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Amyotrophic Lateral Sclerosis: Innovative Therapies for ALS under the Pipeline

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Review Article

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Abstract

Over the last 5 decades, a multiple of experimental drugs compounds have been shown to dissuade disease progression in preclinical animal models of amyotrophic lateral sclerosis (ALS) but failed to show any efficacy in human clinical trials or are still waiting for approval under Phase I–III trials. Only 2 main drug compounds are discovered till date and approved by USA Food and Drug Administration for ALS treatment that show better efficacy, effective against ALS progression in early stages and enhances the survival rate of patients. The riluzole is a glutamatergic neurotransmission inhibitor and edaravone is act as an antioxidants. Various clinical trials carried out for ALS treatment but do not show any effective pharmacodynamic and pharmacokinetic data. We required further study on genetics and pathophysiology of ALS that associated with progression of disease. In this review, we focused on pathological aspects and some important drug molecules that participate in clinical trials.

Keywords: Motor neuron disease (MND); Superoxide dismutase 1 (SOD1); TDP43; Frontotemporal degeneration; Motor Neuron Disease; Reactive oxygen species

Introduction

The Amyotrophic lateral sclerosis (ALS) is a motor neuron disorder in which completely loss of motor neurons in CNS, both upper motor neurons (UMN) and lower motor neurons (LMN) [1]. This is involved in the dysfunction of motor neurons and it also known as Motor neuron disease (MND). In this type of disorder, firstly we observed some physical changes in patient's body like as-fasiculations, muscle weakness, muscle atrophy spasticity and hyper reflexia, along with time lead to paralysis and after that patients die due to respiratory failure within 3-4 years [2]. The main etiology of this disorder is not known but some selected factors are responsible for this disorders such as-Dominant genetic mutation and some environmental factors. This disorder is blocking the some important pathways that are essential for the muscles and neurons functions includes astrogliosis, microgliosis, mitochondrial dysfunction, defects in axonal transport and RNA binding protein processes etc [3,4]. there are two main forms of ALS- Familial ALS (FALS) and Sporadic ALS (SALS), the 90% cases of sporadic ALS and remaining 10% of familial ALS are seen and no evidence for hereditary genetic [5]. The diagnosis of ALS pathogenesis is a critical task in developing stage, but some common methods used for the understanding of ALS pathogenesis such as-molecular mechanisms and genetics [6].

Epidemiology

According to recent epidemiological research, the ALS has existed many different subtypes of stain; these subcategories are based on the phenotype and genetics.

Mostly patients with ALS have ubiquitinated inclusions that stain for trans active response (TAR) DNA-binding protein 43 (TDP43) [7,8]. In 1992, the ALS was very rare about 1.5 to 2.7 per 100,000 in Asia and in 2008 the no. of cases are reported between 1.4 to 1.8 per lakh, no prevalence in disease. This disorder is mostly affected to male than the female (1.5:1 ratio) [9]. The main reason in male excess is possible protective hormonal factors in woman those protect from this disorder. Through recent study, the mortality rate of this disease rise from 1.54 to 2.55 per lakh/year [10]. Although most cases of ALS are sporadic, about 5% of cases have a family history of ALS (fALS) [11].

Factors Associated with ALS

The ALS is a type of disorder that related to the some important factors and these factors are the affect the motor neuron activity/efficacy like as heavy metals, agriculture chemicals, solvents, physical activity, type of diet, dust/fibers/fumes, and electrical magnetic fields etc. These factors are increases the developing risk of ALS [12].

Chemical Exposure and Heavy Metals: The chemicals and heavy metals are associated with the inducing of ALS, the agricultural pesticides/chemicals like as pesticides, fertilizers, insecticides and herbicides are commonly associated with the risk of ALS [13]. If any person's regularly expose these chemicals last 5 years, so induced the risk of acquiring ALS and the death rate are more than two times compare to unexposed persons [13]. Other than pesticides, the heavy metals like as lead are associated with ALS, when exposed in high concentration. The lead are indirectly associated with mitochondria, strengthen glutamate's and oxidative damage those connected with ALS disorder [14]. The manganese metal have neurotoxic properties because they cross the every barrier system at choroid plexus and deposit in the central nervous system. In cerebrospinal fluid (CSF)sample of ALS patients, concentration of manganese were significantly elevated (5.67 mg/L)compared with normal person's (2.08 mg/L).The welders exposed high concentration of manganese through the breathings [15]. Other heavy metals that associated with this disorder and all significantly elevated concentration in CSF in ALS patients, such as-aluminum, cobalt, arsenic, cadmium, copper, zinc, vanadium and uranium [16].

