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ARTICLE

Nanoscience in Multiple Sclerosis

Nitisha Gurav and Susmit Mhatre

Department of Pharmaceutical Sciences and Chemistry, Institute of Chemical Technology, Matunga, Mumbai-400019, India

Multiple Sclerosis (MS) – the most common autoimmune disease of the central nervous system – is traditionally diagnosed by methods mainly using magnetic resonance techniques to detect the lesions. Use of nanoparticles as the contrast agents can help in better diagnosis of the damaged cells. Iron nanoparticles are used in various advanced techniques like superparamagnetic iron oxide nanoparticles (SPIONs) and ultra-small SPIONs (USPIONs). The major challenge in treatment of MS is the delivery of the drug into the brain, crossing the blood brain barrier (BBB). Nanoparticles like liposomes, nanoshells, dendrimers, nanogels, micelles have potential applications in the same. Presently, no significant treatment is devoid of side effects like fever, headache and fatigue, Use of nanoscience in MS in drug delivery and treatment can help solve the prevailing inadequacies. Administered quantum dots conjugated with self-antigens act on lymph nodes and spleen. These assemblies produce regulatory T-cells which prevent degeneration of myelin sheath. New studies study modifications to produce inflammation-resistant myelin by inducing response in lymph nodes during T-cell priming. This review aims to briefly describe the application of nanotechnology in diagnosis, drug delivery and treatment of MS.

1. Introduction

Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system. Inflammation in the central nervous system and plaque of demyelinated neurons are the major indications of MS. It is an autoimmune disease involving the innate immune system i.e. T cells and B cells. Innate immunity plays a vital role in the onset and progression of the disease. T cells have specific myelin protein targets. Presence of different T lymphocytes can indicate attack on specific myelin proteins[1,2]. MS has affected over two million individuals and the count increases. Since its discovery in the 19th century, there has not been any significant development in the clinical management of MS until last 50 years. In the initial stages, MS diagnosed by the medical history of the patient. Recently, blood tests and lumbar puncture tests were used for the diagnosis of MS. The development of magnetic resonance imaging (MRI) greatly simplified the diagnosis of this neurodegenerative disease. Traditional MRI techniques are used for early diagnosis and estimation of its progression and the response of the cells to the treatment. After newer techniques like MR spectroscopy, magnetization transfer imaging, MS has been better studied[3,4]. However, the diagnosis as well as treatment methods are insufficient as they are partially effective and cannot reduce the progress rate of disease.

Nanotechnology deals with materials of particle size of about 1-100 nm. Nanomedicine is the application of nanomaterials in biological systems. Its physicochemical properties offer a hope in developing new therapeutic methods in diagnosis and treatment of several diseases[5]. This review focuses on the application of nanotechnology and nanosciences in multiple sclerosis for diagnosis, drug delivery and treatment.

2. Diagnosis

Diagnosis of multiple sclerosis is difficult due to the nonspecific nature of this disease. A combination of various tests and imaging techniques are generally used for diagnosis. However, they lack sensitivity, and permeability through the blood brain barrier (BBB) and decrease half-life after administration of the diagnostic agent intravenously[6]. The ability of conventional imaging techniques to detect lesions depends upon the dosage of contrasting agent, the imaging parameters and the field strength[7,8,9]. Nanoparticles are nanoscale solid colloidal particles composed of polymers or solid lipids. Those particles with size greater than 100 nm are used for drug delivery. The techniques generally used for diagnosis of such diseases are positron emitted tomography (PET), MRI and single photon emission computed tomography (SPECT). The use of nanoparticles might improve the neuroimaging power if better contrasts and better targeted molecular imaging probes are developed[10,11,12,13,14,15].

The use of nanotechnology in diagnosis of the cellular inflammation is mainly seen in development of new contrast agents which have better reach and activity owing to their small size. The common markers used to detect cellular

inflammation in MS are quantum dots, superparamagnetic iron oxide nanoparticles (SPIONs) and gadolinium-DTPA (Gd-DTPA). Gold nanoparticles (AuNPs) and Silver Nanoparticles (AgNPs) are used in the detection and amplification of molecular signals. For early detection of MS, radio diagnoses such as MRI and SPION are most common in which these contrast agents can be used to detect weak signals even at molecular concentrations. Localized Surface Plasmon Resonance (LSPR) is a cheaper and ultrasensitive technique involving Silver nanoparticles (AgNPs). In this method, the changes in the refractive index of the magnetic field due to interactions AgNPs with protein marker. Scanning tunneling microscopy-based technique can detect concentrations as low as 10fg/mL by indications in pulse-like peaks of tunnel microscope. Gold nanoparticles (AuNPs) conjugated with proteins can detect concentrations as low as 1pg/mL[16].

