REVIEW PAPER

Volume-7 | Issue-9 | September-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

AMYOTROPHIC LATERAL SCLEROSIS (ALS) AND SUPEROXIDE DISMUTASE 1 (SOD1): A LINK BETWEEN THE DISEASE AND ENZYME

Neurology

Miss Aishwarya Padmanabhan M.Sc. Zoology Department of Zoology University of Delhi Delhi, India 110007

Prof. Namita Agrawal * Department of Zoology University of Delhi Delhi, India 110007*Corresponding Author

ABSTRACT

Amyotrophic lateral sclerosis (ALS) is the most common variant of motor neuron disease affecting adults that typically strike during mid to late life. The aetiology of this devastating condition, for which no cure has been developed, is poorly understood. A wide range of genes have been identified through the years which increase susceptibility to the disease, intensify the rate of motor neuron degeneration or are responsible for a given disease symptoms. One of the first and most widely studied gene, involved in pathogenesis of ALS, is Superoxide Dismutase-1 (SOD1). This gene encodes a Cu-Zn binding, anti-oxidizing enzyme that also has a role in extracellular signaling and apoptosis. Mutant SOD1 is associated with cellular pathology caused by protein aggregation, ER stress, Mitochondrial and axonal transport disruption. Potential of SOD1 as a therapeutic target for ALS is being actively researched with drugs that chelate excessively accumulated copper and RNAi strategies to reduce levels of mutant SOD1 being developed to combat this disease. This review deals with the possibilities of SOD1 as a prospective target to tackle this devastating condition.

KEYWORDS

Amyotrophic Lateral Sclerosis (ALS); Motor Neuron Disease; Protein Aggregation; Superoxide Dismutase 1 (SOD1); therapeutics

INTRODUCTION

Neurodegenerative diseases are late onset crippling conditions with no known cure. Many evidences suggest an increase in protein aggregation and inclusion body formation being a common mechanism that tie the various neurodegenerative diseases together [1]. Neurodegenerative diseases encompass a vast array of neurological disorders with diverse pathological and clinical expressions affecting specific groups of neurons in particular functional anatomic systems. The reason behind complex pathological features of most of these diseases are unknown and they progress unsparingly, tremendously decreasing the expectancy and quality of life[2].

ALS is one of the most prevalent motor neuron diseases in adults characterized by progressive degeneration of the large pyramidal neurons in the motor cortex and associated cortico-spinal tracts, the descending axons of which in the lateral spine appear scarred. The disintegration of spinal motor neurons with amyotrophy and secondary denervation clinically manifests itself as upper and lower motor neuron symptoms such as muscle weakness and denervation atrophy [3,4]. Characteristic manifestations include Bulbar ALS ,primary lateral sclerosis, progressive muscular atrophy and pseudo-bulbar palsy (4). 10% ALS cases are of familial type, inherited as an autosomal dominant trait with high penetrance, particularly post the sixth decade of life (5). Cases of autosomal recessive and X-linked dominant Familial ALS (fALS) have also been reported [6].

Oxidative stress is closely linked to ALS and preventing oxidative stress could be one of the strategies to slow down the progression of ALS. An imbalance between Reactive oxygen species (ROS) and the antioxidant defence causes oxidative stress. ROS comprise of oxygen and its reduction products superoxide, hydrogen peroxide and hydroxyl radical [3].

ALS is caused by multiple factors including mutation in specific genes; one of the most frequently affected is Superoxide Dismutase 1 (SOD1). Mutations in SOD1 has been linked to 20% of familial ALS (fALS) cases [7]. SOD1 is a powerful enzyme that binds both copper and zinc ions, catalyses the disproportionation of superoxide to molecular oxygen and hydrogen peroxide and confers antioxidant defence to cells protecting them from the damaging effects of superoxide radicals [8] . SOD1 is found in high abundance, almost 1% of the total protein content of the cell and mainly present in the cytosol with minor presence in the inner mitochondrial membrane space [9].

SOD1 is a 32 kDa protein that acquires an 8-stranded Greek key beta barrel structural motif. It has 2 functional loops, an electrostatic loop which directs the superoxide towards the redox active sites where the Cu2+ is present and a zinc binding loop. A characteristic functional feature of the protein is the presence of a disulfide bond between Cys57 and Cys146 subunits. The coordination of copper as well as the formation of the intra-subunit disulfide bond is carried out by the CCS (Copper chaperone for SOD1) which is a cytosolic copper carrier protein [9,10]. Using NMR and ESI-MS, a SOD1 maturation framework has been proposed which involves Zn loading followed by SOD1 & CCS heterodimerisation after which Copper is transferred from CCS to SOD1, subsequently intra-subunit (Cys57 & Cys146) disulfide bond formation and homodimerisation of SOD1 occurs [9,11].