Smoking: The smoking enhances the chances of ALS due to some factors like as- oxidative stress, inflammation and neurotoxicity, because it contained dangerous heavy metals that affect the motor neuron functions [17]. Those people start the smoking in younger age, ALS risk are highest. Hike the mortality rate in ALS patients due to inhaled the cigarette smoke because it contains formaldehyde that induces the glycolysis and glutathione export in neural cells. Mostly, it changes the metabolism and oxidative stress in brain and may cause neurodegenerative disorders [13].

Physical Activity: Athletes have higher ALS risk compared to the normal population; however, performing passive to concretion physical activity has not shown an increased susceptibility of developing ALS. Some common genes that related with exercise (leukemia inhibitory factor, ciliary neurotrophic factor and vascular endothelial growth factor 2) and these are a risk factor for ALS [18]. But, some studies show the invalidating results about the ALS risk, because the only physical activity is not proven a main cause of ALS, in this type of patients, the genetic profile is responsible for ALS risk. The genetic profile is responsible for physical fitness but not muscles strength [19].

Radiation/electromagnetic fields: When a person's performed their work in a chemical laboratory where electromagnetic waves surrounded it. If they expose very low frequency electromagnetic waves, so generates cellular reactive oxygen (CRO) in very large quantity in own body, and these excessive quantity of CRO produce oxidative stress. Due to this, disable the antioxidant property of cells and it susceptible for ALS [20]. The electromagnetic waves is responsible for breaking DNA strands into brain cells, leading to apoptosis and necrosis and these events are responsible for ALS like risk [21]. Other than radiations, the daily diet are affect the functions of neurons, according to previous studies, those person's that consuming high quantity of glutamate and fat in their diet these affect easily with ALS like symptoms due to the selective neuron death because the overstimulation of glutamate receptor that increase the Ca^{2+} intracellular, and while those consume the high Omega3 fatty acids, Vitamin E, and fibers show the defensive effect against ALS [22].

Symptoms of ALS

The Amyotrophic Lateral Sclerosis (ALS) is a neurological disorder in which damage the functional activity of both neurons, upper motor neuron (UMN) and lover motor neuron (LMN). Some common features that associated with ALS patients like as [23,24]-

- Difficulties in speech and swallowing
- Muscles weakness
- Wasting of Muscles
- Muscle atrophy
- Fasciculation
- Flail arm and flail leg

Molecular Mechanism

Various intra-cellularly mechanism that responsible for degeneration of motor neurons in ALS. The common cause

of ALS is a mutation of gene misreading the antioxidant enzyme superoxide dismutase 1 (SOD1).These SOD1 is show the structural instability in mutated enzyme, lead to death of motor neuron in CNS [25,26]. Instead of this various other biochemical factors which cause ALS like symptoms such as- mitochondrial dysfunction, impaired axonal structure, glutamate excite-toxicity and free radical-mediated oxidative stress etc. These all factors are considered secondary events in ALS generation [27].

Structural and Functional Abnormalities of mitochondria: Mitochondria act as a powerhouse of every cells of body that responsible for converting energy into ATP, these form of energy is required for the metabolism of the cells. The mitochondria are performing the main role in regulating the neurons and other important events in cell. The mitochondrion is a cell membrane bound organelles and it perform the important role in the regulating of cell functions. Some important function of mitochondria are- Cellular respiration, Intracellular energy production, Calcium homeostasis and control of apoptosis etc [27]. According to previous study, if abnormalities in mitochondrial function, i.e. morphological and biochemical, lead to cause of ALS. In ALS patients, observed the changes in morphology of mitochondria of skeletal muscle and spinal motor neurons like as- Magnify cristae, Swelling, Breakage network and abnormally release of neurotransmitters [28]. After carrying autopsy of ALS patients, researchers observed the mutant SOD1 is accumulate at the cytoplasmic surface of mitochondria that responsible for the transport of proteins and nucleotides. After increasing the misfolded mutant SOD1 in spinal cord mitochondria is considered as the main reason for mitochondrial dysfunction that leads to abnormal functioning of ATP production, calcium homeostasis, axonal transport of mitochondria, and apoptotic triggering [29]. Other than ATP generation, the mitochondria help in the regulation of cytosolic calcium level, because the dysregulation of Ca2+ in cytosole lead to neuronal death and pathogenesis of ALS. The calcium is act as intracellular messenger that responsible for the regulation of metabolic pathways, neurochemical transmitter and new neuron generation. Several studies have shown that cytosolic calcium is misregulated in ALS patients. The lower level of calcium in cytosolic contents, it can main factor of neurons damage [30].