Latest research also reveals that ultra-small superparamagnetic particles of iron oxide nanoparticles (USPION) can monitor cell infiltration more accurately over traditional methods. In-vivo techniques have already proven that USPIONs work on CD4+ lymphocytes cell mostly of midbrain and interbrain and CD8+ cells of brainstem region. These USPIONs conjugate with anti-CD4 antibodies and detect pathological regions in MS when injected intravenously in rat brain.

2.1. SPIONs

SPIONs are based on core of magnetite (Fe_3O_4) or maghemite (γ-Fe_2O_3) stabilized with a hydrophilic surface coating[17,18,19]. These nanoparticles are not only biocompatible but also show useful physicochemical properties. These particles contain only a single domain and hence exhibit superparamagnetism[20]. Their properties enable them for their applications in MRI as contrast agents and in multimodal imaging, etc. SPIONs have been in use as contrast agents for MRI for last many years. They have been classified into Standard SPIONs (150-380 nm), very small SPIONs (< 10nm) and ultrasmall SPIONs (10-50 nm) based on the particle size[21]. SPIONs are produced by thermal decomposition of iron salts[22], then are coated with surface complexing agents, and then prevented from agglomeration. SPIONs are injected intravenously to detect the lesions formed in the liver whereas USPIONs, due to their higher plasmic life, their uptake is not as fast as SPIONs, and they can reach macrophages or normal cells in affected tissues[23,24,25,26]. Human transferrin proteins coupled with SPIONs were injected into tumour bearing rats and a 40% change in the signal intensity was observed[27]. Another study was conducted by MRI and electron paramagnetic resonance (EPR) on SPIONs injected in the inflamed muscles. The mean iron oxide concentration in inflamed cells was 0.8% and 0.4% of the dose injected initially. When the same study was done by EPR, it was observed that concentration of the grafted USPIONs was higher by two times in the muscles than the ungrafted ones. Hence, association of SPIONs helps in enhancing the sensitivity of inflamed cell detection. SPIONs are phagocytosed by the macrophages when administered into systemic circulation. Hence, they show their paramagnetic effect by MRI in CNS disorders like MS.

3. Drug Delivery

The blood brain barrier (BBB) is a semipermeable system composed of endothelial cells of brain capillaries, astrocyte foot process and pericyte. Endothelial cells of capillaries in the blood differ from normal capillaries in presence of tight junctions. Astrocyte and pericyte ensure the transfer of essential components like glucose, amino acids including some gases, lipophilic molecules with molecular weight < 600 Dalton. Hydrophilic or larger molecules are prohibited to enter central nervous system and cerebrospinal fluid. Hence crossing the BBB becomes a major challenge in the treatment of multiple sclerosis[28].

Another important factor in selection of suitable drug delivery systems like liposomes is a novel drug carrier system offering a potential approach to resolve this problem.

3.1. Liposomes

Liposome is a sphere of concentric lipid bilayers with an internal aqueous cavity which traps the drug. After several years of research, liposomes have been found to have drug carrying capacities. The structural property of liposome forming barrier between the drug and the non-targeted tissue helps in effective formulation and administration of toxic drugs. Liposomes can penetrate through the blood brain barrier via various mechanisms i.e. triggered drug release, cationation of vectors, targeting ligands etc.[29]. Liposomal complexes of doxorubicin, anthracycline derivative have been tested. Mitoxantrone, an immunosuppressant, can be a suitable candidate for use of liposomal preparations in the treatment of MS[30]. Recently studied glucocorticoids loaded in nano-sterically stabilized liposome (nSSL) showed a therapeutic efficiency in the Proteolipid Protein (PLP) induced Experimental Autoimmune Encephalomyelitis (EAE). In animal models, recovery from acute disease was faster when treated with nSSL than with the MS drugs, Betaferon and Copaxone[31].

Antibodies against Myelin basic protein (MBP) are considered as cause of MS[32]. Mannosylation of liposomes loaded with MBP fragments decrease the rate of anti-MBP antibodies and facilitates their uptake through mannose receptors present on macrophages[33]. Targeting ability of liposomes is enhanced by conjugating its surface with some ligands like glucose, lactoferrin, transferrin, specific peptides etc. Surface modified liposomes efficiently cross the BBB and are able to deliver the drug at the particular site. This advanced nanotechnological approach has proven to reduce the progress rate of MS by preventing plaque formation and removing the already formed amyloid deposits in preclinical studies[34].

