We have recently published a paper in Annals of Neurology entitled “Evidence of Hydrogen Sulphide involvement in Amyotrophic Lateral Sclerosis” [9] where we reported a study performed in patients, and in a genetic model of familial ALS. The outcome of this study is an original finding: the overproduction of hydrogen sulphide (H2S) in the human patients and in the animal model. We also show that H2S is produced, mainly, by glial cells, is toxic to motor neurons and increases significantly cytosolic Ca2+ concentration. Altogether, our data introduce H2S as a new contestant in the ALS-related toxic pathways, which has potential implications for innovative drug design in ALS.

Keywords: Amyotrophic lateral sclerosis; hydrogen sulphide; inflammation; glial cells

Figure 1. H2S can have a “Janus two-faced effect”. Depending on its concentration it can be either protective or harmful to the cellular homeostasis. Hence it is important to clearly understand the effects that the modulation of its production/metabolism may have in brain function.

Hydrogen sulphide (H2S), a gas with a very unpleasant odor, is now considered along with carbon dioxide (CO2) and nitric oxide (NO), a member of the gasotransmitters family\(^\text{[17]}\). To this list, the role played by RNA metabolism, particularly by two proteins, the TAR DNA binding protein 43 (TDP-43)\(^\text{[12, 13]}\) and the fused in sarcoma/translocated in liposarcoma protein (Fus/TLS)\(^\text{[14, 15]}\), has been identified as one of the key pathways leading to the ALS spectrum of disorders\(^\text{[16]}\). Yet, in spite of all the effort that the ALS research has dedicated to the understanding of this disease, many questions are still unanswered.

Historically its toxicity has been primarily ascribed to the inhibition of cytochrome c oxidase (Complex IV) resulting in ATP generation. In striking contrast, more recent data showed that low concentrations of H2S serve as a stimulator of electron transport by acting as a mitochondrial electron donor\(^\text{[18]}\). Cystathionine-β-synthase (CBS), a mainly cytosolic haem-containing enzyme, that accumulates in the mitochondria during hypoxia and has been, so far, identified as the major source of H2S in the mammalian brain\(^\text{[19, 20]}\). Cystathionine-γ-lyase (CSE) also cytosolic and more present in the periphery then CBS\(^\text{[19, 20]}\). Both enzymes metabolize cysteine and (or) homocysteine to release H2S. The third enzyme is 3-mercaptoppyruvate sulftransferase (3MST) that produces H2S from 3-mercaptoppyruvate, a product of the metabolism of cysteine and α-ketoglutarate by cysteine aminotransferase (CAT)\(^\text{[19, 20]}\). The mitochondria are the site of H2S metabolism. Hydrogen sulphide has two opposite effects on the mitochondria: it is an enzymatic substrate at low concentrations and a poison at high concentrations, via the inhibition of Complex IV\(^\text{[18]}\). Increasing evidence suggests that it is a “double-faced Janus”, a molecule with opposite effects (Fig. 1). It participates in the regulation of neuronal functions and signaling\(^\text{[20]}\), but it switches from neuroprotective to neurotoxic as its concentration raises\(^\text{[21]}\) above a certain threshold. In fact, exposure to high µM to mM concentrations are cytotoxic (free radical generation, glutathione depletion, intracellular iron), while nM to low µM concentrations are cytoprotective (antinecrotic and antiapoptotic). Bian and co-workers have demonstrated that H2S suppresses oxidative stress induced by hydrogen peroxide\(^\text{[22]}\), and protects cells against the neurotoxins rotenone\(^\text{[23]}\) and 6-OHDA\(^\text{[24]}\). The protective effects of H2S have been established also “in vivo” in animal models of Parkinson’s disease\(^\text{[25, 26]}\) and Alzheimer’s disease\(^\text{[27]}\), Slivka et al.\(^\text{[28]}\) reported that memory deficiency in Alzheimer’s disease may be related to reduced H2S. More recently Xuan et al.\(^\text{[29]}\) proposed the administration of the H2S donor sodium hydrosulfide (NaHS) as a possible therapeutic approach for Alzheimer’s disease.