Glutamate excitotoxicity: The glutamate is an anions of glutamic acid and it act as a neurotransmitter that used for the signals transmission in nerve cells. Its synthesized in the presynaptic cleft of neurons and it release during the neurotransmission. After releasing neurotransmitters from the synaptic cleft, it activates the postsynaptic receptors that present on postsynaptic neurons [31]. After bursting of synaptic cleft, the release of neurotransmitters, several glial cells and proteins (as excitatory amino acid transporters). Through the release of glutamate, the concentration gradient balance are maintain and prevent the induction of neuronal damage, because the several studies explain, the level of glutamate/astroglalial cells at neuronal junction are very less in spinal cord and motor cortex of ALS patients [32]. Due to excess extracellular glutamate, over stimulating the glutamate receptors which lead to increase the influx of calcium and motor neurons. The high content of calcium at extracellular region, activate the Ca^{2+} dependent enzymatic pathways which helpful for the generation of free radicals. The various glutamate related changes in the biochemical process of cells that cause sporadic type ALS [33].

Changes in axonal structure and transmission process: The motor neurons are major site for reflex action. It has long axons that responsible for the transmission of nerve impulses, RNA, proteins, lipids and other related neurotransmitters. The neuronal transmission through the soma is also called as retrograde while transmission through the synaptic part of neuromuscular junction is called anterograde. The mutant SOD1 is affect the both process of transmission (as retrograde and anterograde). The deregulation of axonal transport is performed a key role in path physiology of ALS [34]. Some important pathways that alter the axonal transport system those relate with mutant SOD1 such as- excitotoxic damage by glutamate, mitochondrial dysfunctions and dissociation of kinesin function through tumor necrosis factors etc. Abnormal axonal transport is responsible for the accumulation of neurofilaments, mitochondria and autophagosomes in degenerated motor neurons and it leads to motor neurons death [35-37]. The reactive oxygen species (ROS) is a byproduct of normal metabolism of oxygen. The reactive oxygen species (ROS) are accumulating in intracellular region of a cell and damage the physiology of cells. The oxidative stress is a difference between the production of ROS and cell antioxidant defenses. When the excess amount of ROS is accumulate in cell it causes changes in normal physiology of cell and due to this effect decrease the defensive ability of cells [38].

Genetic Causes of Amyotrophic Lateral Sclerosis

The both types of ALS are associated with genetic mutations. About 20% cases of ALS with autosomal dominant familial ALS are cause due to the mutations in particular genetic loci and less than 3% cases of sporadic ALS show mutations in the Copper-Zinc superoxide dismutase (SOD1) gene. The antioxidant functions of SOD1 are altering through the gain of toxin and get mutation in genes and produces ALS like symptoms [39]. The various other genes that associated with the causing of motor neuron disease (MND) such as – ALS in (ALS2), TAR DNA binding protein 43 (TDP-43), Vesicle associated membrane protein (VAPB), fusion in sarcoma

(FUS), optineurin (OPTN), dynactin (DCTN1) etc [40-43]. Through the TARDBP mutation approx 5-10% cases are observed of FALS. Both TDP-43 and FUS mutations are show the 5% cases of FALS. Remaining 1% cases are observed by the ANG mutation. Approximately, 50–60% of FALS cases have mutations arising from the 19 genes that have been identified to date [44,45].

Treatment of ALS

Due to clinical and molecular anisomelia of Amyotropic lateral sclerosis and frontotemporal degenration (FTD), a very challenging task for development an effective therapy/treatment. Some different cases, a common class of drugs like as selective serotonin reuptake inhibitors (SSRI) and antipsychotic drugs are useful for the management of mood and behavior [46,47]. Various drugs are used for the clinical trial but mostly decline under phase I to III clinical trials, recently several new potential drugs are being tested in phase I to III clinical trials [47].

At this time only two drugs are available, riluzole and Edaravone. In 1995, the riluzole was approved by the FDA for clinical use on ALS patients. The riluzole shown the 38.6% reduction in mortality rate and 35% improve the survival rate with 100 mg dose. The riluzole are specially show the neuroprotective effect and it mainly block the glutamate receptors [48]. Although it mostly block the voltage dependent Na^+ channels and signal transduction events [49]. After that in 2017, a second drug (Edaravone) was approved for clinical use by FDA of USA. The edaravone is a neuroprotective agent that shown the antioxidant properties and prevent the progression of ALS events. In a double-blind, placebo controlled, Phase 2 study using I.V. Edaravone therapy in ALS patients, Akimoto showed a decrease in primary endpoint in the Revised ALS Functional Rating Scale (ALSFRS-R) scores from baseline to 24 weeks after screening [50].

