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REVIEW ARTICLE

Edaravone May Prevent Ferroptosis in ALS

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Abstract: Radicava™ (Edaravone) was approved the Food and Drug Administration (FDA) as a new treatment for amyotrophic lateral sclerosis (ALS). Edaravone is a synthetic antioxidant that specifically targets oxidative damage interacting with lipid radicals in the cell. ALS is a disease that multiple cell types are involved in the devastating loss of motor neurons. Mutations and biochemical changes in various cell types jointly contribute to motor neuron death, disease onset, and disease progression. The overall mechanism of neurodegeneration in ALS is still not completely understood. Dying motor neu-rons have been reported to exhibit features of apoptosis. However, non-apoptotic features of dying motor neurons have also been reported such as ferroptosis. The role of Edaravone in the prevention of ferroptosis in parallel with other therapeutic approaches to ALS therapy is discussed.

1. INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a form of motor neuron disease characterised by dying nerve cells. The result of which is significant patient disability and death. ALS is still an intractable motor neuron disease, despite at least 150 years of research worldwide. In 1995, the Food and Drug Administration (FDA) approved Riluzole. It modulates glutamate neurotransmission by inhibiting N-methyl-D-aspartate receptors. Unfortunately, Riluzole's impact on the disease is limited. The FDA recently approved Radicava™ (Edaravone) for the treatment of ALS. Even though several mutations underlying rare cases of familial ALS have been identified during the last decade, the pathogenesis of ALS remains poorly understood. Various mechanisms have been suggested to contribute to disease pathology such as excitotoxicity, oxidative stress, protein aggregation, inflammation, vascular endothelial growth factor deficit which leads to hypoxia and ferroptosis. Compelling evidence points nowadays towards ALS to be a multi-system disease. Both the appearance and the intensity of the disease is a combination of structural, physiological, and metabolic changes within the nervous system [1, 2]. As many ALS patients express extrapyramidal deficits, neuropathological findings fully support the notion of multisystem brain degeneration. To treat the multisystemic character of ALS therapeutically is a difficult task especially in the absence of reliable biomarkers and a full understanding of the pathophysiological mechanisms of the disease [3]. Recent results have confirmed that a combinatorial approach co-targeting different pathogenic mechanisms in independent cell types is a beneficial therapeutic strategy for ALS [4].

1.2. Main Pathogenic Mechanisms in ALS

Our previous investigations demonstrated that the catalytic activity of iron in CSF is modulated by exogenous ligands and that there could be some synergy between redox activities of iron and copper in the Fenton system in CSF [5]. Iron accumulation was observed in the CNS of ALS patients by magnetic resonance imaging [6]. More recently, accumulated iron was detected in multiple brain regions, including the motor cortex, Substantia nigra, globus pallidus, red nucleus, and the putamen in ALS patients by quantitative susceptibility mapping [7]. The pathogenic impact of iron metabolism deregulation in ALS has been further supported by the partially protective effects of iron chelators in ALS mouse models [8]. The neuroprotection of iron chelation may result from the attenuation of iron-related oxidative stress, iron accumulation, and prevention of ferroptosis. There are clinical evidence about the neuroprotective potential of a therapeutically safe chelation treatment on early-stage amyotrophic lateral sclerosis (ALS) patients, which respond-ed significantly to treatment in both brain iron deposits and indicators of disease progression [9].

There have been historical attempts to treat ALS with antioxidants. All previous studies demonstrated that antioxidants alone had no significant therapeutic effect in ALS pa-

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tients. Of interest are the findings in an ALS study group which showed that α-tocopherol alone had no effect on the deterioration of function assessed by the modified Norris limb scale [10]. Possible reasons are that α-tocopherol acts in the plasma membrane and is regenerated by vitamin C which has no effect on intracellular membranes. Apostolski and colleagues evaluated the effects of α-tocopherol in combination with selenium, amino acids, and nimodipine, a calcium ion channel blocker [11]. It is particularly important in the geographical regions deficient in selenium.

Recent results compared the Serbian population to the results reported in other population groups worldwide and some authors concluded that the Serbian population had signifi-cantly reduced content of Se (12). Selenium is also important as it supports the activity of glutathione peroxidase-4 (GPx4). GPx4 is also known as phospholipid hydroperoxide-glutathione peroxidase. Within the protein glutathione perox-idase family, GPx4 is the only enzyme with phospholipid hydroperoxydase activity, using glutathione as the reducing agent [13]. Decreased GPx4 activity is also found to potenti-ate Ferroptosis. It seems that events that potentiate Ferroptosis are the main components in the induction of motoneuron death in ALS.

