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Superoxide dismutase and its Mimetics: Role in neurodegenerative diseases– A review

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Abstract
Formation of superoxide ions and oxidation process poses a great threat to the degeneration of neurons. This neurodegeneration may result in the neurodegenerative diseases. Superoxide dismutase is an antioxidant enzyme that can be found in all living cells. And hence it has been considered for the treatment of the neurodegenerative diseases like alzheimers. Synthetic SOD mimetics have been identified which have been tested for their neuroprotective action in many of the neurodegenerative diseases. This review includes a detailed exploration of activity of Superoxide dismutase and its mimetics in the neurodegenerative diseases.

Keywords: superoxide dismutase, antioxidant, SOD mimetics, neurodegenerative disease

Introduction

Superoxide dismutase is an enzyme found in all living cells [1]. It is the most powerful antioxidant which acts as the first detoxification enzyme in the cell. It is an essential anti-oxidant element for the first line of defence against Reactive oxygen species (ROS). This enzyme is responsible for the catalysation reaction between potentially hazardous two molecules of Superoxide anion (O2) into less harmful Hydrogen peroxide as well as oxygen molecules (O2).SOD needs a metal cofactor for its activity since it is a metalloenzyme. also found in chloroplasts and peroxisomes [4, 5].

Physiological Roles

Metabolism of reactive oxygen species (ROS):
Superoxide (O2_2) is formed by NADPHoxidase, xanthine oxidase, nitric oxide synthase (NOS), lipoxygenase,and mitochondrial enzymes. Superoxide istransformed by superoxide dismutase (SOD) to H2O2, and the H2O2 gets converted spontaneously to hydroxyl Based on the kind of metal ion necessary as a cofactor, different forms of SOD enzyme exist [2, 3]. Iron (Fe), zinc (Zn), copper (Cu) and manganese (Mn) arethe normally bound metal ions by SOD. According to this superoxide dismutases are categorized into three different forms

1. (Fe-SOD- normally found in prokaryotes and chloroplast of number of plants
2. Mn-SOD- which is found in prokaryotes and mitochondria of eukaryotes and
3. Cu/Zn-SOD- found in eukaryotes and mostly dispersed essentially in cytosol but radical (OH^-), or associated metal related reactive species, which are highly reactive, in the presence of reduced transition metal (Fe2 +, Cu+). Mainly nitric oxide (NO) will be quickly rendered inactive byreaction with O2_2 and produces the powerful oxidant peroxynitrite (ONOO2). Hence SOD is considered the first line ofdefense against toxicity of superoxide anion radicals. By regulating ROS e.g., O2_2, H2O2) and obtainable NO, this enzyme also takes part in cell signalling [7].

Classification

Isoform	Characteristics	Metal cofactor	Location
SOD1 (Cu/ ZnSOD)	32 kDa, homodimer	Cu2 + (catalytic) Zn2 + (stability	Cytoplasm, mitochondrial IMS, and others (nucleus, lysosomes, peroxisomes)
SOD2 (MnSOD)	96 kDa, homotetramer	Mn3 + (catalytic)	Mitochondria matrix
SOD3 (ecSOD)	135 kDa, homotetrameric secretory glycoprotein	Cu2 + (catalytic) Zn2 + (stability)	Extracellular matrix, cell surface, extracellular fluids

SOD1 (Cu/ZnSOD)

The main intracellular SOD (cytosolic Cu/ZnSOD) is SOD1. It is present as a 32kDa homodimer and is majorly found in the cytosol with small amount in intermembrane space (IMS) of mitochondria [8, 9, 10]. It is also observed that SOD1 is also present in nuclei, lysosomes, and peroxisomes, using immuno cytochemical methods, and shows extensive distribution in different types of cells [11]. The differentiating character of SOD1 from SOD 2 is that it's sensitivity to cyanide, while SOD1 is comparatively resistant. 21q22.1 region of chromosome 21 of the human genome contains the gene for SOD1 [12].
The presence or absence of Cu and Zinc decides the enzymatic activity of SOD1. Zn is responsible for the correct protein folding and the stability. The quantity of Cu that is bound in the original Cu site is proportional to the activity of remetallated derivatives of SOD [13, 14, 15]. Zn can be replaced by cobalt and Cu, and is not required for the activity of the enzyme at low pH, whereas Cu is irreplaceable with other metals [15, 16, 17].

