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Higher susceptibility of cerebral cortex and striatum to sulfite neurotoxicity in sulfite oxidase-deficient rats

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ABSTRACT

Patients affected by sulfite oxidase (SO) deficiency present severe seizures early in infancy and progressive neurological damage, as well as tissue accumulation of sulfite, thiosulfate and S-sulfocysteine. Since the pathomechanisms involved in the neuropathology of SO deficiency are still poorly established, we evaluated the effects of sulfite on redox homeostasis and bioenergetics in cerebral cortex, striatum, cerebellum and hippocampus of rats with chemically induced SO deficiency. The deficiency was induced in 21-day-old rats by adding 200 ppm of tungsten, a molybdenum competitor, in their drinking water for 9 weeks. Sulfite (70 mg/kg/day) was also administered through the drinking water from the third week of tungsten supplementation until the end of the treatment. Sulfite decreased reduced glutathione concentrations and the activities of glutathione reductase and glutathione S-transferase (GST) in cerebral cortex and of GST in cerebellum of SO-deficient rats. Moreover, sulfite increased the activities of complexes II and II-III in striatum and of complex II in hippocampus, but reduced the activity of complex IV in striatum of SO-deficient rats. Sulfite also decreased the mitochondrial membrane potential in cerebral cortex and striatum, whereas it had no effect on mitochondrial mass in any encephalic tissue evaluated. Finally, sulfite inhibited the activities of malate and glutamate dehydrogenase in cerebral cortex of SO-deficient rats. Taken together, our findings indicate that cerebral cortex and striatum are more vulnerable to sulfite-induced toxicity than cerebellum and hippocampus. It is presumed that these pathomechanisms may contribute to the pathophysiology of neurological damage found in patients affected by SO deficiency.

1. Introduction

Sulfite oxidase (SO) is an enzyme located in the mitochondrial intermembrane space responsible for the oxidation of sulfite to sulfate, the terminal step in the catabolism of the sulfur-containing amino acids cysteine and methionine [1,2]. This enzyme also degrades sulfite derived from exogenous sources, including sulfur dioxide and sulfiting agents used extensively in foods and medicaments as preservatives [3–5]. For the oxidation of sulfite, SO depends on a molybdenum cofactor (MoCo) in its catalytic site, where two electrons from sulfite reduce the molybdenum center of the cofactor, which subsequently transfers them to the cytochrome *c* at the respiratory chain through the cytochrome-b5-type heme cofactor, another prosthetic group present in SO [6–8]. Due to a lack of capability to recycle the MoCo and its precursors from nutritional sources, it must be synthesized *de novo* in the body by a four step biosynthetic pathway, using GTP as substrate [9,10].

SO deficiency is an autosomal recessive inborn error of metabolism that can arise either from a mutation in the gene *SUOX*, encoding the apoenzyme (isolated sulfite oxidase deficiency; ISOD) [11–13], or from mutations in genes encoding the enzymes involved in the MoCo biosynthetic pathway (MoCo deficiency; MoCD). So far, mutations have been identified in three of the four genes that encode enzymes of the MoCo biosynthetic pathway: *MOCS1* (type A deficiency), *MOCS2* (type B deficiency) and *GPHN* (type C deficiency) [10,14]. These mutations result not only in the loss of SO activity, but also of other two human molybdoenzymes: xanthine oxidase and aldehyde oxidase. Nevertheless, it has been established that the severe neurological symptoms observed in MoCD are caused by the absence of SO activity, and not by combined deficiency of xanthine oxidase and aldehyde oxidase or the isolated xanthine oxidase deficiency [1].

Abbreviations: CAT, catalase; CS, citrate synthase; DCIP, dichloroindophenol; DTNB, 5,5-dithio-bis (2-nitrobenzoic acid); G6PDH, glucose-6-phosphate dehydrogenase; GDH, glutamate dehydrogenase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione S-transferase; IDH, isocitrate dehydrogenase; ISOD, isolated sulfite oxidase deficiency; MDH, malate dehydrogenase; MoCD, molybdenum cofactor deficiency; MoCo, molybdenum cofactor; SO, sulfite oxidase; SOD, superoxide dismutase; TNB, 5-thio-2-nitrobenzoic acid; $\Delta\Psi m$, mitochondrial membrane potential.

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ISOD and MoCD are clinically indistinguishable [10,15] and are characterized by intractable seizures usually manifesting early in infancy, severe and progressive neurological dysfunction, alterations in muscle tone and failure to thrive, often resulting in early childhood death [1,16–18]. Biochemically, affected patients present accumulation of sulfite, thiosulfate and S-sulfocysteine in tissues and body fluids [1]. Brain imaging and neuropathological studies reveal global white matter and gray matter abnormalities, with demyelination, loss of neurons, gliosis and multicystic lesions [19–21]. Pronounced atrophy of the cerebral cortex, cerebellum and ganglionic structures, thin brain stem and corpus callosum, and secondary microcephaly are also reported [13,22–24].

The mechanisms underlying the severe neurological dysfunction observed in ISOD and MoCD are, at least in part, associated to the elevated levels of the toxic accumulating metabolites sulfite, thiosulfate and S-sulfocysteine. Previous studies showed that these compounds impair redox homeostasis and glutamatergic neurotransmission in rat cerebral cortex [25–27] and reduce the antioxidant capacity in plasma and erythrocytes of SO-deficient rats [28,29]. It was also demonstrated that sulfite generates sulfur radicals and superoxide in the presence of oxygen and/or transition metals [30–33]. Moreover, sulfite also induces mitochondrial dysfunction and bioenergetics failure [34,35].

