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Original article

Aspartame induced cardiac oxidative stress in Wistar albino rats

Stress oxydatif cardiaque induit par l'aspartame chez le rat Wistar albinos

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Abstract

Aspartame (non-nutritive sweetener) is consumed by millions of people in products like beverages, instant breakfasts, desserts, breathe mints, sugar free chewing gum, vitamins, and pharmaceutical. On a weight basis, metabolism of aspartame generates approximately 50% phenylalanine, 40% aspartic acid and 10% methanol. The detailed mechanisms of their effects on cardiac tissue are still unclear. The present study aimed to clarify whether longer time aspartame consumption has any effect on heart of Wistar albino rats. Animals were randomly divided into 4 groups of 6 animals (group-1: control, group-2: folate deficient diet fed animals, group-3: control animals treated with aspartame, group-4: folate deficient diet fed animals treated with aspartame). Aspartame was given orally (40 mg/kg·bw/day), dissolved in normal saline and for 90 days. Since human beings have very low hepatic folate content, the folate deficient diet fed animals were used to mimic the human methanol metabolism. Aspartame consumption increased significantly plasma corticosterone level, suggesting that aspartame may act as a chemical stressor. There was a significant increase in lipid peroxidation, nitric oxide and protein carbonyl, and significant decrease in protein thiol, cardiac membrane bound ATPases (Na^+, K^+, Ca^{++}, Mg^{++}), enzymatic (SOD, CAT, GPX, G6PD, GR) and non-enzymatic antioxidants (GSH, Vit-C, Vit-E) as well as a significant increase in heart rate and heart marker enzymes (CK and CK-MB). It may be due to excessive generation of free radicals, which impairs cardiac function. Aspartame metabolite methanol or formaldehyde may be the causative factors behind these changes. However, up regulation of Hsp70 in immunohistochemical analysis of cardiac tissue might be a protective response to oxidative stress induced by aspartame metabolites and structural damages in cardiac tissue.

Keywords: Aspartame; Cardiac tissue; ATPases; Oxidative stress; HSP70

Résumé

L'aspartame est un édulcorant consommé par des millions de personnes dans des boissons, des préparations pour petits déjeuners, des desserts, des pastilles de menthe, des gommes à mâcher, des vitamines et certains médicaments. Du point de vue pondéral, ses métabolites sont pour environ 50 % de la phénylalanine, 40 % de l'acide aspartique et 10 % du méthanol, dont les effets sur le myocarde sont mal connus. L'étude avait pour but de préciser chez le rat Wistar albinos les effets cardiaques d'une consommation d'aspartame de longue durée. Les animaux étaient randomisés en 4 groupes de 6 (groupe 1 : animaux contrôle, groupe 2 : animaux nourris avec un régime déficient en folates, groupe 3 : animaux contrôles recevant de l'aspartame, groupe 4 : animaux avec aspartame et régime déficient en folates). L'aspartame était donné per os durant 90 jours sous forme dissoute dans du sérum physiologique à la dose de 40 mg/kg/jour. Les animaux avec régime déficient en folates étaient utilisés pour mimer le métabolisme du méthanol chez l'homme. La consommation d'aspartame induisait une augmentation du cortisol plasmatique, suggérant un effet stressant du produit. La peroxydation lipidique, le monoxyde d'azote, les protéines carbonylées étaient augmentés, alors qu'étaient réduites les protéines thiols et les ATPases membranaires cardiaques (Na^+, K^+, Ca^{++}, Mg^{++}), les antioxydants enzymatiques (SOD, CAT, GPX, G6PD, GR) et non enzymatiques (glutathion réduit, vitamines C et E). Il y avait une augmentation de la fréquence cardiaque et des marqueurs enzymatiques cardiaques (CK et CK-MB), qui pouvait être due à une production excessive de radicaux libres, avec une altération de la fonction myocardique. Des métabolites comme le méthanol et l'aldéhyde formique pouvaient être impliqués. La régulation positive de la protéine Hsp70 en analyse immunohistochimique du myocarde pouvait être une réponse au stress induite par les métabolites de l'aspartame et les altérations structurales myocardiques.

