The increased production of hydrogen sulfide in amyotrophic lateral sclerosis is a significant risk factor?

Longone Patrizia[1], Davoli Alessandro[2], Ricciardo Rizzo Giada[2], Guatteo Ezia[3], Spalloni Alida[1], Greco Viviana[4,5], Urbani Andrea[4,5], Mercuri Nicola Biagio[2,3]

[1]*Molecular Neurobiology Unit, Institute of Hospitalization and Scientific Care–Fondazione Santa Lucia, Rome,* [2]*Neurophysiopathology Unit, Department of System Medicine, University of Rome Tor Vergata, Rome,* [3]*Experimental Neurology Unit, Institute of Hospitalization and Scientific Care–Fondazione Santa Lucia, Rome,* [4]*Department of Experimental Medicine and Surgery, Department of System Medicine, University of Rome Tor Vergata, Rome,* [5]*Proteomics and Metabonomics Unit, Institute of Hospitalization and Scientific Care–Fondazione Santa Lucia, Rome*

A B S T R A C T

Amyotrophic lateral sclerosis (ALS) is a motor neuron disease whose pathophysiological deficits causing impairment in motor function are largely unknown. Recently, our group has found significant high levels of H2S in the liquor of 37 ALS sporadic patients and in tissues and media from the spinal cord cultures bearing the familial ALS (fALS) mutation SOD1G93A (Davoli et al., 2015). Hydrogen sulphide (H2S) has been considered a physiological messenger alike the gasotransmitters nitric oxide and carbon monoxide as well as a novel neuromodulator exerting neuroprotective effects in the brain. Experimentally it is evident that the effect of H2S on the cellular homeostasis depends on its concentration. We propose H2S as a new/additional player in the mechanisms of non-cell-autonomous motor neuron death as a product of glial activation. Here we further discuss its potentials as a novel therapeutic target in ALS.

Keywords: Hydrogen sulphide, Amyotrophic Lateral Sclerosis, neuroinflammation, cerebrospinal fluid.

***Corresponding author:** Email: mercurin@med.uniroma2.it

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease of the motor nervous system. Its mechanisms of pathogenesis remain incompletely understood. While most cases are sporadic, the upsurge of genetic studies have found that the familial cases result from mutation in more than 25 genes [1]. The first gene causally linked to ALS was the superoxide dismutase 1 (SOD1) related to oxidative stress; now the ALS-related genes span an unpredictably broad range of molecular functions, including RNA metabolism, axonal transport, autophagy, excitability, and immunity [2]. Moreover, it is commonly accepted that the pathogenic processes take place not only in motor neurons but also in nonneuronal neighboring cells, astrocytes, and microglial cells and even beyond the central nervous system, in skeletal myocytes and, probably, other cells in the body [3]. In this context, one of the greatest challenges in the ALS field is to sort out this puzzle and understand the interactions among these diverse pathological pathways. That is the likely key to optimize both clinical trials and patient treatment because targeting different pathways may ultimately be the necessary approach to cope with this fatal disease.

Cerebrospinal fluid (CSF) analysis is a basic laboratory tool that reflects pathophysiological alterations in the course of the disease and provides an insight into disease pathomechanisms. The CSF is in intimate contact with most regions of the central nervous system (CNS), circulating through the ventricles and the central canal of the spinal cord. Hence measuring the CSF content of molecules originating from the brain is a well-accepted method to evaluate alterations in the CNS contents of molecule of potential pathological interest. In this context, in a paper published early this year [4], we have quantified the CSF levels of hydrogen sulfide (H2S) in ALS patients and found remarkably high levels of this gaseous neurotransmitter compared to age-matched controls. Moreover, higher levels of liquoral H2S in the ALS female population compared to the male were also seen, although this was not significant. Dividing our population into different clinical subgroups, we could emphasize a relationship between H2S levels and the site of disease onset. In fact, ALS patients with limb onset show significantly higher levels of H2S in the CSF compared to ALS patients with bulbar onset. Moreover, our data suggest an additional caudo-cranial direction in H2S concentration. This is characterized by significantly higher levels of the gas in patients with lower limb onset compared to patients with upper limb onset. Moreover, the correlation that we found between CSF ALS H2S values and progression rate of disease indicates a possible prognostic role of the gas, by possibly suggesting the speediness and severity of the pathological process. Furthermore, the finding of higher levels of H2S in the CSF but not in the sera of ALS patients suggests H2S as a marker of pathogenic processes specifically involving the central nervous system degenerative process.

