio-Ap1-40                | Basic research                     | 33)       |
| (vi) | mAb                                               | HIRMAb-IDUA                   | InsR             | mAb, HIRMAb                                         | Linker             | IDUA                          | Phase I-II (NCT03053089, NCT03071341) | 34)       |
| (vii)| mAb                                               | INS-GNPs                      | InsR             | insulin                                             | Nanoparticle Gold  | Basic research                | Basic research                     | 35)       |
| (viii)| mAb                                               | Biotin-labelled                | LRP1             | Angiopep-2                                          | Linker             | Biotin                       | Basic research                     | 36)       |
| (xi) | mAb                                               | Ang2-liposomes                 | LRP1             | Angiopep-2                                          | Linker             | Paclitaxel                   | Phase II (NCT01967810, NCT02048059) | 39)       |
| (xii)| mAb                                               | Bispecific mAb against CD98hc and BACE1 | CD98hc | mAb against CD98hc                                  | Linker             | mAb against BACE1            | Basic research                     | 40)       |
| (xiii)| CPP                                              | GFP-THRRe 2m                   | TIR              | THRRe                                               | Linker             | GFP                           | Basic research                     | 44)       |
| (xiv)| CPP                                              | Insulin and D-R8              | HSPG             | THRRe and D-R8                                      | Linker Electrostatic interaction | Insulin | Basic research                     | 47)       |
antigenicity in the blood stream. Architectural and compositional design of nanoparticles in terms of ligand density and affinity must be tuned step by step for optimal uptake into the brain via in vitro and in vivo assays.

3. Conclusions

Recently, several clinical trials regarding agents for CNS diseases such as AD and PD have been hindered. This is attributed to poor drug cell membrane permeation at the BBB. Thus, accomplishment of selective and effective drug delivery into brain across the BBB is demanded. Doxil (liposome, models. To cure brain diseases sufficiently, more effective local clustering although the molecular construction selectively delivered into the brain by means of RMT due to covered with both TfR ligands and LRP1 ligands might be revealed that ligand–receptor clustering formation trig-

ubiquitous expression can result in ineffective ligand distribution to brain and off-target side effects; however, the fact that TfR and LRP1 are moderately expressed in the brain may provide a useful clue as to molecular design. Furthermore, it was revealed that ligand–receptor clustering formation triggered endocytosis. Nanoparticles (40–50 nm in diameter) covered with both TfR ligands and LRP1 ligands might be selectively delivered into the brain by means of RMT due to effective local clustering although the molecular construction is relatively costly. Moreover, the delivery effectiveness of RMT at the BBB is relatively low in many cases, although it was proven to be efficacious in the in vivo assay using animal models. To cure brain diseases sufficiently, more effective drug delivery across the BBB is demanded. Doxil (liposome, approx. 80 nm in diameter) can contain no less than 10,000 low-molecular drug molecules. Encapsulating the appropriate number of drug molecules inside the nanoparticle might be a solution, when selective delivery at the BBB based on RMT is accomplished without any off-target side effects. Nonetheless, selective and effective RMT methodologies must be developed. We should keep in mind (across the BBB using cognitive approach) that the Bible says, “Strive to enter in at the strait gate: for many, I say unto you, will seek to enter in, and shall not be able.” It is true that drug administration based on brain is a solution, when selective delivery at the BBB based on RMT is accomplished without any off-target side effects. Nonetheless, selective and effective RMT methodologies must be developed. We should keep in mind (across the BBB using cognitive approach) that the Bible says, “Strive to enter in at the strait gate: for many, I say unto you, will seek to enter in, and shall not be able.” It is true that drug administration based on craniotomy procedure is easy to give, but the patients are forced to go through great distress. Thus, the medicinal chemists and pharmaceutical scientists have been expected to develop noninvasive delivery methods to enter drug into the brain across the BBB. The approach by RMT using nanoparticles or mAbs as ligand–drug conjugate will pave the way to cure the patients suffering from refractory symptom such as neurodegenerative diseases.

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Conflict of Interest The author declares no conflict of interest.

