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Research Article

NANO DRUG DELIVERY SYSTEM IN TREATMENT OF AMYOTROPHIC LATERAL SCLEROSIS

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A B S T R A C T

Amyotrophic lateral sclerosis (ALS) is a common late-onset neurodegenerative disorder that affects motor neurons. There is no conclusive etiology of ALS, though approximately 2% of cases have been linked with mutant Cu, Zn-superoxide dismutase (SOD1). A plethora of mechanisms that contribute to ALS disease progression have been discovered in the last two decades, including nonneuronal cell signaling, activation of the apoptotic cascade, and excitotoxicity. Though conclusive diagnosis of ALS remains elusive and though there is still no known effective treatment for this disease, continuously exploring the pathology would provide insight into the creation of novel technologies for the treatment and diagnosis of ALS. In this review, we provide an overview of hypothesized mechanisms and possible biomarkers for ALS pathology, in the first section. In the following section, we introduce recent nano/biotechnological approaches in studying disease mechanism and developing diagno-therapeutic methods for ALS.

INTRODUCTION

The prefix "nano" has found in last decade an ever-increasing application to different fields of the knowledge. Nanoscience, nanotechnology, nanomaterials or nanochemistry are only a few of the new nano-containing terms that occur frequently in scientific reports, in popular books as well as in newspapers and that have become familiar to a wide public, even of non-experts [1]. The prefix comes from the ancient Greek Vavo through the Latin nanus meaning literally dwarf and by extension, very small. Nanotechnology is a barrier of biological and physical sciences by applying nanostructures and nanophases at various fields of science, especially in nanomedicine and nano based drug delivery systems [2]. Nanomaterials can be well-defined as a material with sizes ranged between 1 and 100 nm, which influences the frontiers of nanomedicine starting from biosensors, microfluidics, drug delivery and microarray tests to tissue engineering. Nanoparticle-based medicine has been developed by combining both the treatment and imaging modalities of disease diagnosis. The very first generation of nanoparticle-based therapy included lipid systems like liposomes and micelles, which are now FDA-approved [4]. Nanodrugs show higher oral bioavailability because they exhibit typical uptake mechanisms of absorptive endocytosis [3].

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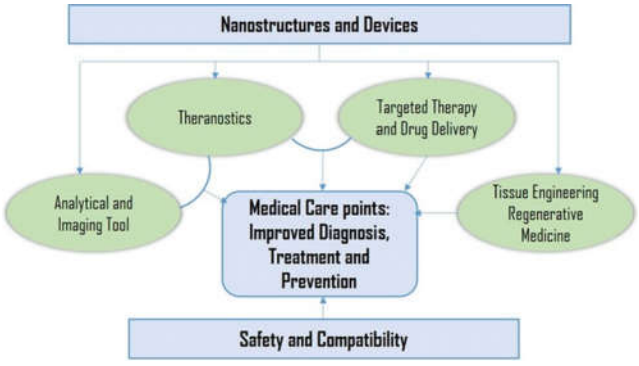

Fig No. 1 Nanostructures and devices

Fundamentals of nanotechnology based techniques in designing of drug

Nanomedicine is the branch of medicine that utilizes the science of nanotechnology in the preclusion and cure of various diseases using the nanoscale materials, such as biocompatible nanoparticle and nanorobots for various applications including, diagnosis, delivery, sensory or actuation purposes in a living organism [5]. Drugs with very low solubility possess various biopharmaceutical delivery issues including limited bio accessibility after intake through mouth, less diffusion capacity into the outer membrane, require more quantity for intravenous intake and unwanted after-effects preceding traditional formulated vaccination process [6]. However, all these limitations could be overcome by the application of nanotechnology approaches in the drug delivery mechanism[7]. This can consequently lead to the improvement

and development of convenient administration routes, lower toxicity, fewer side effects, improved bio distribution and extended drug life cycle. The engineered drug delivery systems are either targeted to a particular location or intended for the controlled release of therapeutic agents at a particular site [8]. Their formation involves self-assembly where in well-defined structures or patterns spontaneously are formed from building blocks. Additionally they need to overcome barriers like opsonization/sequestration by the mononuclear phagocyte system [9].

