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Ammonia role in glial dysfunction in methylmalonic acidemia

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ABSTRACT

Hyperammonemia is a common finding in patients with methylmalonic acidemia. However, its contribution to methylmalonate (MMA)-induced neurotoxicity is poorly understood. The aim of this study was evaluate whether an acute metabolic damage to brain during the neonatal period may disrupt cerebral development, leading to neurodevelopmental disorders, as memory deficit. Mice received a single intracerebroventricular dose of MMA and/or NH_4Cl, administered 12 hs after birth. The maze tests showed that MMA and NH_4Cl injected animals (21 and 40 days old) exhibited deficit in the working memory test, but not in the reference memory test. Furthermore, MMA and NH_4Cl increased the levels of 2′,7′-dichlorofluorescein-diacetate (DCF), TNF-α, IL-1β in the cortex, hippocampus and striatum of mice. MMA and NH_4Cl also increased glial proliferation in all structures. Since the treatment of MMA and ammonia increased cytokines levels, we suggested that it might be a consequence of the glial activation induced by the acid and ammonia, leading to delay in the developing brain and contributing to behavioral alterations. However, this hypothesis is speculative in nature and more studies are needed to clarify this possibility.

1. Introduction

The methylmalonic acidemia (MMAc) is a heterogeneous group of autosomal recessive inborn errors of organic acid metabolism caused by tissue and body fluids accumulation of methylmalonate (MMA) and its metabolites as propionate, metilcitrato and, β-OH propionate, due to deficiency of the enzyme activity of L-methylmalonyl-CoA mutase (MCM) (Royes et al., 2007; Chandler et al., 2009; Fenton WAR, 1995; Chandler and Venditti, 2005).

Most of the patients present an acute life-threatening metabolic crisis in the first months of life, which is usually precipitated by catabolic stress. The biochemical profile is characterized by metabolic acidosis and encephalopathic crises, hyperglycinemia, hypoglycemia, and hyperammonemia (Brismar and Ozand, 1994a). Furthermore, neurological features are also common in this disease, such as hypomyelination, cerebral atrophy, and neurodegeneration (Zwickler et al., 2012; Brismar and Ozand, 1994b; Melo et al., 2012; O'Shea et al., 2012).

Approximately 70% of patients with methylmalonic acidemia are known to present hyperammonemia during the course of the disease (Stewart and Walser, 1980). In inborn errors of metabolism, it is important to note that enzymes of the urea cycle are usually normal. However, patients with methylmalonic acidemia show a reduction in the activity of carbamoylphosphate synthetase and propionyl-CoA accumulation (Wolf et al., 1978). The accumulation of these metabolites in the liver mitochondria of patients with MMA acidemia leads to the inhibition of the biosynthesis of N-Acetylglutamate (Gebhardt et al., 2003). Consequently, the ammonia produced by protein degradation cannot be detoxified in the liver by incorporating it into urea cycle, which leads to high ammonia concentration in the blood. This in turn

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causes an increased accumulation of ammonia in the CNS of methylamlonic acidemia patients (Gebhardt et al., 2003).

In these metabolic diseases, the hyperammonemia is a symptom of metabolic decompensation that may result in chronic neurotoxicity and an impaired neurological outcome. The severity of hyperammonemia can also be observed in other ammonia-metabolism disorders in which the elevated blood ammonia level causes mortality and delay of development (Rodrigo et al., 2010; Cauli et al., 2011). The mechanisms underlying ammonia-induced neurotoxicity, although not completely understood, seem to involve ATP depletion, activation of glutamatergic and GABAergic mechanisms, free-radical generation and inflammation (Royes et al., 2007; Rodrigo et al., 2010; Cauli et al., 2011; Saez et al., 1999; Royes et al., 2016a). In line this view, Shawcross et al. (2004) proposed that systemic inflammation exacerbates the neuropsychological alterations induced by hyperammonemia. They showed that hyperammonemia deteriorates neuropsychological test scores during inflammatory state, but not after its resolution. Hyperammonemia increases also the sensitivity to immune challenges, for example, the injection of lipopolysaccharide (LPS) increases cytokine production similarly in normal or hyperammonemic mice (Marini and Broussard, 2006). However, the cognitive deficits induced by LPS were stronger and long-lasting in hyperammonemic mice. These reports support that hyperammonemia and inflammation cooperate in inducing cognitive deficits.

