Expert Opinion

Various drug delivery approaches to the central nervous system

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Importance of the field: The presence of the blood–brain barrier (BBB), an insurmountable obstacle, in particular, and other barriers in brain and periphery contribute to hindrance of the successful diagnosis and treatment of a myriad of central nervous system pathologies. This review discusses several strategies adopted to define a rational drug delivery approach to the CNS along with a short description of the strategies implemented by the authors' group to enhance the analgesic activity, a CNS property, of chimeric peptide of Met-enkephalin and FMRFa (YGGFMKKKFMRFa-YFa).

Areas covered in this review: Various approaches for drug delivery to the CNS with their beneficial and non-beneficial aspects, supported by an extensive literature survey published recently, up to August 2009.

What the reader will gain: The reader will have the privilege of gaining an understanding of previous as well as recent approaches to breaching the CNS barriers.

Take home message: Among the various strategies discussed, the potential for efficacious CNS drug targeting in future lies either with the non-invasively administered multifunctional nanosystems or these nanosystems without characterstics such as long systemic circulating capability and avoiding reticuloendothelial system scavenging system of the body, endogenous transporters and efflux inhibitors administered by convection-enhanced delivery.

Keywords: blood–brain barrier, brain targeting, central nervous system, colloidal nanosystems, convection-enhanced delivery, delivery approaches

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1. Introduction

Brain, the most important organ of the body, has been studied extensively to find ways for drugs and drug delivery systems to get across the barriers imposed by it in the diagnosis and treatment of neurological disorders. The blood–brain barrier (BBB) (**Figure 1**) is the major barrier, and it always presents a massive obstacle to overcoming these disorders. The BBB separates the CNS from systemic circulation, and its major physiological functions comprise sustaining homeostasis at the brain parenchyma and shielding the brain from potentially harmful substances. The BBB is formed principally from capillary endothelial cells that lack fenestrations and are very closely joined together by tight intercellular junctions [1,2], presenting high trans-endothelial electrical resistance compared with other tissues [3], and thereby efficiently restrict the paracellular diffusion of solutes or drugs [4-7].

The second barrier, the blood cerebrospinal fluid barrier (BCSFB) (**Figure 1**) located at the choroid plexus, separates the blood from the cerebrospinal fluid (CSF), which, in turn, runs in the subarachnoid space surrounding the brain, does not express the classical BBB capillary endothelial cells and is slightly more permeable to solutes/drugs [1-3,6,7].

Besides these, some regions within the CNS lack a BBB but have micro-vessels similar to those of the periphery. These areas are collectively named the

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Article highlights.

- A concise introduction of the physicochemical properties of the barriers offered by the CNS and the general nature of molecules that target the CNS.
- An overview of the various drug delivery approaches to CNS.
- Describes the prodrugs and chemical delivery systems' ability to target the CNS and also briefly discusses the computational approaches to targeting the CNS.
- Evaluating the significance of endogenous transporters and their pathways in felicitating CNS delivery.
- Explaining the efflux transporters' inhibition mechanism, design and use of efflux transporter inhibitors for CNS targeting.
- Describes the design of CPPs, sequential nature and transport mechanism in mediating delivery to the CNS.
- Overview of the methods/agents for BBB modulation in targeting the CNS.
- A detailed description of the various nanosystem formulations to target the CNS.
- Evaluating the role of modified and conjugated nanoliposomes in CNS targeting.
- Considering nanoparticles as targeting agents to the CNS with different modifications and conjugations.
- Assessing the ability of polymeric micelles to target the CNS with modifications and conjugations.
- Illustrates that dendrimers with certain modifications and conjugations assist in CNS drug delivery.
- Visits different methods for bypassing the BBB.
- Depicting direct drug administration into the CNS can be helpful to target the CNS.
- Illustrates the value of drug delivery by the olfactory pathway in targeting the CNS.
- Portrays the recently adopted technique convection-enhanced delivery (CED) as a mode of site-specific delivery of drugs/delivery systems to the CNS.
- Concluding multifunctional nanosystems or nanosystems delivered by means of the CED as prospective CNS drug delivery methods.
- Authors provide their perspective that either multifunctional nanosystems or nanosystems delivered by CED are the potential approaches for site-specific CNS drug delivery.

This box summarises keypoints contained in the article.

circumventricular organs (CVOs), comprising the choroid plexus, median eminence, and so on. The capillaries in CVOs are fenestrated, allowing the free movement of solutes between the blood and the surrounding interstitial fluid [2,3].

In addition to these structural barriers, the CNS also presents functional barriers in the form of influx [8] and efflux transporter mechanisms [9], which are responsible for the inclusion and exclusion of solutes/therapeutics into and out of the CNS. Enzymes expressed by brain also hinder solutes/therapeutics by degrading them and thus limit their passage to the CNS [10]. Owing to all these barriers, cerebral diseases have proved to be most refractory to therapeutic interventions.

Generally, it is assumed that drugs that readily cross the BBB by passive diffusion in pharmacologically significant amounts should be relatively small, having a molecular mass of < 500 Da, a log octanol/water partition coefficient between -0.5 and 6.0 [7], be lipid-soluble, be either neutral or significantly uncharged at physiological pH 7.4, and be capable of forming < 8 H-bonds with water [8].

Recent studies have revealed that the largest substance CINC1-7.8 kDa crosses the BBB by transmembrane diffusion [11] and some octanol/lipohiphilic soluble compounds cannot cross the BBB owing to the lack of required structural and functional characteristics to cross the BBB. Molecules with a polar surface area > 80 $Å^2$, a high Lewis bond strength and a high affinity for hydrogen bond formation do not usually cross the BBB. Furthermore, molecules of a given molecular mass containing rotatable bonds and that are highly branched cannot readily cross the BBB [7].

Therefore, it is of great significance to understand the physicochemical properties of the BBB with different cells and the structural and functional mechanisms involved in the passage of molecules across the BBB to design potential drug candidates or delivery systems for the diagnosis and treatment of CNS diseases.

This review discusses the approaches adopted so far with a view to gaining an insight into developing drug delivery strategies to felicitate a high therapeutic index to the CNS and predicting either non-invasively injected multifunctional nanosystems or these nanosystems without properties such as long systemic circulating capability and avoiding the reticuloendothelial system (RES) scavenging system of the body, endogenous transporters, and efflux inhibitors injected by the convection-enhanced delivery (CED) technique as the future of targeted drug delivery to the CNS with minimum or negligible side effects.

2. Strategies for drug delivery to the CNS

2.1 Prodrug and chemical delivery systems

Typically, lipid-soluble drugs/prodrugs show an ability to cross the BBB in comparison with polar molecules and drugs (**Figure 2**). Prodrugs and chemical delivery systems (CDDS) are pharmacologically inactive compounds resulting from transient chemical modifications of a biologically active species to its active form and is 'locked-in' within the CNS with improved characteristics such that levels of the active drug should remain high in the CNS [7] in comparison with the periphery to show the desired effect at the receptor site. CDDS differs from the prodrug approach only due to the introduction of multistep transformations and targetor moieties [3].

Morphine, an effective analgesic, has a quite low CNS uptake, but its derivatives codeine (methyl derivative) and heroin (diacetyl derivative) show significant increase in brain uptake owing to their lipid solubility, as quantified by the brain uptake index (BUI) technique. Heroin acts as a prodrug for morphine within the CNS. It rapidly penetrates the brain, where it converts to polar morphine and interacts with opioid receptors of brain [7].

Recently, new developments in the prodrug approach been explored. Pignatello *et al.* [12] conjugated flurbiprofen, a

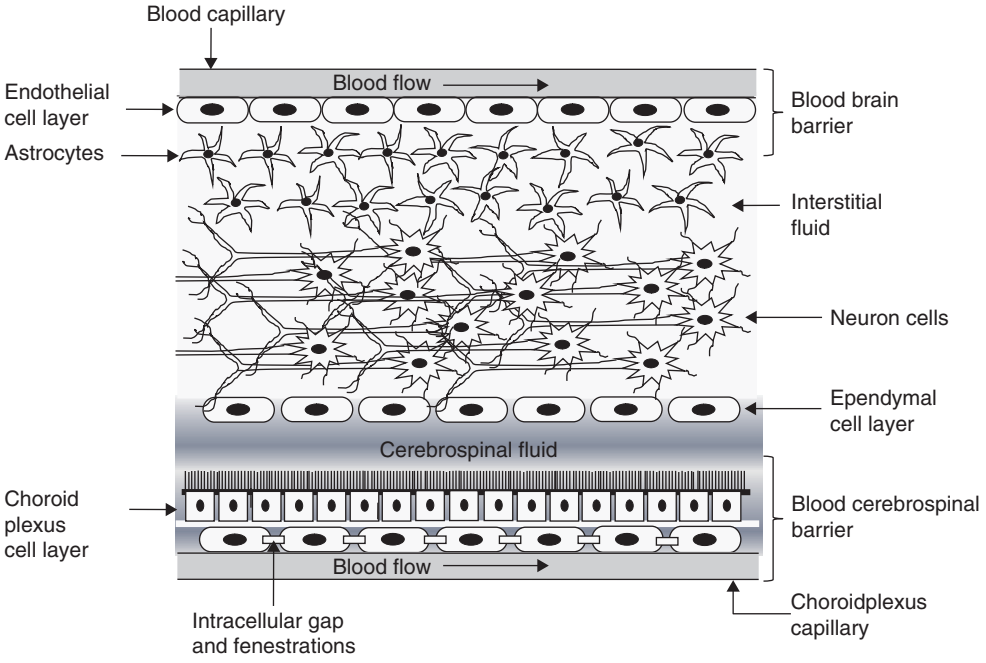

Figure 1. Schematic representation of the two major barriers presented by the brain to solutes, drugs and delivery systems.
Adapted with permission from [2].

potential neuroprotective agent in Alzheimer's disease, with lipoamino acids (LAA) containing alkyl chain and a polar amino acid as promoiety, to obtain amphiphilic derivatives with a membrane-like character that can favor interaction and penetration through biological membranes and barriers such as the BBB. Studies on some of the compounds delivered by the prodrug approach are shown in **Table 1** [12-19].