There are two ways through which nanostructures deliver drugs: passive and self-delivery. In the former, drugs are incorporated in the inner cavity of the structure mainly via the hydrophobic effect [10]. When the nanostructure materials are targeted to a particular sites, the intended amount of the drug is released because of the low content of the drugs which is encapsulated in a hydrophobic environment. Conversely, in the latter, the drugs intended for release are directly conjugated to the carrier nanostructure material for easy delivery [11]. In this approach, the timing of release is crucial as the drug will not reach the target site and it dissociates from the carrier very quickly, and conversely, its bioactivity and efficacy will be decreased if it is released from its nanocarrier system at the right time [14].

Targeting of drugs is another significant aspect that uses nanomaterials or nanoformulations as the drug delivery systems and, is classified into active and passive. In active targeting, moieties, such as antibodies and peptides are coupled with drug delivery system to anchor them to the receptor structures expressed at the target site[13]. In passive targeting, the prepared drug carrier complex circulates through the bloodstream and is driven to the target site by affinity or binding influenced by properties like pH, temperature, molecular site and shape [15]. The main targets in the body are the receptors on cell membranes, lipid components of the cell membrane and antigens or proteins on the cell surfaces. Currently, most nanotechnology-mediated drug delivery system is targeted towards the disease and its cure [12].

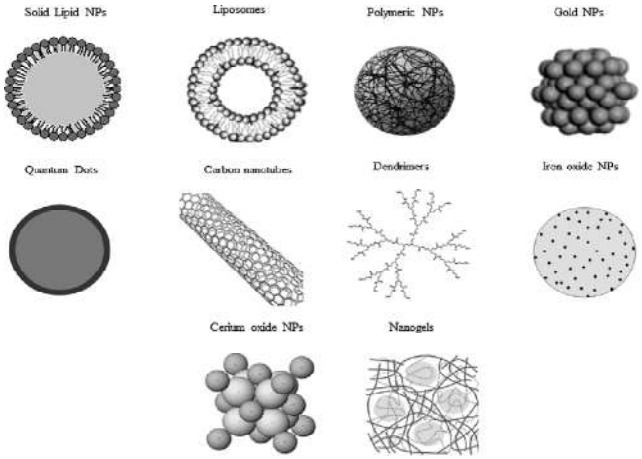

Fig No 2 Different type of nanomaterials for biomedical use. Nanomaterials are commonly defined as objects with dimensions of 1-100 nm, which includes nanogels, nanofibers, nanotube and nanoparticles [17]

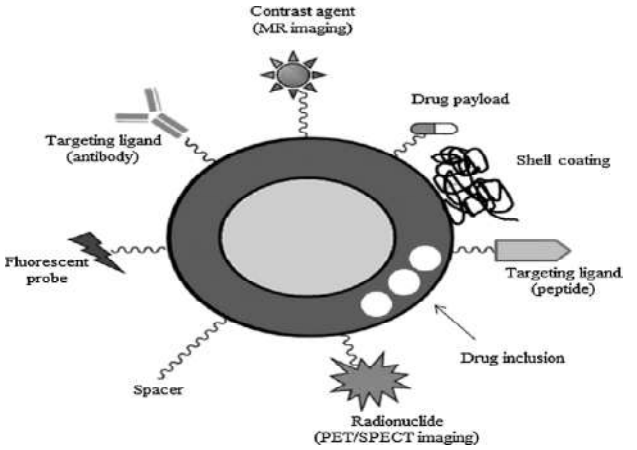

Fig No 3 Structure and targets of nanoparticles [16]

Multi-functionalized nanoparticles: Examples of multifunctional nanoparticles (NP) for (i) molecular imaging, functionalized with contrast agents for MRI or with radionuclides for PET/SPECT imaging; (ii) drug delivery, functionalized with a drug incorporated within the core of NP or conjugated to the surface; (iii) fluorescence detection [18], functionalized with a fluorescent probe; (iv) enhanced half-life in blood circulation, functionalized with a shell coating (e.g. with PEG); (v) specific targeting [19], functionalized with specific ligand molecules. Optionally, spacer/linker molecules could be used.

Polymers used in Nanoparticles

The integration of therapy and diagnosis is defined as theranostic and is being extensively utilized for disease treatment. Theranostic nanoparticles can help diagnose the disease, report the location, identify the stage of the disease, and provide information about the treatment response [20]. In addition, such nanoparticles can carry a therapeutic agent for the tumor, which can provide the necessary concentrations of the therapeutic agent via molecular and/or external stimuli [21].