Furthermore, ammonia metabolism plays a pivotal role in astroglial cells (Neary et al., 1987; Bodega et al., 2015). In fact, glutamine synthetase, the enzyme that detoxifies ammonia by condensing it with glutamate to form glutamine, is mainly found in astrocytes (Norenberg and Martinez-Hernandez, 1979). Astroglial dysfunction might, therefore, lead to nerve cell disease (Albrecht, 2005) and development delay of brain (Barres, 2008). Many astroglial abnormalities have been reported in hyperammonemia, with astroglial edema among the most prominent (Rama Rao and Norenberg, 2007). The many changes in cell physiology induced by ammonia might have an effect on the cell cycle, and consequently on astroglial proliferation. In addition, primary or secondary astroglial damage has been implicated in several developmental or perinatal CNS pathologies (Haynes et al., 2009; De Keyser et al., 2008; Oja et al., 2017), suggesting that a vulnerability of glia and microglia in early stages of development may critically alter cerebral development and cause neurological abnormalities. However, the effects of ammonia on astroglial proliferation have been little documented.

Then, since studies have reported that methylmalonic acid induces cognitive impairment (Ribeiro et al., 2013a), inflammation (Ribeiro et al., 2013b; Salvadori et al., 2012), and oxidative damage (Royes et al., 2016b), it is plausible to verify if a ammonia administration induces cognitive impairment and glial dysfunction during the neonatal period after MMA injection as well as alters the inflammation and oxidative species production after MMA injection in the cerebral cortex, hippocampus and striatum of mice.

2. Experimental procedures

2.1. Ethics statement

Laboratory experiments were performed in accordance with national and international legislations (Brazilian College of Animal Experimentation [COBEA] and the U.S. Public Health Service's Policy on Humane Care and Use of Laboratory Animals-PHS Policy) and approved by the Ethics Committee for Animal Research of Universidad Federal de Santa Maria (UFSM; Permit Number: 2067310115). Indeed, animal handling and laboratory assays were carried out in such a way that all efforts were made to minimize suffering

2.2. Animals and reagents

The present study utilized pup male Swiss mice newborn. Pregnant Swiss mice were housed in individual cages and left undisturbed during gestation. These animals were obtained from a local breeding colony (Federal University of Santa Maria).Twenty-four hours after delivery, litters were culled to six male pups. The mother fed pups since birth until 21 days of life when they were weaned. Animals were divided in order to have the same number of mice for each treatment in each cage. Animals had free access to water and to a standard commercial chow and were maintained on a 12:12 h light/dark cycle in an air-conditioned constant temperature (24 ± 1∘C, 55% relative humidity) with free access to food (Guabi, Santa Maria, Brazil) and water. All efforts were made to minimize the number of animals used and their suffering.

2.3. MMA and ammonia administration and drug treatment

The MMA administration directly into the intraventricularlly cisterna magna was realized 12 hs after birth (P0) with MMA (2.5 μmol/g; pH 7.4) or vehicle sodium chloride (NaCl 0.9%) (Hoffmann et al., 1993; Brusque et al., 2001a) and one dose of ammonium acetate was administered intraperitoneally of 7.5 mmol/Kg on the second day of life for animals and 0.9% saline (0.9% SF) as control (Rangroo Thrane et al., 2012). The external reference point used to locate cisterna magna was the intersection between bones, i.e the meeting point of bone sutures bregma, lambda and the interaural line. The animals were anesthetized (a mixture of local anesthetics (EMLA) cream (2.5% lidocaine/2.5% prilocaine; Ready & Edwards, 1992; Fish et al., 2007) and after injected directly in the 30-gauge needle, the parameters used to inject were: anterior posterior (AP) = -2.7 mm (later the interaural line), vertical (V) = −1 mm (below dura mater), lateral (L) = 0, angle (Θ = 90°) (Consiglio and Lucion, 2000). All drugs are injected within a period of 2 min using a Hamilton syringe. The experimental protocol is described in the Fig. 1.

2.4. Physical development

All mice used in the experiments had assessed their behavioral development. For this, the weight of animals was weekly determined at the appropriate ages by one experimenter that was not aware of the subject condition.

2.5. Test behavioral

2.5.1. Open-field task

The open field was performed as usually conducted to measure spontaneous activity in mice. Briefly, the apparatus consisted of a gray square 60 cm × 40 cm × 50 cm. Its floor was divided by black lines into 12 equal squares which had been drawn in the floor of the box. The test room was dimly illuminated (a 25 W white lighting bulb located 130 cm above the center of the box. A single mouse was placed in the center of the floor and after 30 s of adaptation, the number of squares crossed (with the four paws), the number of rears (posture sustained with hindpaws on the floor) was counted manually for 5:00 min. All behavior was the performed by two people using a timer to compute time and note the number of crossing and rearing of each mouse. After each test the box was cleaned with 90% alcohol solution (Walsh and Cummins, 1976).

2.5.2. Radial arm maze test

The maze consists of a wooden eight radial arms maze (RAM) that was secured to a wooden base and elevated 100 cm from the floor. The radial arms maze were 35 cm in length, with outer arm walls 2.6 cm high, inner arm walls 15 cm high and 5.8 cm wide. The center well of the maze was 16.7 cm in diameter; the maze was situated in the middle with moderate luminosity (Ros-Simo et al., 2013; Olton, 1972), the food