Mutated SOD1 gene can acquire both gain and loss of function mutations. A4V, D90A and G93A are the common mutations that affect protein activity in SOD1. Deleterious mutations have been shown to modify SOD1 activity, which leads to the accumulation of highly toxic hydroxyl radicals. Accumulation of toxic hydroxyl radicals due to modified activity of mutant SOD1 causes degradation of nuclear as well as mitochondrial DNA and protein misfolding [12].

Cognitive impairment and fronto-temporal dementia along with upper and lower motor neuron degeneration in SOD1 related ALS is rare [13]. Sporadic ALS (sALS) and SOD1-mediated fALS are clinically indistinguishable and both affect motor neurons with a similar pathology. Mutant SOD1 animal models and cellular models are thus powerful tools to decipher the mechanism underlying disease progression. Even in the presence of the endogenous enzyme, ALS-like phenotype is observed in transgenic mice overexpressing human G93A-SOD1, whereas despite the toxicity of the superoxide radicals SOD1 knockout mice do not develop ALS. Yet, the exact nature of the toxicity and the cause of the motor neuronal degeneration are still largely debated[14].

Research suggests fALS should be considered a protein misfolding disorder, where the mutant protein attains a toxic, non-native oligomeric form. The series of events initiated by protein accumulation, axonal transport alterations, mitochondrial and/or proteasome dysfunctions could be triggered by this misfolding of the protein. Overproduction of ROS and caspase activation could be an indirect consequence of this cascade of events. Neuronal intranuclear protein inclusions, positive for promyelocytic leukemia gene product, ubiquitin and proteasome subunits, reported in ALS patients indicates a potential intracellular target of SOD1 toxicity. Increased levels of 8-hydroxy-2′-deoxyguanosine (8OH2′dG), an oxidative DNA damage marker has been observed in the DNA of sALS patients and spinal cord, striatum and cortex of SOD1-G93A mice [14]. Aggravation of sALS might also be due to a combination of genetic, environmental and behavioural factors. 2-4% of sALS cases can be attributed to

SOD1 mutation [15].

Even though many new genes linked to ALS have been identified recently, research into the deviant function of mutant SOD1 has played an important role in understanding the pathogenesis of ALS [16]. Additionally, secreted SOD1 also plays an important role in extracellular signalling. Existence of extracellular SOD1 leads to an increase in intracellular Ca2+ by a mechanism that involves activation of phospholipase C/protein kinase C pathway. Presence of increased levels of intracellular calcium has been found to confer neuroprotective effects on cerebral granular neurons vulnerable to dopaminergic toxin (9). Furthermore, WT SOD1 binds to Bcl-2 (N-terminal domain) and prevents apoptosis [17]. Therefore, SOD1 can be a potential therapeutic target for amelioration of ALS.

Mutation in SOD1 – the first known genetic cause of ALS

A tight genetic linkage between a gene that encodes cytosolic Cu/Zn-binding superoxide dismutase 1 (SOD1) and fALS was reported in 1993. 11 different missense SOD1 mutations were identified in 13 different ALS families. 9 out of the 11 sequence changes were found to alter the recognition sites for restriction enzymes [5]. 185 mutations have currently been identified within SOD1 linked to ALS (http://alsod.iop.kcl.ac.uk/)[18].

Mechanism of action of Superoxide Dismutase

SOD catalyses the conversion of superoxide anion radical O2- (toxic) to H2O2 by alternate reduction and reoxidation of Cu2+. Hydrogen peroxide can be further converted to water by glutathione peroxide or catalase. SOD also prevents reaction of superoxide with nitric oxide to form peroxynitrate anion (ONOO-) by reducing superoxide levels, which can further generate toxic hydroxyl radical. It was postulated that in fALS patients either SOD1 activity is reduced, causing accumulation of toxic superoxide radical or the activity of SOD1 is increased leading to excessive levels of H2O2 and hydroxyl radical formed by reaction of H2O2 with a transitional metal like iron [5].