Relatively small chemical modifications to a molecule may augment the circulatory half-life owing to reduced peripheral distribution volume or confrontation to enzymatic hydrolysis in the systemic circulation [1-3]. Increasing merely the lipid solubility of a drug molecule does not account for brain penetration and may cause detrimental effects such as decreasing aqueous solubility, increasing plasma protein binding, enhanced uptake by the liver and RES and thus reduction in bioavailability. Moreover, extremely lipid-soluble substances can partition into the membranes so ardently that they will not repartition into the aqueous environment of the interstitial fluid of the brain.

Delivery of neuropeptides to brain involves chemical transformations of the peptide–CDDS system. Peptide is a part of bulky CDDS system [20] in which it is attached to a redox targetor, a spacer function and lipophilic modifying groups that direct BBB penetration and disguise the peptide nature of the molecule. An opioid peptide (enkephalin) was observed for analgesic activity with this CDDS method. The strategy incorporates a 1,4-dihydrotrigonellinate targetor that undergoes an enzymatically mediated oxidation to a hydrophilic, membrane-impermeable trigonellinate salt. The polar targetor peptide conjugate that is trapped behind the lipoidal BBB is deposited in the central nervous system. Analgesia was observed with 'packaged' enkephalin but not with the unmodified peptide or lipophilic peptide precursors.

In recent years, computational *in silico* approaches have been proved to be a valuable tool to envisage BBB permeability mathematically by taking into account numerous calculated physicochemical parameters for a structure [21]. This approach, to a certain extent, is quite advantageous to exclude undesired structures on the basis of some parameters needed to cross the BBB. However, at present this approach cannot estimate the interaction of a compound on the basis of influx and efflux transporter mechanisms in the BBB. Therefore, this will cause deviation of penetration factor from the predicted value and cannot accurately predict pharmacokinetics of the drug with the BBB.

2.2 Endogenous transporter-mediated delivery

Several highly lipid-soluble molecules do not have the expected high BBB penetration and are excluded from the CNS because of dissimilar physicochemical characteristics. Polar molecules, such as D-glucose, L-amino acids and nucleosides, penetrate the brain far more readily than their lipid solubility might recommend. These molecules, together with many others, which are essential metabolites for the brain, have a carrier-mediated transport (CMT) (**Figure 2**) that is either a facilitated diffusive entry or an energy-dependent concentrative mechanism dependent on specific carrier

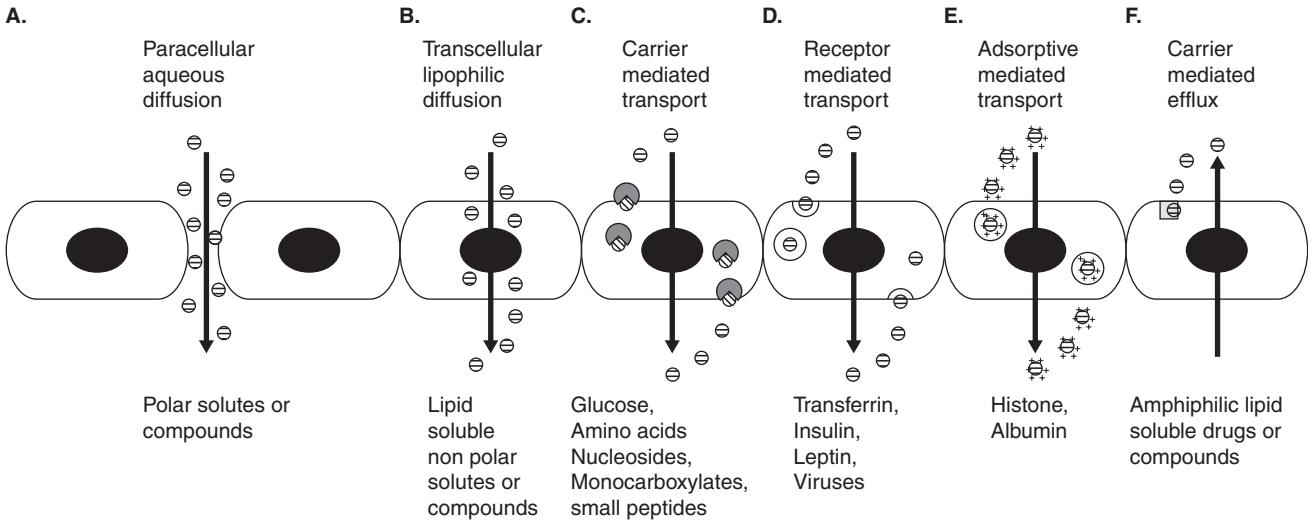

Figure 2. Mechanisms of transport across the BBB. A. Paracellular diffusion of very small amounts of water-soluble compounds. **B.** Transcellular lipophilic diffusion of lipid-soluble agents. **C.** Carrier-mediated transport of essential polar solutes such as glucose, amino acids, nucleosides, monocarboxylates and small peptides. **D.** Receptor-mediated transport of essential proteins such as transferrin, insulin, leptin, and so on. **E.** Adsorptive-mediated transport of cationized molecules such as albumin. **F.** Carrier-mediated efflux transport prohibits the penetration of amphiphilic lipid-soluble drugs and many other substances into the brain.

proteins inserted into the luminal and albuminal membranes of the capillary endothelial cells [1-3].

These transporters require stereochemical specificity for transport. Therefore, several prospective drugs should be designed as pseudosubstrates to resemble structurally the endogenous ligand for specific transport system transporters and thus enhance their uptake across the BBB [22]; but it is not necessary, the BBB hexose transporter GLUT1 is very stringent in its stereochemical requirements and therefore transports D-glucose but does not transport psuedosubstrates and L-glucose [23].

A large neutral amino acid transporter system (or L-system) that accepts a large number of pseudosubstrates transports several drugs into the brain [24]. Neurotransmitter dopamine, because of its hydrophilic nature, is unable to cross the BBB, but its conversion into its α-amino acid, L-DOPA, enables the brain to uptake by means of the large amino acid transporter of type 1, LAT 1 [25,26]. Studies on some of the compounds delivered by endogenous transporters are shown in **Table 2** [27-33].

Smaller drugs or substances with low molecular mass are usually transported by CMT, whereas large peptides or proteins are transported by receptor-mediated transport (RMT) or adsorptive-mediated transport (AMT) by means of an endocytosis process [34] across the BBB (**Figure 2**). RMT involves binding of the peptide/protein to a receptor present at the luminal surface of the BBB, leading to the formation of an endocytic vesicle of the receptor–ligand complex aggregate, and then triggers the internalization of the endocytic vesicle [35]. These vesicles then enter a pathway, during which the peptide/protein dissociates from the receptor resulting in transportation across the cerebral endothelial cells and the receptor is exocytosed at the luminal membrane of the endothelial cell. The RMT pathway can be understood by the linking of drug or peptide/protein to the monoclonal antibodies (mAb; OX26). The OX26 antibody expressed in the luminal membrane of the BBB has affinity towards the transferrin receptor. Therefore, mAb-linked drug or peptide/protein binds to the transferrin receptor and the whole complex is transcytosed across the BBB by the process of endocytosis [35]. Otherwise, drug or peptide/protein not linked to mAb could not cross the BBB. However, not all vesicles have the fate of being transcytosed across cerebral endothelial cells, and some fuse with lysosomes, resulting in hydrolysis of the contained drugs/peptides/proteins [36].

AMT depends on the charge interaction between molecules/peptide/proteins and the luminal surface membrane of the endothelial cells. Substances possessing significantly positive charge with respect to the luminal surface membrane of the endothelial cells induce vesicle formation and then internalization and transcytosis across the BBB [37].

2.3 Efflux transporters inhibition

Uptake of drugs in the CNS is influenced by efflux transporters, which exorcise multiple drugs from the CNS (**Figure 2**). Efflux transporters that belong to members of the ABC cassette (ATP-binding cassette) of transport protein are multi-drug resistance protein (MRP), P-glycoprotein (Pgp) and the multispecific organic ion transporter and breast cancer protein. The drugs that are predicted from physicochemical properties of the drug, such as lipid solubility, to cross the BBB may not necessarily cross it as these transporters continually hydrolyze ATP and are able to extrude drugs from the cerebral capillary endothelial cells and the CNS into the blood against a concentration gradient [9,38].

Table 1. Studies on some of the compounds delivered by a prodrug approach.

Pro-moiety	Main compound	Animals	Dose(s); route	Significant findings	Action on CNS	Ref.
Lipoamino acids (LAA)	Flurbiprofen (FLU)	Mice	100 μg FLU equiv/mouse; i.v.	C6 alkyl chain of FLU showed a small amount of FLU up to 9 h while C10 alkyl chain of FLU displayed an increased amount of FLU up to 9 h in comparison with free FLU, which disappears in 3 h	Alzheimer's disease	[12]
Azomethine	(R)-α-methylhistamine	Mice	24 μmol/kg; oral	Trifluoromethylated benzophenone derivative has 140% and 2-pyrrolyl derivative has 47.4% CNS penetration index, respectively	CNS disorders, inflammatory and acid-related diseases	[13]
Benzyl ester	S-Acetylthiorphan	Mice and rats	1 – 10 mg/kg; parenteral	Extensive inhibition of cerebral enkephalinase and the long-lasting potentiation of analgesia elicited by ICV-administered (D-Ala2,Met5) enkephalin	Nociception	[14]
Triglyceride	Ketoprofen	Mice	40 μmol/kg; i.v.	Threefold increase in the area under the brain concentration–time curve of triglyceride ketoprofen, compared with ketoprofen alone	Nociception	[15]
m-nitrophenyl ester (MNPC)	Nipecotic acid	Mice	400 mg/kg; s.c.	Nipecotic acid brain levels peaked at 30 min after s.c. administration of (-) MNPC	Convulsions	[16]
Probenicid (P)	Tacrine hydrochloride (THA)	Mice	23.5 mg/kg PTHA eq. to 10 mg/kg THA; i.v.	PTHA has 1.76 times higher concentration of THA in brain than THA alone. Overall targeting efficiency of THA was also enhanced from 10.97 to 16.11% with PTHA	Alzheimer's disease	[17]
Lecithin (L)	Indomethacin (ID)	Rats	LID (10 mg/kg) and ID (3.54 mg/kg); oral	Brain-to-plasma concentration ratio was found to be 2.5-fold higher in the case of LID than ID alone	Nociception, Alzheimer's disease	[18]
Chlorine (Cl)	YFa	Mice	10 mg/kg; i.p.	Cl-nation of phenylalanine (Phe) at position 4 in YFa favors enhanced BBB permeability and analgesia of ~ 22.5% MPE at 10 mg/kg [p-Cl Phe4] YFa than YFa alone	Nociception	[19]

i.p.: Intraperitoneal; i.v.: Intravenous; MPE: Maximum possible effect; s.c.: Subcutaneous.

