Neurological disorders and therapeutics targeted to surmount the blood–brain barrier

Jagat R Kanwar
Bhasker Sriramoju
Rupinder K Kanwar

Nanomedicine Laboratory of Immunology and Molecular Biomedical Research, Centre for Biotechnology and Interdisciplinary Biosciences, Institute for Frontier Materials (IFM), Deakin University, Waurn Ponds, Victoria, Australia

Correspondence: Jagat R Kanwar
Nanomedicine Laboratory of Immunology and Molecular Biomedical Research, Centre for Biotechnology and Interdisciplinary Biosciences, Institute for Frontier Materials (IFM), Deakin University, Waurn Ponds, Victoria 3216, Australia
Tel +613 5227 1148
Fax +613 5227 3402
Email jagat.kanwar@deakin.edu.au

Abstract: We are now in an aging population, so neurological disorders, particularly the neurodegenerative diseases, are becoming more prevalent in society. As per the epidemiological studies, Europe alone suffers 35% of the burden, indicating an alarming rate of disease progression. Further, treatment for these disorders is a challenging area due to the presence of the tightly regulated blood–brain barrier and its unique ability to protect the brain from xenobiotics. Conventional therapeutics, although effective, remain critically below levels of optimum therapeutic efficacy. Hence, methods to overcome the blood–brain barrier are currently a focus of research. Nanotechnological applications are gaining paramount importance in addressing this question, and yielding some promising results. This review addresses the pathophysiology of the more common neurological disorders and novel drug candidates, along with targeted nanoparticle applications for brain delivery.

Keywords: blood–brain barrier, neurological diseases, brain delivery, targeted nanoparticles

Introduction

Because the population is currently an aging one, neurological disorders, such as neurodegenerative diseases, inflammatory diseases, and brain tumors are becoming more prevalent. This increasing prevalence has triggered an increase in research focused on developing novel techniques for successful delivery of drugs across the blood–brain barrier. In general, molecules that penetrate the blood–brain barrier are lipophilic and less than 500 Da in size. These unique properties limit the number of potential therapeutic tools able to access the brain. Current research in the area of nanobiotechnology has had an impact on diagnostic tools and drug delivery by developing molecules that are smaller than 100 nm in size and endowed with special properties. These nanosized particles have an influential role in therapeutics for brain disorders, especially in overcoming and facilitating enhanced treatment options. Hence, it is necessary to understand the physiology of the blood–brain barrier along with the pathology of neurological disorders in order to develop brain-specific therapeutics.

Blood–brain barrier

The blood–brain barrier is a protective mechanism that controls cerebral homeostasis and provides the central nervous system with unique protection against all foreign matter. The blood–brain barrier prevents 98% of small molecules and 100% of large molecules from reaching the brain. It is located at the level of the capillaries between the blood and cerebral tissue, and is characterized by the presence of tight intracellular junctions and polarized expression of many transport systems. The blood–brain
barrier is located at the choroid plexus epithelium, which controls the exchange of molecules between the blood and cerebrospinal fluid.⁸

The endothelial cells of the brain differ slightly from other tissues in that they lack fenestrations and are also unique in having tight junctions between them.⁷,⁸ They express membrane receptors responsible for active transport of nutrients to the brain and excretion of potentially toxic compounds from the cerebral and vascular compartments. Brain endothelium in mammals has highly controlled permeability towards plasmatic compounds and ions, and has high transendothelial electrical resistance. Dysfunction of the blood–brain barrier is seen in many neurological disorders. In the absence of the blood–brain barrier, the brain microvasculature is an extraordinary way to access the brain, with the possibility of distributing molecules to all areas within the brain. The volume occupied by the capillaries and endothelial cells is around 1% of the total brain volume and, as a result, the brain microvasculature has a total surface area of approximately 20 m². This highly vascularized network means that every brain cell is located approximately 20 nm from a capillary. This could allow for rapid diffusion of small molecules delivered to the brain. However, this possibility is limited by the physiological characteristics of the blood–brain barrier.⁸

Neurological disorders
In Europe alone, 35% of all the burden of disease come from neurological disorders, and over 1.5 billion people suffer from a pathogenic neurological condition.¹⁰ Diseases that affect the brain and central nervous system can be divided into a number of categories, including neurodegenerative, neuroinflammatory, and neoplastic diseases.

Neurodegenerative disease
Causes of neurodegeneration
The precise causes and mechanisms of neurodegeneration are unknown as yet. Individuals with a family history of neurodegeneration are at higher risk of neurodegenerative disease, suggesting a role for genes in its initiation. A significant risk factor for developing neurodegeneration is increasing age,¹¹ and this has gained special attention because the population aged 65 years and above is increasing in the developed countries. Thus, it can be foreseen that the risk of developing a neurodegenerative disease will increase in the next few years. Abnormalities related to memory and the motor system are seen in the aged, similar to those observed in neurodegeneration. This observation highlights advancing age as a significant risk factor for developing neurodegeneration. Aging has also been found to be associated with neuronal death in various regions of the brain, followed by shrinkage of the brain.¹² Further, at autopsy, aged individuals demonstrate the hallmarks of neurodegeneration in the brain, like Lewy bodies in Parkinson’s disease and the neurofibrillary tangles seen in Alzheimer’s disease.¹³,¹⁴ In light of these observations, the question arises as to whether neurodegeneration occurs during the aging process or whether aging itself represents a presymptomatic phase of degeneration? Environmental factors also tend to provoke neurodegeneration, as evident with the observation of the Parkinson’s disease-amyotrophic lateral sclerosis complex in the Chamorros population after ingestion of a toxic compound obtained from Cyclic circinalis.¹⁵ This is also evident with the toxic compound 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) which produces parkinsonian symptoms upon ingestion.¹⁶ The likely mechanisms of neurodegeneration are described as follows, and are summarized in Figure 1.

Excitotoxicity
Excitotoxicity is mediated via glutamate, the major excitatory amino acid in the central nervous system. Hyperactivity of glutamate receptors may be caused by both endogenous and exogenous stimulation, resulting in deleterious effects on neurons. Excitotoxicity is mediated by repeated stimulation of glutamate receptors, leading to neuronal damage, and possible mechanisms accounting for this could include deregulation of intracellular calcium homeostasis and overproduction of free radicals.¹⁷

Apoptosis
Apoptosis, or programmed cell death, has a convincing role in mediating neurodegenerative diseases, particularly Parkinson’s disease. Animal models of Parkinson’s disease have shown that oxidative stress and mitochondrial dysregulation culminate in activation of the p53-mediated apoptotic cascade, and eventually neuronal death. Neurons that fail to complete this apoptotic phase become necrotic and inflamed which, in turn, leads to neurodegeneration.¹⁸ The role of nitric oxide in mediating neuronal death cannot be ignored because nitric oxide has a significant role in stimulating inflammation, release of glutamate, and respiratory inhibition.¹⁹

Neurodegeneration and cell death
In most of the neurodegenerative diseases, neuropathological changes in the form of neuronal loss are seen, while the remaining neurons tend to show a variety of morphological changes, ranging from normal appearance to a combination
of changes in size, shape, nuclear defragmentation, and chromatin condensation. Intracellular accumulation of proteins is also evident and useful for differentiating some of the neurodegenerative diseases. For example, intracellular inclusion of Lewy bodies is specific to Parkinson’s disease. Earlier, apoptosis and necrosis were considered to be the modes of cell death in neurodegeneration, but the present understanding of neuronal death also includes autophagic and cytoplasmic mechanisms. A brief introduction to the neurodegenerative pathology of Alzheimer’s disease and Parkinson’s disease is now described.