SOD2 (MnSOD)

It is a Manganese-containing enzyme present in mitochondria, consists a 96 kDahomotetramer and contained in the produced by vascular smooth muscle cells and fibroblasts in the vascular tissue [25]. SOD3 is also found in inflammatory cells, in injured tissue and atherosclerosis [26, 27, 28]. It is produced and directed to mitochondrial matrix [18, 19]. At the active site of SOD2, Mn acts to catalyze the disproportionation of O2-to oxygen and H2O2 in a manner that is similar to SOD1 and SOD3(Cu/Zn SODs) [17]. SOD2 is produced in the cytoplasm and transferred to mitochondria by a signal peptide, and there it then dismutates O2 produced by the respiratory chain enzymes. The active site of SOD2 is not similar to SOD1 but shows homology to FeSOD, which is generally not present in eukaryotes [20].

SOD3 (extracellular Cu/ZnSOD and ecSOD)

It is the main SOD in the vascular extracellular space and is a secretory extracellular Cu/Zn- containing SOD (ecSOD). In a large group of species, SOD3 is a 135 kDahomotetramer containing two disulfide-linked dimers. SOD3 can be mainly found in the extracellular matrix and on cell surfaces with less amount in the plasma andextracellular fluids. It is thought that tissue SOD3 accounts for 90%–99% of the SOD3 in the body [21, 22]. Although the tissue distribution of SOD3 varies in different species, in general, it is highly produced in some tissues such as blood vessels, the lung,kidney, uterus, and, to a smaller fraction, in the heart [21, 23, 24, 25]. Similar to SOD1 the SOD3 enzyme is also sensitive to cyanide. SOD3 is mainly theextracellular matrix and endothelial cell surface throughbinding to the heparan sulfate proteoglycan (HSPGs), collagen, and fibulin-5 [29, 30, 31].