The toxicology of high concentrations of H2S as an environmental pollutant has been studied extensively. In recent years, since it was discovered that mammalian and human tissues can synthesize it, H2S has attracted considerable interest as an endogenous gaseous mediator and a potential pharmacological and therapeutic tool [5]. We now know that H2S is involved in diverse physiological and pathophysiological processes, such as regulation of blood pressure, modulation of long-term potentiation, inflammation, neurodegenerative disease, and metabolic disorders, including obesity and diabetes [6–8]. Depending on its concentration H2S can transform its acts from physiological/protective to pathological/harmful. In ALS an increased inflammatory drive has been definitely described, and although the precise role of endogenous H2S in the inflammatory signaling is not yet clear we may infer that its increased levels may represent a response to the acute, as well as chronic neuronal degeneration in ALS. However, at this stage we do not know whether H2S levels were elevated to drive the inflammatory response or to control or limit tissue degeneration/inflammation. We may hypothesize an initial beneficial action of H2S, which has shown neuroprotective (antinecrotic and antiapoptotic) effects through multiple mechanisms, a protective action that subsequently turns deadly when its concentration reaches toxic values as we have shown in patients and in a series of in vitro studies.

What is also noteworthy about our study is the fact that we have found elevated H2S levels in the sporadic ALS patients and in the classical ALS mouse model of the SOD1G93A mouse. This observation pinpoints H2S as a factor concurring to the ALS-related neurodegeneration regardless of its cause.

Neuroinflammation is a common pathological hallmark of ALS and it has been detected in spinal cords from human ALS patients and mouse models of the disease [9,10]. Moreover, innate immunity is implicated in the amplification of the inflammatory response in ALS [11]. For example, chronic infusion with lipopolysaccharide (LPS) augments the innate immune response and exacerbates pathology in presymptomatic ALS mice [12]. In our study, we found that in spinal cord cultures treated with LPS the concentration of H2S increases significantly, particularly in the ALS-mutant cultures. Hence, it is realistic to number H2S as a pro-inflammatory factor, released by glial cells, involved in the non-cell-autonomous degeneration in ALS. In contrast to what it is stated about its role in other neurodegenerative diseases, namely Alzheimer's and Parkinson's disease, where it has been proposed as a potential therapeutic molecule, in ALS H2S seems to operate as a pro-inflammatory risk factor.

There are still strong limitations to identify diagnostic, prognostic, or disease progression biomarkers for ALS because this is a rare condition, characterized by a remarkable phenotypic heterogeneity, without prodromal clinical characteristics and, consequently, with a long latency from onset to diagnosis. Since neuroinflammation has gained a particular focus as a key mechanism of ALS development and in the neuroimaging field, several studies aiming at highlight neuroinflammation in ALS have proliferated.

In particular, novel positron emission tomography/computer tomography (PET/CT) radiotracers have been developed to image activated microglia by binding specific proteins which are highly expressed in activated microglia and astrocytes,

Reliable results indicate (18F-FDG) Positron Emission Tomography/Computed Tomography (PET/CT) as a biomarker to discriminate ALS patients from controls, emphasizing the presence of a relative hypermetabolism in specific brain regions as a mark of neuro-inflammation related to astrocytosis and microglia activation [13–15].

Therefore, our next desirable goal is to explore the neuroinflammatory status of ALS patients either by measuring the levels of H2S in the CSF or by performing PET neuro-imaging. Thus, the correlation of biochemical and neuroimaging results with clinical data could allow an objective assessment of the clinical consequence of the neuroinflammatory processes, in order to create a new model of biomarkers that could result as useful for the diagnosis and prognosis of ALS.