Alzheimer’s disease

Alzheimer’s disease is one of the commonest neurodegenerative diseases, for which only symptomatic therapy is available. Dementia is the primary clinical symptom noticed, along with impaired learning and cognition. As the disease progresses, irritability, confusion, and behavioral changes may persist. The brains of patients with Alzheimer’s disease show a characteristic pattern of β-amyloid (Aβ) plaques and neurofibrillary tangles that are considered to be the pathological hallmarks of the disease and progression. Aβ plaques are peptides consisting of 39–42 amino acids and are the result of impaired metabolism of amyloid precursor protein. Mutations in amyloid precursor protein allow enhanced proteolysis by the β-amyloid precursor protein-cleaving enzyme (β-secretase), followed by increased production of insoluble Aβ plaques by γ-secretases. These Aβ plaques aggregate readily, remaining a focus for generation of disease and tend to be the real culprits in mediating a variety of destructive effects on neurons, including free radical generation and neuronal loss. The neurotoxicity is directly associated with toxic Aβ-derived oligomers and diffusible ligands. Interesting facts have been revealed about the involvement of Aβ plaques in the pathogenesis of Alzheimer’s disease, in particular that either an increase in the ratio of Aβ42/Aβ40 (insoluble/soluble) plaques or Aβ42 alone strongly correlates with the predisposition to the disease.

After diagnosis, the amount of Aβ deposited and its neuroanatomical location reflects the extent of neuronal damage, triggering of tau protein, and dementia. Other proteins associated with the pathology are neurofibrillary tangles, ie, paired helical filaments of microtubule-associated tau protein. In general, these tangles support the growth of neurons, but become cytotoxic if hyperphosphorylated and disrupt the cytoskeleton. Evidence of neuronal loss, specifically of the cholinergic type, is observed with disease progression, leading to the conclusion that the acetylcholine neurotransmitter

Figure 1 Various causes of neurodegeneration. Aging and inflammation due to infiltration of T lymphocytes followed by cytokines are the most common risk factors for neurodegeneration. (A) Apoptosis is considered to be programmed cell death that allows clearing out of old cells by inducing their death. However, if the same mechanism becomes dysregulated as a result of mutations, healthy cells also die, leading to neuronal loss followed by symptoms of disease. (B) Excitotoxicity is the other major cause whereby the N-methyl D-aspartate (NMDA) receptor is excessively activated by the endogenous ligand, glutamate. This drives the influx of extracellular calcium intracellularly, activating caspases which in turn destroy the nucleic acids mediating cell death. (C) Mitochondrial dysfunction due to old age or toxins generates free radicals (reactive oxygen species) that defragment DNA. Note: These processes may occur either independently or collectively under the influence of environmental factors, medications, and infections, precipitate symptoms of neurological disorders.
plays a prominent role in sustaining memory and cognition. \textsuperscript{24} Mutated presenilin 1, presenilin 2, and apolipoprotein E genes have been found to be closely associated with respect to inheritance of the disease. \textsuperscript{28}

Current treatments for Alzheimer’s disease are mainly the acetylcholine esterase inhibitors, which prevent breakdown of acetylcholine and enhance its bioavailability. The drugs approved are donepezil, rivastigmine, and galantamine. \textsuperscript{29} Memantine falls into the category of N-methyl D-aspartate receptor antagonism, with a proposed mechanism involving inhibition of excitotoxicity. It exhibits this action by antagonizing binding of glutamate (an endogenous excitatory neurotransmitter) to the N-methyl D-aspartate receptor. \textsuperscript{30} Other strategies attempted in the treatment of Alzheimer’s disease have included antioxidant and anti-inflammatory drugs, ie, α-tocopherol (vitamin E), \textsuperscript{31} ginkgo biloba plant extracts, \textsuperscript{32} and nonsteroidal anti-inflammatory drugs. \textsuperscript{33} The prospects for future therapy are mainly focused on Ab plaques, aiming either to prevent their formation or solubilize them soon after they have accumulated. Likewise, β-secretase and γ-secretase inhibitors would be potentially useful. Another potentially useful strategy has been immunotherapy against β amyloid, that has been identified to solubilize the plaques. \textsuperscript{34} Drugs in trials for Alzheimer’s disease include semagacestat, a γ-secretase inhibitor, which acts by inhibiting Ab plaque formation. \textsuperscript{35}

Bapineuzumab \textsuperscript{36} and solanezumab, both humanized monoclonal antibodies, were also tried against Ab and were effective in solubilizing them. \textsuperscript{37} Intravenous immunoglobulin antibodies against the tau proteins are also effective and undergoing evaluation. \textsuperscript{38,39} Dimebolin hydrochloride, an antihistamine drug with neuroprotective activity, and brain-derived neurotrophic factor, which has a substantial influence on neuronal survival, are being evaluated in preclinical studies. \textsuperscript{40,41} Finally, a recent potential target identified is the retinoid X receptor and its agonist, bexarotene, which has been found to clear Ab plaques, along with reversal of symptoms, in a murine model of Alzheimer’s disease. \textsuperscript{42}

Parkinson’s disease

PD is a neurodegenerative disease mostly associated with loss of striatal and dopaminergic neurons in the substantia nigra that coordinate motor movements, along with intracellular inclusions of abnormal proteins called Lewy bodies. \textsuperscript{43} Hence, symptoms commonly found in PD are rigidity, tremors, hypokinesia, and impaired balance. Lewy bodies in the diseased neurons are identified as eosinophilic inclusions, and are considered to be the hallmark of PD and its progression. Distribution of Lewy bodies is seen in several regions of the brain, accounting for the severe neuronal loss in affected areas. \textsuperscript{44} The etiology of PD is rather unclear, with a pathology of misfolding and aggregation of proteins, oxidative stress, neuroinflammation, and loss of integrity of the blood–brain barrier. \textsuperscript{7} Mutations are also involved in the disease, and four proteins, ie, α-synuclein, \textsuperscript{45} parkin and DJ-1, \textsuperscript{46} and PINK1, \textsuperscript{47} are found to be associated with its inheritance. Upregulation of synuclein mRNA and its protein expression is noticed in lesions of the substantia nigra, that result in induction of neuronal apoptosis.

A possible mechanism correlated with the disease generation is the ability of Lewy bodies to self-aggregate. \textsuperscript{48,49} Mutated versions of parkin and synuclein genes have a major effect on the functioning of the ubiquitin-proteasome system that is responsible for clearing excess, misfolded, or aggregated proteins, leading to accumulation of abnormal proteins and neuronal death. \textsuperscript{50} Finally, free radicals have a significant role in the pathogenesis of neurodegenerative diseases, including PD. \textsuperscript{51} Interesting facts have been revealed about mitochondrial dysfunction and its role in neurodegenerative diseases. The theory is that neurons are always in great demand for mitochondrial energy, and under the influence of aging, environmental factors, or toxins, mitochondrial functioning becomes deregulated and thus the bioenergetic demand for neuronal functioning is unmet. In order to compensate for this, deregulated mitochondria initially stimulate production of Ab in Alzheimer’s disease and synuclein in PD, that ultimately reaches the stage of aggregation, resulting in neuronal death. Localized inflammation is also responsible for deleterious effects in Alzheimer’s disease and PD because of increased mitochondrial reactive oxygen species. \textsuperscript{52} This finding opens up a potential new avenue of therapeutic investigation into neurodegenerative diseases. The drugs currently available for PD treat the disease symptomatically, either by mimicking dopaminergic actions or by enhancing the duration of action of dopamine. Drugs mimicking dopamine include the combination of levodopa and carbidopa, with the former acting as a dopaminergic agonist and the latter preventing its metabolism in the periphery. The Duodopa\textsuperscript{8} pump (a prolonged duodenal infusion) is a modified formulation of levodopa and was tried in a patient population that showed significant symptomatic relief. \textsuperscript{53} Other dopaminergic agonists that can be administered include dihydroergocryptine, ropinirole, pramipexole, and pergolide.