Table 2. Studies on some of the compounds delivered by endogenous transporters.

Compounds	Endogenous transporter	Animals/ cell line	Dose(s); route	Significant findings	Action on CNS	Ref.
Glycosylated Leu-enkaphalin amide	GLUT 1	Rats	Glycosylated and non-glycosylted: 0.07 and 0.02 nmol; ICV, 46.4 and 11.4 nmol/kg; i.v., 137.2 and 34.3 nmol/kg; i.p. and 20.3 and 7.2 nmol/kg; s.c.	ICV increased the antinociception potency more than 3-fold, i.v. 4.1-fold increase in potency, i.p. and s.c. also showed increase in potency of glycosylated compared with non-glycosylated analogue	Nociception	[27]
[O-Glu-Ser5] YFa	GLUT 1	Mice	40 and 60 mg/kg of all analogues; i.p.	[O-Glu-Ser5] YFa showed enhanced analgesia of ~ 48.66% MPE, and 57.85% MPE in comparison of 44.57% MPE and 50.38% MPE analgesia by [O-Gal-Ser5] YFa and 26.15% MPE and 41.05% MPE analgesia by non-glycosylated analogue [Ser5]YFa and YFa alone	Nociception	[28]
L-4-chloro kynurenine (4-Cl-KYN)	Large amino acid transporter (LAT 1)	Rats	500 mM 4-Cl-KYN; i.v.	4-Cl-KYN converted intracerebrally to 7-Cl-KYNA (7-chlorokynurenic acid). Brain concentrations of 4-Cl-KYN and 7-KYNA were higher after perfusion with 500 mM 4-Cl-KYN	An NMDA receptor antagonist	[29]
Ketoprofen-tyrosine	Large amino acid transporter (LAT 1)	Rats	64 μM; i.v.	Ketoprofen-tyrosine uptake was 0.846 pmol/(mg min), which is higher than when 2 mM 2-aminobicyclo(2,2,1) heptane-2-carboxylic acid (BCH) was added to the perfusion medium.	Nerve pain	[30]
Glutathione adamantamine and glutathione dopamine	Glutathione transporter	Madin Darby canine kidney (MDCK II) cell line	100 μM of both the compounds	Glutathione adamantamine and dopamine have shown higher influx rate than efflux rate	Parkinson's disease	[31]
6-O-Nipecotil-ascorbate	SVCT2	Mice	0.75 mmol/kg; i.p.	6-O-Nipecotil-ascorbate has increased in latency to convulsions to 922 s in comparison with nipecotic acid and saline-treated group	Convulsions	[32]
Peripheral benzodiazepine receptors ligand – gemcitabine (PK 11195-GEM)	Peripheral benzodiazepine receptors (PBRs)	Rats	0.43 mg/kg; i.v.	PK11195-GEM possessed a twofold enhancement in brain tumor selectivity compared with GEM alone	Glioma	[33]

i.p.: Intraperitoneal; i.v.: Intravenous; MPE: Maximum possible effect; s.c.: Subcutaneous.

Extensive knowledge of the structure–activity relationships (SAR) of the ABC efflux transport mechanisms [38-40] with their substrates and inhibitors is needed because they lack interaction in classic enzyme-substrate/lock-and-key manner and therefore Michaelis–Menten kinetics cannot be applied to the action of ABC efflux transport mechanisms. One strategy for counteracting this problem is to develop inhibitors for efflux transporters, and another is to design types of analogue that cannot interact with efflux transporters [9,38]. To increase the CNS delivery of drugs, both competitive and non-competitive inhibitors have been explored to modulate the activity of the major ABC transporters, such as Pgp, MRP, and so on. Thus, P-gp modulators such as verapamil, diltiazem and CSA have been used to increase the brain penetration of several compounds with low BBB permeability, such as antiviral protease inhibitors, the anticancer agent paclitaxel (PCL) and the antifungal agent itraconazole. In addition, some polymers such as pluronic block copolymer (P85) have been shown to enhance the permeability of a wide variety of drugs in an *in vitro* model of the BBB by inhibiting drug efflux transporters. The mechanism by which polymers modulate drug efflux transporter activity involves alterations in membrane fluidity [41]. However, this approach is not quite safe as other toxic substances can move freely into the brain owing to this modulation of ABC transporters.

2.4 Cell penetrating peptide-mediated delivery

Cell penetrating peptide (CPP) vectors are also used to transport molecules into the BBB [42,43]. CPPs comprise of an amphipathic α-helix containing alternating distinct hydrophobic and positively charged domains. Repeating sequences of charged amino acid such as arginine or lysine form positively charged domains that are followed by a sequence of hydrophobic residues to form hydrophobic domain [7,44]. Cell penetrating peptides translocate molecules in a mechanism more similar to that of AMT [45].

Internalization of CPPs does not depend on their specific primary sequences. Structure–activity studies revealed that stereochemically derived analogues of CPPs retained the internalizing properties of their parent peptides [46,47] and thus display non-dependent stereospecific receptor recognition. Translocating ability of CPPs depends on their cationic nature and this signifies the decisive role of basic residues in them. Transactivating-transduction (TAT)-derived peptide, a CPP, induces the formation of reverse micelles as an energy-independent process [48], and does not involve the cell surface receptors in transcytosis [42]. TAT has been shown to carry heterologous proteins into several cell types and across the BBB [49]. Studies on some of the compounds delivered by CPPs are represented in **Table 3** [50-54].

2.5 Blood–brain barrier disruption

The tight junctions between the cerebral endothelial cells can be either partially or completely opened to permeabilize the BBB to enhance drug penetration. Enhanced CNS drug delivery involves the systemic administration of drugs in conjunction with transient BBB disruption. Over several years osmotic opening of the barrier technique has been applied effectively in the treatment of malignant gliomas [55]. An osmotic agent, hypertonic mannitol, when infused with a cannula in the carotid artery of brain, causes endothelial cell shrinkage, and thereby leads to the opening of tight junctions. If a drug is administered through the same cannula it can diffuse freely into the brain. Osmotic opening of the BBB causes a 10- to 100-fold increase in the concentration of methotrexate (MTX) to the tumor [7].

Similar to mannitol, alkylglycerols also cause modulation of the BBB [56]. The concentration of MTX in the CNS is increased appreciably with the help of alkylglycerols. 1-Ohexyldiglycerol and 1-*O*-heptyltriglycerol result in BBB disruption, but 1-Ohexyldiglycerol is the most effective [56]. Retinoic acid or phorbol myristate acetate (PMA) also helps in opening of the BBB via prolonged infusions [57]. Immune adjuvants such as Freund's complete or incomplete adjuvant cause long-term disruption of the BBB to circulating IgG [58].

The mechanism of the opening of BBB is still debatable and needs more investigation. However, opening of the cerebral barrier may cause a hazard to brain as this procedure breaks down the self-defense mechanism of the brain, leaving it for non-selective permeability to circulating chemicals or toxins from blood to brain [1].

Recently, selective opening of the blood–tumor barrier was attained by the intracarotid infusion of leukotriene C4 without concomitant alteration of the normal BBB [59]. Peptide bradykinin acting by means of B2 receptors plays an important role in the permeabilization of BBB. Bradykinin, expressed in the luminal membrane of the endothelium, elevates intracellular free calcium levels, which in turn is considered to modulate the tight junctions by activating the actin/myosin system within the cell. An analogue of bradykinin, RMP7 or Cereport, has been developed as an agent for BBB opening [60], and it is mediated specifically through bradykinin B2 receptors. Enhanced tumor drug delivery and survival in rats with metastatic brain tumor have also been seen with RMP7 [61].

In recent times, modulation of BBB function has also been achieved by using an ultrasound and electromagnetic radiation technique [62,63]. This technique may modify the integrity of the tight junctions and increase BBB permeability to several solutes/drugs. This function is attributable either to the changes in membrane fluidity or to alterations in the molecular conformation of transporters, resulting in changes in their activity. The most important feature of this technique is that a selective brain region or a tumor can be focused with some precision, not the whole brain.

2.6 Colloidal nanosystems

Colloidal carriers as nanosystems represent a valuable approach for enhancing transcellular permeability of therapeutic agents across the BBB [64-71] in comparison with microsystems [72] and other delivery approaches discussed

Table 3. Studies on some of the compounds delivered by CPPs.

