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Scope and Applications of Nanomedicines for the Management of Multiple Sclerosis

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Abstract: Multiple sclerosis (MS) is characterized by inflammation, demyelination and lesions in the central nervous system (CNS) that results in the physical and cognitive disabilities in patients. Immune cells get access into the brain region, after infection in the blood brain barrier (BBB) due to bacteria/virus or by genetic predisposition, where the autoimmune response may induce the demyelination, inflammation as well as neurodegeneration in brain areas. Various types of therapeutics are used worldwide approved by the food and drug administration (FDA) for the management of MS. Hence, side effects of conventional therapy goes hand in hand. The advancement in nanomedicines have opened window for the management of various disorders of neurodegeneration including MS. Various clinical trials are in process to explore the etiology of MS and in this connection animal models like experimental autoimmune encephalomyelitis (EAE) have promising outcomes for the management of MS by using nanomedicines that give new insights. The current review elaborates the scope of nanomedicines with respect to MS patients.

Keywords: Blood brain barrier (BBB), central nervous system (CNS), experimental autoimmune encephalomyelitis (EAE), inflammation, demyelination, lesions, multiple sclerosis (MS), nanomedicine.

INTRODUCTION

Multiple sclerosis (MS) means many scars in the region of brain and spinal cord. It is generally accepted that MS is an autoimmune, inflammatory, neurodegenerative as well as demyelinating disorder of CNS [1, 2]. Studies have shown that researchers put forward many theories which explicate several mechanisms related to the disease progression including immune malfunctioning as a central role player as well as genetics [3]. In addition neurological symptoms, cognitive and physical disability are observed. Several experimental and clinical studies and reviews reflect that the pathophysiology of MS is very complex and not completely understood. One of the most important features in MS is autoimmune attack against the natural fatty insulating covering/layer around the axons of some neurons called myelin [2]. Prevalence of this disease ranges from 2 to 150 patients per 10,000 individuals [4]. Progressive and puzzling onset of MS in white matter appears in young individuals and more common in females [5].

Pathology and disease progression is highly unpredictable. Initially, MS is characterized by reversible neurological discrepancies while in chronic form it becomes irreversible as well as progressive neurodegenerative disorder having lesions in CNS. Progression of disease is considered to be associated with two critical mechanisms having both biochemical and molecular basis like destruction of myelin also called demyelination of neuronal tissues with lack of ability for remyelination and continuous damage of axons with almost no recovery of neuronal cell [6-8].

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According to neurobiologists MS patients can be categorized into four major groups based on severity of disease: 1) relapsing/remitting MS (RRMS), 2) primary progressive MS (PPMS), 3) secondary progressive MS (SPMS) and 4) progressive relapsing MS (PRMS) (Table **1**). MS could be diagnosed by lesions (scars) at least two in white matter, at least two episodes of disease progression and inflammation of CNS chronic in nature. Spectroscopic analysis and magnetic resonance imaging (MRI) are basic diagnostic tools for clinicians to observe the severity and condition of disease in MS patients [2, 9].

The aim of disease modifying agents and/or therapies is to lessen the immune response by targeting the immunological cascades which are responsible for the damaging of myelin sheath of neurons. In broader sense, there is need for management of MS patients not only to treat inflammation of disease but also the salvage of neurons to avoid neurodegeneration at cellular and subcellular levels as well as biochemical and molecular levels [10].

HUMAN AUTOIMMUNE DISORDER

MS is one of the various autoimmune disorders like type 1 diabetes mellitus, psoriasis, rheumatoid arthritis and others that share clinical, epidemiological therapeutic as well as genetic features. Genome-wide association studies (GWAS) revealed that multiple genetic pathways associated with the pathogenesis of autoimmune diseases are common. Moreover, GWAS helped in thoughtful insights in the genetic basis of multifaceted disorders. More than 200 gene loci have been found to be linked with autoimmune disorders like type 1 diabetes [11], systemic lupus erythematosus [12], rheumatoid arthritis [13, 14], Crohn's disease [15], psoriasis [16] and the most important according to the current review [17, 18]. Multiple intracellular signaling pathways are involved that regulates the

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Table 1. The four major groups based on the severity of MS

	Type	Description
1	Relapsing/remitting MS (RRMS)	Most common form, 85% of patients, marked by relapses
2	Primary progressive MS (PPMS)	10% of patients, no relapses, symptoms become worse gradually, resistant to drugs used to treat.
3	Secondary progressive MS (SPMS)	Develops in some patients, relapsing and remitting form, treated with disease modifying agents, if treated then progression is delay
4	Progressive relapsing MS (PRMS)	Rare form with <5% patients, progressive at start, worsening symptoms at irregular intervals, no time of remission

B and T-cells activation, mediation of innate immunity, microbial and viral responses, as well as signaling through cytokines and their receptors (Table **2**).