1.3. Role of Edaravone

Edaravone is an antioxidant targeting oxidative damage particularly intracellular lipid membranes, but can also act in extracellular and intracellular compartments. This synthetic antioxidant molecule (Fig. **1**) is able to quench hydroxyl, peroxyl, and superoxide radicals and showed antioxidant activity against lipid peroxidative damage induced by water- or lipid-soluble radicals [14, 15]. Oxidative stress-induced by Fe^{2+} increased production of hydoxil radicals (Fenton reaction) is the main cause of neuronal damage in ALS [16]. It is characterised by a significant increase in markers of DNA oxidative injury (including 8-hydroxy-2'-deoxyguanosine) and in protein oxidation (for example, free 3-nitrotyrosine and its metabolite 3-nitro-4-hydroxyphenol acetic acid) in CSF of ALS patients. Antioxidants have failed to limit increased oxidative damage in ALS patients. Contrary to other antioxidants advantages and characteristics of Ederavone are:

• Its pKa (7.0) is close to the physiological pH (7.4), and Edaravone exists as both a neutral molecule and an anion in the body [16]

• Edaravone anion exhibits potent radical-scavenging activity *via* an electron-donating mechanism and can react with a wide variety of radical species.

Edaravone is well distributed to both the aqueous phase where radicals are primarily generated and the lipid phase where lipid peroxidation proceeds by radical chain reaction *via* secondarily induced peroxyl radicals, and it should scavenge radicals in both environments [17]. Edaravone succeeds in overcoming the refractory mechanism to exogenous antioxidants by expressing its anti-oxidative, lipid stabilising effects intracellularly, thereby preventing a process termed ferroptosis. There are data that indicate that Edaravone possesses antioxidant properties mainly due to its reactivity with free radicals, such as the hydroxyl radical ($^{\bullet}OH$) and peroxide radicals [18]. The possible mechanism behind Edaravones's antioxidant capability is the transfer of electrons from the Edaravone anion to the peroxyl radical, halting lipid peroxidation. Pertinent to this, Wang and co-workers have shown that Edaravone inhibits the oxidation of unilamellar soybean phosphatidylcholine liposomal membranes [19]. As a strong antioxidant, Edaravone is a free radical scavenger, useful for protection against oxidative stress and neuronal apoptosis [20]. In the mouse focal ischemic model, the antioxidant properties of Edaravone were postulated, crucial for the neuroprotective effects of Edaravone [21, 22]. In China and Japan, Edaravone alleviated the effects of cerebral ischemic stroke [23]. Edaravone efficiency has also been confirmed in the treatment of traumatic brain damage [24]. It is likely that Edaravone can have other effects than acting only as a free radical scavenger. Edaravone improves vascular blood flow through the up-regulation of endothelial nitric oxide synthase and reduction in LDL oxidation, which in turn results in an improvement in ischemic tissues. These findings were subjected to a critical assessment of Edaravone in acute ischemic stroke trials [25]. Edaravone suppressed ferroptosis in cystine–glutamate antiporter (xCT) - knockout mouse-derived embryonic fibroblasts, which usually die in normal cultivating conditions due to the depletion of intracellular Cys and GSH [26].

1.4. Therapeutic Approach in ALS and Way Forward

The current treatment regimen for ALS is Riluzole which slows disease progression modestly and Edaravone recently approved by the FDA to slow ALS progression. There is evidence to support the efficacy of Edaravone in delaying definitive disease progression (the benefits of Edaravone therapy are maximised after early ALS diagnosis [27]. The most recent investigation showed that Edaravone alone was not effective in unselected ALS patients [28] as well as in selected patients in a randomised double-blind trial and in patients with advanced disease [29].

The multifaceted mechanisms of ALS initiation and progression indicate that a suitable therapeutic approach must be complex. Single components have little chance to be therapeutically effective in such a disease. One observation regarding the role of uric acid in cerebrospinal fluid [30] was followed up [31-33] indicating that high urate levels correlate with improved survival in ALS patients and that urate was neuroprotective in several models of neurodegeneration [33-35]. These findings provide the basis for larger clinical trials of inosine testing as a potential therapy for disease modification for ALS [36]. The conclusion is that they demon-strate safety, tolerability, and urate-elevating capability of the urate precursor inosine in ALS patients. Taken together with convergent epidemiological, biological, and clinical data pointing to urate as a potential neuroprotectant, the study supports clinical trials aimed to test the ability of inosine to slow clinical ALS progression [36]. The next phase should be a combination of therapeutics such as Edaravone. Edavarone has some additional effects among antioxidative ones. Cell damage during ischemic reperfusion occurs *via* the opening of mitochondrial permeability transition pores, which leads to overflow of ROS and calcium ions. Edara-vone reduces calcium ion-induced mitochondrial swelling in the rat brain. In addition, Edaravone acts as an apoptotic