Compounds	CPP	Animals	Dose(s); route	Significant findings	Action on CNS	Ref.
TAT-β-Gal	TAT	Mice	1.7 nmol; i.p.	Has shown strong activity in some regions of brain after 2 h and almost all regions of brain after 4 h, whereas control β-Gal showed no activity after 8 h	Enhanced BBB permeability	[50]
TAT–Bcl-xL	TAT	Mice	3 mg/kg and 9 mg/kg; i.p.	Resulted in 2- to 7-fold increase in protein transduction in neurons in various brain regions after 4 h in comparison with Bcl-xL alone, and decreased cerebral infarction (up to 40%) as determined at 72 h after 90 min of focal ischemia at 9 mg/kg	Neuronalapoptosis	[51]
TAT–Bcl-xL	TAT	Mice	0.6 nmol; i.v.	Showed increased level of Bcl-xL after 4 h, reduces infarct volume and neurological deficits after long ischemic insults lasting 90 min	Neuronalapoptosis	[52]
Doxorubicin(DOX)-SynB	SynB vectors	Mice	0.5 μM DOX -SynB, 3μ M DOX; *in situ* brain perfusion	An average of 30-fold increase in brain uptake of [^{14}C] doxorubicin was studied with all SynB vectors	Brain tumor	[53]
Doxorubicin(DOX)-SynB1, DOX-D-penetratin	SynB1, D-penetratin	Rats	1.8 nmol/ml DOX-D-penetratin, 1.8 nmol/ml DOX-SynB1, 5.4 nmol/ml DOX; *in situ* brain perfusion	An average sixfold increase in brain uptake was obtained for both DOX-D-penetratin and DOX-SynB1 than for free DOX	Brain tumor	[54]

i.p.: Intraperitoneal; i.v.: Intravenous.

in this review. Nanosystems are in the 1 – 1000 nm size range, and used as carrier systems in which the drug is dissolved, entrapped, encapsulated, adsorbed or chemically attached to the surface [64-71]. The nanosystems group chiefly includes nanoliposomes, nanoparticles, micelles and dendrimers. These nanosystems characteristically differ in structural make up, drug-loading capacity, ability to encapsulate hydrophobic or hydrophilic molecules, target specificity and stability of the system as a whole.

Nanoliposomes (NLPs) are vesicles composed of lipid bilayers surrounding internal aqueous compartments, and relatively large amounts of drug molecules can be incorporated into the aqueous compartment (water-soluble compounds) or lipid bilayers (lipophilic compounds). Nanoparticles (NPs) are solid colloidal particles made of polymeric materials, which include both nanocapsules (with a core-shell structure – a reservoir system) and nanospheres (a matrix system) [71], in which the drug or biologically active entity is dissolved, entrapped, encapsulated, adsorbed or attached [73]. Polymeric micelles are generated spontaneously in aqueous solutions of amphiphilic block copolymers having a core-shell architecture, which maintains physical properties distinctive of conventional micelles, but with improved thermodynamic stability. The core is composed of hydrophobic polymer blocks and the shell of hydrophilic polymer blocks. Dendrimers are highly branched polymer molecules in which the surface of the central core is surrounded by the shell of the branches. Dendrimer interior cavitie, known as 'dendritic boxes', can entrap various drugs, which can be released under physiological stimulus followed by shell degradation.

In drug delivery, physicochemical properties of the carrier system with encapsulated drug as a whole matter, but not the pharmacokinetic properties of the active molecule of the drug. Nanoencapsulation offers many advantages, such as the protection of sensitive active molecules against *in vivo* degradation, the reduction of toxic side effects, better drug pharmacokinetic behavior, and an increase in therapeutic efficacy. These nanosystems can be taken up actively by CMT, RMT and AMT to reach the cerebral parenchyma, or are degraded within lysosomes to discharge the drug into the brain. These carriers may signify a valuable approach for non-invasive drug delivery to the CNS exploiting active targeting [71].

Nanosystems are cleared rapidly from the circulation by macrophages of the RES following i.v. (intravenous) administration and are not equipped with targeting and imaging moieties for site-specific delivery and localization in brain and may produce toxic effects; these shortcomings limit their usefulness in delivering drug/agents to the brain. Therefore, it is urgent to design nanosystems containing multiple properties to counter these problems. Such types of nanosystem are termed multifunctional nanosystems (**Figure 3**) [74], which should be stable, biocompatible, biodegradable, with optimum surface charge, long-circulating, less immunogenic, coupled with targeting biomolecules/ligands along with the combination of imaging modalities to image the receptors/target tissue and

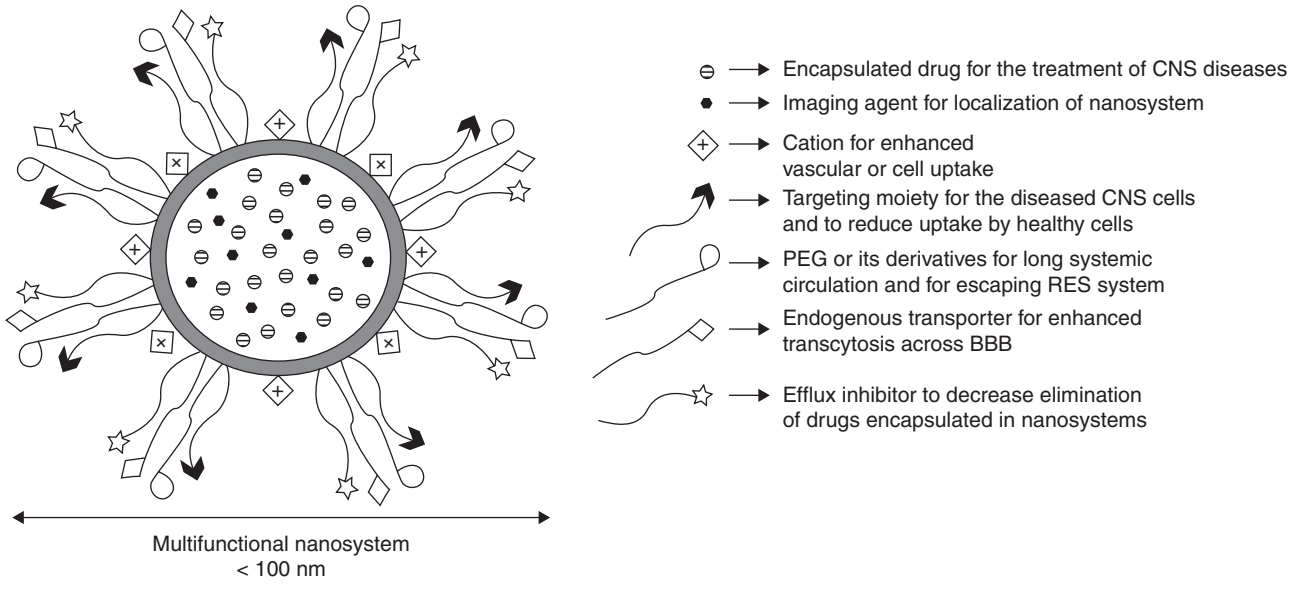

Figure 3. Structure of a multifunctional nanosystem to deliver drugs/agents for the diagnosis and treatment of CNS diseases.

cellular events, incorporation of endogenous transporters for BBB and inhibitors of efflux transporters, can encapsulate high drug pay load, bio or externally stimulated and spatiotemporally release the biomolecules/drugs/imaging agents in a controlled manner at the target site for the diagnosis and treatment of neural disorders.

To a certain extent, researchers have achieved not all but some of the above-mentioned features in one nanosystem or some in other nanosystem by making biologically favorable charged surface [75], selecting biodegradable and biocompatible lipids (soybean lecithin) and polymers (PLA-PLGA poly (DL-lactide-*co*-glycolide), poly(ε-caprolactone)), extended circulation time by decreasing the particle size (< 100 nm) and by surface modification of nanosystems with polyethylene glycol (PEG) or polyethylene oxide chains [76], functionalization with targeting biomolecules/aptamers/ligands [77,78] and imaging agents [79], incorporating influx transporters for BBB permeability [80] and inhibitors of efflux transporters [81,82], can incorporate high drug concentration, bio or externally stimulated and selective spatiotemporal release of drugs/agents [78,83,84]. Most of these nanosystems were administered by non-invasive methods. These administrative techniques are also not fool-proof as they encounter the problem of binding with the blood proteins, degrading enzymes in blood, gastrointestinal track and first-pass metabolism by liver, and barriers imposed by the nasal route. These cause a lack of achieving homogeneous and optimal volume of distribution and concentration of drug in the diseased brain regions. To circumvent these problems, recently a technique called 'convection-enhanced delivery' has been adopted, which is discussed in a later section. However, instability of the nanosystem resulting from batch-to-batch variation, unwanted body reactions such as immunogenicity and other toxicity concerns, and reproducibility of the data in higher animals still persist as roadblocks to achieving biologically favorable nanosystems. This is why the nanosystems are from being approved by clinical trials and received on the market. Below, some of the examples of nanosystems carrying not all but some of the above-described characteristics are discussed.

2.6.1 *Nanoliposomes*

Liposomal nanosystems that have shown excellent promise in delivering drugs to the brain are discussed here. A recent publication demonstrated the improved delivery of anticancer drug 5-fluorouracil (5-FU) with transferrin surface-conjugated liposomes to brain after i.v. administration in animals. (99m)Tc-DTPA-labeled transferrin-coupled liposomes caused a 17-fold increase in the brain uptake of 5-FU, whereas an average 10-fold increase in the brain uptake of the drug was observed with liposomal delivery of 5-FU [80]. It was also proposed that transferrin-conjugated pegylated liposomes achieve tumor-specific delivery of sodium borocaptate (Na210B12H11SH, BSH) to malignant glioma, as an application of boron neutron capture therapy (BNCT) to brain cancer. The transferrin-conjugated pegylated delivery system showed increased tumor–normal brain ratio, that is, 17.85 μg/g and survival time 21.8 days in mice after 72 h in comparison with bare BSH and pegylated liposome-BSH [84].

PEG-based poly(ethyleneglycol)-poly(ε-caprolactone) (PEG-PCL) polymersomes conjugated with mouse-anti-rat monoclonal antibody OX26 (OX26-PO) acquire the greatest BBB permeability surface area product and percentage of injected dose per gram brain (%ID/(g brain)). Moreover, encapsulation of NC-1900 peptide improved the scopolamine-induced learning and memory impairments by

means of i.v. administration even at a very low dose of 1 ng/kg in rats, and thus proved to be a promising carrier for peptide brain delivery [85]. Polyethylenimine/oligodeoxynucleotides polyplexes encapsulated in pegylated liposomes and further modification with monoclonal antibody 8D3 specific to transferrin receptor showed increased circulation time, decreased clearance and 10-fold brain uptake following i.v. bolus injection in mice at a final concentration of 3.0 μM oligodeoxynucleotides [86].