Immune system is divided into two main system; adaptive and innate systems which are highly interdependent and integrated at cellular, biochemical as well as molecular levels. Phylogenetically innate system is designed for immediate response against the pathogens by highly conserved pattern recognition receptors like toll-like receptors (TLR). On contrary, adaptive system consists of basically B and T cells having surface immunoglobulin and T-cell receptors respectively with genetically highly diverse receptor system, responsible for recognizing millions of distinct foreign antigens and also form immunological memory. Various genes and alleles are involved in the auto-reactive mechanisms of B and T cells, but they are kept in check by various processes. These processes are appeared by alteration in genetic loci involved in autoimmune mechanisms in biological system [19]. One such gene example is encoding protein tyrosine phosphatase non-receptor type 22 (*PTPN22*) [20]. We have listed various factors (Table **3**) that shows MS as an autoimmune disorder like other diseases.

Autoimmunity of CNS is regulated by various inflammatory cytokines like interferon-gamma (IFNγ) and interleukin-17 (IL-17) and opposing regulatory cytokines type-1 IFNs and IL-10. Moreover, experimental animal models have elaborated the central role of TLR in the pathogenesis of MS [29]. Immune system respond to the stimuli from environment or pathogens while enter into the biological system by lymphocyte activation through cellular receptors like TLR and maturing the antigen presenting cells (APCs) [30, 31] in normal and/or pathological state like MS. When the integrity of BBB is damaged in case of MS, the leukocytes like B cells, T cells, monocytes, dendritic cells (DCs), natural killer cells (NKs), CD8+ and CD4+, move towards the CNS and regulate the demyelination, damage of axon, neurodegeneration and ultimately death of neurons [32-34]. In case of MS, high expression of TLR in CNS region also involves the protection and restoration of neurons [35-37].

MYELIN SHEATH

Myelination is an essential process for the protection of axons in the neurons. It is a modified form of the cell membrane spirally arranged around the axon. Oligodendrocytes are responsible for synthesis of myelin sheath in the neurons of CNS, while in neurons of peripheral nervous system (PNS) Schwann cells are responsible for the synthesis of myelin sheath. The gaps in myelin are called "nodes of Ranvier" where sodium channels are concentrated for the regulation of electrical impulses. Myelin also has some flanking membranous loops (paranodes) where potassium channels are concentrated in adjacent juxtaparanodal region of the axon. These regions and channels are responsible for rapid saltatory conduction.

Chemically, it consists of lipids and protein (lipoprotein) contents. The distinguishing feature of myelin is high lipid to protein ratio as compared to other biological membranes. Distribution of lipids and proteins (integral and/or peripheral) depends upon the relative molecular weight, hydro- or lipophilicity and charge upon them. The most abundant intrinsic protein in myelin sheath is myelin proteolipid protein (PLP), while myelin basic protein is extrinsic in nature. Moreover, myelin oligodendrocyte glycoprotein (MOG), myelin associated glycoprotein (MAG) as well as 2'3'-cyclic-nucleotide 3'-phosphodiesterase (CNP) are also found but in lesser quantity. Cholesterol, glycosphingolipids and phospholipids, with constant molar proportions 2:1:2, are components of lipids in myelin sheath. Cholesterol and sphingomyelin are responsible for the formation of domain regions in membrane called lipid rafts that are sites of signal transduction [38, 39]. Lipid composition in CNS has been studied for MS to understand the defects in lipid during the progression of disease [40]. Moreover, anti-lipid antibodies have become focus of scientists during the progression of MS [41].

NANOMEDICINES AND MULTIPLE SCLEROSIS

Therapeutic approaches using nanotechnology have been an interesting field for the management of various diseases. Varieties of nanomedicines have been approved by FDA and many of them under the status of clinical trials as well as in the course of experimental models for the evaluation of behavior and metabolism in biological system. The aim of using nanomedicines is programmed drug delivery or targeted drug delivery at the site of interest in biological system, where conventional medicines are unable to reach like in brain through BBB. Moreover, scientists are working on the animal models using nanomedicines for neuronal enhancement as well as neuroprotection in case of brain disorder like Alzheimer's (AD), Parkinson's (PD) and MS. Dr. Pearse and coworkers are working on site-directed nanotherapeutics to abrogate RRMS and repair of myelin sheath and in long-term goal to develop a multifunctional nanoparticle (NP) approach for halting the inflammation and demyelination [42].