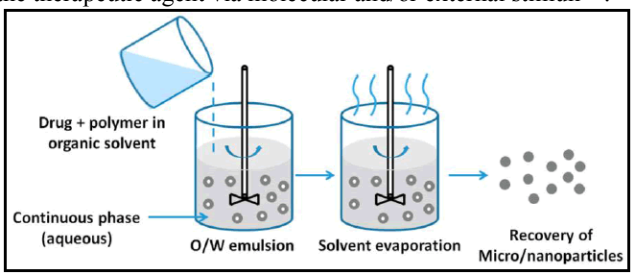

Fig No 4 Solvent evaporation technique [99]

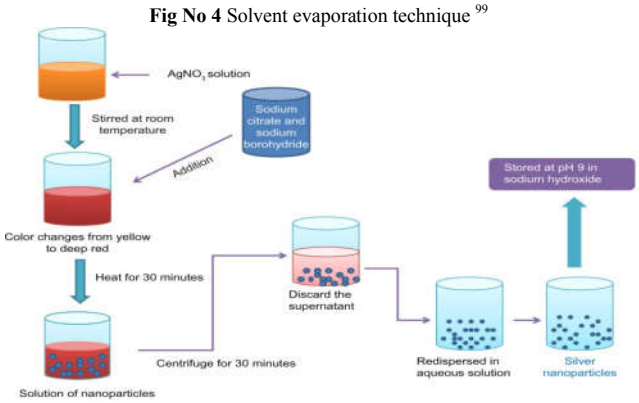

Fig No. 5 Silver nanoparticle formation [100]

Chitosan is a biopolymer which possesses distinctive properties with biocompatibility and presence of functional groups. It is used in the encapsulation or coating of various types of nanoparticles [22], thus producing different particles with multiple functions for their potential uses in the detection and diagnosis of different types of diseases [100].

Another option that can be used is alginate, which is a natural polymer derived from the brown seaweed and has been expansively scrutinized for its potential uses in the biomedical field because of its several favorable characteristics [23], such as low cost of manufacture, harmonious nature, less harmfulness, and easy gelling in response to the addition of divalent cations [25]. Alginate nanogels act as contrast-enhancing agents and can be assumed as an appropriate material for pharmacological application. Also, the polymeric material dextran is a neutral polymer and is assumed as the first notable example of microbial exopolysaccharides used in medical applications [24]. A remarkable advantage of using dextran is that it is well-tolerated, non-toxic, and biodegradable in humans, with no reactions in the body. Photodynamic therapy is a site-specific disease cure with less damage to other healthy cells.

Drugs used to treat ALS	category	Role for treatment in ALS	Polymers used
Arimoclomal	experimental drug developed by CytRx Corporation	Protects against neuronal dysfunction and cell death	
Talampanel	An AMPA glutamate receptor antagonist	talampanel is thought to prevent glutamate from triggering the death of motor neurons.	
Masitinib	tyrosine kinase inhibitor (TKI)	It is an oral therapy that targets cells of the immune system called the mast cells and macrophages. It works by blocking the activation of proteins called tyrosine kinases, which are thought to play a role in inflammation and chronic inflammatory states.	
Nimesulide	anti-inflammatory drug (NSAID)		
diazoxide	Gynecological anti infectives and antiseptics, Hypoglycemic agent.	low doses of diazoxide improve symptoms of ALS and extend the survival rate in transgenic mouse models for ALS.	
Bromocriptine	Hyper prolactemia,hypogonadism.	neuroprotection, sustained motor function and slowed disease progression in mouse models of amyotrophic lateral sclerosis (ALS) .	

Different Drugs used for the treatment of ALS:

Amyotrophic Lateral Sclerosis (ALS)

Also known as motor neuron disease (MND) or Lou Gehrig's disease, is a specific disease that causes the death of neurons controlling voluntary muscles. Some also use the term motor neuron disease for a group of conditions of which ALS is the most common [27]. ALS is characterized by stiff muscles, muscle twitching, and gradually worsening weakness due to muscles decreasing in size. It may begin with weakness in the arms or legs, or with difficulty speaking or swallowing. About half of the people affected develop at least mild difficulties with thinking and behavior and most people experience pain. Most eventually lose the ability to walk, use their hands, speak, swallow, and breathe [26].