Topotecan (TPT) liposomes modified with tamoxifen (TA) and wheat germ agglutinin (WGA) showed dual targeting properties that not only improve significantly drug transport across the BBB but also target brain tumor to reduce spheroid volume change ratio in murine C6 glioma spheroids from day 6 to 10 in the range 65.2 – 45.9% after applying a dose of 10 μM at day 5 in comparison with free topotecan and topotecan liposomes. Higher survival rate of ~ 26 days in a brain tumor-bearing rat model of murine C6 glioma cells was also observed by i.v.-administered topotecan liposomes modified with TA and WGA at 5 mg TPT/kg or TA 4 mg/kg [87]. Immunoliposomes encapsulating BSH in liposomes composed of nickel lipid, and antiepidermal growth factor receptor (EGFR) antibodies were conjugated to the liposomes using the antibody affinity motif of protein A (ZZ) as an adaptor. In immunoliposome-treated mice, the amount of ^{10}B in the tumor reached 28.36 mg/g 24 h and remained high until the 21.38 mg/g 48 h mark, compared with the ^{10}B content of tumors at 24 and 48 h being 3.45 and 2.97 mg/g, respectively, in liposome-treated animals after i.v. injection at a dose of 35 mg ^{10}B/kg in a brain tumor model of U87 EGFR cells in mice [88].

Gupta *et al.* investigated the promise of cell penetrating peptide (TAT)-modified liposomes to enhance the delivery of a model gene (i.e., the plasmid encodes the green fluorescent protein (pEGFP-N1)) to human brain tumor U87MG cells in an intracranial model in nude mice. Moreover, no transfection was prominent in the normal brain area adjacent to tumor [89].

In many neurological diseases, leukocytes can cross an intact BBB. It is possible to exploit these inflammatory cells as targeted delivery systems. RGD peptide (i.e., Arg-Gly-Asp) can combine with integrin receptors that are expressed on the surface of leukocytes (neutrophils and monocytes). RGD peptide-conjugated liposomes loaded with ferulic acid (4-hydroxy-3-methoxycinnamic-FA) showed 6.1-fold maximum brain concentration of FA (7.3 mg/g) as compared with that in FA solution (1.2 mg/g) and 3-fold in comparison with that in uncoated liposome (2.1 mg/g) after caudal vein injection in rats. This nanocarrier can be used as a potential protective agent for the treatment of neurodegenerative disorders such as Parkinson's, Alzheimer's and stroke [90].

2.6.2 Nanoparticles

A variety of NPs formulations have been used for delivery of drugs to the CNS. Kreuter and co-workers [69,79] have demonstrated successful targeting of a Leu-enkephalin analogue dalargin to brain with enhanced analgesic activity of 36.8% MPE (maximum possible effect) at 5 mg/kg and 51.8% MPE at 7.5 mg/kg by i.v. administration of poly(*n*-butylcyanoacrylate) nanoparticles (PBCA NPs) coated with hydrophilic surfactant polysorbate 80. This over-coating leads to a distinguished alteration of the NP surface properties and hence preferential absorption of plasma proteins, especially apolipoprotein E (apo-E), which is mistaken by the cerebral endothelium as low-density lipoprotein (LDL) particles and is internalized by the LDL uptake system [81,82] to felicitate CNS effects. Recent investigation has also shown the potential of PBCA NPs coated with polysorbate 80 in the targeted delivery of rivastigmine into the brain to treat Alzheimer's disease. A 3.82-fold enhanced concentration of rivastigmine in comparison with free drug was observed in i.v.-injected animals [91]. A study from the authors' group also revealed the importance of nanoparticles of sodium salt of polyacrylic acid (PAA) and 10 mg/kg YFa, which showed an increment in analgesic activity of intraperitoneal (i.p.)-administered YFa ~ 36% MPE after 30 min in comparison with the 2.89% MPE analgesia of i.p.-administered 10 mg/kg YFa alone in mice [92].

Chitosan and poly(DL-lactide-*co*-glycolide-PLGA)-based systems have also shown great potential in targeting the brain. Chitosan nanoparticles prepared through ionic gelation with tripolyphosphate and coated with an anti-amyloid antibody polyamine-modified F(ab′) portion of IgG 4.1 capable of permeating the BBB to target cerebrovascular amyloid formed in both Alzheimer's and cerebrovascular amyloid angiopathy. The brain uptake of modified nanoparticles was ~ 8- to 11-fold greater than the control nanoparticles on i.v. bolus injection in mice [93]. Intranasal (i.n.) administration of nanoparticles has also shown a great deal of promise in CNS delivery. Following the i.n. approach, the estradiol (E (2))-loaded chitosan nanoparticles (CS-NPs) displayed significantly higher CSF concentration (76.4 ng/ml; t_{max} 28 ± 17.9 min) than those after i.v. administration (29.5 ng/ml; t_{max} 60 min) at 0.48 mg/kg in rats [94].

Tosi *et al.* [78] demonstrated the capability of opioid similar peptide-derivatized poly(DL-lactide-*co*-glycolide) (PLGA) nanoparticles to cross the BBB by a brain perfusion technique in rats. In a subsequent study the same authors showed enhanced analgesia of ~ 60% MPE at a dose of 2.7 mg/kg and of 46% MPE at 1.8 mg/kg of loperamide by PLGA nanoparticles derivatized with glycosylated peptide-H_2N-Gly-L-Phe-D-Thr-Gly-L-Phe-L-Leu-L-Ser(O-β-D-Glucose)-$CONH_2$ in rats following i.v. administration.

Lipid-based nanocapsules (LNC) prepared by using the non-ionic hydrophilic surfactant Solutol® (BASF, Ludwigshafen, DE) HS15 hydroxystearate of poly(ethylene glycol) grant extended circulation times and P-gp efflux pump inhibiting properties. Furthermore, these LNC were conjugated to OX-26 monoclonal antibodies (OX-26 MAb) and Fab′ fragments, giving rise to immunonanocapsules to provide targeted delivery of lipophilic radionuclides and therapeutic molecules to the brain. At 24 h post-i.v. injection in rats, the

brain concentrations of Fab′-immunonanocapsules and OX-26-immunonanocapsules were 1.5- and 2-fold higher, respectively, than non-targeted nanocapsules [95].

Solid lipid nanoparticles (SLNs) and pegylated SLNs have also been used for enhancing the concentration of antitumor drugs, including camptothecin (CA), doxorubicin (DOX) and paclitaxel (PCL) in CNS [96-99]. Oral administration of CA-SLN at dose of 1.3 CA/kg body wt of mice showed enhanced and sustained release of CA at 38.5 and 70.3 ng/g of brain tissue after 15 min and 4.5 h, respectively, in comparison with the CA concentration of 49.7 ng/g from CA-SOL (CA-Solution) after 15 min but nil after 4.5 h. These results revealed that the CA was protected in CA-SLN nanoparticle formulation which was not favored in CA-SOL [97]. The intracerebral bioavailability of DOX and PCL carried by SLN was evaluated compared with the free solutions after systemic treatment at equivalent therapeutic dosage of 5 and 1.5 mg/kg, respectively. DOX vehiculated by SLN achieved intratumoral concentrations ranging from 12- to 50-fold higher compared with free solutions in a rat brain glioma model after 30 min and 24 h, respectively. PCL incorporated in SLN produced 10-fold higher drug concentrations in brain tissue than PCL free solutions when administered i.v. to normal rabbits [98].

SLNs present some rewarding properties, such as low intrinsic cytotoxicity, physical stability, protection of labile drugs from degradation, controlled release, easy preparation and biodegradability of lipids used in their preparation, which makes them very useful candidates for brain delivery and particularly for the treatment of brain tumors.

Delivery systems containing magnetic properties such as iron oxide nanoparticles also showed a great deal of promise in concentrating the particles at a desired magnetic field and delivering encapsulated drugs into the CNS. Hassan and Gallo [100] proposed that magnetic chitosan microspheres containing OX (MCM-OX) administered intra-arterially to the rats at OX doses of 0.1 and 0.5 mg/kg with a magnetic field of 6000 G applied to the brain for 30 min significantly increased OX brain concentrations to a minimum of 100-fold greater compared with those achieved with OX in solution treatments.

2.6.3 Polymeric micelles

Polymeric micelles composed of pluronic block copolymers containing a three-block ABA structure, where the A block is hydrophilic poly(ethylene oxide) (PEO) and the B block is a lipophilic poly(propylene oxide) (PPO) chain have shown an increased rate of transport of a model polypeptide horseradish peroxidase (HRP) across the BBB and enhanced accumulation of HRP within the brain when injected via jugular vein in mice at a concentration of 1×10^5 c.p.m. Conjugates of HRP with Pluronic P85 appeared to be more promising for the delivery of HRP to brain. The unidirectional influx rate (*K*i) for HRP-P85 was 0.0800 μl/(g.min) and the *K*i for HRP was 0.0206 μl/(g.min), almost a fourfold increase in BBB penetration, and the injection per gram (%inj/g) was almost twice as great for HRP-P85 in comparison with HRP [101]. Polymeric micellar systems modified with TAT may also serve as a promising drug delivery vehicle across BBB and polymeric micelles self-assembled from the TAT poly(ethylene glycol) (PEG)-block-cholesterol (TAT-PEG-*b*-Chol) comes under this category [102,103]. The distribution of FITC in hippocampus brain sections of rats was observed at 2 h after i.v. injection of FITC and FITC-loaded micelles (1 ml, 0.0512 mg/ml of FITC). FITC molecules were unable to cross the BBB; by contrast, FITC-loaded TAT-PEG-*b*-Chol micelles crossed the BBB. The micelles with TAT may provide a potential vehicle for delivering ciprofloxacin or other antibiotics across the BBB to treat brain infections.

