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Case Studies

Cysteine, Sulfite, and Glutamate Toxicity: A Cause of ALS?

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Abstract

Background: Amyotrophic lateral sclerosis (ALS) of nonmutant superoxide dismutase (SOD) type may be caused by toxicity of the reduced glutathione (GSH) precursors glutamate and cysteine, and sulfite (a metabolite of cysteine), which accumulate when one or more of the enzymes needed for GSH synthesis are defective.
Objectives: A case is examined where the patient exhibited elevated sulfur on a hair mineral analysis, elevated blood cysteine, positive urine sulfite, elevated urine glutamate, and low whole blood GSH. During the time when strict dietary and supplement measures normalized the patient's whole blood GSH, blood cysteine, and urine sulfite, the patient did not experience additional physical decline. The possible causes of abnormalities of the patient's laboratory test results, as well as the nutrition measures used to normalize them, are discussed in relationship to the functions and importance of cysteine, sulfite, and glutamate in glutathione metabolism in ALS.
Conclusions: Since elevated plasma cysteine has been reported in other ALS patients, sulfite and cysteine toxicity may be involved in other cases of ALS. Patients with ALS with nonmutant-SOD should be tested for sulfite toxicity, cysteine, glutamate and GSH levels, and whether they have low levels of GSH metabolism enzymes. Since glutamate metabolism appears to be inhibited by sulfite, research on the effect of sulfite on glutamate levels in patients with ALS should be pursued. Life might be prolonged in those patients with ALS with sulfite toxicity by closely monitoring the blood cysteine and urine sulfite levels and minimizing their dietary intake, as well as increasing GSH by using sublingual GSH. A long-term solution might be found through research to determine methods to increase GSH synthesis without using sulfur-containing supplements that may add to the cysteine and sulfite toxicity.

Introduction

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, is a terminal neurologic disorder characterized by progressive degeneration of nerve cells in the spinal cord and brain, resulting in muscle weakness and paralysis. ALS is classified into (1) familial or hereditary ALS, which accounts for about 10% of the cases, and of those, about 20% are linked to a mutation in the superoxide dismutase (SOD-1) gene; and (2) sporadic ALS of unknown cause, which accounts for about 90% of cases. A case is presented of a patient with familial ALS, but with normal SOD-1 gene, thus having nonmutant-SOD ALS, exhibiting cysteine and sulfite and glutamate toxicity. The conclusions from this case appear to apply to both sporadic and familial nonmutant-SOD ALS.

Case Report

A 53-year-old female patient was diagnosed on October 15, 2004 by her neurologist with bulbar-onset ALS, involving both upper and lower motor neurons. First fasciculations were noticed March 2004 (also age 53). The patient was having difficulty with talking, walking, and swallowing by July 2005 when she first sought nutritional assistance. A feeding tube was needed by September 2005 to assist eating, a walker by January 2006, and then a wheelchair by May 2006, but her hands retained enough use to be able to use a computer. According to her neurologist, by September 25, 2006 her Medical Research Council scoring (0–5, normal = 5) for strength was 0 for legs and 2 for arms; her reflexes were hyperreflexia 4 out of 4. A genetic test for mutant SOD-1 gene by Massachusetts General Hospital ordered by her neurologist showed that the patient did *not* have the mutant SOD-1

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gene, but her neurologist told her she definitely had ALS caused by an unknown genetic mutation.

Family history of patient with ALS

The patient's father was diagnosed with ALS at Mayo Clinic in January 1987 and died April 1988 at age 67. The patient's father's mother (who is believed to be the carrier of the family's suspected mutant gene) died at age 45 from breast cancer, so the first incidence of ALS in the family was the patient's father.

Case report details

Cysteine and sulfite. See Table 1 for the patient's nutrition laboratory test results. The patient's hair mineral analysis test on July 22, 2005 showed elevated sulfur. Of course, hair permanents and dyes can affect test results, but this patient did not color or perm her hair so the test was probably valid. Sulfur is measured in this test at the cell level for all sulfur, whether it is elemental sulfur or cysteine or sulfate, etc., so it is not indicative of which form of sulfur is high or the cause of the problem.