Causes

ALS is inherited in 5 to 10 percent of cases, while the rest have no known cause.
Researchers are studying several possible causes of ALS, including: [28]

- ***Gene mutation.*** Various genetic mutations can lead to inherited ALS, which causes nearly the same symptoms as the noninherited form.
- ***Chemical imbalance.*** People with ALS generally have higher than normal levels of glutamate, a chemical messenger in the brain, around the nerve cells in their spinal fluid. Too much glutamate is known to be toxic to some nerve cells.
- ***Disorganized immune response.*** Sometimes a person's immune system begins attacking some of his or her body's own normal cells, which may lead to the death of nerve cells [29].
- ***Protein mishandling.*** Mishandled proteins within the nerve cells may lead to a gradual accumulation of abnormal forms of these proteins in the cells, destroying the nerve cells[99].

Risk Factors

Established risk factors for ALS include:[30]

- ***Heredity.*** Five to 10 percent of the people with ALS inherited it (familial ALS). In most people with familial ALS, their children have a 50-50 chance of developing the disease.
- ***Age.*** ALS risk increases with age, and is most common between the ages of 40 and 60. after age 70.
- ***Genetics.*** Some studies examining the entire human genome (genome wide association studies) found many similarities in the genetic variations of people with familial ALS and some people with non inherited ALS. These genetic variations might make people more susceptible to ALS.
- Environmental factors may trigger ALS. Some that may affect ALS risk include:
- ***Smoking.*** Smoking is the only likely environmental risk factor for ALS. The risk seems to be greatest for women, particularly after menopause. [98]
- ***Environmental toxin exposure.*** Some evidence suggests that exposure to lead or other substances in the workplace or at home may be linked to ALS. Much study has been done, but no single agent or chemical has been consistently associated with ALS.
- ***Military service.*** Recent studies indicate that people who have served in the military are at higher risk of ALS. It's unclear exactly what about military service may trigger the development of ALS. It may include exposure to certain metals or chemicals, traumatic injuries, viral infections, and intense exertion [31].

Pathophysiology of Als

The defining feature of ALS is the death of both upper motor neurons (located in the motor cortex of the brain) and lower motor neurons (located in the brainstem and spinal cord). In

ALS with frontotemporal dementia, neurons throughout the frontal and temporal lobes of the brain die as well [32]. The pathological hallmark of ALS is the presence of inclusion bodies (abnormal aggregations of protein) in the cytoplasm of motor neurons. In about 97% of people with ALS, the main component of the inclusion bodies is TDP-43 protein; however, in those with *SOD1* or *FUS* mutations, the main component is SOD1 protein or FUS protein, respectively [34]. The gross pathology of ALS, which are features of the disease that can be seen with the naked eye, include skeletal muscle atrophy, motor cortex atrophy, sclerosis of the corticospinal and corticobulbar tracts, thinning of the hypoglossal nerves (which control the tongue), and thinning of the anterior roots of the spinal cord [33]. Besides for the death of motor neurons, two other characteristics common to most ALS variants are focal initial pathology, meaning that symptoms start in a single spinal cord region, and progressive continuous spread, meaning that symptoms spread to additional regions over time. Prion-like propagation of misfolded proteins from cell to cell may explain why ALS starts in one area and spreads to others [35].

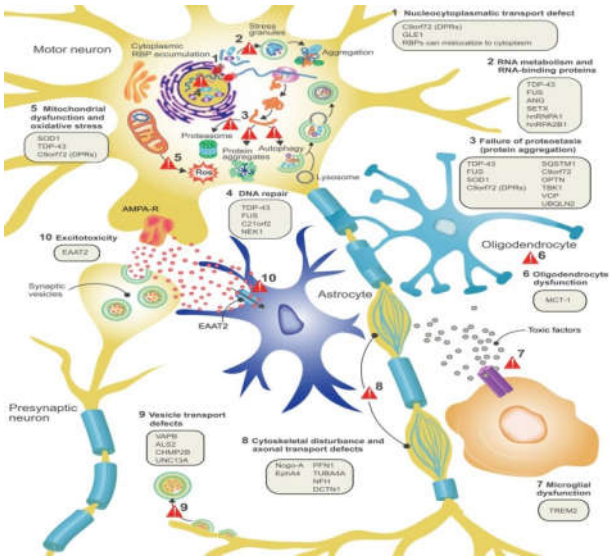

Fig. No. 6 Pathophysiology of ALS [40]