Drugs on the Pipeline

For finding new reliable medication for the treatment of different motor neurons diseases/ALS various clinical trials are progressed. Many different approved drugs that are used for the treatment of various diseases, currently, these drugs are used in clinical development for ALS.

Triumeq: The Triumeq is an antiretroviral drug that used in the treatment of HIV infection. The Triumeq is now investigated in a Phase IIa open-label study to determine its safety and tolerability in Motor Neuron Disease (MND)/Amyotrophic Lateral Sclerosis (ALS) patients. [Clinicaltrial.gov NCT02868580] Observe under clinical trials, it is an integrate strand transfer and nucleotide reverse transcriptase inhibitors. After autopsy observation, the level of reverse transcriptase in serum of ALS patients was same as HIV infected patients [51].

Tirasemtiv: Mostly in ALS patients the muscles degeneration are common due to neuromuscular blockage, so researchers found a new medicine that improve the muscular functions and enhance physical activity. Tirasemtiv is a medicine that regulates muscles contraction because it enhances the calcium releasing rate from troponin C [52,53]. After showing to be safe and tolerable drug for ALS patients, FDA and EMA designated to be an orphan drug (ORPHA2999528). According to this, it's approved for phase III study called VITALITY-ALS at international level (Clinicaltrial.gov NCT02496767). The enrollment for this trial was ended in mid-2016 and results are expected in 2017. This clinical trial was essential for the estimation of longterm safety and tolerability among the patients.

Arimoclomol: Arimoclomol is a small hydroxylamine molecules that discovered by Hungarian researchers for treatment of diabetes, but after further clinical study researchers observe its shown to reduce protein aggregation and improve muscular functions in ALS patients [54]. The arimoclomol have multiple mechanisms of action and several evidences that reduce the level of protein aggregate in motor neurons because it able to increase the expression of chaperons Hsp 70 and Hsp90 which help in synthesis of new protein molecules. It has also shown beneficial effects in muscle denervation in the SOD transgenic ALS mice [Clinicaltrial.gov NCT00706147]. Finally, researchers evaluate the efficacy and safety of this drug after phase II trials but phase III randomized, placebo-controls, double blind trials are in pipeline [55].

Ceftriaxone: The Ceftriaxone is an antibiotic which belong to the third generation of cephalosporin category. It shown the neuroprotective effect because it's easily crosses the blood brain barrier (BBB) [56]. After clinical trials researchers proved that it induced the astrocytic glutamate transporter EAAT2 expression in humans, and GLT1 glutamate transporter expression in rodents, and we know that over expression of glutamate transporters can boost clearance of synaptic glutamate and protect from neuronal damages. Unfortunately, this compound did not show efficacy at Phase III clinical trials [57].

RNS60: The RNS60 is electro kinetically aqueous fluid that composed of 0.9% saline containing charge stabilized oxygen nano bubble based structure. In this fluid, active pharmaceutical ingredient absent, but it active against inflammation and neuronal damages therefore it's also a new option for the treatment of neuro-inflammatory and neurodegenerative disorders. This fluid is composed by

water, sodium chloride and 55 ppm oxygen [58,59]. The researchers observed the beneficial effect of RNS60 in regulation of myelin. According to phase I clinical study, RNS60 has shown safety and tolerability in ALS patients after administration by IV and inhalation routes. (Clinicaltrial.gov NCT02525471)

Cu(II)ATSM: The mutation in metallo-protein Cu/Zn-superoxide dismutase (SOD1) and over expression of mutant SOD1 in animals are the main causes of familiar ALS. The SOD1 is an intermediate metal deficient state that contributes the main role in inducing ALS like symptoms [60]. The Cu(II)ATSM (diacetyl-bis (4-methylthiosemicarbazonato) copperII) is responsible for copper delivery into CNS and maintain the copper homeostasis in SOD1 transgenic mice resulting that improve the locomotors activity and enhance the survival time of an human/animal [61]. Because the deficiency of cu^{2+} in spinal cord shown the symptoms same as ALS patients. Collaborative Medicinal Development Pty Limited (US/Australian) has perform the clinical development of Cu(II)ATSM with a multicenter, open-label, phase I study of Cu(II)ATSM administered by oral route to patients that already suffer with MND/ALS. (Clinicaltrial.gov NCT02870634) Researcher were observed the beneficial effect of Cu(II)ATSM after phase I study on SOD1 animal models.