The elevated blood cysteine and elevated plasma sulfate test results and elevated urine sulfite readings are more indicative of the source of high sulfur in this patient. The plasma cysteine test result on January 30, 2006 was elevated in spite of the patient avoiding (in response to the elevated sulfur on the hair mineral analysis) onions, garlic, and egg yolks, which contain sulfur, for 5 months prior to the plasma cysteine test. In addition, the patient could not drink much wine without a headache, indicating that the patient might not metabolize sulfites well since most wines contain sulfites. Sulfite urine dipstick tests using Merckoquant Sulfite Test EM Science Catalogue No. 10013 (EMD Chemicals Inc., Gibbstown, NJ, an affiliate of Merck KGaA, Darmstadt, Germany) were used on freshly voided urine samples with a pH

Table 1. Amyotrophic Lateral Sclerosis Patient's Nutrition Laboratory Tests, Referred to in the Case Report

Date	*Item*	*Findings*	*Results*	*Reference range*	*Comments*
(1) Hair mineral analysis by Genova Diagnostics Laboratory					
July 22, 2005	Sulfur	Elevated	59,076 μg/g	41,178–59,023	No toxic metals found
May 24, 2006	Sulfur	Elevated	68,556 μg/g	41,781–60,894	Too soon to reflect dietary changes
	Arsenic	Elevated	0.103 μg/g	≤0.080	Probably from atrophying muscles
	Gadolinium	Elevated	0.0009 μg/g	≤0.0005	
	Cadmium	Borderline	0.022 μg/g	≤0.022	
(2) Comprehensive detoxification profile liver function test by Genova Diagnostics Laboratory					
Jan. 30, 2006	Glycine conjugation phase II	Low normal	31% Recovery	30–53	
	Glucuronidation phase II pathway	Low	26% Recovery	27–56	See below[a]
	Sulfation phase II pathway	Elevated	41% Recovery	16–36	Working above normal
	Plasma sulfate	Elevated	5.40 mg/dL	4.80–5.30	Working above normal
	Glutathione conjugation phase II pathway	Low normal	6.9% Recovery	5.6–11.4	Working in low normal area
	Whole blood reduced GSH	Low	29 mg/dL	≥32	
	Cysteine, plasma	Elevated	4.88 mg/dL	3.10–3.90	
	Superoxide dismutase (SOD)	Elevated	2,490 U/g Hgb	1,610–2,162	May reflect the body's attempt to deal with excess free radicals
	Urine lipid peroxides (TBARS test)	Elevated	15.5 nmol/mg	3.0–9.0	Suggesting oxidative damage to lipids
(3) Whole blood reduced GSH and serum cysteine test by Genova Diagnostics Laboratory					
July 17, 2006	Whole blood reduced GSH	Normal	980 μmol/L	≥669	After sublingual GSH
	Cysteine, serum	Normal	0.64 mg/dL	0.61–1.16	After dietary changes
July 19, 2006	Whole blood reduced GSH	Normal	903 μmol/L	≥669	After IV GSH
(4) Glutamate urine test by Neuroscience, Inc.					
July 18, 2005	Glutamate	Elevated	39.0 μmol/g Cr	Day 10–25, night 8–20	While taking riluzole
Dec. 3, 2005	Glutamate	Elevated	46.2 μmol/g Cr	Day 10–25, night 8–20	While not taking riluzole; nutritionally increased serotonin and GABA neurotransmitter levels
July 31, 2006	Glutamate	Elevated	47.9 μmol/g Cr	Day 10–25, night 8–20	While taking riluzole

[a]Glucuronidation phase II pathway probably low due to 2 months of taking amitriptyline, which primarily uses the glucuronidation pathway for detoxification, as well as long-term use of riluzole, which is metabolized mostly by the liver using P450-enzyme-dependent-hydroxylation and glucuronidation, although riluzole had been discontinued 2½ months prior to the test.