Monoamine oxidases are the enzymes responsible for dopamine metabolism, and agents inhibiting these enzymes are found to delay dopamine degradation and thus increase
its bioavailability. Selegiline is one such drug, and rasagiline is proving to be effective both in vitro and in vivo.54 Other modes of treatment that have been tried include stimulation of deeper brain regions and replacement of dysfunctional neurons, but without success.55 Future therapies against PD are targeted to oxidative stress, excitotoxicity, and neuronal degeneration. Coenzyme Q10 has been found to have neuroprotective and antioxidant properties, but has not been proven to have a significant clinical effect as yet.56 Attempts have been made to evaluate the effects of riluzole, an antiglutamate drug, but further clinical evaluation in patients is needed.57 Administration of glial-derived neurotrophic factor has shown promising improvement in restoring dopaminergic neurons in animal models of PD.58

Stroke

In terms of neurologic disability, stroke ranks next to Alzheimer’s disease and, in general, is the third largest killer next to heart disease and cancer. The statistics for stroke are striking, in that five million deaths are reported each year, and 20% of men and 25% of women aged between 45 and 85 years are likely to experience the disease. The prominent risk factors associated with stroke are hypertension, atherosclerosis, diabetes, and heart disease. The main pathology of the disease is vascular occlusion in the brain (ischemic stroke) or vascular rupture (hemorrhagic stroke).59 Ischemic stroke is further categorized as thrombotic (localized occlusion of blood vessels), embolic (vessel occlusion due to clot formed elsewhere), or reduced systemic hypoperfusion (due to reduced cardiac function).

However, all of the etiological factors have a common factor, ie, compromised blood supply, that eventually leads to a reduced glucose and oxygen supply to the brain. Because the brain tissue relies completely on aerobic metabolism, and due to the lack of sufficient respiratory reserve, the brain parenchyma suffers death immediately, while the surrounding areas sustain partial damage. Depending on the severity of the ischemic attack, affected brain tissue is designated as the core region, with a severely compromised blood supply, showing necrosis of neurons and glial cells. Areas less severely affected are considered to be the penumbra, and comprise cells that are capable of being revived upon timely therapeutic intervention.60 The ischemic cascade sets off a series of events that culminate in neuronal death. In general, neuron survival and transmission of impulses requires a continuous supply of oxygen and glucose, but in the event of ischemic attacks, there is energy depletion that triggers apoptosis, followed by neuronal death. Ischemia also causes intracellular deregulation of the ions needed for energy exchange.61 These destructive events lead to activation of glutamate receptors that cause neuronal damage via abnormal activation of enzymes, generation of free radicals, and excitotoxicity.62 The severity of the damage is related to the focus of the origin, duration, and severity of ischemia. Necrosis is predominant in the ischemic core, whereas apoptosis predominates in the surrounding neurons. Ischemic damage initiated by expression of Bcl-2 and p53 is noticed first, followed by release and activation of proapoptotic precursors.63,64 Thus, an ischemic attack culminates in irreversible neuronal loss.

Hemorrhagic strokes are defined as ischemic regions containing a variable amount of blood cells leaked from damaged vessels due to either increased vascular wall permeability or post ischemic vessel rupture. Affected brain regions show continuous blood leakage, precipitating hypoxia (due to reduced blood supply), increased intracranial pressure (which in turn negatively affects cerebral blood flow), and persistent irritation. These manifestations are considered to be even more serious than ischemic stroke. One of the subtypes of hemorrhagic stroke identified is intracerebral hemorrhage, which is the result of blood oozing directly from the leaky vessel wall into the brain parenchyma. The potential risk factors identified are hypertension, followed by traumatic brain injuries, angiopathy, blood vessel malformations, and illicit use of medications like amphetamine. Subarachnoid hemorrhage is a less common subtype of stroke, arising from rapid rupture of a basal brain aneurysm.65

Therapeutic intervention for stroke is primarily focused on either enhancing the blood supply and/or dissolution of the blood clots formed, by using thrombolytic and neuroprotective strategies. With respect to the thrombolitics available so far, alteplase, or tissue plasminogen activator, is used intravenously in emergencies but has the limitation of severe side effects, including cerebral hemorrhage.66 Aspirin, an antiplatelet drug with a possible mechanism of cyclo-oxygenase inhibition,67 and lipid-lowering drugs like statins, eg, atorvastatin and lovastatin, which act to prevent formation of cholesterol plaques and their deposition in the walls of blood vessels, are two classes of drugs considered to be of therapeutic benefit for enhancing blood flow.68

Monoclonal antibodies have also been tried, and the first one to enter clinical use is abciximab, which acts by inhibiting the glycoprotein IIb/IIIa receptor expressed on the surface of platelets and preventing their aggregation.69 The other possible candidate for thrombolysis is heparin, along with fibrinolytic agents like plasmin and microplasmins.
Although these are beneficial, they are hampered by the same side effects seen with tissue plasminogen activator. Angiotensin-converting enzyme inhibitors, eg, enalapril and ramipril, and angiotensin II receptor blockers, eg, losartan and valsartan, regulate altered blood pressure by acting on the renin-angiotensin-aldosterone system. Angiotensin II receptor blockers also have anti-inflammatory effects that are suitable for use in the management of stroke. A novel drug investigated for stroke is edaravone, a free radical scavenger with potential neuroprotective ability, which is used in combination with tissue plasminogen activator for effectiveness. The following drugs with neuroprotective activity in stroke have been investigated in trials. Citicoline has been identified as preventing generation of free radicals by inhibiting lipid metabolism and has shown promising preclinical results. Metal ions have a significant potential in mediating the activities of enzymes. Hence, the DP-b99 metal chelator (chelates zinc, calcium, iron, and copper ions) was investigated preclinically and clinically in Phase II studies, and reduced areas of ischemic infarct were observed. Finally, valproic acid, sodium butyrate, and trichostatin-A, all histone deacetylase inhibitors with potential anti-inflammatory and antiapoptotic activity, have undergone preclinical investigation. These agents were effective in reducing infarct size and are now heading towards clinical trials. A recent update in stroke research is ReN001, ie, neural stem cell therapy, which is undergoing evaluation in a small patient group.

**Neuroinflammation**

According to the published data, neuroinflammation has a significant role in mediating several neurological disorders, and constant vigilance by immune cells is necessary for neuronal defense, repair, and homeostasis. Pioneering research by Moalem et al showed that self-reactive T cells and activated macrophages promote neuronal survival and growth. However, the limitation of exploiting inflammatory processes in the brain is that, unlike epidermal cells in the periphery, neurons cannot be sacrificed because of their limited potential for regeneration. Neuroinflammatory processes are mediated principally by the key players of the brain’s innate immune system, ie, microglial cells, that are only equipped with dedicated antigen-presenting potential, and have been found to be involved in several central nervous system disorders, including Alzheimer’s disease, multiple sclerosis, and infections, which substantiates their pathological role. The term neuroinflammation is denoted by the response of the inflammatory glial cell (particularly the microglia) that is specific to the central nervous system and characterized by formation of lesions, neurite growth disorders, and hyperphosphorylation of tau proteins, which are completely different to the inflammatory events taking place in the periphery. This understanding has led to the hypothesis that the microglia are the primary immune cells in the brain and respond in a unique way to an inflammatory insult by stimulating cytokines and characteristic phagocytic mechanisms. In order to understand neuroinflammation better, the terms “acute inflammation” and “chronic inflammation” have been introduced.

Acute inflammation is basically a protective mechanism enabling recovery from an insult and is seen as an immediate response to the causative agent, lasting only for a short time period. Soon after the insult, activated microglia and astrocytes aggressively stimulate targeted cell activation that is similar to the events seen in the periphery. However, there is no evidence of leukocyte infiltration in the brain unless the blood–brain barrier is severely damaged; but if the leukocyte permeation is seen, severe destruction results. In contrast, chronic inflammation indicates a disease state because the inflammation persists for a longer duration of time. Multiple sclerosis is a classical example of chronic inflammation, the details of which are described as follows.

**Multiple sclerosis**

Multiple sclerosis is a chronic demyelinating inflammatory central nervous system disease characterized by immune attack directed towards myelin, the protective neuronal sheath. It affects more than one million people globally, and patients present with symptoms of weakness, ataxia, fatigue, sensory and vision loss, and impaired memory. The severity of these symptoms is determined by the amount of demyelination present, and the more severe the damage, the less the neurons can communicate with each other. The exact etiology of the disease remains elusive, but it is fascinating to study the infiltration of autoreactive immune cells followed by generation of inflammatory lesions.

