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Full Length Article

Aminoguanidine alleviated MMA-induced impairment of cognitive ability in rats by downregulating oxidative stress and inflammatory reaction

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ABSTRACT

Methylmalonic acidemia (MMA) is the most common organic acidemia in childhood. Many "treated" patients continued to display various degrees of mental retardation and psychomotor delay, which could be caused by brain damage from elevated oxidative stress. Aminoguanidine (AG), a synthetic antioxidant, was tested in a MMA rat model for its potential therapeutic effects on memory impairment. The effects of AG on MMA-induced cognitive impairment in Wistar rats were evaluated with Morris Water Maze. The levels of nerve cell apoptosis and microglial activation were investigated to illustrate the mechanisms of the improvement of cognition with AG treatment in MMA rats. To further explore the mechanism of neuroprotection induced by AG, several biomarkers including free radicals and inflammatory cytokines in the hippocampus were quantified. The results showed that the rats treated with AG exhibited better neurological behavior performances than MMA model rats. The AG-treated rats had a decreased level of apoptosis of the hippocampal neurons, which could be the structural basis of the observed neural behavior protection. In addition, AG treatment significantly inhibited the activation of microglia. The AG-treated rats had decreased levels of IL-1β, IL-6, TNF-α, NO, malonaldehyde and iNOS activities in the hippocampus. The level of glutathione and superoxide dismutase activity in the hippocampus of the AG-treated rats increased significantly. In conclusion, AG could alleviate the MMA-induced cognitive impairment via down-regulating of oxidative stress and inflammatory reaction and provide a basis as a therapeutic potential against MMA-induced cognitive impairment.

1. Introduction

Methylmalonic academia (MMA) is one of the most common congenital abnormal metabolic disorders. The disorder is usually attributed to the deficiency of the methylmalonyl CoA mutase (MCM) or the defect of vitamin B12. The typical biochemical feature of the disease is the accumulation of methylmalonic acid, propionate, 3-hydroxypropionate and 2-methylcitrate (Baumgartner et al., 2014).

One of the most noticeable characteristics of patients with MMA is neurological dysfunction. Many newborns suffered with MMA died in the first few weeks or months of life due to encephalopathic crises. Some patients could survive longer with some effective treatments. The treatments include restricted protein diet for limiting the intake of some methylmalonic acid precursors such as valine, isoleucine and methionine (Manoli et al., 2016a), supplement of L-carnitine and cobalamin (Manoli et al., 2016b). Nonetheless, a notable amount of patients who initially response to the treatments presented different levels of mental retardation and cognitive impairment in the long-term time (Fischer et al., 2014). Some studies reported that the neuron reduction in the hippocampus was observed in some children with methylmalonic acidemia, which was the possible reason of cognitive impairment induced by MMA (Sum et al., 1993; Yamaguchi et al., 1995; Kanaumi et al., 2006). Although the underlying mechanism of neuronal damage in this disorder is still elusive (Baumgartner et al., 2014), some evidence showed that disruption of redox homeostasis, neuroinflammation and increased oxidative stress played a critical role in the neuropathology of this disease (Viegas et al., 2014; Fernandes et al., 2011; Ribeiro

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et al., 2013). Up to date, no effective and specific therapy is available to protect the neurons against the MMA-induced damage. There is unmet medical need to develop novel drugs to improve the treatment effects of the patients. Thus, we hypothesized that antioxidants and anti-inflammatory drugs may potentially protect neurons from MMA-induced neural damage.

Aminoguanidine (AG), an artificial compound, is a potential treatment for some kinds of diseases, primarily due to its role as an antioxidant (Alipour et al., 2014), and inhibition of nitric oxide synthase (Barmaki and Khazaei, 2015). In this study, we explored the mechanism of AG on alleviating MMA-induced cognitive impairment in rats.

2. Materials and methods

2.1. Animals

Male 5-day-old Wistar rats were used ($n = 160$). The newborns and maternal rats were purchased from Beijing Vital River Laboratory Animal Technology, Co. Ltd. (Beijing, China). The animal feeding-room environment conditions included $24 \pm 1\,^{\circ}C$ and 55% relative humidity. The rats were placed with free food and water. All rats after weaning at 21 days of age were housed five per cage. Animal experiments were permitted from the ethics committee of Xuanwu hospital, Capital Medical University. The report about animal experiment was written according to the ARRIVE guidelines (Kilkenny et al., 2010).