GSH, glutathione; IV, intravenous; GABA, γ-aminobutyric acid.

above 6.0. Sulfite testing is not usually done because it requires a freshly voided urine sample and thus cannot be done on a specimen sent to a laboratory, and it also requires a pH above 6.0. According to the Merckoquant Sulfite Test package insert, "The pH value of the solution to be tested should lie between pH 6 and pH 12. At low pH values, less sulfite is indicated than is actually present."[1] Wright and Littleton report that "A fresh random urine sample may be preferred . . . due to the possibility of spontaneous non-enzymatic oxidation of sulphite to sulphate ion."[2] Sulfite urine dipstick tests were done starting in April 2006 at multiple times during the day for at least 120 tests over a 4–5 month period, showing that the patient did indeed have sulfite toxicity with continuously fluctuating urine sulfite levels. The patient's urine sulfite readings were often 0 but rose to 15 ppm (for example, 2.5 hours after consumption of various foods such as flounder and tilapia and other foods that were found to contain sulfite), and then declined to 0–6 ppm by the next morning. By way of comparison, another patient without ALS had sulfite readings of 0 at 1.5 and 2.5 hours after drinking a 3-ounce glass of wine that had a sulfite reading of 200 ppm.

As a result of the elevated urine sulfite readings and the elevated blood cysteine test result, dietary changes were made to decrease the patient's dietary intake of methionine- and cysteine-containing foods. In addition, foods were checked before consumption for sulfite with the sulfite urine dipsticks, and any food testing positive for sulfite was discarded. Any sulfur-containing supplements such as *N*-acetyl-cysteine and α-lipoic acid were discontinued, because they might add to the cysteine and sulfite toxicity. After these changes, a subsequent serum cysteine test result was within the normal range on July 17, 2006 and most urine sulfite readings were reduced to zero.

One food, which unfortunately was not tested for sulfites before consumption until August 2006, and no urine sulfite tests were done during consumption, was Vivonex Plus by Novartis Nutrition Corporation (subsequently purchased by Nestlé Health Nutrition Minnetonka, MN), which was recommended by a hospital dietitian for tube feeding. Sulfite readings on Vivonex Plus were 450 ppm, which is appreciably higher than a red wine reading of 270 ppm. This food was not used except for convenience on several vacations. However, the patient did experience considerable decline during and after relying on Vivonex Plus.

Glutathione. Glutathione (GSH, reduced glutathione) is an important antioxidant and antitoxin; its metabolism is illustrated in Figure 1. The patient's whole blood GSH test level was below normal on January 30, 2006 after 5 months of supplementing *N*-acetyl-cysteine 200 mg twice daily and α-lipoic acid 40 mg twice daily to try to increase GSH levels. So intravenous GSH (containing *no* sulfite additives) was obtained and administered through another neurologist in his recommended doses for ALS. When it was determined that there was an apparent dose-related correlation between the intravenous GSH dosage and the elevated sulfite readings, then the intravenous (IV) GSH dose was quickly reduced. See Table 2 for IV GSH doses and urine sulfite tests done between May 2006 and June 2006. Perhaps the body breaks down excess GSH into its component parts of glycine and glutamate and cysteine, thus adding to the cysteine load. When the intravenous GSH dose was low enough to result in sulfite readings of 0, then a subsequent whole blood reduced glutathione test at $1\frac{1}{2}$ hours after administration of 250 mg of intravenous GSH showed a result within the normal range. Another whole blood GSH test at $1\frac{1}{2}$ hours after administration of 50 mg sublingual GSH (Chem-Defense by Source Naturals, Scotts Valley, CA) also showed a result within the normal range. Note that taking GSH orally is not effective because it is broken down in the intestinal tract, but sublingual GSH bypasses that problem. The sublingual GSH was easier to use and able to be taken three times daily for more uniform blood levels; thus, the IV GSH was discontinued.

Glutamate. The glutamate urine tests were elevated while taking riluzole, which is a drug approved by the U.S. Food and Drug Administration to help counteract the known glutamate toxicity in ALS (Table 1). Whether riluzole had any effect on the patient's glutamate toxicity by other mechanisms is not known. All food was prepared fresh after August 2005, so no food additive excitotoxins were being ingested with the exception of Vivonex Plus as discussed above. Glutamate also remained elevated with nutritionally increased serotonin and γ-aminobutyric acid neurotransmitter levels and without riluzole.

Patient outcome. During part of the summer 2006 when the patient's whole blood GSH, blood cysteine, and urine sulfite test results were normal, the patient did not experience additional physical decline and had strong breathing per her own assessment.