3.2. Nanocapsules and Nanospheres

Nanocapsules and nanospheres are systems of size range 10-1000 nm[35]. They are composed of natural nanoparticles like chitosan, alginate and/or synthetic nanoparticles like poly(lactide-co-glycolide) (PLGA), poly-lactic acid (PLA), polymethacrylic acid (PMA) and polyethylene glycol (PEG)[36]. Nanocapsules are thin polymeric envelopes enclosing oil-filled cavities while nanospheres have solid core with a dense polymeric matrix[37]. They are highly stable, easy to synthesize and can escape without being recognized by macrophages. In application, indomethacin-loaded nanocapsules were found to protect in-vitro hippocampal cultures against inflammation[38].

3.3. Dendrimers

Dendrimers are polymeric, three dimensional, monodisperse and highly branched materials which can entrap and/or conjugate with active molecules. Dendrimers show high water solubility, stability, permeability of drug[39,40], biocompatibility[41], polyvalency[42] and precise molecular weights[43], which makes them an ideal carrier for drug delivery. Various types of dendrimers such as polyamidoamine (PAMAM), poly(propylene imine) (PPI), poly-L-lysine, melamine, poly(etherhydroxylamine) (PEHAM), poly(esteramine) (PEA) and polyglycerol have been synthesized and studied as drug delivery vehicles[44,45]. Neutral G3-G4 phosphate dendrimers are recently explored for their ability to reduce secretion of proinflammatory cytokines from mice and human monocyte-derived macrophages[46]. To increase the activity of therapeutic agents in brain tissue, dendrimers are conjugated with CNS-targeting molecules such as transferrin[47,48], lactoferrin[49], D-glucosamine[50] and DGL-PEG-leptin30[51]. *In vitro* and *in vivo* studies have demonstrated the low toxicity of dendrimers[52]. Dendrimers can act on both astrocytes and microglial cells which are involved in inflammation in diseases like MS[68].

The activation and proliferation of CD4+ T lymphocytes in Interleukin-2 (IL-2) stimulated Peripheral Blood Mononuclear Cells (PBMC) is inhibited by phosphorus-containing dendrimers with an N_3P_3 (cyclotriphosphazene) core and PMMH (phenoxymethyl-methylhydrazine) branches[53,54].

3.4. Micelles

Micelles are spherical arrangement of lipid molecules of 20 - 200 nm due to the amphipathic nature of lipids composed of polyethylene glycol (PEG), polypropylene glycol, etc. In normal micelles, hydrophobic tails are arranged inside and hydrophilic heads form the outer surface. Micelles may have reverse structure, i.e. hydrophobic core and hydrophilic surface, in water-in-oil conditions[53].

3.5. Nanogels

Nanogels are cross-linked networks which can encapsulate oligonucleotides, siRNA, DNA, proteins, and low-molecular-mass drugs delivering them across the BBB. The drug-loading capacity of nanogel is up to 40–60%[54]. *In vivo* studies suggested that nanogels increased brain uptake of oligonucleotides while decreasing uptake in the liver and spleen[55,56]. Poly N-isopropylacrylamide (PNIAM) is a thermoresponsive polymer which has water retaining capacity. Hydrogel formed with PNIAM has been studied successfully in Zebrafish model for sustained drug release and compatibility. PNIAM hydrogel is a functionalized nanogel loaded with donepezil with polysorbate-80[57].

3.6. Pomegranate Seed Oil

PSO has higher levels of a unique polyunsaturated fatty acid, punicic acid. Punicic acid is a natural antioxidant. Its higher concentrations are needed for its antioxidant action. Nanodroplet formulation of PSO, labelled as nano-PSO has been tested in mice induced for EAE and gives desirable results at relatively lower concentrations reducing the oxidation of lipids and demyelination in EAE mice. An addition of other antioxidants and beta-sitosterol enhanced the activity over individual ingredients as it accumulates in plasma membrane of brain cells. Nano-PSO can be used in combination with other MS medications such as natalizumab[58,59].

4. Treatments

Nanoscience is a promising approach in treatment of MS[60]. Nanoparticles can be readily synthesized at laboratory scale. They can carry nano medicines to the site of action by protecting

the degeneration of the API, enhancing its bioavailability[61]. It has gathered attention as it involves action at the cellular and subcellular levels. Cytobots and karybots are some examples of synthetic magnetic nanomaterials which work on electrochemical principles and interact directly with the lesions in the nervous system formed during MS[62,63]. Due to nanotechnological advancements like quantum dots used in nanoimaging, surgical tweezers, and AFM tips as sharp needle nano scissors, newer approaches like single cell surgery are also possible[64].

