Copper, zinc superoxide dismutase (SOD1) and its role in neuronal function and disease with particular relevance to motor neurone disease/amyotrophic lateral sclerosis

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Superoxide dismutase and motor neurone disease/amyotrophic lateral sclerosis

Motor neurone disease/amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative condition characterized by muscle weakness and wasting (for a review see [2]) which leads to severe disability. There is also spasticity, hyperreflexia, and commonly loss of bulbar function affecting speech and swallowing. The prevalence is approximately 4–10/100 000 with an incidence of 1–2 per 100 000. The main sites affected in the disease are the large motor neurones of spinal cord, motor cortex and brain stem. The lateral corticospinal pyramidal tracts arising from the motor cortex also degenerate giving rise to lateral sclerosis from which the most commonly used term for the disease arises. No cognitive decline normally occurs in this disease although some frontal atrophy may be present in a small proportion of cases. Microscopically, motor neurones of the spinal cord atrophy, undergo chromatolysis, inclusions accumulate (e.g. neurofilament conglomerations, Bunina bodies, Hirano bodies) and spheroids (up to 50 µm in diameter) appear, particularly in the proximal axons. In the motor cortex, there is atrophy and loss of Betz cells, and considerable loss of dendritic arborization is evident from Golgi-stained sections. In peripheral nerves, the number of myelinated axons is markedly reduced.

The greatest advance in understanding the aetiology of ALS has come with the discovery of mutations in SOD1 in a subset of familial cases of ALS. Only 5–10% of cases are familial and only ~20% of these familial cases have mutations in SOD1. The remaining gene defects still need to be elucidated.

There are 59 missense mutations known covering all five exons of the gene [3–10]. Until recently exon 3 was thought to be devoid of mutations as it contributes significantly to the region of the active site. However, we and others have now reported mutations in this exon [11]. The most common mutation in the U.S.A. (Ala-
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Over-expression of SOD1 and SOD1 containing FALS mutations

The functional properties of wild-type SOD1 and SOD1 containing FALS mutations have been elucidated using a range of techniques from transfection studies in cell lines to animal models of cerebral ischaemia and transgenic mouse lines. In neural cells, the over-expression of SOD1 inhibits apoptosis induced by serum withdrawal and growth factor withdrawal or by calcium ionophore. In contrast, over-expression of SOD1 containing FALS mutations promotes neural apoptosis [15]. These cell lines show reduced viability and are more vulnerable to oxidative stress. SOD1 down-regulation by use of antisense oligonucleotides causes apoptosis which is reversed by interleukin-1β-converting enzyme (ICE) inhibitors [16].

Over-expression of SOD1 in transgenic mice provides resistance to reperfusion injury after focal cerebral ischaemia and results in greatly reduced infarct volume [17]. Although over-expression of wild-type SOD1 never produces motor neurone disease, there is evidence of increased peroxidation. Over-expression of SOD1 mutations in transgenic mouse lines (e.g. Gly-93Ala, Gly-85Arg, Gly-37Arg) provides an excellent model of 'motor neurone disease'. In the first such transgenic line, Gly-93Ala, animals showed motor neurone degeneration, hind limb
weakness at 3–4 months of age, loss of large myelinated axons from ventral motor roots and neurofilament accumulation in surviving motor neurones [18]. The full profile of ALS pathology affecting both 'upper motor neurones' in motor cortex as well as 'lower motor neurones' in spinal cord has never been demonstrated. Intense SOD1 immunoreactivity in astrocytic inclusions and other pathogenic changes that occur prior to motor neurone pathology have also been shown in astrocytes in the Gly-85Arg SOD1 transgenic line [19] and suggest a role for glial cells in disease development.

**SOD1 gene deletion**

In *Drosophila melanogaster* the null mutation for SOD1 confers a syndrome including reduced lifespan, infertility and toxic hypersensitivity to oxygen stress. FALS mutations expected to destabilize SOD1 subunits and their assembly impair the activity of normal subunits in heterozygotes resulting in significantly lower enzyme activity than expected [20]. The effect of disrupting the interactions responsible for the binding of Cu and Zn at the active site would be the loss of Cu and Zn and the destabilization of the subunit structure. Neurospora crassa mutants null for SOD1 are sensitive to paraquat and conditions of elevated oxygen [21].

Deletion of the entire coding region of the SOD1 gene in mammalian cells is surprisingly free of severe pathology. Development is normal; motor neurone size, density and lipid peroxidation are also all normal. However, motor neurones do appear to be more vulnerable to facial nerve axotomy, showing a 50% loss of motor neurones in animals homozygous for the gene deletion compared with 30% loss in normal animals [22]. In mice bearing a disruption of SOD1 (either SOD1 −/− or +/−) there is an exacerbation of neuronal cell injury and oedema formation following cerebral ischaemia [23].

**Gain of function properties demonstrated in SOD1-bearing FALS mutations**

The overriding conclusion from these transgenic studies is that the mere loss of SOD1 enzyme activity in deletion studies is not sufficient to produce severe neuropathology whereas the presence of SOD1 mutations does produce a motor neurone disease-like condition or exacerbates the effects of cerebral ischaemia. The nature of the toxic effect of the mutant protein has been suggested to reside with the observation that SOD1 enzymes containing FALS mutations also have the property of being able to generate free radicals in addition to their dismutase activity.

