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Commentary

Selenium-based compounds: Emerging players in the ever-unfolding story of SOD1 in amyotrophic lateral sclerosis.

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Amyotrophic lateral sclerosis (ALS) is the most common of the Motor Neurone Diseases and is commonly called Lou Gehrig's disease in North America. ALS is a rapidly progressive disease that attacks both upper and lower motor neurons responsible for controlling voluntary muscles, consequently leading to muscular paralysis and invariably death, most often within three years of diagnosis. The prevalence of ALS internationally is estimated to be between 3 – 7 people per 100,000, but the rapid progression and poor prognosis of the disease result in lifetime risk of around 1:300–1:400. Although the underlying cause of sporadic ALS remains a mystery, there is a rapidly growing list of genes in which mutations have been linked to inherited forms of familial ALS (fALS) which accounts for 5–10% of all ALS cases.

The proportion of fALS kindreds that are linked to mutations in *SOD1* depends on the geographical location and ranges from 13% in Australia to 20% in the USA and up to 50% in China. Behind C9ORF72 repeat expansions, mutations in *SOD1* is by far the second leading known cause of ALS. Globally, there are over 150 disease-causing mutations in *SOD1* associated with ALS. It is well established that the mutations cause a gain of function rather than loss of function, and the mutations have in common an ability to destabilize the native structure of the SOD1 protein [1]. It is likely that much of the newly synthesized SOD1 never reaches its properly folded native state, since it is particularly supersaturated in the cellular milieu [2] and that the disease-associated fibrillary aggregate structures it forms are thermodynamically favourable [3]. In stark contrast, once it reaches its mature tertiary structure, it is amongst the most stable in the human body with a thermal melting point of around 90 C. The consequence of such destabilization are the SOD1 inclusions found in post mortem tissue, and downstream consequences such as ubiquitin-proteasome dysfunction, endoplasmic reticulum stress, mitochondrial dysfunction, and calcium dyshomeostasis leading to subsequent motor neuron death. The misfolding, and thus also the downstream consequences, can be propagated throughout the nervous system [4]. Misfolded wild type SOD1 has been detected in sporadic ALS tissue [5], of uncertain significance. It is likely that promoting the correct folding and maturation of SOD1 will address the underlying causes of SOD1 familial ALS, and perhaps will prove beneficial in sporadic ALS.

Although limited to a handful of studies, previous attempts at developing SOD1 pharmacological chaperones have predominantly targeted the maintenance of the SOD1 dimeric state [6] which is the starting point in misfolding from the native dimer. Whilst effective at preventing SOD1 aggregation of purified protein *in vitro*, the use of chemical cross-linkers as a therapeutic is limited by the fact that they typically have off-target effects such as toxic non-specific protein-protein crosslinking, and by the fact that much of newly synthesized protein in the cell never achieves the dimeric state. Previous work has shown that the selenium-based antioxidant compound ebselen pushes the folding equilibrium towards the native dimer in several SOD1-FALS mutants *in vitro* and in cells [7]. There is an important distinction between the dimer-promoting activity of ebselen and a mere tethering of already interacting subunits. In addition, the same work also demonstrated that ebselen could significantly increase the amount of disulfide oxidized SOD1 in cells [7]. Together these effects increase the thermal stability of the protein that should result in a protective effect in SOD1 ALS.

It is at this point that the current work of Hasnain and colleagues [8] weighs in. Their paper, recently published in *EBioMedicine*, reports that feeding G93A SOD1 mice ebselen as part of their diet from 70 days old delayed disease onset by 10 days. The mice were fed food mixed with or without 0.016% w/w powdered ebselen at 24 mg/kg.

They found that while ebselen significantly delayed the onset of ALS in mice, the treatment did not significantly alter mean survival time, body weight, or motor performance. While the *ad libitum* diet drug delivery method could be improved upon in future study, the *in vivo* drug efficacy is encouraging. To this end, the authors synthesized ebselen analogues and put them through a barrage of tests, from dimer dissociation, x-ray crystalization, and cell-based SOD1 toxicity experiments. Amongst the panel of analogues were a few that had enhanced activity when compared to ebselen. Future studies should determine the proportion of the drug that reaches the brain and the maximum tolerated dose of ebselen. It will be important to see if the

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lead analogues from the current work improve on the efficacy of ebselen *in vivo*.

Importantly, ebselen is part of the National Institutes of Health Clinical Collection, a chemical library of bioavailable drugs considered clinically safe but without proven use, enabling rapid translation to the clinic. Apart from the implication for future clinical practice, the study also demonstrates that pharmacological chaperones discovered through *in vitro* studies can translate to *in vivo* rodent studies and hopefully to humans with ALS.

Authors disclosure

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Contributors

Drs. Yerbury and Cashman shared the writing and editing of this commentary.

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