An August 2006 vacation, during which the patient used Vivonex Plus extensively for food, was accompanied and followed by decline. That was immediately followed by a serious car accident in which she was not injured but suffered considerable stress; her husband (and principal caregiver) was incapacitated, requiring new caregivers, which caused additional stress and somewhat disrupted her strict diet and supplement plan. No further blood cysteine or urine sulfite tests were able to be done. Then in October 2006 she contracted a bad cold, which worsened her already compromised breathing, and her decline continued until she could only move two fingers and she died in November 2006.

Discussion

Cysteine and sulfite

According to Pean et al., "[c]ysteine and its metabolites cysteine sulphinic acid and taurine were shown to be toxic to human and rat neuronal cell lines. . . . ALS patients have high plasma and CSF cysteine levels."[3] Taurine was found to be "increased constantly and most markedly in the motor cortex of all the 3 ALS cases" in one study.[4]

There appear to be no other case reports in the literature of patients with ALS having sulfite toxicity, probably because sulfite tests can be difficult to do as discussed above and are not usually done. Sulfite can be deadly, as in sulfite oxidase deficiency; sulfite should not be found in the urine. "Sulfur is excreted as sulfate, the urinary excretion of sulfate generally reflecting input from either inorganic (sulfate and sulfite) or amino acid sources (methionine, cysteine, cystine, and taurine)."[5] As stated by Arnold, "[p]ositive sulfite dip-