Recently it was found that this activity is enhanced in FALS mutants, e.g. Gly-93Ala, whereas dismutase activity is identical to that of the wild-type enzyme [24]. In the U.S.A. the Ala-4Val mutation is the most prevalent and also among the most clinically severe having a duration of 1.2 years [10]. When the cDNA for this SOD1 mutation was over-expressed in insect cells it was found to have similar dismutase activity to the wild-type enzyme but was found to show even greater free-radical-generating activity than the Gly-93Ala mutant in the presence of low concentrations of H₂O₂. This was assayed by a spin trap method in which transient free radical generation is detected by conversion to a stable free radical adduct with 5,5 dimethyl-1-pyrroline N-oxide (DMPO) which is detected by EPR spectroscopy. The Ala-4Val SOD mutation showed a decreased $K_m$ for H₂O₂ compared with wild-type enzyme which correlated with the clinical severity observed in these patients [25]. Wiedau-Pazos et al. [26] have also shown that several FALS mutations produce higher levels of DMPO-OH adducts compared with the wild-type enzyme. The consequences of the enhanced free radical-generating activity of the FALS mutants could have a direct effect on the SOD1 enzyme, accelerating the inactivation of the mutant SOD and causing the release of its metal ions, which in turn could promote peroxide generation by the Fenton reaction. In this way the damaging effects would be localized to sites, such as motor neurones, where high concentrations of the mutant enzyme are present.

The SOD1 mutant protein can also generate a nitronium intermediate which nitrates tyrosine [27] and evidence of increased nitrotyrosine residues in ALS spinal cord has been demonstrated.

In order to determine whether SOD1 mutations give rise to novel protein interactions relevant to ALS pathogenesis which are not shown with wild-type SOD1, the yeast interaction trap system has been used. Two proteins present in ventral spinal cord, lysyl-tRNA synthetase and translocon-associated protein δ were found to interact with mutant forms of SOD1 but not the wild-type enzyme [28].
SOD1 distribution in spinal cord and involvement in ALS pathology

SOD1 immunoreactivity studies show areas of intense labeling in spinal cord but this seems to be localized mainly to Lewy-body-like hyaline inclusions and spheroids and is not uniformly distributed across motor neurons [29]. Analysis of SOD1 mRNA in motor neurons of ALS cases shows that although the total number of motor neurons is substantially reduced, the level of expression within preserved cells is normal. However, expression in atrophic neurons is reduced [30]. As is the case for protein levels, the level of SOD1 mRNA in motor neurons is significantly greater than any other neuronal population emphasizing the potential importance of SOD for the motor neuron. A marked change in levels of SOD is seen between the neonate and young adults. In later adulthood a decrease in SOD1 appears to develop. We quantified SOD1 mRNA in control human spinal cord and found a significant age-dependent decline between 26 and 90 years of age (P<0.01). This change may be of importance in defining the late onset of the disease in sporadic cases. We also reported abnormal straight-chain filaments in dense tangles in a FALS case with the SOD1 mutation (Ile-113Thr), indicating a link between SOD1 mutations and pathological cytoskeletal changes [31].

Pathogenic mechanisms leading to motor neuron degeneration and the role of SOD1 in this cascade of events

A key factor involved in many neurodegenerative events such as those induced by stroke, trauma or seizures is the release of the major excitatory neurotransmitter glutamate. It is present at millimolar concentrations in the central nervous system and readily reaches pathological extracellular concentrations at which it is neurotoxic following such tissue damage. This mechanism has also been implicated in ALS as levels of the glutamate transporter are severely depleted in spinal cord [32]. This could lead to elevated synaptic levels of glutamate. Glutamate is known to lead to a now well-established cascade of reactions that can cause both necrotic and apoptotic cell death. Initially the normal intracellular signalling pathway mediated by Ca^{2+} is used to induce immediate early genes (IEGs), e.g. c-jun which in turn, when activated, regulate the transcription of other genes. In parallel, cellular 'stress' induces activation of protein kinases which are responsible for activation of transcription by phosphorylation e.g. c-jun N-terminal kinases. The IEG c-jun has been shown to play an important role in the central nervous system, in particular with reference to the function of motor neurons in which it is constitutively expressed. c-jun plays a pivotal role in cellular stress as it can promote either repair and recovery or apoptosis. In ALS, we have shown a massive induction of c-jun in ventral spinal cord particularly in neurons of the intermediate grey matter which further implicates this bifurcate pathway in the pathogenic mechanism [33]. Similar changes occur in experimental lesions [34]. The subsequent apoptotic route involves ICE and free-radical-mediated reactions that can be countered by the anti-oxidant properties of bcl-2. In addition, the free-radical-generating reactions of the SOD1 enzyme containing FALS mutations can potentially promote apoptosis. In support of this model, c-jun was found to be strongly induced in neurons of the intermediate grey matter (Rexed's laminae V-VIII and X) of transgenic mice carrying a FALS mutation (Gly-93Ala) [35].

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