In the year 2003, liposomes were prepared by a research group with diameter of 90 to 100nm for encapsulation of prednisolone (a drug used for MS) and were injected intravenously in animal model (mice) with autoimmune encephalomyelitis (model for MS using animals). The liposomes were concentrated to high level in CNS within 2 hours of administration, showing that liposomes crossed the BBB. Moreover, drug-loaded liposomes exhibited reduced in-

Table 2. Association of molecular signals in autoimmune diseases like MS

Chromosome	Candidate gene	Possible function	Disease
6	Major histocompatibility complex	Antigen presentation, disease specific association signals	Most autoimmune disorders
19	Tyrosine protein kinase 2 (TYK2)	Downstream of cytokine receptor (Janus kinase)	MS
10	Interleukin-2 receptor, subunit α (IL2RA)	Interleukin-2 receptor signaling	MS
5	Interleukin-7 receptor, (IL7R)	Activation and differentiation of T cells	MS

Table 3. Shared factors between MS and other autoimmune disorders

Sr. No.	Shared features between MS and other disorders	References
1	An human leukocyte association (HLA)	[21]
2	Presence of oligoclonal auto-reactive B and T cell expansion	[22, 23]
3	Association of auto-antibodies	[24]
4	Therapeutic effectiveness of corticosteroids	[25]
5	Plasmapheresis	[26]
6	Anti-lymphocyte globulin	[27]
7	Cure by lymphoid excision	[28]
8	Cure by autologous haematopoietic cell transplantation	[28]

flammation, reinstatement of BBB integrity and alleviation of macrophage permeation. It concluded that such treatment to be better one to the administration of free glucocorticosteroid (conventional treatment for MS) [43].

Targeting Strategy by Nanomedicines

The targeting strategy by NPs in which receptor-specific ligand binding is involved helps in enhanced penetration through damaged BBB as well as also facilitates the understanding of bioavailability, pharmacokinetics and pharmacodynamics of brain related medicines [44, 45]. As compared to the issues related to conventional liposomes, the PEGylated liposomes help in better transport through BBB by receptor-mediated mechanisms [46]. Moreover, nanoliposomes, consisting of monoclonal antibodies and/or human insulin receptors help the nanomedicines to cross the BBB as well as escaping the complex mechanism of pericytes, endothelial cells and astrocytes (Reticulo-Endothelial system, RES) and delivering therapeutic genes of interest [47, 48].

For nanotechnologies to be useful in targeting the neuronal tissues, three main features must be in consideration;

1) After the systemic administration of nanomedicines, it must find BBB without affecting the other cells of biological system,
2) It must be able to cross the BBB and
3) Must be able to target the specific cells in brain after crossing the BBB as well as release of drug molecules. PEGylated NPs showed the ability to cross BBB and capable to release drug molecule in animal models of Parkinson's disease [49, 50].

Stability and Biocompatibility of Nanomedicines

In the nervous system, cell gradually lose their regenerative ability following *in vivo* damaging. Nanomedicines are designed in such a way that they support the stability and biocompatibility of drug molecule as well as assist to pass through BBB, results in safe transferring of drug molecule at damaged site of brain. BBB could be seen along the capillaries with tight junctions around the capillaries in brain [51]. Endothelial lining does not allow the diffusion of micro-organisms and large hydrophilic molecules into the cerebrospinal fluid (CSF). On the other hand, small hydrophobic molecules like hormones, O_2 and CO_2 are allowed to diffuse [52]. The association between BBB damaging and MS progression could be observed due to molecular and histopathological changes among the patients.

Conjugation of Drug Molecules

Conjugation of drug molecules (antibodies, peptides) to the surface of nanomedicines helped in targeting the site of interest in biological system particularly in brain pathology. Such techniques may be used for targeting lesions with growth factor, neuroprotection at site of sclerotic lesions and imaging in MS [53]. Presence of BBB and complex endothelial cell system including pericytes and astrocytes has been a challenge for delivery of conventional drug to CNS disorders. The use of nanomedicines with site-specific ligand binding enhances biocompatibility and penetration in cell medium by crossing the cell membrane and other barriers like BBB in CNS.