References

1) Editorial. Nat. Med., 24, 247 (2018).
2) André P., Debray M., Scherrmann J.-M., Cisternino S., J. Cereb. Blood Flow Metab., 29, 1293–1304 (2009).
3) Shimomura K., Okura T., Kato S., Couraud P.-O., Scherrmann J.-M., Terasaki I., Deguchi Y., Fluids Barriers CNS, 10, 8 (2013).
4) Tashima T., Bioorg. Med. Chem., 23, 4119–4131 (2015).
5) Tashima T., Bioorg. Med. Chem. Lett., 27, 121–130 (2017).
6) Tashima T., Bioorg. Med. Chem. Lett., 28, 3015–3024 (2018).
7) Rashed M. H., Bavarakar E., Helal G. K., Abd-Elilah M. I., Amero P., Chavez-Reyes A., Rodriguez-Aguayo C., Int. J. Mol. Sci., 18, 258 (2017).
8) Heinzel N. P., Llorente A., Cell. Mol. Life Sci., 75, 193–208 (2018).
9) Harding C., Heuser J., Stahl P., J. Cell Biol., 97, 329–339 (1983).
10) Pan B. T., Johnstone R. M., Cell, 33, 967–978 (1983).
11) Valadi H., Ekström K., Bossios A., Sjöstrand M., Lee J. J., Löttvall J. O., Nat. Cell. Biol., 9, 654–659 (2007).
12) Christianson H. C., Svensson K. J., Kuppevetel T. H., Li J.-P., Belting M., Proc. Natl. Acad. Sci. U.S.A., 110, 17380–17385 (2013).
13) Weidle U. H., Birzele F., Kollmorgen G., Rüger R., Cancer Genomics Proteomics, 14, 1–15 (2017).
14) Tominaga N., Kosaka N., Ono M., Katsuda T., Yoshioka Y., Yamura K., Löttvall J., Nakagama H., Ochiya T., Nat. Commun., 6, 6716 (2015).
15) Paget S., Lancet, 133, 571–573 (1889).
16) Hirose H., Takeuchi T., Osakada H., Pujals S., Katayama S., Nakase L., Kobayashi S., Haraguchi T., Futaki S., Mol. Ther., 20, 984–993 (2012).
17) Nakase L., Noguchi K., Aoki T., Takatani-Nakase T., Fuji I., Futaki S., Sci. Rep., 1991, 7 (2017).
18) Albanese A., Tang P. S., Chan W. C., Annu. Rev. Biomed. Eng., 14, 1–16 (2012).
19) Wagner T., Novick P. J., Physiol. Rev., 91, 119–149 (2011).
20) Zhen Y., Stenmark H., J. Cell Sci., 128, 3171–3176 (2015).
21) Fradette W. M., BioDrugs, 31, 503–519 (2017).
22) Hallevis G., Sväntner S., Fang Y. T., Lammfelt L., Sehlin D., Thrombosis, 5, 308–318 (2019).
23) Grub F., Maris-Ariza M., Herline K., Peyer D., Boutajangout A., Mehta P., Drummond E., Fielli P., Winiewski I., Alzheimer’s Res. Ther., 10, 10 (2018).
24) Haqqani A. S., Thom G., Burrell M., Delaney C. E., Brunette E., Baumann E., Sedja C. J., J. Neurochem., 146, 735–752 (2018).
25) Thom G., Burrell M., Haqqani A. S., Yogi A., Lessard E., Brunette E., Delaney C., Baumann E., Callaghan D., Rodrigue N., Webster C. J., Stanimirovic D. B., Mol. Pharm., 15, 1420–1431 (2018).
26) Yu Y. J., Atwal J. K., Zhang Y., Tong R. K., Wildsmith K. R., Tan C., Bie-Lyn N., Herson M., Malone J. A., Meilandt W. J., Bumbaca D., Gadkar K., Hoyte K., Luk W., Lu Y., Ernst J. A., Seerage-Levie K., Couch J. A., Denis M. S., Watts R. J., Sci. Transl. Med., 6, 261ra154 (2014).
27) Bie-Lyn N., Yu Y. J., Bumbaca D., Elstrott J., Boswell C. A., Zhang Y., Luk W., Lu Y., Denis M. S., Weiner R. M., Chung I., Watts R. J., J. Exp. Med., 211, 233–244 (2014).
28) Falanga A., Melone P., Cagliani R., Borbone N., D’Errico S., Piccialli G., Netti P. A., Guarnieri D., Molecules, 23, 1655 (2018).
29) Okuyama T., Kosuga M., Hamazaki T., Shintaku H., Seo J.-H., Mol.
30) Björkhem I., Meaney S., Arterioscler. Thromb. Vasc. Biol., 24, 806–815 (2004).
31) Molino Y., David M., Varini K., Jabès F., Gaudin N., Fortoul A., Baklouk K., Masse M., Bernard A., Drobecq L., Lécorché P., Temsamani J., Jacquot G., Khrestchatisky M., FASEB J., 31, 1807–1827 (2017).