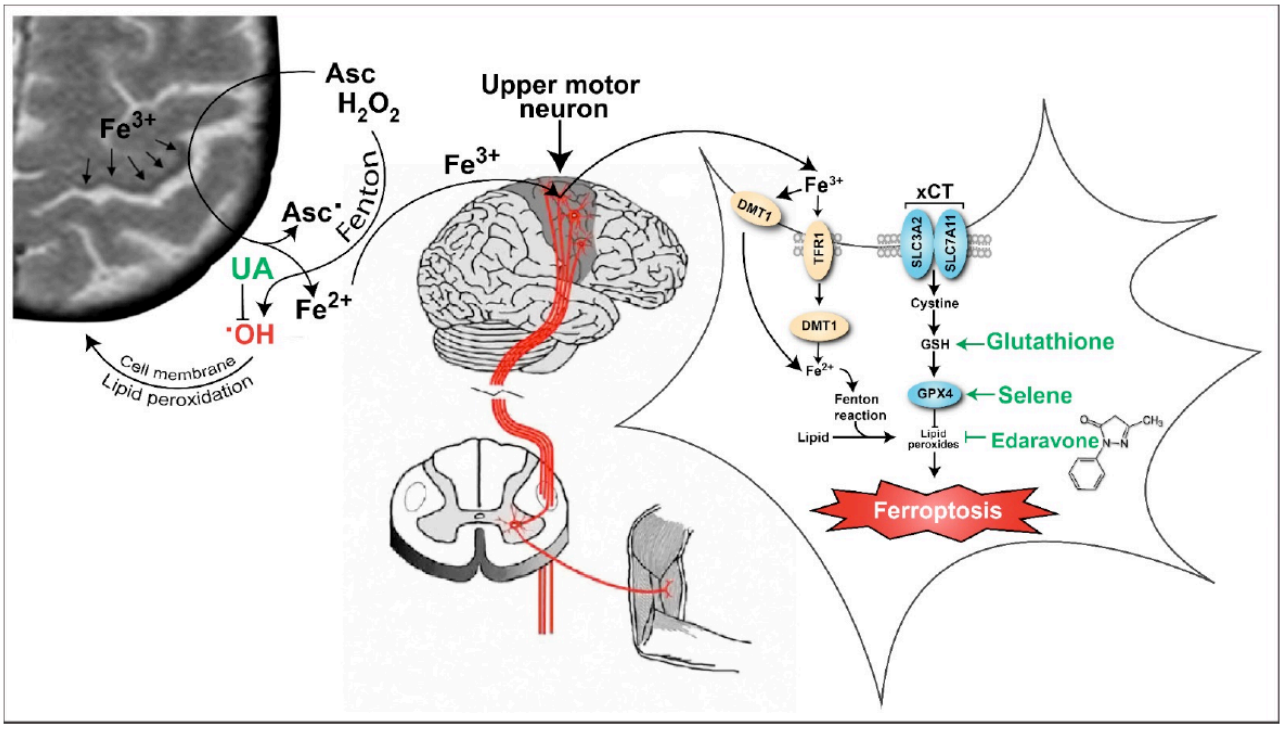

Fig. (1). A scheme showing the key steps of ferroptosis induction in motor neurons. A possible role of Edaravone in ferroptosis inhibition is presented. Asc – Ascorbate; DMT1-Divalent metal (ion) transporter 1; GSH-Glutathione; GSSG-oxidised glutathione; •OH-hydroxyl radical; TFR1-Transferrin receptor; UA-uric acid; xCT-cystine–glutamate antiporter. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

shield within ischemic neurons by reducing the expression of Fas-associated death domain protein, death-associated protein and caspase-8 immuno-reactivity in a rat MCAO model [37]. During cerebral ischemia provoked by calcium ions, ROS overload, and iNOS expression, Edaravone attenuated apoptosis and inflammation. Edaravone has also been used in combination therapy with other thrombolytic drugs to protect the integrity of neurons and blood vessels.

1.5. The Molecular Base for Combinatorial Approach in ALS Therapy

Motor neurons challenged by an increased presence of iron protect themselves by oxidising ferrous ions (Fe^{2+}) to ferric ions (Fe^{3+}) in the extra-cellular space and depositing them in the precentral gyruses gray matter (Fig. **1**) [6]. Fe^{3+} may be reduced to Fe^{2+} by ascorbate. Electron paramagnetic resonance spectroscopy identified ascorbyl radicals in the CSF from ALS patients, but not in controls [5]. The addition of H_2O_2 to CSF from ALS patients provoked the further formation of ascorbyl radicals and the formation of •OH radicals *ex vivo*. In contrast, the addition of uric acid to CSF from ALS patients *ex vivo* diminished the production of •OH radicals. Fe^{2+} may participate in the Fenton reaction extracellularly in CSF of ALS patients as H_2O_2 is continuously released by neurons (Fig. **1**).

