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Original article

Aspartame induces alteration in electrolytes homeostasis of immune organs in wistar albino rats

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ABSTRACT

Aspartame is rapidly and completely metabolized in humans and experimental animals to aspartic acid (40%), phenylalanine (50%) and methanol (10%). Methanol, a toxic metabolite is primarily metabolized by oxidation to formaldehyde and then to formate. These processes are accompanied by the formation of superoxide anion and hydrogen peroxide. This study is focused to understand whether the oral administration of Aspartame (40 mg/kg bw) for 15 days, 30 days, and 90 days have any effect on immune organs. Damage to plasma membrane was assessed by levels of membrane-bound ATPases. Oxidative stress status was assessed by alterations in level of lipid peroxides, protein carbonyls, protein thiol and lipid-soluble antioxidant vitamin E. To mimic human methanol metabolism, folate-deficient animals were used. There was decrease in all membrane-bound ATPases activities in immune organs. Aspartame administration to rats inducing excess free radical generation is confirmed by increase in lipid peroxidation, obvious which is also again substantiated by the elevated protein carbonyl and decrease in protein thiol in this study. These excess free radical generations also decrease the cellularity (reduction in organ weight and cell count) of immune organs.

1. Introduction

Aspartame (L-aspartyl-L-phenylalanine methyl ester) is a high intensity sweetener added to a large variety of food, most commonly found in low calorie beverages, desserts and table top sweeteners added to tea or coffee [1,2]. It is composed of substances normally found in the diet and the body, i.e. the amino acids aspartic acid and phenylalanine and the alcohol methanol [3] After oral administration to humans and experimental animals, Aspartame is rapidly and completely metabolized to aspartic acid, phenylalanine and methanol [4,5]. On weight basis, metabolism of Aspartame generates approximately 50% phenylalanine, 40% aspartic acid and 10% methanol [6,7]. It forms methanol when the methyl group of aspartame encounters the enzyme chymotrypsin in the small intestine [7]. A relatively small amount of aspartame can significantly increase plasma methanol levels [3]. Methanol is being increasingly recognized as a substance that damages the liver cells where it is oxidized to formaldehyde and later to formate [4]. These processes are accompanied by elevation of NADH level and the formation of superoxide anion, which may be involved in lipid peroxidation [5,8].

Membrane ATPases may play an important role in ionic gradients between the intracellular/extracellular compartments of the cell. Membrane ATPases are intimately associated with the plasma membrane and participates in the energy requiring translocation of sodium, potassium, calcium and magnesium [9]. Na^+K^+ ATPase plays an important role in active transport of Na^+ and K^+ ions, across the plasma membrane. Similarly, Ca^{2+} ATPase is clearly linked with Ca^{2+} pump and transport of Ca^{2+}. The Mg^2 ions forms Mg^{2+} ATPase complex, which is the substrate for the enzyme Mg^{2+} ATPase, to control the intracellular Mg^{2+} concentration changes which can modulate the activity of Mg^{2+}dependent enzymes and regulate rates of protein synthesis and cell growth [10].

Oxidative stress was originally defined as the disequilibrium between pro-oxidants and antioxidants in biological systems [11]. However, oxidative stress is a broad descriptor and so the biological response will vary depending on the specific ROS involved. Two broad modes of action can be identified for ROS. One involves irreversible damage to macromolecules such as DNA, membrane lipids and proteins. A number of analytical techniques have been developed to measure the oxidation products directly (e.g. carbonyl assay for oxidized proteins) or the resultant degradation products (e.g. malondialdehyde for lipid peroxidation [12].These oxidation products can be used as biomarkers in tissue to monitor the irreversible consequences of oxidative stress in animal models [13,14]. Alternate modes of action of ROS involve altering protein function through reversible thiol modifications of protein cysteine

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residues. Reversible protein thiol oxidation has been demonstrated in proteins with a range of functions including signal transduction, ion transport, contractility, metabolism, protein synthesis and protein catabolism [15,16]. Alterations in protein function caused by protein thiol oxidation can profoundly affect cell function.