Human genome encodes 2 other SOD proteins (1) Mitochondrial Mn-dependent SOD2 and [2] Extracellular EC SOD3. SOD2 and SOD3 are homotetrameric proteins [9]. The possibility that mutations in the genes encoding the SOD2 and SOD3 proteins may also be responsible for incidence of fALS not linked to SOD1 is being explored [5]. The cytoprotective role of SOD2 has clearly been established by the very short life-span of MnSOD knockout mice, that died soon after birth exhibiting neurodegeneration and dilated cardiomyopathy [19,20]. EC SOD3 has been shown to be a major regulator of nitric oxide (NO) bioavailability and bioactivity, which acts by regulating generation of the toxic peroxynitrite in the vasculature (20,21).

Role of mutant SOD1 in disease pathogenesis

Though the exact mechanism of SOD1 mutations is not yet clear, the mutant protein misfolds and attains a plethora of toxic function that can cause ER stress, disruption of axonal transport, mitochondrial dysfunction and extensive amount of unfolded protein response. Mutation induced misfolding of SOD1 leads to formation of insoluble aggregates, which can be seen as a distinct pathological feature of human fALS. These inclusions containing aggregates of mutant SOD1 forms have also been reported in the lower motor neurons of SOD1 mouse models (22). Aggregation of mutant SOD1 forms occur through an oxidation-mediated mechanism followed by recruitment of WT SOD1 by cross-linking of intermolecular disulphide bonds [22,23]. It still remains debated if these aggregates have a deleterious or protective function in disease progression.

Mutant SOD1 disrupts secretory protein transport in the ER-Golgi network. This leads to ER stress, protein aggregation, fragmentation of Golgi and apoptosis in mutant SOD1 expressing cells. Overexpression of COPII subunit Sar1 safeguards against formation of inclusion bodies and apoptosis thus defining the functional relationship between pathology of ALS and ER-Golgi transport (24).

Effects of mutant SOD1 on mitochondria

Mitochondrial dysfunction is one of the trademark effects of formation of toxic protein related to neurodegenerative diseases. Aberrant mitochondrial morphology predisposes the motor neurons to degenerate [25]. The intermolecular covalent bond formed by the apoprotein in the inter membrane space of the mitochondria in SOD1 mouse models (G93A, A4V, L126X) prelude the onset of the disease symptoms [5]. A comprehensive knowledge about the effect of SOD1 on the mitochondria gives a valuable insight into understanding the progression and pathogenesis of ALS.

In vivo study of mitochondrial morphology and dynamics in nervous system was done by developing a transgenic mouse mitoDendra, which expresses Dendra fluorescent protein in mitochondria under the control of neuronal-specific Thy1.2 promoter (26). This transgenic line permits direct imaging of mitochondria in living neurons. The line was created and sustained in a B6SJLF1 background and crossed in the same genetic background with wild type SOD1 and SOD1G93A mice. The mitoDendra-SOD1G93A double transgenic mice showed a mean age of onset of the disease at 106.7 days and average survival of 140.7 days. No disease phenotype was observed up to 2 years in mitoDendra-SOD1WT double transgenic mice. Reduced frequency of mitochondrial movement and dwindling proportion of mitochondria moving in retrograde fashion was observed at the age of 45 days in SOD1G93A mice. Remarkable reduction in the proportion of mitochondrial movement in both retrograde and anterograde direction was observed by 90 days in SOD1G93A mice which edged towards symptomatic stage of the disease. The mean length of the mitochondria in the sciatic nerve of the SOD1G93A mice displayed a progressive reduction in size with advancing age. Clustering and swelling of mitochondria was also observed in SOD1G93A mice. Prominent reduction in the size of stationary as well as mobile mitochondria was observed in the SOD1G93A mice as compared to the mitoDendra control mice. No significant morphological abnormalities were noticed in the ventral roots of mice with fALS. Fragmentation of mitochondria occurs early in the motor axon terminals, after which diminution of mitochondria in NMJS and motor terminals occurs followed by obvious denervation in the SOD1G93A mice [25].

Sporadic ALS and SOD1

Presence of misfolded WT SOD1 protein in the spinal cord of sALS and non-SOD1 FALS was confirmed by using conformation specific antibody. This incriminates aggregation of WT SOD1 in sALS pathogenesis [9,23]. The initial proven case of SOD1 mutation i.e., H80A which depicted the first de novo mutation identified in SOD1 in a patient with true sALS was elucidated by Alexander and his colleagues in 2002. H80A mutation was identified in a 24 year old man who showed rapid progression and exclusive lower motor neuron symptoms. The aggressive phenotype of the disease was linked to the position of the H80A mutation, which influences the zinc binding attributes of SOD1 protein. Apart from identifying the only proven de novo mutation, the study also brought into focus, the role of Zn-binding in neuronal death mediated by mutant SOD1 [27].