On the other hand, Kurokawa et al.\(^\text{[30]}\) described an H2S-induced NMDA-independent neuronal death, via the activation of the ERK-pathway. Moreover, the murine cortical neurons death induced by the addition of 100 µM glutamate was exacerbated by the co-incubation with the H2S donor NaHS\(^\text{[21]}\). The same group performing a transcriptomic profile provided evidence of an NMDAR involvement and ubiquitin-proteasome recruitment in the H2S-induced neuronal death\(^\text{[31]}\). In cerebella granule neurons (CGN) in culture, H2S raises intracellular calcium to toxic range in a dose dependent manner with a 50 % cell death within 2 hours after treatment\(^\text{[32]}\). L-type Ca2+ channels and NMDAR blockers protected CGN against H2S-induced death and largely attenuated the rise of cytosolic calcium. Hence, H2S via the modulation of the Ca2+ homeostasis, affects neuronal viability.

Staying on the issue of a biphasic biological action, the effects of H2S are highly divergent on the mitochondrial respiratory chain depending on its concentration\(^\text{[18]}\). Historically its toxicity has been primarily ascribed to the inhibition of cytochrome c oxidase (Complex IV) resulting in a shutdown of mitochondrial electron transport and cellular ATP generation. In striking contrast, more recent data showed that low concentrations of H2S serve as a stimulator of electron transport by acting as a mitochondrial electron donor\(^\text{[33]}\).

Hence, we have sought to understand if H2S could have a role in the ALS pathophysiology and measured its concentrations in ALS patients and in a fALS mouse model\(^\text{[1]}\). Developing a specific and sensitive HPLC test to measure H2S in body fluids, we found significantly higher levels of H2S in the CSF of male and female ALS patients, compared with the ones measured in age-matched healthy control\(^\text{[1]}\). We have also observed a remarkable relationship between H2S content in the CSF and the site of disease onset, with significantly higher levels in the limb onset (LO)
neurodegeneration. Our results show that H₂S levels in the liquoral H₂S in the ALS female population but it significantly increased when inflammation was the cytosolic to the mitochondrial fraction, a phenomenon described in ALS. The H₂S blood levels were similar in the ALS patients and in the control population. Such high liquoral levels indicate that the neuronal tissues are in a hypoxic state, which has been described in ALS [34].

In line with these observations, we detected increased levels of this gas in the spinal cord, brain stem and cortex of the SOD1G93A mouse, a fALS murine model which over-expresses the SOD1 mutated at position 93. Similar to the findings in the human patients, we measured higher levels of H₂S in the female mouse population. We also found a significant increased translocation of the CBS enzyme from the cytosolic to the mitochondrial fraction, a phenomenon known to occur in hypoxic conditions [35]. Furthermore, “in vitro” primary spinal cord cultures obtained from the fALS mouse model showed an increased production of H₂S (as measured in the culture media). Remarkably, the inhibition of glial cell proliferation decreased H₂S media concentration, but it significantly increased when inflammation was activated by Lipopolysaccharide (LPS). In addition, when H₂S was administered to control cultures, via the H₂S donor NaHS, we observed a dose-dependent increase in SMI-32 positive neurons death (motor neurons), while GABAergic interneurons were more resistant to the H₂S-mediated toxicity. Finally, measuring cytosolic Ca²⁺ concentration ([Ca²⁺]) in response to NaHS in spinal motor neuron cultures, we found a significant rise in [Ca²⁺] that was strongly attenuated when the intracellular ATP concentration was increased to 2 mM.

In summary our study, using patient’s samples as well as pre-clinical “in vivo” and “in vitro” models identifies H₂S as an additional pathological player in the ALS-related neurodegeneration. Our results show that H₂S levels in the central nervous system of ALS patients reaches supra-physiological concentrations, which appear to be toxic primarily to motor neurons. We have also demonstrated that the main sources of H₂S are glial cells, thus identifying it as a factor involved in the non-cell autonomous motor neurons death. On the other hand, it raises new and unanswered questions. Which is the primary source of H₂S? As inhibitor of the mitochondrial respiratory chain, does it contribute to the mitochondrial distress described in ALS? Why motor neurons are more vulnerable to it than other type of neurons? Is a neuroinflammatory factor the primus movens in ALS? Future studies addressing these and other questions will help to assess if the control of H₂S production could be a valuable approach in a multidrug therapeutic strategy aiming at slowing down and/or cure this deadly disease.

References

15. Vance C, Rogelj B, Hortobagyi T, De Vos KJ, Nishimura AL,