Methylcobalamin: It also used for the treatment of muscular atrophy and neuromuscular pain. It shows the protective effect against glutamate-induced cytotoxicity that affects the cortical neurons [62]. According to Eisai Co., Ltd. (Japan), clinical trial of this compound was finished and researchers observed the positive effect against MND/ALS. (Clinicaltrial.gov NCT00444613) The aim of this study was to investigate the efficacy and confirm the safety of high dose, 50 to 100 times the approved dose of methylcobalamin in ALS patients. After this clinical trial, induced the survival rate and reduced the neuronal damages and progression in ALS patients if daily administration [63]. Whenever, administered high dose of this compound to wobblers mouse motor neuron disease, results observed retardation in progression of MND and neuropathplogical changes. It was not sufficient for approval, so company withdraw it. Recently, Eisai is start further study for development with new strategies. A high dose of vitamin B12 derivative, Biotin is formulated for clinical trials. It's a water soluble form of vitamin B12 and the main goal of this trial to explore the safety of biotin for ALS patients. (Eudra CT Number: 2015- 005810-31)

Future Perspective

The clinical and neuropath logical heterogeneity of ALS represents only the tip of the iceberg of these multifaceted diseases and many key issues remain to be completely explained, such as main reason for the selective vulnerability of cell types like specific motor neurons compared to frontal and temporal neurons as well as the influence of exogenous and endogenous modifier factors that help in progression of disease. Still now, find no any suitable biomarkers that capable for accurate diagnose and predict disease progression. About 90% of patients are affected with ALS that adult-onset forms of unknown etiology [64,65]. Remaining 10% patients are suffered with genetic form of ALS. Other various factors are associated with progressive of this disease and influence the rate of progression has been identified.

There is a need to explore more clearly the incidence, nature, and progression of language changes in the ALS/MND and to establish the exact role that executive dysfunction has on language processing. For cognitive phenotyping in ALS study require a large no. of people for cohort study.

Discussion

Clinical trial design changes may not only result in significant efficiency benefits and cost savings, but they may also broaden eligibility criteria and reduce the amount of time patients are exposed to ineffective medicines or placebo [66,67]. Given the high probability of failure in prior ALS clinical trials and the large number of intriguing therapies, it is vital that future studies are so well. Our proposed design changes might be a significant step forward, with the concepts serving as a model for future clinical studies in ALS using time-to-event outcomes [68].

Clinical and pathophysiologic heterogeneity are major factors in ALS clinical trials, making it difficult to identify therapy effects. We used individual risk profiles to quantify prognostic heterogeneity in clinical trials, and then used the risk profile as an eligibility criterion to decrease the found heterogeneity [69]. The risk profile, on the other hand, may be used to investigate between-trial variations and enhance between-study comparability, while its distribution can reveal the generalizability of trial results and aid in the development of a label for market approval [70]. Risk profiles can be employed in trial design to enhance randomization, investigate risk-based subgroup analyses, or boost statistical power as a covariate in the final analysis [71]. Clinical outcomes and survival time can be combined to improve the suggested design. These composite endpoints might provide researchers more information about the predicted treatment effect, lessen the impact of (informative) missing data, and help them make better decisions about whether to stop a study early or continue it [72]. Furthermore, the regulator's role may vary in the future when the focus shifts to intermediate goals such ALSFRS-R or respiratory function. In these situations, group-sequential designs, longer randomised follow-up, and information-based design

may all be useful, and a continual interaction with regulatory agencies will be necessary to bring innovation to clinical trials in ALS [73-75].

Conclusion

According to this review article, ALS is major neurodegenerative disease that also affects the motor neurons, both upper and lower motor neurons due to blocking the some associated pathways like as astrogliosis, microgliosis, mitochondrial dysfunction, defects in axonal transport and RNA binding protein processes. At this time, no any medicaments or therapies are available in market that completely cures of ALS like disorders. It's a greatest challenging work for those who working in MND drug discovery due to lack of validated targets and animal models. Recently, some private and public researches are working on ALS due to their great efforts currently several clinical trials ongoing to fight against ALS. Some researchers/ clinical trials are focused on development of diagnosing agents which exactly diagnose a causing agents and targeting sites. There are various research ongoing on cell based therapies to treat neuro motor disorders. We mention above some important drugs that are ongoing under clinical trials at this time. At present, more than 20 new drugs are under pipeline for ALS treatment. Although the great number of drugs currently in clinical trials have the main goal of protecting the motor neuron and modifying the course of the disease.

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Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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