It is still not fully understood why neurons die in ALS, but this neurodegeneration is thought to involve many different cellular and molecular processes The genes known to be involved in ALS can be grouped into three general categories based on their normal function: protein degradation, the cytoskeleton, and RNA processing. Mutant SOD1 protein forms intracellular aggregations that inhibit protein degradation [36]. Cytoplasmic aggregations of wild-type (normal) SOD1 protein are common in sporadic ALS It is thought that misfolded mutant SOD1 can cause misfolding and aggregation of wild-type SOD1 in neighboring neurons in a prion-like manner Other protein degradation genes that can cause ALS when mutated include *VCP*, *OPTN*, *TBK1*, and *SQSTM1*[37]. Three genes implicated in ALS that are important for maintaining the cytoskeleton and for axonal transport include *DCTN1*, *PFN1*, and *TUBA4A* [38].

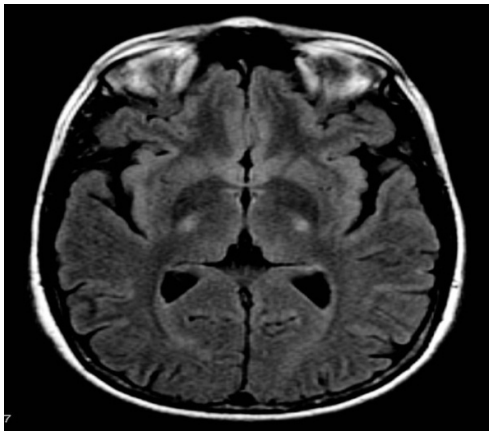

Fig No 7 MRI (axial FLAIR) demonstrates increased T_2 signal within the posterior part of the internal capsule, consistent with the diagnosis of ALS. [39]

There are a number of ALS genes that encode for RNA-binding proteins. The first to be discovered was TDP-43 protein, a nuclear protein that aggregates in the cytoplasm of motor neurons in almost all cases of ALS; however, mutations in *TARDBP*, the gene that codes for TDP-43 [41], are a rare cause of ALS. *FUS* codes for FUS, another RNA-binding protein with a similar function to TDP-43 [97], which can cause ALS when mutated It is thought that mutations in *TARDBP* and *FUS* increase the binding affinity of the low-complexity domain, causing their respective proteins to aggregate in the cytoplasm [42]. Once these mutant RNA-binding proteins are misfolded and aggregated, they may be able to misfold normal protein both within and between cells in a prion-like manner This also leads to decreased levels of RNA-binding protein in the nucleus, which may mean that their target RNA transcripts do not undergo the normal processing [43]. Other RNA metabolism genes associated with ALS include *ANG*, *SETX*, and *MATR3* [44].

C9orf72 is the most commonly mutated gene in ALS and causes motor neuron death through a number of mechanisms [45]. The pathogenic mutation is a hexanucleotide repeat expansion (a series of six nucleotides repeated over and over); people with 30 repeats are normal, while people with hundreds or thousands of repeats can have familial ALS, [46] frontotemporal dementia, or sometimes sporadic ALS. The three mechanisms of disease associated with these *C9orf72* repeats are deposition of RNA transcripts in the nucleus, translation of the RNA into toxic dipeptide repeat proteins in the cytoplasm, and decreased levels of the normal C9orf72 protein [47].

Excitotoxicity, or nerve cell death caused by high levels of intracellular calcium due to excessive stimulation by the excitatory neurotransmitter glutamate, is a mechanism thought to be common to all forms of ALS [48]. Motor neurons are more sensitive to excitotoxicity than other types of neurons because they have a lower calcium-buffering capacity and a type of glutamate receptor (the AMPA receptor) that is more permeable to calcium. In ALS, there are decreased levels of excitatory amino acid transporter 2 (EAAT2), which is the main transporter that removes glutamate from the synapse; this leads to increased synaptic glutamate levels and excitotoxicity. Riluzole [49], a drug that modestly prolongs survival in ALS, inhibits glutamate release from pre-synaptic neurons; however, it is unclear if this mechanism is responsible for its therapeutic effect [96].