Unravelling the genetic backdrop of SALS

High-resolution melting (HRM) analysis is a technique to scan mutation which tracks progressive changes in fluorescence prompted by release of an intercalated DNA dye from a DNA duplex as slight increase in temperature denatures it. The shapes and shifts of the melting curves, which are procured as fluorescence difference plots are used to differentiate between controls and mutations. Akimoto and his colleagues used this pioneering technique to screen SOD1 mutations in 2 groups of Japanese sALS cases. In group 1, 184 cases of sALS were screened, out of which 3 cases showed novel SOD1 mutation (C6Y, Q22H and S134T). In group 2, SOD1 mutations were observed in 8 out of the 256 cases of ALS. 4 out of the 8 were cases of familial type and the rest 4 were cases of sALS. K3E was a novel mutation found in one woman and the rest three showed G93S mutation. Clinical characteristics of mutant SOD1 associated sALS included young age of onset and high incidence of limb involvement at the onset of disease. The prevalence of SOD1 mutations in Japanese sALS cases was calculated to be 1.6%. This group of scientists used HRM to elucidate the genetic background of sALS caused by mutant SOD1 and further hope to screen and identify other causative genes involved in ALS using the same technology [28].

SOD1 – A potential therapeutic target

Many published reports strongly support that mutation in SOD1 could be a major cause of the disease and disease progresses by gain of toxicity and not loss of physiological functions [29]. For the treatment of the disease, only two drugs are available. Riluzole was the first drug to be approved by the FDA that has been observed to slow down disease progression in some patients may be by reducing levels of glutamate which is often present in patients at very high level. This drug is taken orally and reported to have many side effects such as dizziness, tachycardia, gastrointestinal conditions and liver function changes [30].

In 2017, Edaravone (Radicava) was approved by FDA for treatment of ALS. It is an effective scavenger of oxygen radicals and has shown to suppress motor function decline in ALS mouse models [31].

Pathological role of atypical copper build up in SOD1-ALS cases can be correlated with the disease progression by elevation of non-SOD1 copper levels in mice expressing hSOD1G93A (humanSOD1 G93A) which is observed in the spinal cord and not in the brain [32,29]. The evidence of copper imbalance in human is not as convincing as in mice; efficiency of copper-lowering therapy to alleviate symptoms of the disease has thus been studied using mouse models. Treating $hSOD1^{G93A}$ mice with tetrathiomolybdate (TTM) which chelates and reduces levels of accumulated copper ions has resulted in marked decrease and normalization in the levels of intracellular copper, leading to delayed onset of disease and increased lifespan of the $hSOD1^{G93A}$ mice. TTM was also observed to be effective in reducing the rate of disease progression even when it was administered after the onset of disease (32). Dissociation of metal ions leads to increased susceptibility to misfolding of SOD1 because of reduced structural or thermal stability (29,33). Thus, supplying copper ions to active sites of SOD1 could have been a useful therapeutic option to prevent misfolding, and therefore reducing the pathogenicity of SOD1. But tight control of bioavailable concentration of copper hinders any improvement mediated by supplementation of a copper rich diet [29]. Copper complex diacetylbis(N(4)-methylthiosemicarbazonato) copper(II) (CuII(atsm)) administration has recently been proved to remarkably ameliorate acute phenotype of CCS and G93A SOD1 double transgenic mice [34,29]. This copper complex has been found to notably slow down the onset of paralysis and extend the duration of life. One proposed way to productively correct the intracellular copper levels is to develop drugs which can chelate abnormally accumulated copper ions and then transfer them to the apo-SOD1 or CCS. The first steps for developing such a drug is to understand where and how copper ions build up in the spinal cords of mouse models [29].

Reducing the level of SOD1 is another proposed method to treat SOD1-mediated ALS. Several gene silencing strategies such as RNA interference and antisense oligonucleotides have been developed in recent times that enhance degradation of RNA. Artificial short hairpin RNA (shRNA) or microRNA (miRNA) with viral vector delivery systems that are capable of infiltrating the CNS and silencing the toxic genes in brain and spinal cord, are being studied in rodent models and primates apart from humans. Adeno-assosciated viral vectors (AAVrh10 and AAV9) are the preferred treatment vehicle for neurological diseases as they are safe, long term immune-silent gene transfer agents. Artificial miRNA have been proven to be a better option to induce RNAi than artificial shRNA for human patients (35).