32) Rhea E. M., Rask-Madsen C., Banks W. A., J. Physiol., 596, 4753–4765 (2018).
33) Wu D., Yang J., Pardridge W. M., J. Clin. Invest., 100, 1804–1812 (1997).
34) Giugliani R., Giugliani L., Poswar F. O., Donis K. C., Corte A. D., Schmidt M., Boado R. J., Nestrasil I., Nguyen C., Chen S., Pardridge W. M., Orphanet J. Rare Dis., 13, 110 (2018).
35) Betzer O., Shilo M., Motiei M., Popovtzer R., Proc. SPIE, 10891, 108911H (2019).
36) Li Y., Zheng X., Gong M., Zhang J., Oncotarget, 7, 79401–79407 (2016).
37) Sakamoto K., Shinohara T., Adachi Y., Asami T., Ohtaki T., Biochem. Biophys. Rep., 12, 355–359 (2017).
38) Buchanan C. F., Verbridge S. S., Vlachos P. P., Rylander M. N., Cell Adhes. Migr., 8, 517–524 (2014).
39) Papademetriou I., Veduta E., Charest J., Porter T., PLOS ONE, 13, e0205158 (2018).
40) Zuchero Y. J., Chen X., Bien-Ly N., Bumbaca D., Tong R. K., Gao X., Zhang S., Hoyte K., Luk W., Huntley M. A., Phu L., Tian C., Kallop D., Weiner R. M., Lu Y., Kirkpatrick D. S., Ernst J. A., Chib B., Dennis M. S., Watts R. J., Neuron, 89, 70–82 (2016).
41) Di Spiezio A., Sandin E. S., Dore R., Müller-Fielitz H., Storck S. E., Bernau M., Mier W., Oster H., Jöhnk O., Pietrzik C. U., Lehner H., Schwaninger M., Molecular Metabolism, 8, 13–22 (2018).
42) Moura R. P., Martins C., Pinto S., Sousa F., Sarmento B., Expert Opin. Drug Deliv., 16, 271–285 (2019).
43) Lee J. H., Engler J. A., Collawn J. F., Moore B. A., Eur. J. Biochem., 268, 2004–2012 (2001).
44) Diaz-Perlas C., Oller-Salvia B., Sánchez-Navarro M., Teixidó M., Giralte E., Chem. Sci., 9, 8409–8415 (2018).
45) Ceccelli R., Aday S., Sevin E., Almeida C., Culoš M., Dehouck L., Coisne C., Engelhardt B., Dehouck M. P., Ferreira L., PLOS ONE, 9, e99733 (2014).
46) Christianson H. C., Beltin M., Matrix Biol., 35, 51–55 (2014).
47) Kamei N., Yamaoka A., Fukuyama Y., Itokazu R., Takeda-Morishita M., Biol. Pharm. Bull., 41, 546–554 (2018).
48) McCully M., Sanchez-Navaarro M., Teixido M., Giralte E., Curr. Pharm. Des., 24, 1366–1376 (2018).
49) Muñoz, M., Garcia-H hueco C., Champion J. A., Leferovich J., Gajewski C., Schuchman E. H., Mitragotri S., Muzykantov V. R., Mol. Ther., 16, 1450–1458 (2008).
50) Su Y.-C., Burnouf P.-A., Chuang K.-H., Chen B.-M., Cheng T.-L., Roffler S. R., Nat. Commun., 8, 15507 (2017).
51) Gerbal-Chaolu S., Gondeau C., Aldrian-Herrada G., Heitz F., Gauthier-Rouviere C., Divita G., Biol. Cell, 99, 223–238 (2007).
52) Fujii M., Kawai K., Egami Y., Araki N., Sci. Rep., 3, 2385 (2013).
53) Liu A. P., Aguet F., Danuser G., Schmidt S. L., J. Cell Biol., 191, 1381–1393 (2010).
54) Coreton D. K., Harbison C. F., Cocucci E., Parrish C. R., Kirchhausen T., J. Virol., 86, 3330–3340 (2012).
55) Kibbey R. G., Rizo J., Giersch L. M., Anderson R. G. W., J. Cell Biol., 142, 59–67 (1998).
56) Hofman E. G., Bader A. N., Voortman J., van der Heuvel D. J., Sigismund S., Verkleij A. J., Gerritsen H. C., van Bergen and Hengouwen P. M., J. Biol. Chem., 285, 39481–39489 (2010).
57) Emmerson C. D., van der Vlist E. J., Braam M. R., Vanlandschoot P., Merchier P., de Haard J. H., Verrips C. T., van Bergen and Hengouwen P. M., Dolk E., PLoS ONE, 6, e26299 (2011).
58) “The Human Protein Atlas.” ‹https://www.proteinatlas.org/›
59) Papademetriou I. T., Porter T., Ther. Deliv., 6, 989–1016 (2015).