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Anti-neuroinflammatory and antioxidant effects of N-acetyl cysteine in long-term consumption of artificial sweetener aspartame in the rat cerebral cortex

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Abstract This study specifically focuses to investigate whether N-acetyl cysteine (NAC) has potential ameliorative effects against aspartame-induced brain pathophysiology in rats. Thirty adult male Wistar rats weighing 200–220 g were randomly divided into three groups as follows: the first group was administered with distilled water and served as the control group; the second group was given aspartame at a dose of 75 mg/kg b.wt. and the third group was given both aspartame and N-acetyl cysteine at dose of 75 mg/kg b.wt. and 600 mg/kg b.wt. respectively. Oral administration was done in the morning daily for 90 days.

Long term consumption of the artificial sweetener aspartame (ASP) induced large increments in cortical inflammation and oxidative stress. Daily oral NAC administration can significantly reverse brain-derived neurotrophic factor (BDNF) levels, blocked the cyclooxygenase-2 (COX-2) and prostaglandin E_2 (PGE_2) production with selective attenuation in expression of proinflammatory cytokines of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) in the rat cerebral cortex. Also, NAC can significantly replenish and correct intracellular glutathione (GSH) levels, modulate the elevated levels of total nitric oxide (TNO) and lipid peroxidation (LPO). Conclusions: The present results amply support the concept that the brain oxidative stress and inflammation coexist in experimental animals chronically treated with aspartame and they represent two distinct therapeutic targets in ASP toxicity. The present data propose that NAC attenuated ASP neurotoxicity and improved neurological functions, suppressed brain inflammation, and oxidative stress responses and may be a useful strategy for treating ASP-induced neurotoxicity.

Introduction

ASP is one of the most widely used artificial sweeteners in the world. It is found in more than 6000 products including

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carbonated and powdered soft drinks, hot chocolate, chewing gum, candy, desserts, yogurt, and tabletop sweeteners, as well as some pharmaceutical products like vitamins and sugar-free cough drops. Artificial sweeteners, such as ASP, are therefore often included in the diet of diabetic patients because these compounds sweeten foods without adding calories or raising blood glucose levels (Choudhary and Devi, 2015). In the European Union, it is codified as E951. Currently, the acceptable daily intake for humans is set at 50 mg/kg in the United States and 40 mg/kg in Europe. It was previously shown that ASP consumption, frequently used by diabetic patients, is a potent inductor of cytochrome P450 (CYP) enzymes in the brain of juvenile non diabetic rats (Vences-Mejía et al., 2006). Recently, ASP consumption and insulin treatment in a juvenile diabetic rat model leads to increase in CYP 2E1 and CYP3A2 isozymes in brain (Nosti-Palacios et al., 2014).

ASP is a methyl ester of the aspartic acid/phenylalanine dipeptide. Aspartyl phenylalanine diketopiperazine, a type of diketopiperazine (DKP), is created in products as ASP breaks down over time. The safety of the long-term consumption of ASP (75 mg/kg/day) for 24 weeks at doses equivalent to the amount of ASP in approximately 10 L of beverage per day was documented (Butchko et al., 2002). On the other hand, some researchers found that 6 months after ASP was put into carbonated beverages, 25% of the ASP had been converted to DKP (Lim et al., 2006). This form of DKP would undergo a nitrosation process in the stomach producing a type of chemical that could cause brain tumors (Davis et al., 2008). Additionally, long-term consumption of ASP leads to an imbalance in the antioxidant/pro-oxidant status in the brain, mainly through the mechanism involving the glutathione-dependent system (Abhilash et al., 2013).

