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Research Report

Creatine monohydrate supplementation for 10 weeks mediates neuroprotection and improves learning/memory following neonatal hypoxia ischemia encephalopathy in female albino mice

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ABSTRACT

Currently there are no uniform standard treatments for newborn suffering from cerebral hypoxia-ischemia (HI) and to find new and effective strategies for treating the HI injury remains a key direction for future research. Present study was designed to demonstrate that optimal dose (1 or 3%) of creatine monohydrate (Cr) for the treatment of neonatal HI in female albino mice. On postnatal day 10, animals were subjected to left carotid artery ligation followed by 8% hypoxia for 25 minutes. Following weaning on postnatal day 20, mice were divided into three treatments on the basis of diet supplementation (Normal rodent diet, 1% and 3% creatine supplemented diet) for 10 week. A battery of neurological tests (Rota rod, open field and Morris water maze) was used to demonstrate effect of Cr supplementation on neurofunction and infarct size following HI. Open field test results indicated that Cr supplementation had significantly improved locomotory and exploratory behavior in subjects. It was observed that Cr treated mice showed better neuromuscular coordination (rota rod) and improved spatial memory (Morris Water Maze test). A significant affect of creatine supplementation in reducing infarct size was also observed. Post hoc analysis of post hoc multiple comparisons revealed that mice supplemented with 3% Cr for 10 weeks performed better during Morris water maze test while 1% Cr supplementation improved the exploratory behavior and gain in body weight than control group indicating that Cr supplementation has the potential to improve the neurofunction following neonatal brain damage.

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1. Introduction

Cerebral ischemia is a situation in which brain lacks enough blood supply to maintain normal neurological functions (Neufeld et al., 2005; Cotton and Shankaran; 2010). After few minutes of reduced or lack of blood supply, events occur causing failure of ion gradients across cell membrane of neurons ultimately leading to their death. To maintain electrical membrane potentials, a high turnover of ATP is necessary (Tatsumi et al., 2003). Creatine (Cr) serves as energetic buffer and through the Creatine/Phospho creatine (PCr)/Creatine kinase (CK) system, it plays a critical role in ATP metabolism of neurons (Wyss and Kaddurah-Daouk, 2000; Brosnan and Brosnan, 2007). Cr also has a potential to suppress the generation of free oxygen radicals in central nervous system (CNS) that causes cell damage and inactivation of CK (Andres et al., 2008).

Hippocampal pyramidal cells are involved in learning and memory, these cells showed high expression of creatine kinase isoenzymes indicating critical role of Cr/PCr system in learning and memory (Kaldis et al., 1996). In addition, improved working memory performance and better results to solve complex central executive tasks in creatine supplemented subjects as compared to controls supports the evidence that Cr supplementation affects the cognitive process (McMorris et al., 2006, 2007). The detection of elevated brain Cr levels, after oral administration of Cr, provides the evidence that Cr can pass the brain blood barrier (BBB). Hence, potential neuroprotective benefits of creatine supplementation can be expected in both normal and diseased neurological models (Andres et al., 2008). Creatine is also known to be protective in a variety of neurological disorders. Ferrante et al. (2000) had reported that oral creatine administrations enhanced survival significantly, declined the development of brain deterioration and deferred striatal neurons atrophy and the formation of Huntington-positive aggregates in R6/2 mice. Klivenyi et al. (1999) had documented that the oral Cr supplementation made a dose dependent enhancement in motor performance and increased survival in transgenic mice G93A and it protected mice from loss of both substantia nigra neurons and motor neurons at four month of age.

Protective effects Cr against ischemic brain injury in animal models remained a subject of interest for several reported studies (Berger et al., 2004; Lensman et al., 2006; Prass et al., 2007, Beard and Braissant, 2010). Various studies have been carried out with varying protocols differing in dosage and duration regarding the effect of dietary Cr supplementation following HI (Balestrino et al., 1999; Tarnopolsky et al., 2003 and Prass et al., 2007). Creatine is known to prevent the hypoxic damage in vitro. Balestrino et al. (1999) have reported that incubation of brain slices with varying doses of creatine increases intracellular phosphocreatine and delays anoxic depolarization (AD) in a dose-dependent manner. They have reported that addition of 1 mM creatine to the incubation medium significantly increased AD latency during hypoxia and prevented irreversible neuronal damage. Adding 0.5 mM creatine had no significant effect. Higher concentrations of creatine (up to 25 mM) also did not provide any better protection. It has been reported that the most pronounced neuroprotective effect of Cr is observed at intermediate dosages, while supplementation with more or less Cr yields inferior results (Matthews et al., 1999; Ferrante et al., 2000). The determination of optimal Cr dosage that can be applied following HI injury is crucial to reduce the brain damage. This is particularly true for humans for whom no meaningful dose-response data is currently available (Wyss and Schulze, 2002). Therefore, present study was designed to demonstrate the effect of two different doses of Creatine monohydrate (1 and 3%) on behavior and brain infarct volume in female albino mice following neonatal hypoxic ischemic encephalopathy.

2. Results

2.1. Rota rod test

Rota rod test results indicated that mice supplemented with 3% Cr spent more time on rotating drum than control and mice supplemented with 1% Cr but the difference in test performance between the treatments did not reached statistical significance ($P=0.246$) (Fig. 1).

2.2. Open field test

Results of open field test indicated that diet had significant affect on mobile ($P=0.013$), immobile ($P=0.019$) and freezing episodes ($P=0.003$) when compared between the three dietary treatments indicating that creatine monohydrate supplementation is improving locomotory and exploratory behavior in female albino mice following hypoxic-ischemic insult (Table 1). Results of the post hoc test revealed that all the three above parameters had statistically non significant variations when compared between 1 and 3% Cr supplemented groups while mobile ($P=9.3$), immobile (P 9.5) and freezing episodes ($P=17.0$) were found significantly higher in 1% Cr treated group when compared to the untreated control group indicating that 1% Cr supplementation had improved the locomotor and exploratory behavior in female albino mice following hypoxic ischemic insult.

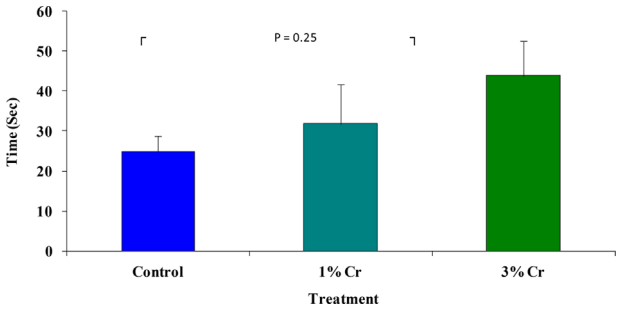

Fig. 1 – Rota rod test result comparison after ten weeks of special diet supplementation in HI group of female albino mice. N=10 for all the experimental treatments. One way ANOVA revealed no significant affect of diet supplementation on rota rod performance.