2.2. Drug administration protocol

Both methylmalonic acid (IUPAC name: 2-Methylpropanedioic acid) and aminoguanidine (IUPAC name: 2-Aminoguanidine) were purchased from Sigma (St. Louis, MO, USA). To evaluate the possible effects of AG against MMA-induced cognitive impairment, the rats were randomized to eight groups (n = 20 for each group): group I (animals were injected with saline alone), group II (MMA group, animals were administered with MMA), group III (AG50 group, animals were injected with AG (50 mg kg^{-1} body weight) alone), group IV (AG100 group, animals were injected with AG (100 mg kg^{-1} body weight) alone), group V (AG150 group, animals were injected with AG (150 mg kg^{-1} body weight) alone), group VI (MMA + AG50 group, animals were injected with a mix of MMA and AG (50 mg kg^{-1} body weight)), group VII (MMA + AG100 group, animals were injected with a mix of MMA and AG (100 mg kg^{-1} body weight)), and group VIII (MMA + AG150 group, animals were injected with a mix of MMA and AG (150 mg kg^{-1} body weight)).

MMA, AG or a mixture of MMA + AG was dissolved in saline and adjusted to pH 7.4 with 6 mmol L^{-1} NaOH. The rats of saline group, MMA group, AG alone group or MMA + AG group were subcutaneously injected twice a day (with 8 h of interval between the injections), from 5th day to 28th day after birth. MMA dosages were used according to previous study (Dutra et al., 1991): during the 5th day to 12th day after birth, the rats were administrated with 0.72 μmol of MMA by gram of body weight, during the 13th day to 19th day after birth, the rats were given 0.89 μmol g^{-1} body weight, and during the 20th day to 28th after birth, the rats were given 1.67 μmol g^{-1} body weight. The rats were injected 10 μl g^{-1} body weight.

2.3. Morris water maze

2.3.1. Spatial memory training test

We filled a circular tank (diameter 150 cm, height 60 cm) with water ($23 \pm 1\,^{\circ}C$). A different shaped geometric figure was provided as a spatial clue on the every wall of the room. An invisible platform, submerged 1.0 cm below the surface of the water, was placed in the central of a quadrant of a pool and remained in the same location during the training. The rats were trained four trials (4 different starting points: N, S, W, and E) a day for 5 continuous days to spend 60 s to search the hidden platform. The sequence of the starting points was randomized during the trials without repeating in each of four trials. If the rats could successfully identify the platform, they were allowed to stay there for 10 s; if the rats could not locate the platform by themselves, they would be guided to the platform and remain there for the same time.

2.3.2. Probe trial

The spatial space training test was followed by a probe trial in the following day of the last training session, in which the platform was removed. In probe trial, the rats were given 60 s to find the target location of the platform. Important parameters to indicate the spatial memory of the rats were measured. These included the time stayed in the target and opposite quadrant, the latency to cross over the platform place for the first time and the frequency of crossing the platform.

2.4. Detection of nerve cell apoptosis in hippocampus

The rats were injected intraperitoneally with 10% chloral hydrate and were allowed to rest in a cage in a dark and quiet environment. Intracardial perfusion began after the withdrawal reflexes were absent in both lower limbs. The rats were subjected to intracardial perfusion with 0.9% saline followed by 4% paraformaldehyde. The rat brains were removed and post-fixed. Apoptosis of nerve cells in hippocampus was measured using the terminal deoxynucleotidyl transferase mediated dUTP nick end labeling (TUNEL) assay kit (Roche Company, Switzerland) according to the manufacturer's instruction. Briefly, 10 rat brains in each group were sectioned and stained. For clear contrast, the cell nucleus was stained with hematoxylin. The hippocampus region was photographed and analyzed with Image Pro plus 6.0 software. A blind fashion was used for analyzing the stained field.

2.5. Measurement of microglial activation in hippocampus

The rats were perfused intracardially with saline and followed by 4% paraformaldehyde. To detect microglial activation, ionized calcium binding adaptor molecule 1 (Iba1) was chosen as the microglia marker. Sections were incubated with primary antibody (rabbit anti-Iba1, Wako Pure Chemical Industries, Ltd., Japan, dilution 1:500). The sections were rinsed by 0.01 M phosphate buffered saline (PBS) containing 0.1% Tween-20 and incubated with the secondary biotinylated antibody followed by avidin-biotin horseradish peroxidase complex (Zhongshan Goldenbridge Ltd, China). Each batch of tests was co-setup with the negative control. 10 rat brains in each group were stained. The hippocampus region was photographed and analyzed with Image Pro plus 6.0 software. A blind fashion was used for analyzing the stained field.

2.6. Detection of IL-1β, IL-6 and TNF-α by ELISA

The levels of IL-1β, IL-6, TNF-α in hippocampus were quantified by ELISA kits (eBioscience, Austria) according to the manufacturer's instruction. Briefly, the hippocampus was removed from the rat brain under anesthesia and washed with chilled saline. Each hippocampus was weighed and homogenized (10% w/v) in 0.9% cold saline and centrifuged at $4\,^{\circ}C$ for 10 min and 12,000 rpm. The supernatant was collected. The methods for detection of IL-1beta, IL-6, and TNF-alfa were as follows: IL-1β, IL-6 and TNF-α present in the supernatant bound respectively to antibodies adsorbed to the microwells. A biotin-conjugated anti-rat IL-1β, IL-6, TNF-α antibody was added. Following incubation, unbound biotin-