Nanoparticles are mainly applied in two ways – by neuroprotection or as anti-inflammatory agents, or both[65] (e.g. fullerene and its derivatives are used as neuroprotective anti-inflammatory agents). Gold NPs stabilized with a layer of PEG molecule administered in mice has been shown to attenuate disease and population expansion of regulatory T-cells.

Nano-suspensions and nanoemulsions are well-known for their target drug delivery by establishing an interaction with the cell membrane[69]. Few nanoparticles can also permeate through the gaps present between the blood brain barrier by transcytosis[70,71]. Carbon nanotubes and nanowires are being used for neural regeneration by showing cellular signal transmission[72].

4.1. Force Tip Microscopy

The AFM tip can directly penetrate through the cell membrane into the nucleus, with the membrane returning to its original shape. This technique can deliver specific monoclonal antibodies (MAB) by coating it onto the tip which will interact with the intracellular protein traffic and track the cell in real time to study its chemistry[60].

4.2. Exosomes

Exosomes are RNA or proteins that are derived from and are created by the dendrites which help in regeneration of myelin sheath[73]. These are similar to liposomes but enriched with adhesive molecules, cytoplasmic enzymes, signal transduction, functional mRNA microRNA. Being natural particles, they have an edge over artificial NPs. Exosomes are capable of transferring specific immunosuppressive molecules to the brain[74,75]. Serum exosomes also increase the myelin content, oligodendrocyte-precursor cell and neural stem cell levels[76].

4.3. Cerium Oxide NPs

A new study suggests that the combination of lenalidomide and cerium oxide nanoparticles reduced demyelination[66]. Cerium oxide NPs fluctuate between +3 and be +4 valence states. It has recently been shown that the intravenous administration of cerium oxide NPs into mice reduced reactive oxygen species levels and disease attenuation. Thus, cerium oxide nanoparticles may be effective in the MS therapy in part through reduction of oxidative stress process[67], which is the primary observation in any neurodegenerative disease. They either receive or donate an electron and hence reduce ROS and enhance brain activity[77,78].

4.4. Nanocurcumin

Curcumin has been studied for its pharmacological activities like its anti-inflammatory, anti-tumor, antifungal, antibacterial, antioxidant and antiprotozoal activity[79,80,81]. Even though curcumin has a large variety of therapeutic activities, its use was limited due to its poor solubility and bioavailability. Addition of dendrosome nanoparticles unfolds its application. Polymerised nano curcumin (PNC) has been studied in EAE model; it inhibits neuroinflammation by blocking cytokines pathway. Initial PNC administration has reduced development of EAE score while daily dose decreased the relapsing symptoms. According to recent studies, nanocurcumin can be a massive treatment to inhibit disease progression in MS patients[82,83,84]. Curcumin is a polyphenolic non-enzymatic antioxidant which removes free radicals[82]. Its use is limited due to insolubility in body fluids, low intrinsic activity and rapid clearance from the body and overall low bioavailability. To overcome these problems, a number of solutions have been examined by adjuvants, liposomes and other NPs[85].

5. Conclusion

In the last few decades, a lot of new studies have been conducted to find out new potential methods for drug delivery in MS. The applications of nanotechnology in MS have resolved the traditional problems, and promise better imaging, drug delivery and treatment. Use of these nanomaterials will not only improve the efficiency of the therapeutic applications but also provide an easy, cost-efficient and potentially a widely accepted method for the same. These techniques can track a particular disease at the cellular or molecular level. Nanosurgery and nano neuroprotection have gained a lot of attention due to some promising applications. However, there are still some challenges to fully study the implications of nanomaterials and a lot of research on their application in disease diagnosis and treatment has to be done. Their safety should also be evaluated.

6. Future Scope

There have been a lot of developments in drug delivery and treatment of MS in last few years. Many new drugs have been developed and approved in diagnosis and treatment of MS but still a lot needs to be done. The use of nanoscience has greatly influenced the methods by which we can treat MS and in upcoming years with more advancement we can develop better models. The development of nanodrug carriers has allowed better targeted action of the drug, site specificity, real time monitoring, effective action and higher sensitivity, which are some advantages that we have already achieved. There are still many limitations on the application of nanotechnology in neuroscience and one must also study the potential hazards of the administration and safety of nanomedicines. The upcoming decade will hold a lot of progress in nanotechnology and its applications is a wide range of diseases.

7. Keywords

Multiple sclerosis, nanoparticles, blood brain barrier, quantum dots.

8. References

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