Conclusion

Significant progress has been made since the discovery of involvement of mutant SOD1 in pathogenesis of ALS. With the development of integrative databases such as ALSoD and presence of vast amount of research and literature, the understanding of the onset and pathogenesis of ALS has increased many folds. Since SOD1 mutations have been observed in only about 20% of FALS families and about 2% of the SALS cases, it implies that additional gene defects may be responsible for ALS. Till date, the primary factor responsible for ALS has not been elucidated. Understanding the intricacies of this devastating disease requires active participation of researchers, doctors, family members and most importantly the individuals suffering from it. Collecting authentic data and appropriate interpretation can serve as a boon to first differentiate cases of FALS and SALS and also to devise more effective and personalized therapeutic alternatives. More extensive and exhaustive study of ALS cases is thus the need of the hour. Though the exact mechanism of SOD1 involved in pathogenesis of ALS continues to evade us, thereby existing information about the mechanism of action of SOD1 is being actively explored as a viable treatment option. Apart from genetic studies, understanding the effects of environment and the Epigenetics of the disease is equally important to characterize and develop SOD1 as a therapeutic target for better mode of management and treatment for this debilitating disease.

REFERENCES

1. Ross CA, Poirier MA. Protein aggregation and neurodegenerative disease. Nat Med. 2004; 10.
2. Przedborski S, Vila M, Jackson-Lewis V. Series Introduction: Neurodegeneration: What is it and where are we? J Clin Invest. 2003;111(1):3–10.
3. Franco MC, Dennys CN, Estévez FHR and AG. Superoxide Dismutase and Oxidative Stress in Amyotrophic Lateral Sclerosis. Curr Adv Amyotroph Lateral Scler [Internet]. 2013 .
4. Taylor JP, Brown Jr RH, Cleveland DW. Decoding ALS: from genes to mechanism. Nature. 2016 ;539(7628):197–206.
5. Rosen DR, Siddique T, Patterson D, Figlewicz DA, Sapp P, Hentati A, et al. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature. 1993;362(6415):59–62.
6. Boylan K. Familial ALS. Neurol Clin. 2015 Nov;33(4):807–30.
7. Chen S, Sayana P, Zhang X, Le W. Genetics of amyotrophic lateral sclerosis: an update. Mol Neurodegener. 2013;8:28.
8. McCord JM, Fridovich I. Superoxide Dismutase AN ENZYMIC FUNCTION FOR ERYTHROCUPREIN (HEMOCUPREIN). J Biol Chem. 1969;244(22):6049–55.
9. Rotunno MS, Bosco DA. An emerging role for misfolded wild-type SOD1 in sporadic ALS pathogenesis. Front Cell Neurosci [Internet]. 2013;7.
10. Furukawa Y, Torres AS, O'Halloran TV. Oxygen-induced maturation of SOD1: a key role for disulfide formation by the copper chaperone CCS. EMBO J. 2004 ;23(14):2872–81.
11. Banci L, Bertini I, Cantini F, Kozyreva T, Massagni C, Palumaa P, et al. Human superoxide dismutase 1 (hSOD1) maturation through interaction with human copper chaperone for SOD1 (hCCS). Proc Natl Acad Sci U S A. 2012;109(34):13555–60.
12. Kaur SJ, McKeown SR, Rashid S. Mutant SOD1 mediated pathogenesis of Amyotrophic Lateral Sclerosis. Gene. 2016;577(2):109–18.
13. Harms MB, Baloh RH. Clinical Neurogenetics: Amyotrophic Lateral Sclerosis. Neurol Clin [Internet]. 2013;31(4).
14. Sau D, De Biasi S, Vitellaro-Zuccarello L, Riso P, Guarnieri S, Porrini M, et al. Mutation of SOD1 in ALS: a gain of a loss of function. Hum Mol Genet. 2007 ;16(13):1604–18.
15. Kim M-J, Bae J-H, Kim J-M, Kim HR, Yoon B-N, Sung J-J, et al. Rapid Progression of Sporadic ALS in a Patient Carrying SOD1 p.Gly13Arg Mutation. Exp Neurobiol. 2016 ;25(6):347–50.