This reaction generates •OH radicals which cause lipid peroxidation of cell membranes. Fe^{3+} from CSF can be absorbed in motor neurons by the transferrin receptor (TFR1) and reduced to Fe^{2+} by divalent metal (ion) transporter 1 (DMT1). Thus Fe^{2+} may promote the Fenton reaction inside motor neuron cells inducing intracellular lipid peroxidation. Under conditions of inadequate quantities of glutathione and low GPx4 (selenium-dependent) activity, the induction of cell death termed ferroptosis may occur.

Even if Edaravone prevents ferroptosis and there is adequate GSH and GPx4 activity, the induction of apoptosis is still possible. Oxidation products promote both DNA and protein damage and cause activation of p53-dependent apoptosis which can also lead to neuron cell death. This could explain the efficiency of Edaravone treatment of ALS in the early stages of disease more so than in later stages of the disease.

Ascorbate has unfavourable effects in ALS patients as it promotes pro-oxidative circulation of iron between CSF and iron deposits in the cortex [38]. In 2009, Keizman and colleagues found that ALS patients had lower serum uric acid levels compared with healthy individuals [39]. The decreased uric acid levels correlated with the rate of disease progression, supporting the role of oxidative stress in the induction and propagation of the disease.

Various forms of regulated cell death may exist in populations of motor neurons, one being dominant when some specific mutation is the cause of disease. Our published work suggested that the misfolded L144P SOD1 mutant protein forms pores that render lipid membranes permeable to ionic flow [40]. Such long-term cellular membrane disturbance could cause irreversible damage to neuronal cells and cell death in ALS patients through a variety of mechanisms in-

cluding defective calcium ion buffering capacity and increased metabolic demands causing oxidative stress and mitochondrial dysfunction leading to another form of cell death in ALS. Even wild-type superoxide dismutase type 1 protein, normally active in healthy individuals as a soluble metalloenzyme, can be transformed with respect to its structure/function into a "toxic channel" by mutation or by environmental stress [41]. Furthermore, these toxic channels in the lipid membranes of motor neurons (such as the outer cell and mitochondrial membranes) can create ionic imbalances (such as calcium ion overload) resulting in the neurotoxicity that characterises ALS. Evidence from *in vitro*, cell culture, and *in vivo* studies has provided strong evidence to support the involvement of a prion-like mechanism in ALS [42].

Motor neuron degeneration underlies motor neuron diseases such as ALS. Therefore, ferroptosis generally refers to an iron-dependent, non-apoptotic form of regulated cell death. Ferroptosis inhibition may be essential for good motor neuron health and survival *in vivo*. Deletion of GPx4 induces ferroptosis-induced motor neuron degeneration and paralysis, strongly indicating that GPx4 is crucial for motor neuron health and survival *in vivo* [43]. The presumption is that ferroptosis requires three critical events: (1) accumulation of redox-active iron, (2) glutathione depletion, and (3) lipid peroxidation. It is proposed that these three events must occur simultaneously as inhibiting just one prevents ferroptosis. The three events are hypothesised to amplify in severity through positive feedback loops. Therefore, the cause of death in ferroptosis is the synergistic combination of antioxidant depletion, iron toxicity, and membrane denaturation. Edaravone, an intracellular antioxidant, may prevent ferroptosis in motor neurons and it may be its primary mode of action.

CONCLUSION

The multifaceted mechanisms underlying both the initiation and progression of ALS indicate that a suitable therapeutic approach must be wide-reaching. The mechanism of Edravone action in ALS is the prevention of oxidative damage in intracellular lipid membranes which prevents ferroptosis (a form of programmed cell death) which can kill motor neurons. It is not likely to influence the wide ALS population. Early stage sporadic ALS patients may react to Edravone treatment before pathophysiological processes are advanced and before the induction of other forms of motor neuron programmed cell death. Edaravone may be one component within a complex treatment regime for ALS. If so, Edaravone could be a new player in a complex playground.

LIST OF ABBREVIATIONS

$^{\bullet}OH$	=	Hydroxyl radical
ALS	=	Amyotrophic lateral sclerosis
CSF	=	Cerebrospinal fluid
DMT1	=	Divalent metal (ion) transporter 1
GPx4	=	Glutathione peroxidase-4
H_2O_2	=	Hydrogen peroxide
iNOS	=	Inducible nitric oxide synthase
ROS	=	Reactive oxygen species
SOD1	=	Copper Zinc superoxide dismutase
TFR1	=	Transferrin receptor
xCT	=	cystine–glutamate antiporter

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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