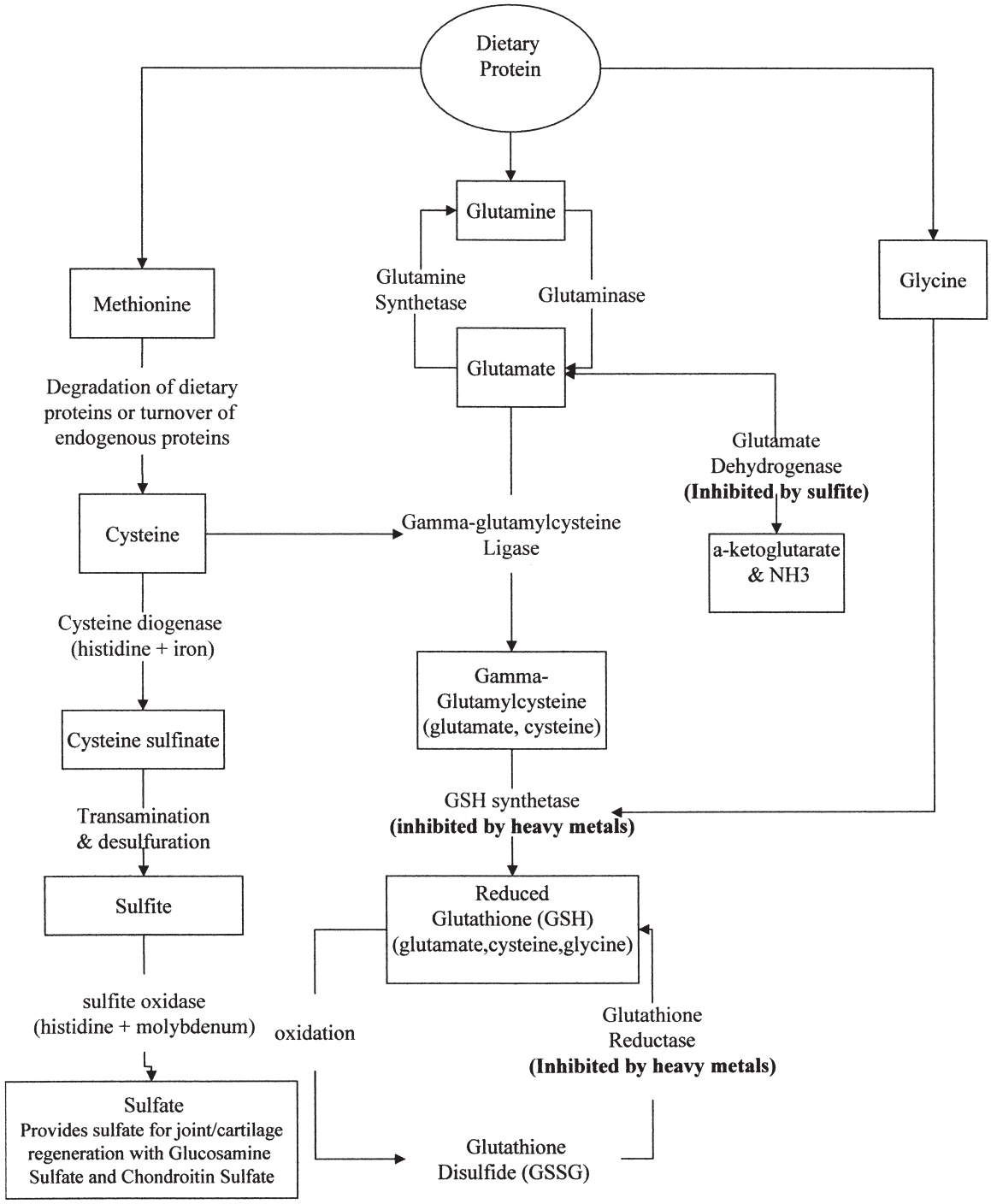

FIG. 1. This figure shows glutathione metabolism and its relationship to cysteine, sulfite, glutamate, glycine, and their enzymes. Glutathione exists in two forms: (1) the antioxidant reduced glutathione, which consists of the amino acids glutamate, cysteine, and glycine, usually called glutathione and abbreviated GSH; and (2) the oxidized form known as glutathione disulfide or GSSG.

stick of very fresh urine is highly suggestive of this disorder (sulfite oxidase deficiency). . . . In most cases, this disorder is fatal in infancy or early childhood. . . . No medical treatments that improve neurologic outcome are known, particularly for those with neonatal presentation of this disorder. When clinical suggestion of sulfite oxidase deficiency is sufficient, inform caregivers of an extremely poor prognosis for the individual with the disorder. Offer the option of discontinuing life support or abstaining from heroic medical interventions."[6] The short lifespan of patients with sulfite oxidase deficiency indicates how deadly sulfite toxicity must be, and is approximately the same length of survival time as patients with ALS after symptoms begin. However, in this patient the elevated plasma sulfate test result and the elevated sulfation phase II pathway result seemed to rule out a compromised sulfite-to-sulfate conversion, such as sulfite oxidase defi-

Table 2. Intravenous (IV) Glutathione Doses and Urine Sulfite Readings

IV Glutathione dose	*Urine sulfite reading*	*Free sulfite ppm*
1,400 mg	10	15
2,000 mg	10	15
2,400 mg	10–35	15–52.5
1,000 mg	8	12
500 mg	4	6
250 mg	0	0

ciency. Rather, with the elevated blood cysteine level, the patient's body perhaps could not process all of the available sulfite quickly enough to prevent toxicity. Figure 1 illustrates the sulfite and cysteine relationship to GSH. Zhang et al. note that "Neuronal cells are highly susceptible to sulfite toxicity. . . . Increased toxicity of sulfite was observed when intracellular reduced glutathione was compromised. . . . Under normal physiological *in vivo* situations, accumulation of sulfite is more likely to occur in neuronal cells compared with hepatic and renal cells whose high SO (sulfite oxidase) activity would facilitate its oxidation to sulfate more efficiently. This could possibly contribute to the more pronounced decrease in ATP (adenosine triphosphate) in neuronal compared with hepatic cells."[7] "Cysteine, besides being an excitotoxin itself, is converted to homocysteic and homocysteine sulphinic acid, both very powerful excitotoxins. Sulfite, a metabolite of cysteine, is also a powerful neurotoxin," according to Blaylock.[8]

Glutamate

Perhaps the sulfite contributed to the elevated glutamate by interference in the metabolism of glutamate: "The biosynthesis of ATP in intact rat brain mitochondria from the oxidation of glutamate was inhibited by micromolar sulfite. . . . Glutamate dehydrogenase (GDH) in rat brain mitochondrial extract was inhibited dose-dependently by sulfite."[7] Figure 1 illustrates the glutamate, sulfite, and GSH relationship.