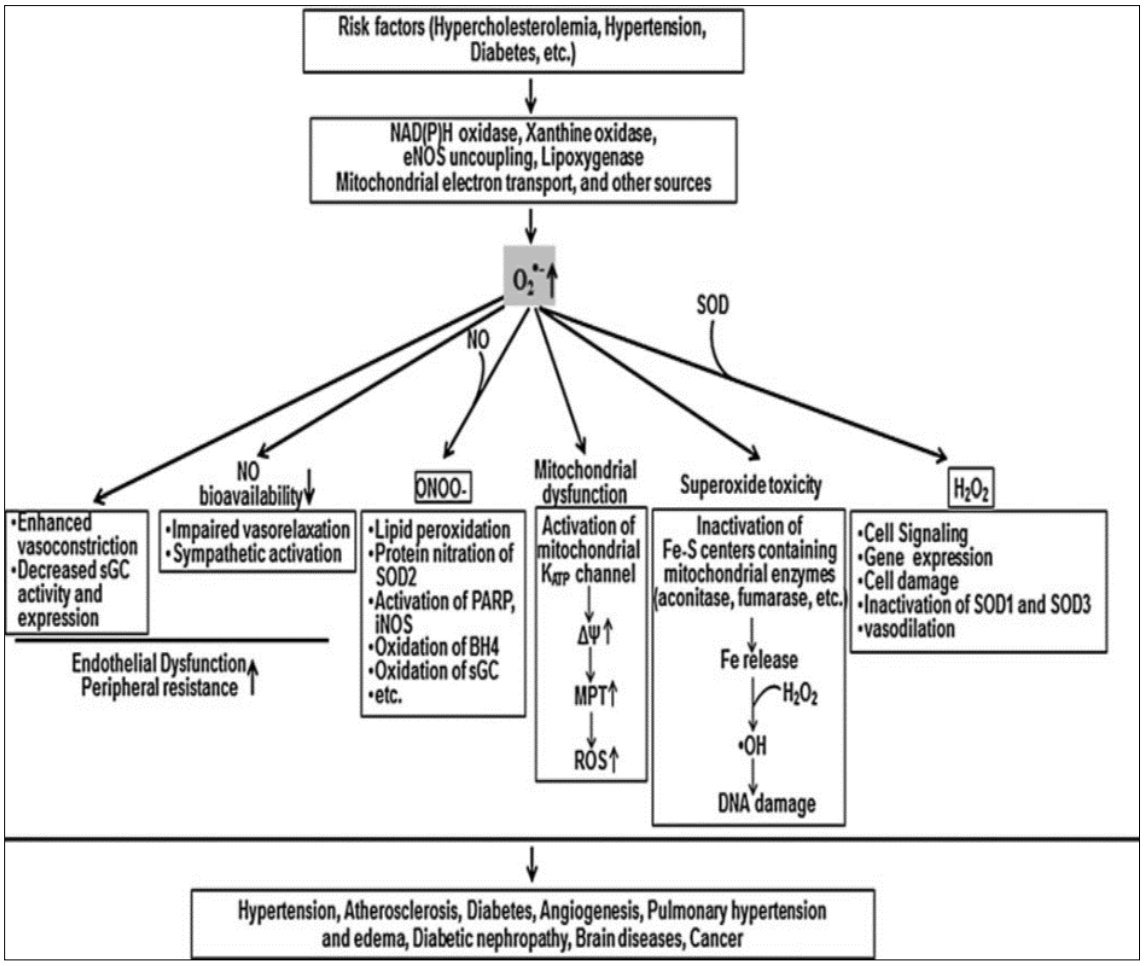

Fig 1: Role of superoxide in various physiological and pathophysiological functions. SOD has a potential impact on various biological function and pathogenesis by regulating NO signaling, ROS (O2_2, H2O2) signaling, and mitochondrial function [7].

SOD in brain

In case of Central Nervous system (Adults),i.e. in hippocampal CA pyramidal neurons and granule neurons of the dentate gyrus, cortical neurons, especially pyramidal cells, neurons of the substantia expression of astroglial cells of this enzyme is still under study. Some studies have shown that the presence of Cu/Zn SOD is highly expressed in astrocytes, but some have failed to identify or have partially identified it in the form of scattering [39, 40]. The expression of reactive astrocytes has been well documented and reported in cases of brain injury, like transient cerebral ischemia, kainate treatment, quinolinic treatment, or Alzheimer's and Down's Syndrome [34, 35, 37, 39].

Though the brain occupies about 2% of the body mass and uses up to 15-20% of the body's energy, it is the most prone organ to oxidative stress. [41-43]

Omega-3- polyunsaturated fatty acids present in brain tissue is mainly responsible for the mass-specific metabolic rate and the phospholipids present are responsible for its susceptibility to peroxidation [44]. It's vulnerability to oxidative stress is further increased in the presence of higher levels of redox-active iron and copper. Since the neurons

and glia present in the brain are terminally differentiated, there is a lower potential of restoration [47].

SOD and neurodegenerative diseases

Oxidative stress was found to be one of the predominant causes of neurodegenerative encoding alterations. A common research interest among scientists is that equal amount of study is being carried out on mechanism of SOD1 mutation which eventually results in autosomal governing phenotype of ALS. Though, not much data has been obtained on the exact molecular pathways resulting in death of motor neurons, certain studies point to the possibilities of accumulation of abnormal SOD1 protein accumulation, effects of mutant SOD1 protein nitrosylation on improvement of proteins, increased peroxidase activity, Glutamate mediated excitotoxicity, unusual intracellular calcium management, disruption of neurofilaments, Toxic copper contact on the active site [49].

Alzheimer's Disease

The main pathogenesis of this disease involves the proteolytic processing of β-amyloid precursor protein (APP) and amyloid-β peptide (Aβ) and which may result in vascular dysregulation due to injury to endothelial lining of the vascular rings and ROS was identified as a possible cause. This may in turn lead to brain functional damage since the cellular energy and flow dependent substrate delivery may get altered. This process can be inhibited by nullifying the noxious effects of Aβ addition of SOD1 scavengers. Studies carried out on transgenic mice showed that mice expressing APP in excess resulted in an intense destruction in endothelium regulations of neocortical micro circulation which was inhibited when the expression of both APP and SOD1 was seen or when SOD was applied topically on cerebral cortex.

When studies of oxidative phosphorylation were performed, it was observed that in AD there was a decrease in mitochondrial complex IV activity which in turn increases the production of ROS. Therefore, the total inhibition of this complex lead to the increased generation of superoxide, though some studies have shown contradictory reports. In normal subjects, it was observed that the levels of SOD1 were greater in large pyramidal neurons of association cortex and hippocampus whereas in patients with neurofibrillary tangles and plaques, it was visible that the immunoreactivity of SOD1 and SOD2 and catalase were increased.

This is of importance since these cells are vulnerable to deterioration in Alzheimer's [49].