**T cell infiltration in multiple sclerosis**

A group of self-reactive T cells directed against myelin crosses the blood–brain barrier under a strong chemokine influence. The autoreactive T cells gain entry to the central nervous system after binding of their cell surface molecules, ie, P-selectin glycoprotein ligand-1, very late antigen-4, and leukocyte function-associated antigen-1, with the corresponding cell adhesion molecules, ie, mucosal addressin cell adhesion molecule (MadCAM), vascular cell adhesion molecule, and intracellular cell adhesion molecule, expressed...
on endothelial cells of the brain capillary walls. Soon after their entry, T cells unleash an immune attack on myelin, which becomes sustained by involvement of microglial cells, macrophages, dendrites, and astrocytes, which attract immune cell trafficking.\textsuperscript{85,109} In particular, a specific subset of T helper cells (Th-17) has been identified to be the leading cause of multiple sclerosis, because they secrete interleukin-17 and are endowed with an ability to open the blood–brain barrier, thereby driving the neuronal insult.\textsuperscript{96} In addition, the immune attack towards myelin is boosted by recruitment of T cells from the periphery and activation of antibody-secreting B cells by reactive T cells, culminating in an assault on myelin, followed by demyelination.\textsuperscript{87,88} Based on severity of symptoms, multiple sclerosis is classified into four subtypes. The first subtype is relapsing-remitting multiple sclerosis, whereby patients show recovery following periods of disability. The second form is secondary progressive multiple sclerosis, whereby symptoms of disability are seen between periods of relapse and recovery. The third subtype is primary progressive multiple sclerosis, which, as its name suggests, involves only symptoms of disease progression without any remission. The last subtype is progressive relapsing multiple sclerosis, which is rather rare, with unique symptoms of disease exacerbation during periods of remission.\textsuperscript{89}

The advent of biomarkers has paved the way for better understanding of the pathology of multiple sclerosis. These biomarkers include the cytoskeletal proteins of astrocytes and axons, ie, glial fibrillary acidic protein and neurofilament light protein, which are found to be increased in the cerebrospinal fluid of patients with neurological disability and disease progression.\textsuperscript{90} A longitudinal cross-sectional study conducted by Hennies et al\textsuperscript{91} reported elevated levels of pentosidine, a biomarker of advanced glycation end products, in patients with multiple sclerosis when compared with healthy volunteers. The same was also confirmed by the finding of downregulation of pentosidine upon treatment, substantiating the therapeutic potential of advanced glycation end products as inhibitors in the future.

**Multiple sclerosis and its treatment**

Therapeutic interventions for multiple sclerosis mainly target immune suppression to slow disease progression and ameliorate its symptoms. First-line drugs currently used for relapsing-remitting multiple sclerosis are the interferon (IFN) agents, ie, IFN-1, IFNb1a, and IFNb1b, along with glatiramer acetate. The precise mechanism by which these drugs act involves downregulation of T cell proliferation and migration, reduction in antigen presentation, and even shifting the immune response to Th2 cells.\textsuperscript{92,93} Corticosteroids also exert negative effects on the immune system, and are commonly used in the treatment of relapsing-remitting multiple sclerosis. These include prednisone, prednisolone, methylprednisolone, and dexamethasone; however, these drugs have limited application in the treatment of other forms of multiple sclerosis because they are associated with many side effects.\textsuperscript{94} Chemotherapeutics targeting the mechanism of immune suppression have also been attempted. For instance, mitoxantrone and cyclophosphamide have been found to be effective against relapsing-remitting multiple sclerosis and secondary progressive multiple sclerosis, with the former inducing apoptosis of lymphocytes and the latter showing activity against both T and B cells.\textsuperscript{95,96}

Strenuous efforts are being made to identify better therapeutics for multiple sclerosis, and drugs entering preclinical and clinical trials are gaining momentum. Several drug candidates are being tried preclinically, of which methylthioadenosine has been particularly promising in experimental autoimmune encephalomyelitis, an in vivo model of multiple sclerosis. This molecule, derived from a natural source, shows characteristic immunomodulatory activity and synergistic efficacy when given in combination with IFN or glatiramer acetate.\textsuperscript{97} Recently, it was found that a cytokine known as midkine, upon binding with heparin, triggered an inflammatory cascade attracting T cell traffic. Animal studies have found that midkine inhibitors are potent enough to reverse experimental autoimmune encephalomyelitis.\textsuperscript{98} Sodium phenylbutyrate, a histone deacetylase inhibitor, has also been shown to ameliorate symptoms of multiple sclerosis in an experimental autoimmune encephalomyelitis model.\textsuperscript{99} A possible mechanism by which the histone deacetylase inhibitors exert a therapeutic effect is inhibition of inflammation and promotion of neuroprotection.\textsuperscript{100} Recent reports by Zhao et al concluded that matrine, a quinolizidine alkaloid obtained from radix *Sophorae flavescentis*, reduces infiltration of lymphocytes into the central nervous system, thereby ameliorating experimental autoimmune encephalomyelitis.\textsuperscript{101} Drugs included in the Phase II clinical trials were fingolimod and BG00012. The former was found to prevent entry and trafficking of lymphocytes in the central nervous system by encouraging complexation of lymphocytes within the lymph nodes, while the latter showed neuroprotective anti-inflammatory activity when administered orally.\textsuperscript{102} Drugs that have reached Phase III investigation include laquinimod, which shifts the cellular immune response to a Th2 type, and teriflunomide, which inhibits the pyrimidine synthesis essential for immune cell proliferation and expansion.
Cladribine is another candidate drug which is effective against multiple sclerosis, with the proposed mechanism being inhibition of the adenosine deaminase enzyme followed by suppression of dividing and resting T cells.\textsuperscript{103,104} Dalfampridine was recently approved by the US Food and Drug Administration and produces symptomatic improvement of ambulation in patients with multiple sclerosis. The probable mechanism by which this drug acts is via blockade of potassium channels and improving axonal conduction.\textsuperscript{105} Promising results were also seen after introduction of monoclonal antibodies that downregulate the immune response. One is alemtuzumab, which specifically targets CD52 expression, lowers lymphocyte, monocyte, and dendritic cell counts,\textsuperscript{106} and another is rituximab, which has unique activity against CD20, reducing the B cell population in cerebrospinal fluid of patients with multiple sclerosis.\textsuperscript{107}

Natalizumab, a humanized monoclonal antibody, was found to be effective in patients with relapsing-remitting multiple sclerosis by inhibiting α-integrin, a cell surface molecule, and blocking permeation of T cells into the central nervous system.\textsuperscript{108} Stem cell transplantation was also attempted in a group of patients with multiple sclerosis, with encouraging results. This strategy now warrants further evaluation.\textsuperscript{109}

**Neuro oncology**

Neuro oncology refers to a wide range of brain tumors growing inside the central nervous system. There is a multitude of neoplasms that originate in the brain, arising either from neural elements or resulting from metastasis of primary tumors situated elsewhere in the body. A variety of malignant brain tumors exist, including gliomas, medulloblastomas, and primary central nervous system lymphomas, as well as tumors that have metastasized to the brain.\textsuperscript{110} Age and gender seem to be associated with certain types of central nervous system malignancy. For instance, malignant astrocytoma and glioblastoma multiforme are the most commonly identified primary brain tumors in male adults, whereas astrocytoma, medulloblastoma, and glioblastoma are predominantly found in children. Risk factors associated with brain tumors include a genetic predisposition to inherited abnormalities, adenomatous polyps, tuberous sclerosis, ionizing radiation, radiofrequency signals emitted from mobile phones, retroviral infection, adenoviruses and papaviruses, carcinogenic chemicals (alkylating agents), and traumatic head injury.\textsuperscript{111} Gliomas are the commonest form of aggressive brain malignancy, accounting for more than 60% of primary tumors in the central nervous system, and are further classified as astrocytoma, oligodendroglioma, ependymoma, and glioblastoma multiforme. Based on the extent of cancerous growth, they are considered as low-grade or high-grade gliomas, with the survival rate decreasing upon detection of high-grade glioma. Astrocytomas are considered to be relatively benign, but often undergo mutation, advancing to high-grade astrocytoma or glioblastoma multiforme. Oligodendrogliomas are low-grade tumors, seen usually in middle-aged individuals with a history of seizures. These tumors can be diagnosed very accurately and are best treated by surgical excision. Ependymomas typically arise from cells covering the ventricles and may produce hydrocephalus. The most aggressive and malignant form of astrocytoma is considered to be glioblastoma multiforme. It has the typical characteristics of malignancy, with aggressive growth, invasion, and metastasis.\textsuperscript{112} Gold standard therapy for high-grade gliomas is a combination of surgery and external beam radiation. However, use of chemotherapeutics is also encouraging in these patients. Alkylating agents (procarbazine, temozolomide, carboplatin), nitrosoureas (lomustine, carmustine), vincristine, and topoisomerase inhibitors, eg, irinotecan, are the most promising cytotoxic agents that have been used so far to treat glioma. The mechanisms of these drugs include DNA alkylation, cell cycle phase-specific inhibition, and DNA strand fragmentation.

