



Research report

Antioxidant administration prevents memory impairment in an animal model of maple syrup urine disease

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ABSTRACT

Maple syrup urine disease (MSUD) is an autosomal recessive metabolic disorder resulting from deficiency of branched-chain α -keto acid dehydrogenase complex leading to branched chain amino acids (BCAA) leucine, isoleucine, and valine accumulation as well as their corresponding transaminated branched-chain α -keto acids. MSUD patients present neurological dysfunction and cognitive impairment. Here, we investigated whether acute and chronic administration of a BCAA pool causes impairment of acquisition and retention of avoidance memory in young rats. We have used two administration protocols. Acute administration consisted of three subcutaneous administrations of the BCAA pool (15.8 μ L/g body weight at 1-h intervals) containing 190 mmol/L leucine, 59 mmol/L isoleucine, and 69 mmol/L valine or saline solution (0.85% NaCl; control group) in 30 days old Wistar rats. Chronic administration consisted of two subcutaneous administrations of BCAA pool for 21 days in 7 days old Wistar rats. N-acetylcysteine (NAC; 20 mg/kg) and deferoxamine (DFX; 20 mg/kg) co administration influence on behavioral parameters after chronic BCAA administration was also investigated. BCAA administration induced long-term memory impairment in the inhibitory avoidance and CMIA (continuous multiple-trials step-down inhibitory avoidance) tasks whereas with no alterations in CMIA retention memory. Inhibitory avoidance alterations were prevented by NAC and DFX. BCAA administration did not impair the neuropsychiatric state, muscle tone and strength, and autonomous function evaluated with the SHIRPA (SmithKline/Harwell/ImperialCollege/RoyalHospital/Phenotype Assessment) protocol. Taken together, our results indicate that alterations of motor activity or emotionality probably did not contribute to memory impairment after BCAA administration and NAC and DFX effects suggest that cognition impairment after BCAA administration may be caused by oxidative brain damage.

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1. Introduction

Maple syrup urine disease (MSUD) is an autosomal recessive metabolic disorder resulting from deficiency of branched-chain α -keto acid dehydrogenase (BCKAD, E.C. 1.2.4.4) complex [1] leading to branched chain amino acids (BCAA) leucine, isoleucine and valine accumulation as well as their corresponding transaminated branched-chain α -keto acids (BCKA) in tissues and body fluids [1,2]. Patients with MSUD present convulsions, ketoacidosis, apnea,

hypoglycemia, coma, ataxia, psychomotor delay and mental retardation. Moreover, MSUD neuropathological brain changes include cerebral edema, atrophy of the cerebral hemispheres, white matter spongy degeneration and delayed myelinization [1–3].

Mechanisms underlying MSUD neurotoxicity are poorly known although neurological sequelae are common in most MSUD patients. It has been demonstrated that tMSUD accumulating metabolites may cause significant alterations of glutamate, aspartate and γ -aminobutyric concentrations in the brain [4–7] possibly related to decreased brain uptake of essential amino acids [8]. In addition, these metabolites affect energy metabolism in rat brain [7,9–12] and induce oxidative stress [13–17]. Recently, Glaser et al. [18] showed that single leucine intra-hippocampal injection in adult rats impairs memory consolidation and long-term potentiation generation. Additionally, Vasques et al. [19] also

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reported that intra-hippocampal administration of MSUD accumulating alpha-keto acids provokes learning disabilities in aversive and non-aversive behavioral tasks. Furthermore, chronic subcutaneous administration of high doses of leucine to young rats induces learning/memory deficits verified in the open field and in the shuttle avoidance tasks during adult age [20]. A major limitation of these studies was to show only leucine and its alpha-keto acids effects failing to analyze effects of other BCAAs such as isoleucine and valine which also accumulate in MSUD.

Previous observations suggest that oxidative stress may be involved in the pathophysiology of the neurological dysfunction of MSUD [13–17] raising questions whether antioxidants may help in the therapeutic management of these patients. It has been described that NAC supplementation was found to reduce oxidative stress by improving thiol redox status, to inhibit oxidative metabolism and to scavenge superoxide, hydrogen peroxide and hydroxyl radicals [21,22]. However, NAC isolated use could have some limitations due to its pro-oxidant effects probably by its interaction with iron [23]. NAC oxidative metabolism can generate thyl free radicals and NAC can reduce Fe^{3+} ions participation in the generation of hydroxyl radical via Fenton reaction. Given this, deferoxamine (DFX), a powerful iron chelator that can inhibit iron dependent free radical reactions, co administration has been used to improve NAC response [24–26]. Here, we have undertaken an evaluation of behavioral parameters after BCAA administration, a chemically induced model of MSUD [27], to better understand non-leucine BCAA may contribute to behavioral changes seen in MSUD patients. Furthermore, we have also investigated whether antioxidant administration (NAC and DFX) were able to modulate BCAA effects.

2. Materials and methods

2.1. Animals

Male Wistar rats of 7 and 30 days old were obtained from Central Animal House of Universidade do Extremo Sul Catarinense. All rats were caged in groups of five with free access to food and water and were maintained on a 12-h light–dark cycle (lights on 7:00 am) at a temperature of 23 ± 1 °C. All experimental procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Brazilian Society for Neuroscience and Behavior recommendations for animal care with the approval of the Ethics Committee of the Universidade do Extremo Sul Catarinense (protocol number 60/2010).