Huntington's Disease

It is a genetic disease in which a patient shows visible signs of regular involuntary movements, cognitive damage as well as a noticeable reduction in mitochondrial complex II (succinate dehydrogenase) caused due to the damage of striatal spiny neurons. It was observed that there were Ultrastructural defects in mitochondria and reduced oxygen consumption in cortex and basal ganglia, though no direct relationships were obtained between HD and oxidative damage. In the rat models of HD, it was seen that rats which were injected with mitochondrial toxin 3-nitro propionic acid (3-NP) were selectively lesioned and in some cases where the rats lacked SOD2, the lesions produced were much larger hence proving the fact that ROS play an important role in neurotoxicity studies of HD.

Apoptotic cell death, a course that involves ROS generation, has also been concerned for the neuronal deterioration that takes place in the brain of HD patients [50].

Stroke and Ischemia-Reperfusion Injury

Ischemic injury to the brain is generally caused due to the decreased anti-oxidant defence systems in the brain, which makes it more vulnerable in oxidative injury as well as due to free radical reactions resulting in increased liberation of intracellular iron present in the brain.

The speed of these reactions was also found to be further increased by Epinephrine, Norepinephrine and dopamine which produce superoxide and iron ions on interaction.

One of the main intentions to treat ischemia induced brain injury was to reduce the harmful actions of ROS which has been mainly studied in cerebral infarction by means of various therapeutic approaches such as administration of ROS scavenging drugs and upregulation of endogenous ROS- scavenging drugs as well as circumvention of leukocyte infiltration into damaged brain tissue. The pattern involves reversible middle cerebral artery occlusion followed by rupture of ROS within the first 10-12 minutes. As a result of ischemia, the expression of SOD1 and SOD2 is enhanced and the subsequent activity of enzymes are affected [51].

SOD in PD

Parkinson's disease (PD) is a neurodegenerative disease caused by the deterioration of cells caused by the Reactive oxygen metabolites (ROMs), mitochondrial energy crisis as well as exogenous and endogenous neurotoxins which result in loss of nigrostriatal dopaminergic neurons in substantia nigra pars compacta (SNc) and striatal dopamine(DA) reduction[50,51]. Other causes of neurodegeneration may be due to altered anti-oxidant defense system, enhanced DA turnover, increased ROM production. ROMs are responsible for the pathogenicity of the disease though not majorly and may be caused by local inflammation in brain tissue leading to release of cytokine, resulting in changes in copper and zinc concentrations both intracellularly as well as extracellularly [51]. Sufficient proof have been obtained on the upregulation of Cu:Zn- SOD and Mn- dependent SOD activity in brain and similar outcomes were observed for plasma SODs of the same. Some studies have also shown that the Mn-dependent SOD activity resulted in an increase in PD. All these studies have shown the protective mechanisms of SODs against ROMs [52].

SOD Mimetics

These are synthetic compounds which mimic the actions of the original SOD enzyme. They are highly efficient catalysts in superoxide anion dismutation, and their design is based on the design and subsequent production of Mn (II) and Fe (III) complexes [54]. The stability as well as high SOD value has been determined by means of computer aided modelling studies and they have also paved the way for design and development of newer categories of highly stable and highly active SOD catalysts [55]. These synthetic SOD mimetics are exemplified by the prototype complexes, M40403 and M40401,derived from the 15-membered macrocyclic ligand,1,4,7,10,13-pentaazacyclopentadecane, consisting the added bis (cyclohexyl pyridine) functional groups[52]. An important feature of these SOD mimetics is that they can

selectively remove superoxide anion (O2-) by catalysation without reacting with other reactive species such as nitric oxide, peroxynitrite, hydrogen peroxide, hypochlorite or oxygen [51,52]. They also possess similar catalytic activity as the original enzymes as well as also benefit by being much smaller in size. (eg.MW483-M40403 vsMW30000 for the mimetic and native enzyme respectively) [54, 56].

Manganese Porphyrin

A single porphyrin ring connects the manganese (III) centers in the porphyrin SOD mimetics [55].

Manganese (II) Penta-Azamacrocyclic: M40401/3

M40403 and M40401 are Manganese (II) Penta-Azamacrocyclic complexes containing SOD mimetic properties [56].

Properties of superoxide dismutase mimics, e.g. M40403.

- Manganese-containing biscyclohexylpyridine
- Shows catalytic activity which is equal, if not greater to that of the original enzyme
- Non-peptide, small molecule, non-immunogenic
- Ability to Penetrate cells
- Selectively acts on superoxide (no reaction with biologicallyimportant molecules)
- Stability in in-vivo, no Manganese dissociation
- Does not get deactivated by peroxynitrite
- Found to be protective in different models of acute and chronic inflammation, reperfusion injury and also shock [59].