Cytostatic agents are now gaining popularity, and drugs like tamoxifen which inhibit protein kinase C have shown promising effects. Retinoic acid, with its ability to induce differentiation followed by apoptosis, and thalidomide, which decreases angiogenesis, are the other drugs included in this category.\textsuperscript{113} Primary central nervous system lymphoma is reasonably infrequent but aggressive, and constitutes 4% of all primary brain tumors. It takes the form of B cell lymphoma and is confined to the brain and/or spinal cord regions, without systemic lymphocytic involvement.\textsuperscript{114} Whole brain radiation therapy was initially the standard treatment for primary central nervous system lymphoma, but this has become used less frequently with the advent of effective chemotherapeutics. The maximum therapeutic outcome was observed for a combination of chemotherapy with cranial irradiation. A Phase II clinical trial has demonstrated the best patient response using a regimen of methotrexate, procarbazine, and vincristine given intravenously in combination with intrathecal methotrexate followed by whole brain radiation therapy.\textsuperscript{115,116}
Medulloblastoma is a common brain malignancy in the pediatric population but accounts for only about 1% of all brain tumors in adults, so there is a need to optimize the therapeutic regimen as per the pediatric setting.\textsuperscript{114} First-line therapy for medulloblastoma is surgical excision of the tumor bed followed by cranial irradiation. Chemotherapy in combination with radiotherapy is worthy of consideration for patients with unresponsive disease. The chemotherapeutic regimen includes cyclophosphamide, lomustine, cisplatin, carboplatin, vincristine, and etoposide.\textsuperscript{117,118} Tumors metastasizing to the brain are the most commonly diagnosed brain tumors. Autopsy studies have indicated that approximately 25% of patients diagnosed with systemic cancers have metastases to the brain.\textsuperscript{119}

Although chemotherapeutics are effective, the side effects are significant, so the present research is focused on small molecule inhibitors targeting the intracellular kinases, farnesyl transferases and matrix metalloproteinases. Protein kinases are of particular importance because they regulate cell proliferation and are overexpressed in malignant cells.

Intracellular signaling pathways are mainly regulated by phosphorylation via protein kinases, and several protein kinase inhibitors, including ZD1839 (gefitinib), OSI774 (erlotinib), and STI-571 (imatinib), are undergoing clinical evaluation. Farnesyl transferases are the enzymes responsible for farnesylation of Ras proteins, which are essential for cellular proliferation, survival, and angiogenesis. Hence, by blocking the farnesyl transferase enzyme, farnesyl transferase inhibitors inhibit the farnesylation essential for proliferation of cancer cells. R115777 is another drug included in this category.\textsuperscript{120} Another attractive target is the matrix metalloproteinases, which are overexpressed in glioma, allowing tumor cell invasion and metastatic spread. This is because of the role of matrix metalloproteinases in digestion of the cellular basement membrane and breakdown of the extracellular matrix. Hence, drugs like marimastat and prinomastat that inhibit matrix metalloproteinases are being considered for therapeutic screening.\textsuperscript{121}

**Blood–brain barrier and neurological disorders: therapeutic options**

The blood–brain barrier is a complex network of vasculature comprised of a tight layer of capillary endothelial cells along with clusters of enzymes, efflux pumps, receptors, and transporters acting in concert to limit access of molecules to the central nervous system. However, if the molecules are permeabilized, they become distributed throughout the brain as a result of its rich vasculature.\textsuperscript{122} As stated earlier, the major limitation in the treatment of neurological disease is the inability to deliver drug molecules across the blood–brain barrier. Therefore, the main focus of research is the development of treatment strategies targeting specific markers on the capillary endothelium associated with the various pathologies. The targeting agents could be antibodies or substrates for receptors, or could be drug-loaded nanoparticles.\textsuperscript{123,124}

Conjugating drug molecules to brain-specific transporters will enhance their penetration into the central nervous system. These include transporters of glucose, oligonucleotides, amino acids, and monocarboxylic acid, for which the receptor-mediated transport system has been the most extensively studied. The transferrin receptor, for example, is found to be more selectively expressed on the capillary endothelium in the brain than in the rest of the body, so represents a potential transport system to deliver drug molecules to the brain.\textsuperscript{125,126} In terms of delivery of therapeutics, enhancing lipophilicity by chemical means would increase drug distribution in the central nervous system. Utilizing biology-based approaches, eg, designed carriers resembling the in situ milieu would also increase penetration of drug molecules into the brain. These tailor-made carriers can be either drugs alone or polymeric nanocarriers modified with the special ability of recognition. Customizing drugs to resemble the in vivo substrates of a normal transporter would also facilitate transport across the blood–brain barrier. However, the real challenge lies in drug modifications which have the probability of losing functionality.\textsuperscript{122} A list of conventional and novel therapeutic interventions is shown in Table 1.

**Why nanotechnology?**

Nanoparticles are typically characterized as colloidal drug delivery systems with a size measuring not more than 100 nm, and have significant potential for delivering drugs across the blood–brain barrier. The main reason behind the introduction of nanoparticles into the medical field is that they have characteristic features not possessed by conventional therapeutic agents. Because of their small size, nanoparticles tend to have a large surface to volume ratio, providing a huge surface area that favors absorption. This large surface area also imparts the unique ability to absorb/bind a variety of substances, such as therapeutic and diagnostic agents. The unusually small size of the nanoparticle allows easy cellular permeation and evasion of the
Table 1  Conventional and future therapeutics for neurological disorders