It is not necessary that all polymeric micelle formulations show effective delivery of antibiotics to the CNS. A study by Yáñez *et al.* [104] demonstrated that the macrolide antibiotic rapamycin incorporated in PEG block-poly(α-caprolactone) (PEG-*b*-PCL) micelles formulated with and without the addition of α-tocopherol observed reduced brain uptake compared with control rapamycin dissolved in Tween 80/PEG 400/*N*,*N* dimethyl acetamide. Rapamycin in PEG-*b*-PCL revealed a significant decrease in concentration in brain by 75%. Rapamycin in PEG-*b*-PCL + α-tocopherol resulted in a significant decrement in concentration in brain by 75%, whereas the concentration increased significantly by 69% in urinary bladder. In conclusion, polymeric micelles with Pluronic and those with TAT-PEG*b*-Chol provide excellent opportunities for the treatment of various brain pathologies.

2.6.4 Dendrimers

Dendrimers have also been studied for the treatment of CNS diseases. Polyamidoamine (PAMAM) dendrimer modified with a transferrin (Tf)-conjugated PEG has been also shown to deliver gene/DNA into the brain. PAMAM/DNA, PAMAM–PEG/DNA and PAMAM–PEG–Tf/DNA complexes were injected through the tail veins of mice, and measured for gene expression. The brain gene expression of the PAMAM–PEG–Tf/DNA complex was approximately twofold higher than that of the PAMAM/DNA and PAMAM–PEG/DNA complexes. Moreover, body distribution analysis also showed 2.25-fold increased uptake of PAMAM–PEG–Tf/DNA complex in comparison with PAMAM complex and PAMAM–PEG complex [105]. Hildgen and co-workers [106] demonstrated an excellent study with D-glucosamine-conjugated polyether-copolyester (PEPE) dendrimers loaded with methotrexate (MTX), which showed not only increased delivery across the BBB but also tumor specificity through a facilitative glucose metabolism by the glucose transporters (GLUT) in the tumors. The extent of internalization of dendrimers was three to five times greater in U343MGa cells than in U87MG cells. Conjugation of glucosamine significantly increased the endocytosis of the dendrimer eightfold in U87MG and approximately twofold in U343MGa cells. In

U87MG and U343MGa cells, MTX loaded in dendrimers had 1.5 – 5 times lower IC_{50} value than the free MTX. Against MTX-resistant U87MG cells, MTX encapsulated in the dendrimers had IC_{50} values 9 – 15 times lower than that of the free MTX. The conjugation of glucosamine to dendrimers reduced further the IC_{50} of MTX, but the extent of reduction was dependent on the cell line. MTX-loaded dendrimer–glucosamine conjugates were 3.5 – 4.5 times more potent than dendrimers alone towards U87MG cells and 2 times more toxic towards U343MGa cells. Even in the MTX-resistant cell lines, glucosylated dendrimers were two to five times more effective in inhibiting the cell growth. The influence of various treatments on the growth of tumor spheroids was also studied. Free MTX was able to inhibit the growth of tumor spheroids for a few days but produced only marginal reduction in their volume. On the other hand, MTX-loaded dendrimers produced considerable reduction in the size and volume. In the tumor spheroids treated with glucosylated dendrimer loaded with MTX, this effect was enhanced further.

2.7 Bypassing the BBB

2.7.1 *Direct targeting*

BBB can be circumvented by directly injecting a drug either into the brain parenchyma or intraventricularly or intrathecally into CSF or with an implant into the brain. Directly targeting the BBB theoretically has several advantages in comparison with vascular and other delivery routes. This route bypasses the BBB, drugs encounter minimal protein binding in the CSF, and decreased enzymatic activity in plasma leading to longer drug half-life in the CSF, thereby resulting in immediate high drug concentration in CSF to provide better CNS effects and effectively minimizing systemic toxicity. Nerve growth factor (NGF) was transported in the brain interstitium with the help of implanted controlled release polymers in rats. Levels of radioactivity > 50 pg NGF per section were detected within thick (1 mm) coronal slices of the hemisphere ipsilateral to the site of implantation up to 3 mm rostral and caudal to the edge of the polymer, whereas lower levels of radioactivity (> 5 pg but < 50 pg NGF per section) were detected throughout the rest of the brain [107].

Direct targeting also encounters disadvantages. Implantation into the brain can damage brain tissue around the implant as well as along the track of the catheter introduced. A direct rapid volume injection into the brain parenchyma may definitely damage a volume of brain equal to or more than the volume introduced. Slow infusion of drugs for a long period might be beneficial with this technique, but probable infection due to the introduced cannula is an impediment. Direct injection into the brain is also not useful as the drug may not reach in proper concentrations to the desired site in the CNS as it may be subjected to dilution and flushed out of the brain owing to the continuous production of new CSF by the brain [40,41,108]. A recent technique, convection-enhanced delivery, which uses positive hydrostatic pressure to deliver a fluid containing a therapeutic substance, has been utilized to overcome the disadvantages of direct targeting and is discussed later on.

2.7.2 *Olfactory route*

The olfactory pathway is another route for bypassing the BBB and to introduce drugs into the brain. The CSF fluid, which appears to move out of the arachnoid space, recirculates back into the subarachnoid CSF and may carry drug applied to the olfactory mucosa back into the subarachnoid space of the CNS [1,2,7]. A route into the CNS through the olfactory epithelium and nerves is a viable and interesting possibility for the delivery of drugs to the brain [109].

Lipophilic molecules are easily transported by means of a transcellular mechanism, but molecules with molecular mass between 20 and 40 kDa cannot [110,111]; whereas polar or hydrophilic molecules pass by means of a paracellular mechanism by this route. To obtain therapeutic concentrations of drugs in the CNS, the drugs should be designed to overcome the barriers presented by the mucus epithelium owing to its low pH, inadequate aqueous solubility of drugs, mucociliary clearance, enzymatic barrier and possibility of large variations caused by the nasal pathology, such as the common cold.

Modification of drugs to esters is a good approach for nasal delivery as it permits easy introduction of different functionalities at hydroxyl or carboxyl groups of drugs, altering their octanol/water partition coefficient and their stability in physiological fluids, and, furthermore, their chemical or enzymatic bioconversions can be controlled by suitable substitutions [2,112,113].

Therapeutic agents do not necessitate any modification for CNS delivery by the intranasal route. This technique is advantageous as it is non-invasive relative to other approaches but the possibility of barriers produced by nasal mucosa, nasal pathologies and non-homogenous distribution of drugs in brain may limit the optimum therapeutic concentration of drugs in the brain [114]. Several drugs have been delivered successfully to the brain after introduction into the nasal cavity and their studies are shown in **Table 4** [115-124].

2.7.3 *Convection-enhanced delivery*

Diffusion-based delivery approaches, oral delivery and even systemic delivery of drugs is limited by a lack of achieving high drug concentration and optimal and homogenous distribution of drug/molecules/drug delivery vehicles/nanosystems in brain tissues. A new delivery approach, 'convection-enhanced delivery', has been adopted, which uses positive hydrostatic pressure to deliver a fluid containing a therapeutic substance (small or large molecules, independent of molecular mass) by bulk flow directly into the interstitial space within a localized region of the brain parenchyma. This recent technique using convection or 'bulk flow' was proposed to supplement simple diffusion, which characterizes local intracerebral delivery by stereotactic injections. CED circumvents the BBB and provides a wider, more optimal and homogenous distribution, high drug concentration within brain tissue and diseased brain

Table 4. Several drugs have been successfully delivered to brain after introduction into the nasal cavity.

Compounds	Animals	Dose(s); route	Significant findings	Action on CNS	Ref.
Ethoxyzolamide and acetazolamide	Rats	50 μM; superfusion	Blocked the evoked depolarizing response after the 10 Hz train	Epilepsy	[115]
Progesterone	Mice	8 mg/kg: i.n.	Neuroprotective in both transient and permanent ischemia. Decreases the amount of edematous tissue and the lesion volume	Cerebral injury	[116]
Butyl ester of L-DOPA	Rats	20 mg/kg; i.n., i.v.	9 μg/ml and negligible amount in CSF and 100 μg/ml and ~ 1 μg/ml in the olfactory bulb after 120 min by i.n. and i.v., respectively	Parkinson's disease	[117]
17β-estradiol	Rats	0.48 mg/kg; i.n., i.v.	C_{max} value in CSF (54.76ng/ml) by i.n. after 20 min and C_{max} value in CSF (26.48ng/ml) at 60 min by i.v.	Neuroprotective action	[118]
Neurotrophin (NGF)	Mice	10 μM; i.n.	Mice spent more time with the new object after the 1-h and 24-h delay and greater exploration time dedicated to the new object compared with the familiar object at 10 min time interval as compared with the control group	Alzheimer's disease	[119]
Insulin-like growth factor-I (IGF-I)	Rats	5 nmol i.n.; 0.011 nmol i.v.	IGF-I has >100-fold higher concentration in CNS areas by i.n. than by i.v.	Neurodegenerative diseases and acute CNS injury	[120]
Deferoxamine (DFO)	Rats	6 mg; i.n., i.v.	DFO showed higher brain concentrations -0.9 to 18.5 μM by i.n. and -0.1 to 0.5 μM by i.v. at 30 min and decreased infarct volume by 55% versus control	Ischemic stroke, Intracerebral hemorrhage	[121]
Recombinant human erythropoietin (rhEPO)	Rats	4.8, 12 and 24 U; i.n.	Reduced infarct volume, brain swelling and cell damage in the ischemic hemispheres, and improved behavioral dysfunction 24 h after cerebral ischemia	Brain injury, cerebral ischemia	[122]
Neuroprotective peptide (NAP)	Mice	0.5 μg NAP; i.n.	Reduced both Aβ 1 – 40 and 1 – 42 to 20% and phosphorylated tau at Ser^{202}/Thr^{205} to 31% and Thr^{231} to 39%	Alzheimer's disease	[123]
Insulin	Human	10, 20, 40,or 60 IU; i.n.	Recall on two measures of verbal memory in memory-impaired ε4- adults, with performance generally peaking at 20 IU	Alzheimer's disease	[124]

i.n.: Intranasal; i.v.: Intravenous.

regions, and encounters minimal protein binding in brain in comparison with systemic circulation. In practice, drugs are delivered continuously through a catheter connected to a syringe pump, thus enabling the distribution of large volumes of high drug concentrations with minimum systemic toxicity [125].