Potent inhibition of transport of glutamate by excitatory sulfur amino acids such as cysteine sulfinic acid and cysteic acid has been reported in rats.[9]

Possible causes of low enzymes

Perhaps the glutamate and cysteine were both elevated and the GSH was low due to inherited deficiency of one or more of the enzymes needed for GSH synthesis, which include γ-glutamylcysteine ligase (γ-glutamylcysteine synthetase) to combine glutamate and cysteine to form γ-glutamylcysteine, and glutathione synthetase to combine glycine with the γ-glutamylcysteine, and glutathione reductase. As noted by Kidd, "Inherited deficiency of the enzyme gamma-glutamylcysteine synthetase, the first of the two enzymes necessary for GSH synthesis, has been described in two human siblings. They exhibited generalized GSH deficiency, hemolytic anemia, spinocerebellar degeneration, peripheral neuropathy, myopathy, and aminoaciduria, and severe neurologic complications as they moved into their fourth decade of life. Their red cell GSH was less than 3% of normal, their muscle GSH less than 25%, and their white cell GSH less than 50% normal. One of them may have been hypersensitive to antibiotics, having developed psychosis after a single dose of sulfonamide for a urinary tract infection."[10] Note the sensitivity to sulfur in this case as well.

In addition, it is possible that toxins in the patient's living environment may have put stress on her GSH synthesis over the years. As stated by Gilbère and Hom, "Environmental toxins are known to cause depletion of glutathione."[11] The patient's hair mineral analysis on July 22, 2005 showed no toxic elements, but a subsequent hair mineral analysis on May 24, 2006 showed some arsenic, cadmium, and gadolinium probably released from storage sites in atrophying muscles, so such toxic metals from known air pollution in her environment were probably impacting her GSH synthesis in the past. Cadmium and arsenic are two of the sulfhydryl-reactive metals (mercury, cadmium, lead, and arsenic) that bind irreversibly with GSH for excretion, thus decreasing the supply of available GSH; the more studied mercury "not only directly removes GSH from the cell, but also inhibits the activities of two key enzymes involved in GSH metabolism: GSH synthetase and GSH reductase."[12] Since these environmental toxins inhibit the GSH synthesis enzymes, they might play an even greater role in other ALS cases that are not familial. For example, "The observed incidence of ALS in young Gulf War veterans exceeded the expected, suggesting a war-related environmental trigger."[13]

Conclusions

The most important findings in this case were (1) discovery of sulfite toxicity with continuously fluctuating urine sulfite levels in the patient, and (2) during part of the summer of 2006 when the patient's whole blood GSH and blood cysteine and urine sulfite test results were normal, the patient did not experience additional physical decline and had strong breathing per her own assessment.

ALS (of nonmutant-SOD type) may be caused by toxicity of the GSH precursors of glutamate and cysteine, and sulfite (a metabolite of cysteine), which accumulate when one or more of the enzymes needed for GSH synthesis are defective. The presence of high levels of cysteine may present more sulfite than the body is able to rapidly process, particularly when cysteine and sulfite are further increased by dietary intake, thus resulting in sulfite toxicity with continuously fluctuating urine sulfite levels. The combination of sulfite and glutamate toxicity together is made worse by low GSH levels.

Since elevated plasma cysteine has been reported in other patients with ALS, perhaps sulfite and cysteine toxicity is also a factor in other ALS cases. Patients with nonmutant-SOD ALS should be tested for sulfite toxicity as well as cys-

teine, glutamate, and GSH levels and whether they have low levels of γ-glutamylcysteine ligase and/or glutathione synthetase and/or glutathione reductase. Since glutamate dehydrogenase and glutamate oxidation in the biosynthesis of ATP appear to be inhibited by sulfite, research on the effect of sulfite on glutamate levels in patients with ALS should be pursued if other ALS cases are found to have sulfite toxicity.

A way to prolong life in those patients with ALS with sulfite toxicity may be to carefully monitor the blood cysteine and urine sulfite levels and minimize their dietary and supplement intake, as well as increase GSH by using sublingual GSH. While it is difficult to counteract sulfite toxicity, a better solution might be found through future research to determine methods to increase GSH synthesis without using sulfur-containing supplements that may add to the cysteine and sulfite toxicity.

Disclosure Statement

No competing financial interests exist.

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