<table>
<thead>
<tr>
<th>S no</th>
<th>Drug molecule</th>
<th>Mechanism</th>
<th>Effective against</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Donepezil, rivastigmine, and galantamine</td>
<td>Acetylcholinesterase inhibition</td>
<td>AD</td>
<td>29</td>
</tr>
<tr>
<td>2</td>
<td>Memantine</td>
<td>N-methyl D-aspartate receptor antagonism</td>
<td>AD</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>α-tocopherol, ginkgo biloba plant extracts</td>
<td>Antioxidant and anti-inflammatory</td>
<td>AD</td>
<td>32</td>
</tr>
<tr>
<td>4</td>
<td>L-dopa and carbidopa, Duodopa® pump, dithydropyroglycine, ropinirole, pramipexole, and pergolide</td>
<td>Dopaminergic agonism</td>
<td>PD</td>
<td>53</td>
</tr>
<tr>
<td>5</td>
<td>Selegline and rasagline</td>
<td>Monoamine oxidase inhibition</td>
<td>PD</td>
<td>54</td>
</tr>
<tr>
<td>6</td>
<td>Alteplase</td>
<td>Tissue plasminogen activation</td>
<td>Stroke</td>
<td>66</td>
</tr>
<tr>
<td>7</td>
<td>Aspirin</td>
<td>COX inhibition</td>
<td>Stroke</td>
<td>67</td>
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<tr>
<td>8</td>
<td>Statins</td>
<td>Cholesterol synthesis</td>
<td>Stroke</td>
<td>68</td>
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<tr>
<td>9</td>
<td>Heparin, plasmin, and micoplasmin</td>
<td>Thrombolysis and fibrinolysis, respectively</td>
<td>Stroke</td>
<td>70</td>
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<tr>
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<td>Edaravone</td>
<td>Anti-oxidant and neuroprotection</td>
<td>Stroke</td>
<td>72</td>
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<tr>
<td>11</td>
<td>IFN-1, IFNβ1a, and IFNβ1b</td>
<td>Immune modulation</td>
<td>MS</td>
<td>92</td>
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<tr>
<td>12</td>
<td>Glatiramer acetate</td>
<td>Immune modulation</td>
<td>MS</td>
<td>93</td>
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<td>13</td>
<td>Corticosteroids</td>
<td>Immune suppression</td>
<td>MS</td>
<td>94</td>
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<td>14</td>
<td>Mitoxantrone</td>
<td>Lymphocyte apoptosis</td>
<td>MS</td>
<td>95</td>
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<tr>
<td>15</td>
<td>Cyclophosphamide</td>
<td>Anti-T and anti-B cell activity</td>
<td>MS</td>
<td>96</td>
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<tr>
<td>16</td>
<td>Dalfampridine</td>
<td>K+ channel blockade</td>
<td>MS</td>
<td>105</td>
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<tr>
<td>17</td>
<td>Alkylating agents and nitrosoureas</td>
<td>DNA alkylation followed by apoptosis</td>
<td>Gliomas and medulloblastoma</td>
<td>113</td>
</tr>
<tr>
<td>18</td>
<td>Vincristine</td>
<td>Cell cycle phase-specific inhibition</td>
<td>medulloblastoma</td>
<td>117</td>
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<tr>
<td>19</td>
<td>Topoisomerase inhibitors</td>
<td>DNA strand fragmentation</td>
<td>Primary central nervous system lymphoma</td>
<td>114</td>
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Drugs in preclinical studies

<table>
<thead>
<tr>
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<th>Drug molecule</th>
<th>Mechanism</th>
<th>Effective against</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Immunotherapy against β amyloid</td>
<td>Plaque dissolution</td>
<td>AD</td>
<td>34</td>
</tr>
<tr>
<td>2</td>
<td>Brain-derived neurotrophic factor</td>
<td>Neuronal survival and development</td>
<td>AD</td>
<td>41</td>
</tr>
<tr>
<td>3</td>
<td>Bexarotene</td>
<td>Dissolution of Aβ plaques</td>
<td>AD</td>
<td>42</td>
</tr>
<tr>
<td>4</td>
<td>Gliar-derived neurotrophic factor</td>
<td>Dopaminergic neuron revival</td>
<td>PD</td>
<td>58</td>
</tr>
<tr>
<td>5</td>
<td>Citocline</td>
<td>Lipid metabolism inhibition</td>
<td>Stroke</td>
<td>73</td>
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<td>6</td>
<td>DP-699</td>
<td>Metal chelation</td>
<td>Stroke</td>
<td>75</td>
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<tr>
<td>7</td>
<td>Valproic acid, sodium butyrate, and trichostatin A</td>
<td>Anti-inflammatory and antiapoptotic activity</td>
<td>Stroke</td>
<td>77</td>
</tr>
<tr>
<td>8</td>
<td>Methylthioadenosine</td>
<td>Immunomodulatory action</td>
<td>MS</td>
<td>97</td>
</tr>
<tr>
<td>9</td>
<td>Midkine inhibitors</td>
<td>Anti-inflammatory action</td>
<td>MS</td>
<td>98</td>
</tr>
<tr>
<td>10</td>
<td>Histone deacetylase inhibitors</td>
<td>Anti-inflammatory and neuroprotective activities</td>
<td>MS</td>
<td>100</td>
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<tr>
<td>11</td>
<td>Matrine</td>
<td>Reduced lymphocyte infiltration</td>
<td>MS</td>
<td>101</td>
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</table>

Drugs in clinical trials

<table>
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<th>Mechanism</th>
<th>Effective against</th>
<th>Reference</th>
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<tr>
<td>1</td>
<td>Semagacestat</td>
<td>γ-secretase inhibitor</td>
<td>AD</td>
<td>35</td>
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<tr>
<td>2</td>
<td>Intravenous immunoglobulin</td>
<td>Passive immunization against amyloid</td>
<td>AD</td>
<td>38</td>
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<tr>
<td>3</td>
<td>Dimebon</td>
<td>Neuroprotection</td>
<td>AD</td>
<td>40</td>
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<tr>
<td>4</td>
<td>Riluzole</td>
<td>Ant glutamatergic drug</td>
<td>PD</td>
<td>57</td>
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<td>5</td>
<td>ReN001</td>
<td>Neutral stem cell therapy</td>
<td>Stroke</td>
<td>78</td>
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<tr>
<td>6</td>
<td>Fingolimod and BG00012 (phase II)</td>
<td>Lymphocyte complexation and neuroprotective and anti-inflammatory</td>
<td>MS</td>
<td>102</td>
</tr>
<tr>
<td>7</td>
<td>Laquinimod (phase III)</td>
<td>Shifting the T cell response to T helper 2 type</td>
<td>MS</td>
<td>103</td>
</tr>
<tr>
<td>8</td>
<td>Teriflumomide (phase III)</td>
<td>Interrupting pyrimidine synthesis</td>
<td>MS</td>
<td>104</td>
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<tr>
<td>9</td>
<td>Cladribine</td>
<td>Acts against resting and dividing T-cells</td>
<td>MS</td>
<td>105</td>
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<tr>
<td>10</td>
<td>ZD1839 (gefeinib), OSI774 (erlotinib), and STI-571 (imatinib)</td>
<td>Protein kinase inhibition</td>
<td>Glioblastoma</td>
<td>120</td>
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<tr>
<td>11</td>
<td>R115777</td>
<td>Farnesyl transferase inhibition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Marimastat and praminostat</td>
<td>Matrix metalloproteinase inhibitor</td>
<td>Brain tumors</td>
<td>121</td>
</tr>
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</table>

Monoclonal antibodies

<table>
<thead>
<tr>
<th>S no</th>
<th>Drug molecule</th>
<th>Mechanism</th>
<th>Effective against</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bapineuzumab, solanezumab</td>
<td>Plaque dissolution</td>
<td>AD</td>
<td>37</td>
</tr>
<tr>
<td>2</td>
<td>Abciximab</td>
<td>Glycoprotein Iib/IIa antagonism</td>
<td>Stroke</td>
<td>69</td>
</tr>
<tr>
<td>3</td>
<td>Natalizumab</td>
<td>Inhibits entry of T cells</td>
<td>MS</td>
<td>108</td>
</tr>
<tr>
<td>4</td>
<td>Alemtuzumab</td>
<td>Depletion of lymphocytes</td>
<td>MS</td>
<td>106</td>
</tr>
<tr>
<td>5</td>
<td>Rituximab</td>
<td>Depletion of lymphocytes</td>
<td>MS</td>
<td>107</td>
</tr>
</tbody>
</table>

Abbreviations: AD, Alzheimer’s disease; COX, cyclo-oxygenase; IFN, interferon; MS, multiple sclerosis; PD, Parkinson’s disease; S no, serial number.
reticuloendothelial system, so avoiding unnecessary drug loss. To summarize:

• Nanoparticles deliver therapeutics to the desired site, overcoming the limitations of membrane barriers that are considered impermeable to conventional drug delivery systems.

• Surface-modified nanoparticles are advantageous in terms of site-specific delivery, reducing peripheral toxicity and enabling controlled drug release kinetics.

• Stability of the drug molecule is retained, and after reaching the desired site, drug release is attained by diffusion or erosion from the polymeric nanoparticle wall.\(^{127-129}\)

The advent of nanotechnology has revolutionized therapeutic delivery, and enabled the manufacture of tailor-made materials that can interact with targets selectively and achieve the desired physiological response.