Following oral administration to humans and experimental animals, ASP is rapidly and completely metabolized by intestinal esterases and dipeptidases to aspartic acid, phenylalanine and methanol, substances normally found in the diet and body (Mourad and Noor, 2011). The aspartic acid in ASP is a well-documented excitotoxin (Prakash et al., 2014). Excitotoxins are usually amino acids, such as glutamate and aspartate. Aspartate is a highly excitatory neurotransmitter and phenylalanine is a precursor of catecholamines in the brain, increased levels of these molecules could change the basic activity level of the brain to an unhealthy state (Nosti-Palacios et al., 2014). The methanol metabolized from ASP is converted to formaldehyde and then formic acid (Choudhary and Devi, 2015). Besides, ASP ingestion is implicated in neurological problems and chronic exposure of aspartame resulted in detectable methanol in blood (Simintzi et al., 2008). Moreover, methanol per se and its metabolites are responsible for the generation of oxidative stress in the brain region (Iyyaswamy and Rathinasamy, 2012). Furthermore, Chronic formaldehyde exposure at very low doses has been shown to cause immune system and nervous system changes and damage as well as headaches, general poor health, irreversible genetic damage, memory loss, and a number of other serious health problems (Sun-Edelstein and Mauskop, 2009; Abdel-Salam et al., 2012b).

Excitotoxins are substances that react with specialized receptors in the brain in such a way as to lead to destruction of certain types of neurons. Despite evidence that glutamate, aspartame and other food neurotoxic amino acids are excitotoxins that can destroy central neurons following oral intake by animals of various species, this amino acid continues to be one of the most vilely and heavily used food additives in the world (Blaylock, 2002). Excitotoxins have a devastating effect on formation of the brain (wiring of the brain) and such an exposure can cause the brain to be miswired (Soffritti et al., 2006). Also, the new-born rats prenatally treated with ASP showed significant increase of the LPO rate and depletion of SH groups in the brain homogenate. Further, ASP is a potential angiogenic agent that can induce ROS production that stimulates induction of cytokines and growth factors as it enhances IL-6, vascular endothelial growth factor and their soluble receptors release from the endothelial cells (Alleva et al., 2011) as well as brain oxidative stress increased by repeated ASP administration (Abdel-Salam et al., 2012a,b).

NAC is an acetylated variant of the amino acid L-cysteine, is an effective antidote able to increase cell protection to oxidative stress (Fries and Kapczinski, 2011). Moreover, NAC is an effective scavenger of free radicals as it interacts with ROS such as OH**.** and H2O2 as well as a major contributor to maintenance of the cellular GSH status and can minimize the oxidative effect of ROS through correcting or preventing GSH depletion. Further, NAC has been used successfully to treat GSH deficiency in a wide range of infections, genetic defects and metabolic disorders, including HIV infection and chronic obstructive pulmonary disease (COPD) (Atkuri et al., 2007). In addition, NAC has a broad spectrum of actions and possible applications across multiple conditions and systems (Duarte et al., 2012). As a drug, NAC represents perhaps the ideal xenobiotic, capable of directly entering endogenous biochemical processes as a result of its own metabolism (Sahin and Alatas, 2013). Recent research has also highlighted the fact that NAC may cross the blood–brain barrier (BBB) and the most "exciting" work is in the ability of NAC to heal brain dysfunctions and neuropathies and it is now emerging as a treatment of vascular and nonvascular neurological disorders, modulates glutamatergic, neurotrophic and inflammatory pathways (Shahripour et al., 2014).

This study aims to evaluate the neuroameliorative effect of NAC in response to inflammatory and oxidative stress caused by chronic administration of ASP to rats.

Material and methods

Animals

The experimental animals were carried out after approval from the Department of Zoology Council, Women's College, Ain Shams University, Egypt, which is an ethical authority. Thirty adult male Wistar rats weighing 200–220 g were used as experimental animals after being procured from the Animal House of El-Nile Company for Pharmaceutical Products, Cairo, Egypt. The animals were acclimatized for 2 weeks in the Animal House of Zoology Department, Women's College, Ain Shams University prior to the experiment. They were fed to appetite on standard laboratory animal rodent feed and water was available for animals *ad libitum*. They were housed in a well ventilated animal house kept under standard managerial and environmental conditions (12 h light/dark cycles at 25 ± 2 °C).