Delivery by CED depends on many key factors that should be taken into account to get enhanced therapeutic efficacy, such as: catheter placement: should be short to target site for better volume of distribution (V_d); catheter design: reflux-free glued infused silica tubing, single end port for spherical distribution, bone wax fixed for increased pressure of injected fluid and avoiding back flow, primed cannula to prevent air bubbles; and catheter size: ≥ 32 gauge for preventing the occurrence of backflow or leakage and ≤ 0.5µl/min rate of infusion to ensure optimal V_d in rodents, infusion in brain regions such as gray and white matter as these regions are different in the molecular transport mechanism because of internal structure. White matter displays a better ability to lodge infusate as it is more densely packed and has less extracellular space [126], osmotic and hydraulic dilation of brain interstitial spaces, enzymatic degradation of the brain extracellular matrix by hyaluronic acid by co-administration of hyaluronidase, and an increase in heart rate enhancement, thereby causing an increase in brain fluid circulation and thus an increase in V_d; to achieve optimum drug concentration at the desired brain tissue, co-infusates such as mannitol, heparin, and so on are used to reduce the affinity of infusates to the brain environment [125]. Above all, the elimination route has to be controlled to prevent a rapid elimination by blood capillaries in brain extracellular matrix. This technique allows the local delivery of a wide range of substances, such as conventional chemotherapeutic agents [127], monoclonal antibodies [128], targeted toxins [129], other proteins [130], viruses [131] and nanocarriers [125,132-134].

A prospective use of CED is for epilepsy treatment in which the antiseizure agents are administered locally at the diseased area, avoiding the systemic toxicities of orally administered antiepileptic drugs and undue effects on non-epileptic brain regions. Recent findings showed that brief CED infusions of non-diffusible peptides that inhibit the release of excitatory neurotransmitters, including omega-conotoxins and botulinum neurotoxins, can produce long-lasting (weeks to months) seizure protection in the rat amygdala-kindling model [135]. For example: CED infusion of the N-type calcium channel antagonists omega-conotoxin GVIA (omega-CTX-G) and omega-conotoxin MVIIA (omega-CTX-M) at 0.005, 0.05 and 0.5 nmol over a 20-min period resulted in a dose-dependent seizure protection in the rat amygdala-kindling model for 1 week [136].

For first time direct CNS delivery of siRNA at 0.56 nM (equivalent to a dose of ~ 1.2 mg/(kg day)) to a different cell type – oligodendrocytes – using CED demonstrated robust silencing of an endogenous oligodendrocyte-specific gene, 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNPase) in non-human primates. Moreover, it was found that CNPase mRNA reduction is dose-dependent and mediated by an RNAi mechanism. Increasing the flow rate of siRNA infusion increased the distribution of mRNA suppression to encompass white matter regions distant from the infusion site. These results revealed the significant potential of siRNAs in the treatment of CNS disorders involving oligodendrocyte pathology [137].

Seunguk Oh *et al.* [138] reported that brain tumor established intracranially with U87 glioma in nude rats resulted in tumor eradication in 50% rats after the CED delivery of a bispecific ligand-directed toxin DTEGF13 that consists of human interleukin-13, epithelial growth factor, and the first 389 amino acids of diphtheria toxin at 1 µg. CED efficiency was also assessed to treat brain tumors using salirasib (farsnesyl thiosalicylic acid), a highly specific Ras inhibitor shown to exert its suppressive effects on growth and migration of proliferating tumor cells. In this study, CED of salirasib is efficient and non-toxic for the treatment of glioblastoma in a rat model [139].

Not only the drug molecules but also nanosystems loaded with drugs or biologically active substances can be infused by CED at the diseased site in brain to achieve greater therapeutic efficacy. Nanosystems such as liposomes [140-143], nanoparticles [132,144], dendrimers [133] and polymeric micelles [134] have already been injected by CED. Optimal convection delivery of nanosystems in brain parenchyma needs understanding of their characterstics, such as their size, charge, composition, surface properties and physicochemical characteristics. Size of the nanosystems should be < 100 nm, surface coating by PEG and dextran significantly reduce interactions with proteins, they should have neutral or negative surface charge, increase in osmolarity of nanosystems by co-infusion with mannitol and, to avoid binding with brain matrix, increase in viscosity of the nanoparticle suspension by incorporating PEG (3 – 6%) or sucrose is required for improved distribution [125].

Allard *et al.* [144] demonstrated the efficacy of lipid nanocapsules (LNC) entrapping lipophilic complexes of ^{188}Re ($^{188}Re(S_3CPh)_2(S_2CPh)$ [^{188}Re-SSS]) by CED on a 9L rat brain tumour model at doses 12, 10, 8 and 3 Gy in comparison with blank LNC, perrhenate solution (4Gy) and untreated rats. LNC have displayed prolonged retention of ^{188}Re with only 10% of the injected dose being eliminated at 72 h, whereas there was rapid and almost complete elimination of ^{188}Re after CED of ^{188}Re perrhenate solution. Prolonged survival time of rats of ~ 45 days was found with an effective dose of 8 Gy LNC in comparison with control and other treatment groups.

CED of nanoliposomal topotecan (nLs-TPT) at 0.5 mg/ml ~ 10 µg in rats showed retention in brain tissues and subsequent disruption of brain tumor vessels. The CED of nLs-TPT inhibited growth or completely eradicated orthotopic U87MG or U251MG xenografts and led to significant survival benefits, whereas free drugs exerted almost no effect and thereby no survival benefits at an equivalent dose of 10 µg [142]. Two

liposomal formulations can also be mixed together for enhanced antitumor effect. The combination of nanoliposomal topotecan (nLs-TPT) at 0.25 mg/ml and pegylated (polyethylene glycol-coated) liposomal doxorubicin (PLD) at 0.1 mg/ml in conjunction with CED in U87MG intracranial xenograft rat model demonstrated a significant improvement in survival that was significantly longer, that is, up to 90 days, than the rats in the single-agent therapy groups [143]. Similarly, combination therapy of nanoliposomal CPT-11 (nLs-CPT-11) (0.8 mg, 40 mg/ml) and pegylated liposomal doxorubicin (Doxil) (2 mg, 0.1 mg/ml) in conjunction with CED in U251MG and U87MG intracranial xenograft rat models displayed survival times of 100 and 70 days, respectively, in comparison with single-agent counterparts [140].

Non-pegylated liposomes that can encapsulate TPT and gadodiamide (gado) on CED injection not only revealed better survival rates at high dose of TPT 1.0 mg/ml + gado 1.15 mg/ml but also at low dose of TPT 0.5 mg/ml + gado 1.15 mg/ml in U87MG brain tumor xenografts [141].

On combination with CED, maghemite nanoparticles (Fe_2O_3-MNPs) coated with dextran have led to the increment in infusate viscosity and thus provide increased distribution volume and better prospects in the treatment and monitoring of brain tumors. To study the clearance rate, dextran-MNPs (0.2 mg/ml) was infused into the striatum of rats. The clearance rate was repeatedly monitored by MRI which displayed a close to linear rate down to ~ 80 – 90% clearance after 40 days and the rest stayed in the brain for more than 4 months [132].

Doxorubicin in the polymeric micellar constructs infused by CED displayed high therapeutic efficacy against intracranial 9L brain tumor models. Seven days after tumor cell implantation, a single CED infusion was performed for each group of rats with free DOX, liposomal DOX and micellar DOX (each containing 0.2 mg/ml). The study was concluded 90 days after tumor implantation. Rats were monitored daily for survival rates. Nine of the rats that received micellar DOX were euthanized at 15 – 43 days after tumor cell implantation because of neurological symptoms, but the other two rats survived until termination of the study after 90 days. Survival time for this group was 36 days, which is significantly higher than the survival time of 19.6 and 16.6 days for rats receiving free DOX and liposomal DOX [134].

Boronated polyamidoamine dendrimer (BD) chemically linked to epidermal growth factor (EGF), designated as BD-EGF, was assessed as a molecular targeting agent for BNCT of the human *EGFR* gene transfected F98 rat glioma model. At 24 h, 43% more radioactivity was observed in EGFR(+) tumors following intracerebral (i.c.) CED compared with intratumoral injection with ^{125}I-labeled or unlabeled BD-EGF (40 µg 10B/10 µg EGF) to F98 glioma bearing rats and a doubling of the tumor boron concentration (22.3 µg/g versus 11.7 µg/g). On CED, higher survival time was observed with BD-EGF (54.1 days) as compared with its intratumoral injection (43.0 days). This study also showed the significance of i.v. injection of boronophenylalanine (BPA) at 500 mg/kg body weight in conjunction with CED BD-EGF on survival time of rats. Rats that received the combination displayed 86.0 days as compared with 39.8 days for i.v. BPA alone, 30.9 days for irradiated controls, and 25.1 days' survival time for untreated controls [133].

The CED method has some disadvantages too, such as leakage or backflow and improper infusion rate resulting from bad catheter placement and design, absorption by brain extracellular matrix and enzymatic degradation due to the matrix, leakage of drugs in CSF and non-preferred brain regions, causing brain infections or hampering the treatment of brain diseases due to low therapeutic concentration at the preferred location.