**Why targeted nanodelivery?**

Ideally, for successful treatment, the drug molecule encapsulated in a nanoparticle should reach its optimum concentration at the desired site of action, crossing the barriers presented by the body, eg, the blood–brain barrier, and should also retain its therapeutic activity, resisting the extremities while in the circulation. Most importantly, the ideal nanoparticle should be able to act only on abnormal cells, and should spare healthy cells. This can be achieved only if the nanoparticle is aimed at its target selectively and modified accordingly. These two strategies, if applied to a drug-loaded nanoparticle, will have significant potential to improve patient survival without compromising quality of life. Hence, targeted nanodelivery systems have gained increasing acceptance in modern therapeutics (Figure 2).

**Brain-targeted polymeric nanodelivery**

Although the blood–brain barrier poses a serious limitation for delivery of therapeutics to the brain, this barrier is often surmountable when its integrity is disrupted or when the characteristics of a drug molecule are modified. For instance, administering analogs of bradykinin generates osmotic pressure, thus opening the tight junctions of the blood–brain barrier. This allows paracellular drug transport through the blood–brain barrier. Drug permeation...
has also been reported to be enhanced after intracarotid administration of a hyperosmotic solution of arabinose.\textsuperscript{130} However, these modifications are not always compatible because they disturb homeostasis and allow entry of undesirable toxic molecules along with the therapeutic molecule, leading to potential side effects. Hence, targeted delivery of nanoparticles has become a focus of considerable research attention. The primary objective of a targeted delivery system is to deliver a diagnostic or therapeutic agent to a specific site. Because it is associated with localized delivery, targeted nanoparticles have attracted immense attention over the past 15 years. The potential advantages of site-specific delivery include reduced dosage of the drug molecule, enhanced bioavailability at the desired site, and reduced likelihood of peripheral side effects. Accumulation of nanoparticles at a specific site depends on various factors, and, in general, biodistribution is primarily affected by chemical properties, size of the nanoparticle, and availability of the targeted ligand. Knowledge of the pharmacokinetics of targeted nanoparticles is extremely valuable in evaluating the dose, because although targeted delivery is considered to be site-specific, few nanoparticles seem to distribute to nonspecific sites.\textsuperscript{131}

**Surface modifications**

**Conjugation with antibodies**

Effective targeting requires primarily an understanding of the physiology and anatomy of the blood–brain barrier. One of the approaches is to modify the surface of the drug-loaded nanoparticle by conjugating it with an antibody that is particularly specific for the brain, eg, the OX26 antibody and the transferrin receptor. The antibody-drug conjugate recognizes a specific ligand (the transferrin receptor in this case) and crosses the blood–brain barrier by receptor-mediated endocytosis. Unfortunately, it is hard to identify specific ligands relative to a target tissue.\textsuperscript{132} Antibodies are most promising tools for blood–brain barrier targeting because of their capacity for specific binding. For instance, antibodies targeted to transferrin and insulin receptors can act as transporters across the blood–brain barrier and deposit the drug molecules via receptor-mediated endocytosis. According to the published data, chemotherapeutic drugs like methotrexate and proteins like basic fibroblast growth factor, nerve growth factor, and brain-derived neurotrophic factor, are successfully transported into the brain upon conjugation with the transferrin receptor antibody.\textsuperscript{133} Several other therapeutics, like nerve growth factor and brain-derived neurotrophic factor, are also transported across the blood–brain barrier upon binding with the transferrin receptor antibody.\textsuperscript{134,135} Similarly, megalin ligands patented by Starr et al are specific to the brain and can be used for successful delivery of therapeutics upon surface modification.\textsuperscript{136} Another instance of surface modification being used for delivery of radiopharmaceuticals is [\textsuperscript{125}I]-\textsuperscript{Aβ}\textsuperscript{1-40}, the uptake of which is significantly increased upon conjugation with a monoclonal antibody against the human insulin receptor following intravenous administration.\textsuperscript{137}

A peptide specific to the brain invented by Forni et al can also be suitably modified for conjugating with nanodelivery systems and achieving brain-specific delivery.\textsuperscript{138} A specific method for delivery of this peptide conjugate was developed by Roberts et al,\textsuperscript{139} whereby the peptide intended for delivery was conjugated with a nonpeptide water-soluble polymer. Covalent attachment of the peptide to the polymeric nanoparticle could be achieved if the polymer had a surface modified with water-soluble polyethylene glycol (PEG) or copolymers of PEG and polypropylene glycol.

**Surface modification by surfactants**

Interaction of a nanoparticulate system with biological cells is very much influenced by the functional nature of the nanoparticle surface. In order to achieve high specificity, the nanoparticle surface can either be modified by conjugating it with an antibody as already discussed, or coating it with different surfactants.\textsuperscript{140,141} Bioadhesive surfactants, such as PEG-D-α-tocopheryl, polyethylene glycol 1000 succinate or polyvinyl alcohol can certainly enhance adhesion and adsorption by intestinal cells, while masking efflux mechanisms. However, targeting specificity will primarily depend on the physicochemical and biochemical properties of the surfactant. Previous reports underscore the importance of polysorbate 80-coated nanoparticles for brain-specific delivery. Polysorbate 80 surface-modified doxorubicin-loaded and gemcitabine-loaded polybutylcyanoacrylate nanoparticles showed enhanced brain specificity and efficacy compared with unmodified nanoparticles, highlighting the importance of surface modification by surfactants.\textsuperscript{142,143} Another surfactant reported to have the ability to enhance brain permeability is poloxamer 188 (F68) which was found to increase the delivery of drug-loaded polybutylcyanoacrylate and poly(lactide-co-glycolide) (PLGA) nanoparticles to the brain in rats.\textsuperscript{144,145}

A study conducted by Kulkarni et al demonstrated more effective delivery of F68-modified PLGA nanoparticles than F127 and polysorbate 80-modified nanoparticles into the brain. The plausible mechanism behind enhanced brain permeation by these surfactants is that they inhibit the efflux action of P-glycoprotein and solubilize the cell membrane lipids of brain endothelial cells for entry.\textsuperscript{146} Also, polysorbate 80
and F68 surface-modified nanoparticles adsorb apolipoprotein E, apolipoprotein B, or both, mimic low-density lipoproteins, and enter the brain via receptor-mediated endocytosis. Thus, it can be understood that surface modification will essentially influence the pattern of nanoparticle uptake by cells.

**Applications of targeted delivery in neurological disorders**

Targeted nanoparticle delivery has gained paramount importance for delivery of therapeutics to the brain, and a few of the recent successes are described. The transferrin receptor has gained attention because it favors receptor-mediated endocytosis across the endothelium of brain capillaries. Although expressed in the liver, intestines, and erythroblasts, it is almost undetectable in normal tissues. This approach was used by Gan and Feng, who showed that doxorubicin-loaded poly (lactide)-d-α-tocopheryl polyethylene glycol succinate nanoparticles conjugated with the transferrin receptor have enhanced uptake both in vitro and in vivo. Similar results were obtained with the antioxidant, tempol, loaded into PLGA nanoparticles surface-modified with transferrin, and the investigators suggested that this particular formulation may have applications in Alzheimer's disease and PD. Substantial results were also obtained upon conjugation of lactoferrin with the surface of the polyamidoamine (PAMAM) dendrimer, with enhanced uptake by the brain capillary endothelium via lactoferrin receptors.

The significance of this study was that lactoferrin-conjugated nanoparticles were not in competition with endogenous lactoferrin for binding, corroborating the potential of lactoferrin-mediated surface modification. Polymeric chitosan nanoparticles modified with an antiamyloid antibody, IgG4.1, were reported to be taken up significantly in an in vitro model of the blood–brain barrier, indicating the future potential of targeted drug delivery for Alzheimer's disease.