Symptoms

Early signs and symptoms of ALS include [50]:

- Difficulty walking or doing your normal daily activities
- Tripping and falling
- Weakness in your leg, feet or ankles
- Hand weakness or clumsiness
- Slurred speech or trouble swallowing
- Muscle cramps and twitching in your arms, shoulders and tongue
- Difficulty holding your head up or keeping good posture [95]

ALS often starts in the hands, feet or limbs, and then spreads to other parts of your body. As the disease advances and nerve cells are destroyed, your muscles progressively weaken. This eventually affects chewing, swallowing, speaking and breathing.

Diagnosis

Amyotrophic lateral sclerosis (ALS) is difficult to diagnose early because it may mimic several other neurological diseases. Tests to rule out other conditions may include: [51]

- Electromyogram (EMG): The test evaluates the electrical activity of muscles when they contract and when they're at rest.
- Nerve conduction study.
- Magnetic resonance imaging (MRI)
- Blood and urine tests
- Spinal tap (lumbar puncture) [52]
- Muscle biopsy. If doctor believes you may have a muscle disease rather than ALS, you may undergo a muscle biopsy.

Treatment

Two medications are currently approved by the Food and Drug Administration for the treatment of ALS [94].

Riluzole (Rilutek)

Riluzole is used to treat amyotrophic lateral sclerosis. Riluzole delays the onset of ventilator-dependence or tracheostomy in some people and may increase survival by two to three months. Riluzole is available in tablet and liquid form[53].

This drug appears to slow the disease's progression in some people, perhaps by reducing levels of a chemical messenger in the brain (glutamate) that's often present in higher levels in people with ALS [54]. Riluzole is taken as a pill and may cause side effects such as dizziness, gastrointestinal conditions and liver function changes.

Mechanism of action

Riluzole preferentially blocks TTX - sensitive sodium channels, which are associated with damaged neurons. Riluzole has also been reported to directly inhibit the kainate and NMDA receptors [55]. The drug has also been shown to postsynaptically potentiate $GABA_A$ receptors via an allosteric binding site. However, the action of riluzole on glutamate receptors has been controversial, as no binding of the drug to any known sites has been shown for them. In addition, as its antiglutamatergic action is still detectable in the presence of sodium channel blockers, it is also uncertain whether or not it acts via this way. Rather, its ability to stimulate glutamate uptake seems to mediate many of its effects. In addition to its role in accelerating glutamate clearance from the synapse, riluzole may also prevent glutamate release from presynaptic terminals [56]. These effects combined could significantly reduce glutamate signaling and cause indirect antagonism without acting at glutamate receptors themselves [93].

Adverse Effects: [57]

- Very common (>10% frequency): nausea; weakness; decreased lung function
- Common (1-10% frequency): headache; dizziness; drowsiness; vomiting; abdominal pain; increased
- amino transferases.
- Uncommon (0.1-1% frequency): Pancreatitis, interstitial lung disease.
- Rare (<0.1% frequency: Neutropenia; allergic reaction (including Angioedema, anaphylactoid reaction). [92]
- Symptoms of overdose include: neurological and psychiatric symptoms, acute toxic encephalopathy with stupor, coma and methemoglobinemia.
- Severe methemoglobinemia may be rapidly reversible after treatment with methylene blue.

Further studies

A number of recent case studies have indicated that riluzole may have use in mood and anxiety disorders[91]. A reformulation of riluzole that originated at Yale University and is known by the code name BHV-0223 is under development for the treatment of generalized anxiety disorder and mood disorders now by Biohaven Pharmaceuticals [58].

Edaravone (Radicava)

The FDA approved edaravone in 2017 based on six-month clinical trial that showed it reduced the decline in daily functioning associated with ALS [60]. The drug is given via intravenous infusion (typically 10-14 days in a row, once a month), and side effects may include bruising, gait disturbance, hives, swelling and shortness of breath. Edaravone is used to help people recover from stroke in Japan, and is used to treat ALS in the US and Japan [59].