16. McGoldrick P, Joyce PI, Fisher EMC, Greensmith L. Rodent models of amyotrophic lateral sclerosis. Biochim Biophys Acta BBA - Mol Basis Dis. 2013;1832(9):1421–36.
17. Pasinelli P, Belford ME, Lennon N, Bacskai BJ, Hyman BT, Trotti D, et al. Amyotrophic Lateral Sclerosis-Associated SOD1 Mutant Proteins Bind and Aggregate with Bcl-2 in Spinal Cord Mitochondria. Neuron. 2004;43(1):19–30.
18. Abel O, Powell JF, Andersen PM, Al-Chalabi A. ALSoD: A user-friendly online bioinformatics tool for amyotrophic lateral sclerosis genetics. Hum Mutat. 2012 ;33(9):1345–51.
19. Lebovitz RM, Zhang H, Vogel H, Cartwright J, Dionne L, Lu N, et al. Neurodegeneration, myocardial injury, and perinatal death in mitochondrial superoxide dismutase-deficient mice. Proc Natl Acad Sci U S A. 1996 ;93(18):9782–7.
20. Miao L, St. Clair DK. Regulation of Superoxide Dismutase Genes: Implications in Diseases. Free Radic Biol Med. 2009 ;47(4):344–56.
21. Gongora MC, Qin Z, Laude K, Kim HW, McCann L, Folz JR, et al. Role of extracellular superoxide dismutase in hypertension. Hypertens Dallas Tex 1979. 2006 ;48(3):473–81.
22. Bruijn LI, Houseweart MK, Kato S, Anderson KL, Anderson SD, Ohama E, et al. Aggregation and motor neuron toxicity of an ALS-linked SOD1 mutant independent from wild-type SOD1. Science. 1998;281(5384):1851–4.
23. Ajroud-Driss S, Siddique T. Sporadic and hereditary amyotrophic lateral sclerosis (ALS). Biochim Biophys Acta. 2015 ;1852(4):679–84.
24. Atkin JD, Farg MA, Soo KY, Walker AK, Halloran M, Turner BJ, et al. Mutant SOD1 inhibits ER-Golgi transport in amyotrophic lateral sclerosis. J Neurochem. 2014 ;129(1):190–204.
25. Magrané J, Cortez C, Gan W-B, Manfredi G. Abnormal mitochondrial transport and morphology are common pathological denominators in SOD1 and TDP43 ALS mouse models. Hum Mol Genet. 2014;23(6):1413–24.
26. Caroni P. Overexpression of growth-associated proteins in the neurons of adult transgenic mice. J Neurosci Methods. 1997 ;71(1):3–9.
27. Alexander MD, Traynor BJ, Miller N, Corr B, Frost E, McQuaid S, et al. 'True' sporadic ALS associated with a novel SOD-1 mutation. Ann Neurol. 2002 ;52(5):680–3.
28. Akimoto C, Morita M, Atsuta N, Sobue G, Nakano I. High-Resolution Melting (HRM) Analysis of the Cu/Zn Superoxide Dismutase (SOD1) Gene in Japanese Sporadic Amyotrophic Lateral Sclerosis (SALS) Patients. Neurol Res Int. 2011 ;2011:e165415.
29. Tokuda E, Furukawa Y. Copper Homeostasis as a Therapeutic Target in Amyotrophic Lateral Sclerosis with SOD1 Mutations. Int J Mol Sci [Internet]. 2016 ;17(5).
30. Ltd BPG. Rilazole for amyotrophic lateral sclerosis. Drug Ther Bull. 1997 ;35(2):11–2.
31. Sawada H. Clinical efficacy of edaravone for the treatment of amyotrophic lateral sclerosis. Expert Opin Pharmacother. 2017 ;18(7):735–8.
32. Tokuda E, Okawa E, Watanabe S, Ono S-I, Marklund SL. Dysregulation of intracellular copper homeostasis is common to transgenic mice expressing human mutant superoxide dismutase-1s regardless of their copper-binding abilities. Neurobiol Dis. 2013;54:308–19.
33. Rodriguez JA, Shaw BF, Durazo A, Sohn SH, Doucette PA, Nersissian AM, et al. Destabilization of apoprotein is insufficient to explain Cu,Zn-superoxide dismutase-linked ALS pathogenesis. Proc Natl Acad Sci U S A. 2005 ;102(30):10516–21.
34. Williams JR, Trias E, Beilby PR, Lopez NI, Labut EM, Bradford CS, et al. Copper delivery to the CNS by CuATSM effectively treats motor neuron disease in SOD(G93A) mice co-expressing the Copper-Chaperone-for-SOD. Neurobiol Dis. 2016 ;89:1–9.
35. van Zundert B, Brown Jr. RH. Silencing strategies for therapy of SOD1-mediated ALS. Neurosci Lett. 2017;636:32–9.