Trimethylated chitosan-modified coenzyme-Q10 loaded PLGA nanoparticles were found to have enhanced neuroprotective activity in an in vivo mouse model of Alzheimer’s disease, and were internalized via adsorptive-mediated transcytosis. Similarly, diagnostic improvement was shown by conjugation of the antitau monoclonal antibody to gold nanoparticles. This conjugate showed an extreme sensitivity of detecting 1 pg/mL of tau protein, thus demonstrating the potential of targeted nanoparticles. PAMAM dendrimers were modified with Angiopep-2 for selective gene delivery in vitro and in vivo glioma models. The therapeutic efficiency was reported to be promising, without untoward cytotoxicity and maximum permeation into the brain tumor site. The authors claim that Angiopep-2 can be suitably ligated to nanoparticles for brain-specific delivery, because they adsorb low-density lipoproteins and undergo receptor-mediated endocytosis, mimicking endogenous lipoproteins. Similarly chlorotoxin-conjugated DNA-loaded PAMAM nanoparticles showed significant uptake in an in vivo glioma model. A slight modification in ligand binding to PEG-PCL nanoparticles was attempted with Angiopep-2 and EGF-P-EGF1. EGF-P-EGF1 is a fusion protein derived from coagulation factor VII, and binds specifically to tissue factor, which is overexpressed in neural cells, and this conjugate facilitates enhanced brain penetration and selective accumulation of nanoparticles in these cells. Thus, this strategy, if applied, can be successfully translated for treatment of glioma as well.

Polybutylcyanoacrylate nanoparticles coated with polysorbate 80 have shown enhanced delivery of temozolomide targeting brain tumors in vivo. An interesting study reported by Guo et al showed enhanced antiglioma therapy using paclitaxel-loaded PEG-PLGA nanoparticles conjugated with DNA aptamer (AS1411) against nucleolin, which is overexpressed in glioma. Further, conjugation of interleukin-13 peptides with the nanoparticle surface could enhance targeted delivery towards glioma cells, which overexpress the interleukin-13 receptor. Similarly, PEG-PLGA nanoparticles covalently modified with Pep TGN (a 12-amino acid peptide) showed enhanced brain penetration along with reduced accumulation in the liver and spleen in vivo. The significance of this study was that brain-targeted delivery of Pep TGN has never been reported before. Targeted nanodelivery for neuroinflammation was made possible with the fabrication of PLGA nanoparticles conjugated with leukemia inhibitory factor. Leukemia inhibitory factor was found to have a potential immunoregulatory role, with anti-inflammatory effects driving the maturation of T regulatory cells and the inhibition of interleukin-6-mediated progression of Th17. This nanoformulation should prove to have substantial application for therapeutics in multiple sclerosis. Diagnostic applications for multiple sclerosis include glyconanoparticles formulated from cross-linked iron oxide nanoparticles modified with sialyl Lewis X, a ligand specific for selectins. Magnetic resonance imaging has shown increased accumulation of these targeted nanoparticles in an experimental autoimmune encephalomyelitis rat model. A serious limitation of stroke therapy is systemic hemorrhage due to the nonspecificity of tissue plasminogen activator. Hence, nanodevices have been developed, consisting of tissue plasminogen activator-bound polystyrene latex nanoparticles modified with antifibrin antibody that is selective for clots, thus avoiding nonspecific activity.
Simultaneous conjugation of fibrin-specific urokinase (thrombolytic clot buster) and antifibrin antibodies onto perfluorocarbon nanoparticles has shown specific clot dissolution which was found to be effective in an in vivo stroke model. Table 2 lists the known targeted nanoapplications for neurological disorders.

**What's next?**

As already discussed, the strategies needed for brain disorder therapeutics are rather complicated, both in terms of brain penetration and stability of drug molecules following penetration. Hence, applying the principles of neuroprotection or/and neuroproliferation coupled with nanodelivery would be the most reliable technique for resolving some of these challenges. Likewise, we have identified that the mutant form of survivin, SurR9-C84A (baculovirus inhibitor of apoptosis repeat motif) has solid neuroprotective activity when tested in SK-N-SH cells subjected to oxidative stress induced by hydrogen peroxide. Following this, we also identified the potential of SurR9-C84A in stimulating neuronal proliferation, survival, and expression of neuronal markers when tested in SK-N-SH and HCN-2 cell lines. Further to this, we also noted the neuroprotective and mitogenic effects of BARF1 epitopes of Epstein-Barr virus. As already discussed, the impact of neuroinflammation can be drastic because inflammation in itself is toxic to neurons, along with potentiation of neurodegenerative effects. Our previous work has demonstrated promising results in inhibition of inflammation when mice with experimental autoimmune encephalomyelitis were treated with an anti-MAdCAM-1 monoclonal antibody that prevented binding and subsequent entry of T cells.

Surprisingly, neuroprotection was amplified in the experimental autoimmune encephalomyelitis mouse model when the mice were administered a combination of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate/kainate antagonist, 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(f) quinoxaline, and the neuroprotector, glycine (N-terminal tripeptide of insulin-like growth factor). The results included a drastic reduction in disease scores. Following on from these preliminary achievements, we administered a combination of monoclonal antibodies for MAdCAM-1, vascular

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**Table 2 Targeted nanotechnological applications**

<table>
<thead>
<tr>
<th>No</th>
<th>Nanoparticle</th>
<th>Surface modification</th>
<th>Targeted mechanism</th>
<th>Application</th>
<th>Reference</th>
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<tr>
<td>1</td>
<td>PLA-TPGS</td>
<td>Transferrin</td>
<td>Receptor-mediated endocytosis</td>
<td>Enhanced brain uptake</td>
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<td>PLGA</td>
<td>Transferrin</td>
<td>Receptor-mediated endocytosis</td>
<td>Enhanced brain uptake in AD and PD</td>
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</tr>
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<td>Chitosan</td>
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<td>Binds selectively with amyloid-β</td>
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<td>5</td>
<td>PLGA</td>
<td>Trimethylated chitosan</td>
<td>Absorptive-mediated transcytosis</td>
<td>Neuroprotector drug uptake in AD</td>
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<tr>
<td>6</td>
<td>Gold</td>
<td>Anti-tau monoclonal antibody</td>
<td>Binds selectively with tau proteins</td>
<td>Diagnostic in AD</td>
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**Abbreviations:** Ab, antibody; AD, Alzheimer’s disease; IL, interleukin; LRP, low-density lipoprotein receptor-related protein; PAMAM, polyamidoamine; PBCA, polybutylcyanoacrylate; PD, Parkinson’s disease; PEG-PCL, polyethylene glycol-polycaprolactone; PEG-PLGA, polyethylene glycol-poly (lactide-co-glycolide); PLA-TPGS, poly(lactide)-D-α-tocopheryl polyethylene glycol succinate.
cell adhesion molecule-1, intracellular adhesion molecule-1, and leukocyte function-associated antigen-1 molecule, and, not surprisingly, maximum remission of disease symptoms were observed in the mice with experimental autoimmune encephalomyelitis. Successful results were also achieved by administration of anti-integrin (α4β7 and αEβ7) antibodies, when integrins were supposed to drive T cell adhesion in inflammatory conditions. We also demonstrated that lactoferrin-conjugated nanoparticles showed enhanced brain uptake via low-density lipoprotein receptor-related protein-mediated endocytosis, confirming selectivity of lactoferrin for the brain (unpublished data). To conclude, the advent of locked nucleic acid delivery could revolutionize gene therapeutics for brain disorders. Locked nucleic acids are a class of modified ribonucleic acids that can potentially improve the stability of DNAs and RNAs, thereby enhancing their bioavailability. Such agents, that can act as neuroprotectors, neuroproliferators, or both, can be tailor-made when coupled with targeted nanoparticles directing brain-specific delivery and effective therapeutics (Figure 3).

Conclusion
As the prevalence of the aforementioned neurological diseases increases, so too does the research now developing new therapeutic regimens. In the recent past, targeted delivery of nanoparticles to the brain has gained significant attention, as evidenced with the published data. After thorough investigation, nanotechnological administration has been found to be the most reliable mode of drug administration, given that the challenges of drug delivery to the brain are unmet by conventional therapeutics. Therefore, research into targeted nanoparticle delivery represents a significant area of interest for addressing future research questions.

Acknowledgments
The authors would like to thank the Australia-India Strategic Research Fund (BF 030016) and Deakin University for their financial support.

Disclosure
The authors report no conflicts of interest in this work.
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