It was approved for ALS in the US in 2017 based on a small randomized controlled clinical trial with people who had early-stage ALS in Japan, who were administered the drug for 6 months [64]; it had failed two earlier trials in people with all stages of ALS. It is given by intravenous infusion. [90] There is no data on whether it is safe for pregnant women to take, and it is unknown if Edaravone is secreted in breast milk. [61]

Edaravone contains sodium bisulfite, which may cause serious allergic reactions in people with sulfite sensitivity. Edaravone [62], sold as under the brand names Radicava and Radicut, is an intravenous medication used to help with recovery following a stroke and to treat amyotrophic lateral sclerosis [63].

The label carries a warning about the potential for hypersensitivity reactions to edaravone, and adverse effects include bruising, gait disturbances, headache, skin inflammation, eczema, problems breathing, excess sugar in urine, and fungal skin infections [65].

Mechanism of action

The mechanism by which Edaravone might be effective in ALS is unknown. The drug is known to be an antioxidant, and oxidative stress has been hypothesized to be part of the process that kills neurons in people with ALS [66].

The half-life of Edaravone is 4.5 to 6 hours and the half-lives of its metabolites are 2 to 3 hours. It is metabolized to a sulfate conjugate and a glucuronide conjugate, neither of which are active. It is primarily excreted in urine as the glucuronide conjugate form [67].

Adverse effects[68]

- Hypersensitivity reactions
- bruising,
- gait disturbances,
- headache,
- skin inflammation, [90]
- eczema,
- problems breathing,
- excess sugar in urine, and
- fungal skin infections.

Therapeutical Care: [69]

- Breathing care -
  Doctors insert a tube in a surgically created hole at the front of your neck leading to your windpipe (tracheostomy), and the tube is connected to a respirator. [70]
- Physical therapy
- Occupational therapy
- Speech therapy
- Nutritional support
- Psychological and social support
- Potential future treatments

Research Models For ALS

Fig No. 8 Some of the most common models used to study ALS. [71]

Many different organisms are used as models for studying ALS, including *Saccharomyces cerevisiae* (a species of yeast). *Caenorhabditis elegans* (a roundworm), *Drosophila melanogaster* (the common fruit fly), *Danio rerio* (the zebrafish), *Mus musculus* (the house mouse), and *Rattus norvegicus* (the common rat).None of these models perfectly represents ALS in humans, [72] partly because most animal models are based on gene overexpression, meaning that multiple copies of the mutant human gene are inserted into the transgenic model, and partly because the human nervous system is very different from that of other animals [89].

The first animal model for ALS was the *SOD1*^{G93A} transgenic mouse, which was developed in 1994 [73]. It expresses about 20-24 copies of the mutant human *SOD1* gene and reproduces most of the clinical and pathological findings seen in ALS [74].

Although there are now over 20 different *SOD1* mouse models, the *SOD1*^{G93A} model remains both the most widely used *SOD1* mouse model and the most widely used ALS mouse model overall. Much of the present understanding of ALS pathophysiology came from studying mouse models that overexpress mutant *SOD1*, especially *SOD1*^{G93A} mice. However, many drug targets that were shown to be effective in the *SOD1*^{G93A} transgenic mouse failed in clinical trials in humans; other *SOD1* models have had similar problems [76]. Most of these drugs were identified as potentially effective based on a single study in a rodent *SOD1* model and then failed in clinical trials in patients who primarily had sporadic ALS. It is thought that these clinical trials failed because *SOD1* mutations account for only 2% of all ALS cases and because the pathology of *SOD1* ALS is thought to be distinct from all other types of ALS; it lacks the abnormal aggregations of TDP-43 protein and/or FUS protein seen in nearly all other cases of ALS [75].

As of 2018, there are about 20 *TARDBP* mouse models, a dozen *FUS* mouse models, and a number of *C9orf72*, *PFN1*, and *UBQLN2* mouse models [77]. There are also new methods of developing animal models, including viral transgenesis, in which viruses are used to deliver mutant genes to an animal model, and CRISPR/Cas9, which can be used to give an animal model multiple mutated genes. Both of these methods are faster and cheaper than traditional methods of genetically engineering mice; they also allow scientists to study the effects of a mutation in mice of different genetic backgrounds, which better represents the genetic diversity seen in humans [79].