3. Conclusion

Diagnosis and treatment of CNS pathologies is quite challenging as the delivery of drug molecules to the CNS is often prohibited by a variety of physiological, metabolic and biochemical obstacles that collectively comprise the BBB, BCSFB, CVOs and other physiological factors. To meet this challenge different strategies have been taken into account and also discussed in this review. Although these strategies form a preliminary basis for CNS drug delivery, some drawbacks are experienced, such as low therapeutic concentration at the target site and toxicity-related concerns due to the pharmacokinetics and pharmacodynamics of drugs or drug delivery systems. Prodrug approaches such as lipidization or chemical modifications of drugs were done with a view to permitting transendothelial passage, but their entry into the CNS is limited owing to low concentration at target site, nonspecific cell uptake, shorter blood circulation time, uptake by the RES system, and enzymatic degradation. Structural modifications in a drug that is essential to act as a substrate for endogenous influx transport systems of the BBB often result in a lower affinity for the transporter than the endogenous ligand. This reveals that transporters are highly selective systems in allowing the passage of substances across the BBB. CPPs lost their cell penetrating ability owing to a lack of one or two Arg residues. Chemical inhibition of efflux systems or non-selective opening of the BBB for a long time allow access to a broad range of potential neurotoxins and other agents that adversely affect CNS homeostasis and generate unwanted side effects. Even the colloidal nanosystems approach encounters problems of instability, nonspecific cell uptake, uptake by the RES system, immunogenicity, shorter blood circulation time, rapid clearance, enzymatic degradation and hence low bioavailability at the target site in CNS on administration by non-invasive procedures. Approaches such as bypassing the BBB involving direct injection or implanting a device into the CNS are too invasive and lead to microbial infections and damage to brain volume. Olfactory administration, although it does not involve any invasiveness, may suffer with the problem of non-homogenous distribution of drugs to the brain and barriers

imposed by nasal mucosa and pathologies. Colloidal nanosystems as multifunctional nanosystems armored with the assets of stability, biodegradability and biocompatibility, longer blood circulation, escape from the RES system, non-immunogenicity, high drug encapsulation, targeting specific ligands, incorporation of influx transporters, inhibitors of efflux transporters, imaging agents for diagnosis, bio or externally stimulated, slow and controlled drug delivery, represent a potential approach for target-specific CNS drug delivery by non-invasive techniques; but they encounter the problem of binding with the blood proteins, degrading enzymes in blood in the case of the systemic, gastrointestinal track and liver metabolism in the case of oral, non-homogenous and low volume of distribution at the target site and barriers imposed by the nasal route. The recent methodology of convection-enhanced delivery enabling convective distribution of high therapeutic concentrations over large volumes of brain provides better prospects in drug delivery to the CNS while avoiding systemic toxicity. Therefore, either delivery of multifunctional nanosystems by non-invasive techniques or delivery of these nanosystems with biologically acceptable and targeting properties in combination with the CED technique may provide one of the most interesting solutions for target-specific delivery to the brain in future, but one has to take into account problems such as leakage or backflow, catheter placement and design, infusion rate, absorption and enzymatic degradation due to the brain extracellular matrix, leakage of drugs in the CSF and non-desired brain sites.

4. Expert opinion

Considering various aspects of drug delivery to the CNS, the prospect of efficacious CNS drug targeting in future lies either with the non-invasively administered multifunctional nanosystems or these nanosystems without some characterstics (described below) administered by convection-enhanced delivery.

Multifunctional nanosystems by the non-invasive technique and the nanosystems by the CED method consider some common aspects in their composition, except properties such as long systemic circulating capability and avoiding the RES scavenging system of the body, endogenous transporters and efflux inhibitors, which are excluded in the case of nanosystems for CED. The common aspects are as follows.

1. Biodegradable and biocompatible lipids and polymers. At present, PLA-PLGA, poly(DL-lactide-*co*-glycolide), is one of the few approved polymers, together with chitosan, poly (ε-caprolactone) or other natural polymers. Lipids such as soybean lecithin are generally biologically favourable, but lipids modified with polymers and other substances should be used cautiously. A good awareness of the polymers and modified lipids, their biodegradation rate, their by-products, toxicity and their capability to cause adverse immunological responses, should be taken into account for successful nanosystems.
2. Coupling of targeting moieties such as antibodies, aptamers, new synthetic peptides derived from opioids or ligands for endogenous receptors to nanosystems, which can recognize the diseased site or the agents expressed by the diseased site to avoid nonspecific delivery to healthy cells.
3. Optimum surface charge on the nanosystem that should avoid binding with blood proteins and brain extracellular matrix, thus avoiding toxicity. The charge on the nanosystem could be due to the polymer itself, derivatization of the polymer, or due to the whole nanosystem with the encapsulated drug/agents.
4. Adopting procedures that should involve less or no utilization of organic solvents, heat or vigorous agitation, sonication for synthesizing new polymers or derivatizing polymers for the preparation of nanosystems, which may possibly be detrimental to sensitive biomolecules/drugs and should allow a high amount of drug loading.
5. Inclusion of imaging agents such as fluorescent dyes, magnetic materials and quantum dots to image the diseased CNS location.

Properties which are included by nanosystems administered by non-invasive procedures but not by nanosystems administered by CED, are as follows:

1. Nanosystems should have long systemic circulating capability and avoid the RES scavenging system of the body. This can be achieved by decreasing the particle size (< 100 nm) and modification of nanosystem surfaces with PEG or its derivatives.
2. Incorporation of endogenous BBB transporter moieties such as glucose (transported by GLUT1) or substrates transported by LAT 1 or apolipoproteins, and so on, to nanosystem surfaces.
3. Inhibitors of efflux transporters such as Pgp and MRP inhibitors should be included in the surface make-up of nanosystems to resist their passage from brain to blood. The efflux inhibitor should be linked by a locally hydrolysable bond. For example, surfactants such as polysorbate 80 and pluronic 85.

Overall, these nanosystems should be biologically favourable, that is, stable in biological conditions, < 100 nm in size, protect the drug from elimination or metabolism, non-toxic, should show the property of bio-stimulated release of drugs/agents due to pH-sensitive nature or by microenvironment of the diseased site or externally stimulated release of drugs/agents by ultrasound, electromagnetic radiation, and so on, from the nanosystems, and should have the ability to release multiple drugs/agents at the target site in a controlled or time-specific manner. Release of the multiple drugs/agents can be achieved by encapsulating drugs/agents in two distinct regions of the nanosystems, that is, the outer envelope and the inner core, with their release influenced by bio or externally stimulated methods.

To engineer a system with the inclusion of these characteristics may cause increased complexity in the preparation and the possibility of competition in between them if not considered in an appropriate amount/ratio, and this may result in unstable and toxic nanosystems. Antibodies have the potential to be selective; however, their size (of the same order as that of nanosystems) and potential immunogenic properties are constraints to their diffusion into tissue and use. Protein ligands, such as transferrin and its receptor, which have been assessed extensively for targeting, experience similar problems. Batch-to-batch variation in synthesized nanosystems causes impact on the size, zeta-potential, shape, chemistry and surface properties. Degradation, amount of encapsulating drugs/agents, nonspecific drug delivery, stability in biological conditions, the potential for delivery of multiple agents at the same site and reproducibility of data in higher animals are the other preventive factors to deal with nanosystems.

Delivery of nanosystems through non-invasive procedures, however, encounters the problem of binding with the blood proteins and degrading enzymes in the blood, gastrointestinal track and first-pass metabolism by liver, barriers imposed by the nasal route and low and non-homogenous distribution of drugs at the target site. To counter these problems nanosystems delivered by recently adopted technology CED may provide better results to target the CNS.

CED-administered nanosystems should possess the following characterstics: size of the nanosystems should be < 100 nm, surface coating by PEG and dextran significantly reduce interactions with proteins, they should have neutral or negative surface charge, increase in osmolarity of nanosystems by co-infusion with mannitol, increase in viscosity of the nanoparticle suspension by incorporating PEG (3 – 6%), or sucrose is required for improved distribution. High viscosity of the infusate may also reduce backflow, thus increasing the possibility of efficient convection. An increase in concentration increases the volume of distribution (V_d). Furthermore, use of co-infusates, for example, heparin, basic fibroblast growth factor or mannitol, reduces the affinity of infusates to the brain environment and thus augments V_d. General CED factors should also be taken into account (discussed in Section 2.7.3 Convection-enhanced delivery) for efficient targeting of nanosystems to CNS, such as catheter placement, design, size, rate of infusion and infusion in brain regions such as gray and white matter, osmotic and hydraulic dilation of brain interstitial spaces, enzymatic degradation of the brain extracellular matrix, and increase in heart rate enhancement.

Formulation of drugs as in the case of nanoencapsulation may increase brain half-life and thereby V_d. Above all, the elimination route has to be controlled in order to prevent a rapid elimination by blood capillaries in brain extracellular matrix. The intention for using CED for drug delivery of nanosystems, first, is to get the diffusion of nanosystems into the brain parenchyma to obtain an optimal V_d due to its specific structural properties, and, second, the nanosystems would be internalized by diseased cells to show their CNS effects.

Moreover, scientists and researchers from all over the world working in the field of nanobiotechnology should create a database on nanosystems and provide information about their chemical nature, and biophysical, biochemical and biological mechanisms (pharmacokinetics, pharmacodynamics and toxicity) associated with the interaction of nanosystems with the diseased and healthy tissues with their administration routes and submit them in a central depositary unit such as NanoBank, as proposed by the FDA. It is also required by the central unit to release guidelines to utilize nanosystems in clinical trials while considering their efficacy and toxicity in alleviation of diseases. The availability of information about nanosystem-based experiments will need to be better understood to allow widespread adoption of this technology as nowadays nanosystems are considered not only as drug delivery systems but also as therapeutics as a whole and are termed nanotherapeutics. Last but not the least, thorough understanding of physicochemical characteristics of the CNS blockades, receptors and sites of action in relation to the structural and formulation approaches of nanotherapeutics along with close collaboration among researchers of several scientific areas including pharmaceutical sciences, biological chemistry, immunology, physiology and pharmacology is of crucial importance to designing potential nanotherapeutics to target CNS pathologies.

Declaration of interest

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