In order to better understand the pathomechanisms involved in the onset and progression of ISOD and MoCD, the present study evaluated the *in vivo* effect of sulfite on redox homeostasis and energy metabolism in cerebral cortex, striatum, cerebellum and hippocampus of SO-deficient rats and rats with normal SO activity. The redox homeostasis parameters examined were reduced glutathione (GSH) concentrations and the activities of antioxidant enzymes, including catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione S-transferase (GST) and glucose-6-phosphate dehydrogenase (G6PDH). Regarding bioenergetics, we investigated the activities of respiratory chain complexes, glutamate dehydrogenase (GDH) and of the tricarboxylic acid cycle enzymes citrate synthase (CS), isocitrate dehydrogenase (IDH) and malate dehydrogenase (MDH), as well as the mitochondrial mass and mitochondrial membrane potential ($\Delta\Psi m$).

2. Materials and Methods

2.1. Reagents

All chemicals were purchased from Sigma (St. Louis, MO, USA), except for MitoTracker® Green and MitoTracker® Red that were obtained from Invitrogen, Molecular Probes (Eugene, OR, USA).

2.2. Animals

Twenty-one-day-old male Wistar rats, obtained from the Central Animal House of the Department of Biochemistry, ICBS, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil, were used. The animals were maintained on a 12:12 h light/dark cycle (lights on 07:00–19:00 h) in air conditioned constant temperature (22 ± 1 °C) colony room. The experimental protocol was approved by the Ethics Committee for Animal Research of the Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, and followed the NIH Guide for the Care and Use of Laboratory Animals (NIH publication 85–23, revised 1996). All efforts were made to minimize the number of animals used and their suffering.

2.3. Experimental design – Sulfite oxidase (SO) deficiency model

Male Wistar rats were divided after weaning (21-day-old animals) in four groups: control, sulfite, SO-deficient and SO-deficient + sulfite. SO-deficiency was induced according to Herken et al. [29], with slight modifications. All the groups received standard rat chow (Nuvilab CR-1® - Nuvital) *ad libitum*. Tungsten, in the form of sodium tungstate, (200 ppm; Na_2WO_4) was added to the drinking water of the 21-day-old rats of SO-deficient and SO-deficient + sulfite groups for 3 weeks, whereas control and sulfite groups received tap water *ad libitum* (Fig. 1). Tungsten is an element that competes with molybdenum [36] and thereby impairs the biosynthesis of the functional molybdenum cofactor. Thereafter, sulfite (70 mg/kg/day), in the form of sodium metabisulfite, was added to the drinking water of sulfite and SO-deficient + sulfite groups for 6 weeks, after which the rats were euthanized for the measurement of the biochemical parameters. SO-deficient and SO-deficient + sulfite animals continued to receive tungstate until the end of the treatment. The water containing sodium metabisulfite and/or sodium tungstate was prepared and changed three times per week.

2.4. Sulfite oxidase (SO) activity and urinary sulfite levels

SO activity was measured according to Cohen and Fridovich [37], with slight modifications. Animals were euthanized by decapitation and had their livers removed, placed on an ice-cold Petri dish and cleaned with 50 mM phosphate buffer, pH 7.4. Livers were then homogenized (1:10 *w*/*v*) with 50 mM phosphate buffer, pH 7.4, and the homogenates were centrifuged at 3500 *g* for 10 min at 4 °C. The pellet was discarded and the supernatant containing mitochondria was separated. An aliquot of the supernatant was incubated with 5% Triton X-100 for 10 min on ice and diluted (1:10) with 50 mM phosphate buffer (pH 7.4) afterwards. The diluted sample was used for the measurement of SO activity. The reaction medium contained 10 mM sodium sulfite, 0.04 mM cytochrome *c*, 10 mM KCN, 100 mM Tris-HCl buffer, pH 8.5, and tissue supernatant (approximately 10 μg of protein). SO activity was monitored spectrophotometrically through cytochrome *c* reduction at 550 nm at 25 °C. The results were expressed as nmol reduced cytochrome *c* / min / mg protein. Furthermore, during the last two weeks of the experimental model, urinary sulfite levels were measured using sulfite test strips (Quantofix sulfite test strips, Sigma-Aldrich).

2.5. Sample preparation for the measurement of biochemical parameters

Animals were euthanized by decapitation after 9 weeks of treatment. The brain and cerebellum were rapidly removed, placed on an ice-cold Petri dish and had the vessels and blood removed. The cerebral cortex, striatum and hippocampus were dissected, weighed and kept chilled until homogenization.

For oxidative stress parameters evaluation, the four encephalic structures (cerebral cortex, striatum, cerebellum and hippocampus) were homogenized (1:10 *w*/*v*) in 20 mM sodium phosphate buffer, pH 7.4, containing 140 mM KCl, and centrifuged at 750 *g* for 10 min at 4 °C. The pellet was discarded and the supernatant containing mitochondria and other organelles was separated and used for the measurements.

Regarding the determination of respiratory chain complexes II, II-III and IV activities, the structures were homogenized (1:20 *w*/*v*) in SETH buffer, pH 7.4, that contains 250 mM sucrose, 2.0 mM EDTA, 10 mM Trizma base and 50 $IU.mL^{-1}$ heparin. The homogenates were centrifuged at 800 *g* for 10 min at 4 °C and the supernatant was collected and submitted to three subsequent freeze-thawing procedures before performing the experiments.

For the measurement of the activities of GDH and of the tricarboxylic acid cycle enzymes CS, IDH and MDH, cerebral cortex mitochondria

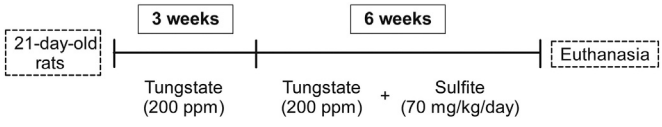

Fig. 1. Experimental design for sulfite oxidase (SO) deficiency induction.