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1. Introduction

Cardiovascular disease (CVDs) is a major problem on every nation's health [1]. Worldwide non-nutritive sweeteners are extensively added to a number of foodstuffs in which aspartame is one of the latest. Aspartame offers an alternative sweetening choice to dieters, diabetics, and others who must bound sugar consumption, It is consumed by millions of people in numerous products like instant breakfasts, beverages, sugar free chewing gum, breath mints, desserts, pharmaceuticals and vitamins [2]. Aspartame is completely metabolized in the gut and absorbed as the two amino acids (aspartic acid [40%], phenylalanine [50%]) and methanol (10%) [3]. Aspartame subsequent consumption may lead to increase in concentrations of its metabolites in the blood [4]. Aspartame is unstable on prolonged heating and become inappropriate for use in cooking and baking [5]. Aspartame also decomposes in liquids during storage for longer time. Above (86 °F = 30 °C), the methanol in aspartame breaks down in to formaldehyde and formic acid. Previous research supports an association between formaldehyde exposure and multiple adverse health effects [6]. Oxidative stress is cumulative damage in the body by free radicals, incompetently neutralized by antioxidants [7]. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) may react with proteins, carbohydrates and lipids, with consequent alteration both in the intracellular and intercellular homeostasis, leading to oxidative stress. Our assumption was based on methanol metabolite of aspartame, which may possibly cause oxidative stress in the cardiovascular centers in the brain stem and/or sympathetic centers in the hypothalamus [8]. These mechanisms may affect cardiac function in experimental animals.

The experimental and epidemiological data currently available to evaluate the above toxigenic risks of aspartame are insufficient and often unreliable, due to the inadequate planning and conduct of previous experiments. Recently from previous studies on safety dose of aspartame (40 mg/[kg bw·day]) as recommended by European food safety authority (EFSA) and food and drug administration (FDA) and its metabolite to alter the oxidative status of the cells, via ROS generation and modulation of intracellular antioxidant enzymes levels, was investigated. Oral aspartame (75 mg/[kg bw·day]) consumption caused oxidative stress in brain and liver [8,9] and oral aspartame (40 mg/[kg bw·day]) consumption caused oxidative stress in brain [10] in liver and kidney [11] and in immune organs [12]. The limited knowledge about the safety/potential toxigenic effects of aspartame widely present in the industrialized diet motivated the design of this experiment. The detailed mechanisms of the effects of aspartame on cardiac tissue are still unclear. Therefore, we aimed for present study to clarify whether longer time oral consumption of aspartame has any effect on heart of Wistar albino rats.

2. Methods

2.1. Chemicals

Pure aspartame powder and methotrexate was purchased from sigma Aldrich chemical, St. Louis, USA and all other chemical used were of analytical grade obtained from Sisco research laboratory, Mumbai, India.

2.2. Animal model

Animal experiments were carried out after getting clearance from the Institutional Animal Ethical Committee (IAEC No: 01/21/2014) and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The experimental animals were healthy, inbreed adult male Wistar albino rats, weighing approximately 200–220 g (12 weeks of age). The animals were maintained under standard laboratory conditions and were allowed to have food and water ad libitum (standard rat feed pellets supplied by M/s. Hindustan Lever Ltd., India) for animals. Animals of aspartame treated groups were daily administered aspartame (40 mg/kg·bw) dissolved in normal saline for 90 days by orally (using gavage needle) as recommended by EFSA [13] and FDA [14]. All the rats were housed under condition of controlled temperature (26 ± 20 °C) with 12-h light and 12-h dark exposure.

2.3. Folate deficient model and treatment

Animals were randomly divided into 4 groups and each group consists of 6 animals. Group 1 were control animals which were administered normal saline orally (by means of gavage needle) thought out the experimental protocol. Group 3 were control animals treated with aspartame orally for 90 days (40 mg/kg·bw). Since human beings have very low hepatic folate content [15] compared to rats, the folate deficient diet fed animals were used to mimic the human methanol metabolism in rats. In methanol metabolism conversion of formate to carbon dioxide is folate dependent. Hence in the deficiency of folic acid, methanol metabolism could take the alternate pathway (microsomal pathway) [16]. To simulate this, rats were made folate deficient by feeding them on a special folate deficient dietary regime for 37 days and after that methotrexate (MTX) in sterile saline were administered by every other day for two weeks [17] before euthanasia. MTX folate deficiency was confirmed by estimating the urinary excretion of formaminoglutamic acid (FIGLU) as recommended by Rabinowitz and Pricer [18] prior to the experiment. Rats on a folate deficient diet excreted an average of 90–100 mg FIGLU/kg body weight/day (Range 25–125) while animals on the control diet excreted an average of 0.29 mg/kg body weight/day (Range 0.15–0.55). These folate deficient animals showed a significant increase in FIGLU excretion when compared to the control animals ($P < 0.05$). The folate