NPs have multiple physico-chemical characteristics and exhibit electrical, chemical and mechanical features, one such example is

carbon nanotubes (CNTs). These particles (nanotubes) are considered better option for using in the field of neurobiology, having the ability to stimulate neurons as well as recording ability for activity of neurons [44-55]. Another type of nanoparticle (polymeric shell) enhances drug release by avoiding nonspecific interactions with serum proteins, non-targeted cells and enzymes of biological system and drug is released from NP by diffusion process at specified target [56]. Using polymeric NPs glatiramer acetate as an active ingredient has been formulated by Teva Pharmaceuticals, Tikva, Isreal, for the management of RRMS [57].

Neuroprotection

Neuroprotection is crucial concept for recovery or regeneration of neurons after their dysfunctioning and/or death in case of chronic CNS damaging that could be observed in MS, AD and PD [58, 59]. Many nanomaterials exhibit antioxidant properties to eliminate the reactive oxygen species (ROS) in various cells and tissues including brain region. NPs like cerium oxide (CeO_2) and yttrium oxide (Y_2O_3) have ability to alleviate ROS in *in vitro* environment using hippocampal neuronal cells [60]. However, diffusion of drug via BBB in all neurodegenerative disorders including MS is major challenge. Moreover, inflammation of neurons is another undeniable aspect in MS patients and experimental models exhibit shared relationship among the T cells, astrocytes and microglia other than demyelination of neuronal cells in CNS. Therefore, targeting the inflammatory neurons in advanced stages of MS showed promising strategy using NPs [61-63].

MRI and NPs have been used in biomedical applications for prognosis and diagnosis of diseases, in which iron particles act as contrast agents. Particles are coated with biocompatible layer/coating to lessen the toxicity of particles and enhancing their imaging probing sensitivity. One such example is superparamagnetic iron oxide (SPIO) particles that are used in MRI for studying the destiny of transplanted cells in biological system [64]. Dextran-coated SPIO ferumoxide NPs have been approved by FDA as intravenous contrast agents. SPIO-labeled cells have been used for the study of bio-distribution of cells after transplantation into CNS to evaluate the treatment of neurological disorders like MS. Animal models of EAE has been used to evaluate the physiological response of biological systems when labeled cell of glial- committed neuronal precursor cells (NPCs) with SPIO. It was observed that labeled cells were migrated as well as differentiated into glial cell lineages in the same way as unlabeled cell [65]. It is known fact that SPIO labeling NPCs are not affected to perform their physiological function and showed their continued existence and differentiation within EAE brain region [66, 67]. One thing very crucial about NPCs is they have immune-modulating features that are neuroprotective in nature [68, 69].

EAE and MS

EAE is an experimentally induced inflammatory and demyelinating disease that behaves like relapsing MS in human and at the same time exhibit ROS mediated damage and destruction of neurons [70-74]. Infiltration of Th1 cells into brain region via damaged BBB, leads to secretion of IFN-γ that activates macrophages which generate inflammatory cytokines, tumor necrosis factor-α (TNF-α) and ROS in neuronal tissues. EAE model in animals may not expresses all the pathological features of the human disease MS, but this model enabled the researchers to develop the currently available therapies for MS. Avonex and Betaferon, which are glatiramer acetate which are immune modulator and beta interferon that may shift T-helper cells from Th1 inflammatory to Th2 phenotype. Moreover, GEMSP, liposomes having tempamine and edavarone act as antioxidants and could be used in MS patients as co-therapy for the elimination of ROS [75-79].

Toxicological Idea

The toxic nature of NPs in the form of cytotoxicity has been studied by various researchers [80-82]. The toxic effect of NPs could be analyzed by studying the uptake mechanisms, transport within the biological system and exposure rate of NPs as well as inflammation. The idea of toxicity is associated with metallic surfaces of NPs without biocompatible corona. Those particles covered with organic biocompatible material are relatively much less toxic. However, particles having layer of starch have shown reduction in ATP contents, mitochondrial dysfunction, DNA damage, production of reactive oxygen species (ROS) as well as cell cycle arrest [83].

CONCLUSION

Physical and cognitive abnormalities are appeared in the patients suffering from MS due to the inflammation, demyelination and lesions in the CNS. Neurobiologists have divided the MS patients into four groups depending upon the severity of disease. Damage in the BBB leads to the neurodegenerative disorders. Conventional therapies are not better option for the management of MS, while advancements in nanomedicines have opened new horizons for the management of brain disorders like MS. Various researches have employed the animal models to investigate the multiple aspects of MS. However, nanoparticles-based treatment has been proved better management option for MS patients. Neurobiologists are working on MS to make better lifestyle of the patients.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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