In conclusion, our findings demonstrate that H2S has pathophysiological effects in ALS and it should be considered an important risk factor for this disease. Hence, knowledge of the H2S biology in ALS raises the possibility that manipulating its system for therapeutic benefits to the patients should be fully explored leading to new treatment approaches. Bearing this in mind, we think that extensive studies should be conducted to achieve a more comprehensive knowledge of H2S in ALS and more in general in CNS, helping to seek promising and more effective therapeutic agents for neurodegenerative disorders.

REFERENCES

1. Andersen PM, Al-Chalabi A. Clinical genetics of amyotrophic lateral sclerosis: what do we really know? Nature Reviews Neurology. 2011;7(11):603–615. doi: 10.1038/nrneurol.2011.150.
2. Rothstein JD. Current hypotheses for the underlying biology of amyotrophic lateral sclerosis. Annals of Neurology 2009;65(suppl 1):S3–S9.
3. Ilieva H, Polymenidou M, Cleveland DW. Non-cell autonomous toxicity in neurodegenerative disorders: ALS and beyond. Journal of Cell Biology. 2009;187(6):761–772. doi: 10.1083/jcb.200908164.
4. Davoli A, Greco V, Spalloni A, Guatteo E, Neri C, Rizzo GR, et al. Evidence of hydrogen sulfide involvement in amyotrophic lateral sclerosis. Annals of Neurology. 2015;77(4):697–709. doi: 10.1002/ana.24372.
5. Kimura H. Hydrogen sulfide: its production, release and functions. Amino Acids. 2011;41(1):113–121. doi: 10.1007/s00726-010-0510-x.
6. Desai KM, Chang T, Untereiner A, Wu L. Hydrogen sulfide and the metabolic syndrome. Expert Review of Clinical Pharmacology. 2011;4(1):63–73. doi: 10.1586/ecp.10.133.
7. Kimura H. Hydrogen sulfide: from brain to gut. Antioxidants and Redox Signaling. 2010;12(9):1111–1123. doi: 10.1089/ars.2009.2919.
8. Kamat PK, Kalani A, Tyagi N. Role of hydrogen sulfide in brain synaptic remodeling. Methods in Enzymology. 2015;555:207–29. doi: 10.1016/bs.mie.2014.11.025.
9. McGeer PL, McGeer EG. Inflammatory processes in amyotrophic lateral sclerosis. Muscle and Nerve. 2002;26(4):459–470.

10. Hensley K, Mhatre M, Mou S, Pye QN, Stewart C, West M, et al. On the relation of oxidative stress to neuroinflammation: lessons learned from the G93A-SOD1 mouse model of amyotrophic lateral sclerosis. Antioxidants and Redox Signaling. 2006;8(11-12):2075–2087.
11. Letiembre M, Liu Y, Walter S, Hao W, Pfander T, Wrede A, et al. Screening of innate immune receptors in neurodegenerative diseases: a similar pattern. Neurobiology of Aging. 2009;30(5):759–768.
12. Nguyen MD, D'Aigle T, Gowing G, Julien JP, Rivest S. Exacerbation of motor neuron disease by chronic stimulation of innate immunity in a mouse model of amyotrophic lateral sclerosis. Journal of Neuroscience. 2004;24(6):1340–1349.
13. Corcia, P., Tauber, C., Vercoullie, J., Arlicot, N., Prunier, C., Praline, J., et al. Molecular imaging of microglial activation in amyotrophic lateral sclerosis. PLOS ONE. 2012;7(12):e52941. http://dx.doi.org/10.1371/journal.pone.005294123300829
14. Turner, M.R., Cagnin, A., Turkheimer, F.E., Miller, C.C., Shaw, C.E., Brooks, D.J., et al. Evidence of widespread cerebral microglial activation in amyotrophic lateral sclerosis: an [11C](R)-PK11195 positron emission tomography study. Neurobiology of Disease.2004;15(3);601–609. http://dx.doi.org/10.1016/j.nbd.2003.12. 01215056468.
15. Zürcher NR, Loggia ML, Lawson R, Chonde DB, Izquierdo-Garcia D, Yasek JE, et al. Increased in vivo glial activation in patients with amyotrophic lateral sclerosis: assessed with [(11)C]-PBR28. Neuroimage: Clinical 2015;19;7:409–414. doi: 10.1016/j.nicl.2015.01.009.