Cellular models used to study ALS include the yeast *Saccharomyces cerevisiae* and rat or mouse motor neurons in culture. Small-animal models include the fruit fly, the roundworm *C. elegans*, and the zebrafish. Of the three, the fruit fly is the most widely used; it has a rapid life-cycle, short lifespan, a sophisticated nervous system, and many genetic tools available [78]. *C. elegans* has a short life-cycle, is easy to manipulate genetically, and has a simple but well-understood nervous system. The zebrafish has transparent embryos that can be injected with DNA or RNA and has a lifespan of up to two years. Induced pluripotent stem cells (iPSCs) can be used to convert skin fibroblasts into motor neurons. It is now possible to generate iPSCs from people with ALS, which can then be converted into spinal motor neurons, which are useful for studying disease mechanisms and for testing potential drugs for ALS. iPSCs allow sporadic ALS to be modeled, which cannot be done with animal models [80].

Nanotechnology for Amyotrophic Lateral Sclerosis

ALS is a fatal ND affecting 1-2/10, 0000 person-year. The hall-mark of the disease is the selective death of motor neurons in the brain and spinal cord, leading to paralysis of voluntary muscles [81]. Approximately 20% of familial ALS cases are caused by mutations in SOD1 gene, encoding superoxide dismutase enzyme. Mutated SOD1 generates toxic free radicals. Additionally, mutant SOD1 forms intracellular deposits that inhibit chaperone and/or proteasome activity [85], with subsequent misfolding and insufficient clearance of numerous proteins. Only one report has been published concerning the preparation of solid lipid NP containing riluzole for the specific treatment of ALS. These NP showed high drug loading, a greater efficacy than free riluzole, a high capability to carry the drug into the brain and a lower

indiscriminate biodistribution in rats, opening the way to the use of NP for ALS therapy. Recently, MRI has been utilized to follow Tcells labeled ex vivo with USPIO NP and injected in a rat model of ALS. The study revealed an infiltration of CD4+ lymphocyte in the midbrain/interbrain[82], while CD8+ cells were more confined to the brainstem region. Machtoub *et al*. injected i.v. USPIO NP conjugated with anti- CD4 antibodies, and were able to detect the pathological regions in ALS rat brain [84].

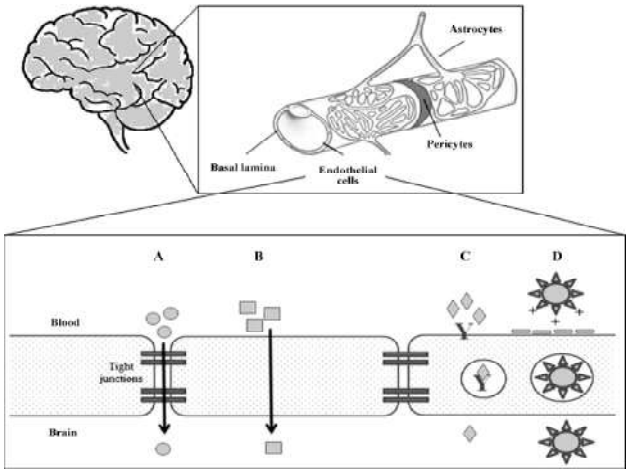

Fig No 9 Blood-brain barrier. Graphical representation of the endothelial cells that form the blood-brain barrier (BBB) and their associations with pericytes and the astrocyte end-processes of brain capillaries. The main routes for molecular traffic across the BBB are shown: (A) paracellular aqueous passage; (B) transcellular lipophilic pathway; (C) receptor-mediated transcytosis; (D) adsorptive-mediated transcytosis. [83]

Future prospects

Scientists are unraveling molecular, cellular and circuit functions of the nervous system and are identifying genes and pathways that cause neurodegeneration. In the last years, revolutionary pro-gresses resulted from the development of nanotechnology, opening the way for a nano-based therapy and diagnosis of ND [86]. However, more investigation will be necessary in this field to allow the trans-lation from preclinical to concrete clinical applications. An intriguing challenge will be the use of NM for combined ther-apy and diagnosis strategies (theranostics) [87]. For this purpose, the most suitable NP are currently magnetic ones, that may be utilized for MRI, targeted drug and gene delivery, tissue engineering and cell tracking, for their unique ability to be guided by an external magnetic field [88]. Given the exponential growth of nanotechnologies, novel tools will emerge, triggering the development of new insights in ND treatment and diagnosis.

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