2.2. Acute administration of BCAA pool

Animals received three subcutaneous administrations of the BCAA pool (15.8 μ L/g body weight at 1-h intervals) containing 190 mmol/L leucine, 59 mmol/L isoleucine and 69 mmol/L valine in saline solution (0.85% NaCl) or saline alone (control group). BCAA pool and saline solution were given to rats on postnatal day PD 30 ($n = 12$). Behavioral tests were carried out 1 h after last injection. Dosage and animal age were based on a previous study [27] showing that BCAA administration lead to Leu, Ile, and Val increase in rat blood and brain, mimicking the main biochemical finding observed in MSUD.

2.3. Chronic administration of BCAA pool and treatment with antioxidants

Animals were divided as follows: (1) control (saline); (2) MSUD (induced by BCAA pool); and (3) MSUD treated with the combination of NAC (20 mg/kg) and DFX (20 mg/kg). Animals received two subcutaneous BCAA administrations (15.8 μ L/g body weight at 12-h intervals) containing 190 mmol/L leucine, 59 mmol/L isoleucine and 69 mmol/L valine in saline solution were administered for 21 days starting at PD 7 (last injection at PD 27) [27] ($n = 12$). NAC was administered subcutaneously twice a day (at 12-h intervals) while DFX was administered once every two days for a total of 21 days [26]. Behavioral tests were carried out 12 h after last injection.

2.4. SHIRPA (SmithKline/Harwell/ImperialCollege/RoyalHospital/Phenotype Assessment) test

The SHIRPA protocol was used to evaluate behavioral changes during the course of MSUD. The SHIRPA was conceived as a multi-test battery used for longitudinal studies with standardized guidelines and materials [28] and its primary screen consists of a series of reflexes and basic sensorimotor function observations providing

a behavioral and functional profile by observational assessment of individual performance. For the purpose of analysis, individual parameters assessed by SHIRPA were grouped into five functional categories (neuropsychiatric state; motor behavior; autonomic function; muscle tone and strength; and reflex and sensory function) according to Lackner et al. [29], determining an overall score and five domain scores. The reflex and sensory domain involves visual placing, pinna reflex, corneal reflex, toe pinch, and righting reflex. The neuropsychiatric state involves spontaneous activity, transfer arousal, touch escape, positional passivity, fear, biting, irritability and vocals. The motor behavior involves locomotor activity, body position, shivering, gait, pelvic elevation, tail elevation, trunk curl, limb grasping, wire maneuver, and negative geotaxis. Autonomic function involves respiration rate, palpebral closure, ruffled fur, skin color, heart rate, tears, and salivation. Muscle tone and strength involves grip strength, body tone, limb tone, and abdominal tone.

2.5. Inhibitory avoidance tests

Inhibitory avoidance conditioning is a well-established model of emotionally motivated memory in rats. Inhibitory avoidance apparatus is a 50 cm \times 25 cm \times 25 cm acrylic box with floor made of parallel stainless steel bars (1-mm diameter) spaced 1 cm apart. A 7-cm wide, 2.5-cm high platform was placed on the floor of the box against the left wall. Animals were placed on the platform and their latency to step down on the grid with all four paws was recorded with an automated device [30,31]. In the training session, immediately after stepping down on the grid, animals were given a 0.6-mA, 1.0-s foot shock. Two retention tests were carried out: 1.5 h (short-term retention) [32] and 24 h (long-term retention) after training [30,31]. During these tests, no foot shock was given and the step-down latency (maximum 180 s) was used as a measure of retention, as previously described. Retention latency refers to the time at which the training session started.

2.6. Continuous multiple-trials step-down inhibitory avoidance (CMIA) task

This task evaluates aversive memory in the test section and learning when analyzing the number of training trials required for the acquisition criterion. It was performed in the same step-down inhibitory avoidance apparatus; however, in the training session, animal was placed on the platform and immediately after stepping down on the grid, received a 0.3 mA, 2.0 s foot shock. This procedure continued until the rat remained on the platform for 50 s. Animals were then returned to the home cage. The number of training trials required to reach the acquisition criterion of 50 s on the platform was recorded. The retention test was performed 24 h later (long-term memory) [33].

2.7. Statistical analyses

Paired *t* tests were applied when comparing groups exposed to two different conditions. Comparisons between three groups were done using one-way analysis of variance (ANOVA), followed by Tukey multiple-comparison procedure that was derived from the interaction error term for the mixed-model ANOVA, these data were expressed as mean \pm SEM. Since the variables being analyzed from inhibitory avoidance test do not follow a normal distribution and its variance does not fulfill the assumption of homoscedasticity, these data were expressed as median and inter-quartile ranges and analyzed by the non-parametric test; Mann–Whitney U. Differences between the groups were rated significant at $P < 0.05$. All analyses were carried out in an IBM-compatible PC computer using the Statistical Package for the Social Sciences (SPSS) software.

3. Results

There were no significant differences between groups after acute and chronic administration of BCAA pool in the five distinct functional categories: reflex and sensory function, neuropsychiatric state, motor behavior, autonomous function, and muscle tone and strength (Table 1). Likewise, we observed no significant difference between groups in the step-down latencies during training trial in rats submitted to acute BCAA administration (Fig. 1). However, we observed statistically significant difference in both short- and long-term inhibitory avoidance retention when compared to the control group suggesting impaired aversive memory.

We demonstrated a significant increase in the number of training trials required to reach the acquisition criterion in the MSUD group compared with the control group during CMIA after acute BCAA administration thus indicating learning impairment (Fig. 2A). Results showed that the MSUD group required approximately two times more stimulus to reach the acquisition criterion. Summarizing, both control animals and animals receiving BCAA learned the task, but animals that received BCAA needed more attempts

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