Superoxide radical dismutation and therapeutic implications

SODs have the capacity to convert superoxide radicals into oxygen and hydrogen peroxide and hence regarded as the first line of defense against ROS. Two main types of isozymes are responsible for the removal of superoxide anions namely SOD1 which is made of copper or zinc protein, located along the mitochondrial intermembrane space, cytosol, peroxisomes as wellas nucleus and SOD2 a mitochondrial manganese enzyme which is generated only during mitochondrial oxidative phosphorylation. It was found that SOD1 and SOD2 were not affected by MPTP induced neurotoxicity and hence it was hypothesized that they may play an important role in etiology of PD [55, 56]. Since these SODs cannot cross the BBB, they may not be used directly as therapeutic agents and hence came the concept of SOD mimetics. So far four main classes of SOD mimetics have been discovered namely

- Metalloporphyrin,
- Nitroxides,
- Mn(III)-Salen complexes and
- Mn(II)-cyclic polyamines [57].

In the study of PD there have been no changes in the activity of cytosolic isoforms (SOD1) and increased activity in mitochondrial isoform (SOD2). Since there is an induce in SOD2 in PD patients [80, 83], it may be the proposed site of ROS generation which is in contrary to catalase and glutathione peroxidase activities which are comparatively decreased. The importance of antioxidant small molecules has also been evaluated in PD brains [60].

Porphyrin-based SOD mimetics

Porphyrin-based SOD mimetics

This class of mimetics consisting of more than 50 molecules were first discovered when there was an extensive search to find an effective water soluble molecule with lower toxicities and more catalytic property, and resulted in identification of such compounds with varying physicochemical as well as functional properties [55]. Mn-poryphyrins which belonged to this class of mimetics was regarded as a rather complex molecule due to its ONO2 scavenger activity and its regulation of redox sensitive cellular transcriptional activity [56]. But the main limitations of this class of mimetics in clinical trials were found to be its Lack of specificity and pro-oxidative activity.

The latter activity may be exploited in cancer research but may prove ineffective in PD patients [56]. Newer molecules of Mn- poryphyrins are still being researched on and a compound known as MnTDE-2-ImP5+ (AEOL 10150) has shown some progress in Phase I of clinical trials on Amyotrophic Lateral Sclerosis patients. Aeolus pharmaceuticals have worked on this compound and have brought about a radio protector and is also working on another molecule AEOL11114- which is another Mn-porphyrin which has shown considerable progress in PD in MPTP based mouse model including increased absorption in GIT, longer plasma half life, BBB penetration and attainment of active concentration in brain [57].

Mn(II)-cyclic polyamines-based SOD mimetics

A relatively newer class of mimetics which were found to be effective against inflammation as well as some disease related conditions in animal models of SOD. A compound of this class M40403 a derivative of 15-membered macrocyclic ligand 1,4,7,10,13 pentaazacyclopentadecane has shown considerable protection activity against rat models of inflammation and ischemia reperfusion injury models and was observed to exhibit resistance to oxidative degradation and is relatively stable. On oral administration, it was shown to exhibit water solubility and penetration through BBB [57] as well as catalysing superoxide radicals and other species such as Nitric oxide, hypochlorite, hydrogen peroxide, peroxynitrite.

In clinical trials it has shown a significant decrease in the management of pain in about 700 patients of phase I and phase II clinical trials and has demonstrated the safety and tolerance and hence newer derivatives are being synthesized with increased stability [58].

Salen–manganese complexes

M40403

These complexes contain aromatic rings, synchronized with manganese center, which are more lipid soluble, pass through the cellular membrane and were found to be more stable than other mimetics [58].

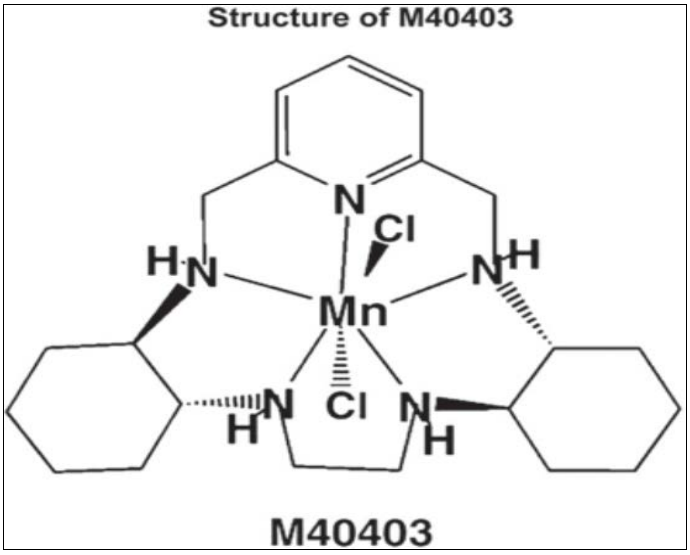

Fig 2

The center of these SOD mimetics which contain an Mn (II) is responsible for its selectivity property. It undergoes oxidation into Mn (III) by protonated superoxide which is reactive whereas when it reduces to its manganese form in cases of diffusion-controlled rates, the complex shows no reactivity with reducing agents. Oxidants containing one electron have not shown oxidative properties against these associated complexes (including nitric oxide and oxygen) since they are relatively difficult to oxidize. M40403and other complexes of this class of SOD mimetics can act as selective probes for taking up the role of superoxide anion in biological systems where other such applicable biological oxidants may be present [59].

EUK-134

EUK- 134 is a synthetic superoxide dismutase mimetic, with testing carried out on rat models showed a significant decrease in seizure activity which may eventually lead to neuronal damage in limbic structure [59].

It comes under the category of Salen-Manganese complex, possesses low molecular weight and exhibits superoxide dismutase (SOD) and catalase activities thereby eliminating hydrogen peroxide and superoxides [60].

In Rat models, Kainic acid (KA) was used to induce seizure activity due to the increased expression of AP-1 and NF-κB in the hippocampus and piriform cortex. Since the transcription factors were not induced after the seizure, this resulted in neuronal damage.

It was observed that all markers of oxidative stress were prevented completely by means of EUK- 134. Since it did not alter the intensity, onset or duration of Seizure activity it may have acted by eliminating oxygen free radicals, and hydrogen peroxide generating by activation of NMDA receptors. In addition, it prevents accumulation of peroxynitrite, and inhibition of nitrosylation of proteins by removal of precursor superoxide. Most of the findings suggest that EUK- 134 protects vulnerable neurons from excitotoxic cell death and may be useful in studying the neurodegenerative diseases caused due to β amyloid toxicity [60].

Nitroxides (Tempol)

Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-Noxyl) is an aqueous soluble as well as redox cycling nitroxide SOD mimetic agent. It is a potential anti-oxidant and metabolizes several reactive oxygen and nitrogen species, and due to its low molecular weight passes through biological membrane. In general, tempol exhibits protective effects against radiation, metabolic syndrome and shock as well as heart, kidney and CNS protection.

In a study that was conducted using Tempol, its mechanism involved was found to be by activating the iron regulatory protein. It is known that Iron Regulatory Protein 1(IRP 1) and Iron Regulatory Protein 2(IRP 2) which senses and regulates iron metabolism genes.

In preclinical studies on mice models, IRP 2 gene was knocked out thus making the mice more susceptible to neurodegenerative diseases along with microcytic Anemia as IRP 1 knockout has not significant effect on the same. This neurodegeneration progressed along with age. A diet consisting of Tempol and stable nitroxide were fed to the mice and it was observed that there was gradual progression of neuro muscular impairment.

In cell line studies, the cell lines obtained from IRP 2 knockout animals as well as the cerebellum, brainstem, and forebrain of animals maintained on the Tempol diet, showed that IRP 1 was converted to IRE binding protein and ferritin synthesis was repressed.

It was hence hypothesized that tempol disassembled the iron sulphur cluster of IRP 1, activates IRE binding protein, Stabilized the TfR 1, Repressed the ferritin synthesis and thus exerting a protecting effect on IRP 2 knockout mice by restoring the normal iron homeostasis of the brain[60].

Conclusion

From long ago it was known that the reactive oxygen species and the oxidation process plays an important role in the generation of neurodegenerative diseases. Superoxide dismutase, the antioxidant enzyme and its mimetics are considered to be a potential treatment for the treatment of these neurodegenerative diseases. With the help of animal models it can be concluded that it can be used to prevent the generation of these diseases. Further human studies has to be conducted and the marketable products are needed to be produced so that these can be available for the treatment of these diseases.

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