Despite numerous toxicological studies of Aspartame; its effect on immune organs have be given little attention. Therefore, the present study was designed to investigate cellular damage, by altering membrane ATPases, lipid peroxidation, and protein carbonyl and protein thiol in immune organs of wistar albino male animals on exposure of Aspartame (40 mg/kg bw).

2. Method

2.1. Animal model

Animal experiments were carried out after getting clearance from the Institutional Animal Ethical Committee (IAEC No: 02/03/11) 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 wk 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 control animals and but for folate-deficient and Aspartame-treated animals were given special folate-deficient diet (FD) for 37 days [17] and MTX (0.1MG/100g bw) i.p every other day for two week [18] before euthanasia. Animals of Aspartame-treated groups were daily-administered Aspartame (40 mg/kg bw) [19] dissolved in normal saline orally (by means of lavarge needle) for 15, 30 & 90 days. All the rats were housed under condition of controlled temperature ($26 \pm 2\,^{\circ}C$) with 12 h light and 12 h dark exposure.

2.2. Experimental design

Group I were the control animals that were administered normal saline orally (by means of lavarge needle) thought out the experimental protocol. Since human beings have very low hepatic folate content [20]. 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) [21]. To simulate this, rats were made folate-deficient by feeding them on a special dietary regime for 37 days and after that, methotrexate (MTX) in sterile saline was administered every other day for two week [18] before euthanasia. MTX folate deficiency was confirmed by estimating the urinary excretion of formaminoglutamic acid (FIGLU) [22] prior to the experiment. Rats on a folate-deficient diet excreted an average of 70 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-deficient animals were further divided into 4 groups. Group II was folate-deficient diet fed control, Group III was folate-deficient animals treated with Aspartame for 15 days (40 mg/kg bw), Group IV was folate-deficient animals treated with Aspartame (40 mg/kg bw) for 30 days. Group V was folate-deficient animals treated with Aspartame (40 mg/kg bw) for 90 days.

2.3. Sample collection

Blood samples and isolation of spleen, thymus and lymph node was performed between 8 and 10 a.m. to avoid circadian rhythm induced changes. Stress-free blood samples were collected as per the technique described by [23]. At the end of the experimental period all the animals were exposed to mild anesthesia and blood was collected from internal jugular vein, plasma and serum was separated respectively by centrifugation at 3000 r.p.m at $4\,^{\circ}C$ for 15 min. Later, all the animals were sacrificed under deep anesthesia using Pentothal sodium (40 mg/kg bw). The spleen, thymus and lymph node was excised, washed in ice-cold saline and blotted to dryness. Quickly weighed and the spleen, thymus and lymph node sample were homogenized by using Teflon glass homogenizers. Ten percent homogenate of spleen, thymus and lymph node was prepared in phosphate buffer (0.1 M, pH 7.0) and centrifuged at 3000 g at $4\,^{\circ}C$ for 15 min to remove cell debris and the clear supernatant was used for further biochemical assays.

2.4. Biochemical determinations

The activity of (ATPase) Na^+/K^+ ATPase (EC 3.6.1.3) was estimated by the method of Bonting [24]. Ca^{2+} ATPase (EC 3.6.1.3) by the method of Hjerten and Pan [25] and Mg^{2+}ATPase (EC 3.6.1.3) by the method of Ohnishi et al. [26], in which the liberated phosphate was estimated according to the method of Fiske and Subbarow [27]. Protein was estimated as per the method described by Lowry et al. [28]. Electrolytes levels in serum were done by auto-Analyzer (Beckman Coulter India, Ltd., Mumbai, India). We studied in the immune organs, thiobarbituric acid-reacting substances (TBARS) as lipid peroxidation and protein thiol and protein carbonyl content (PCC) as protein oxidation markers. TBARS levels (lipid peroxidation) were measured by Ohkawa et al. [29]. Protein carbonyl by Levine et al. [30] and protein thiol by Sedlack and Lindsay [31] was determined. Vitamin E estimation was performed using the method proposed by Desai. et al. [32] and organ cell count by Cross et al. [33].

2.5. Statistical analysis

Data are expressed as mean ± standard deviation (SD). All data were analyzed with the SPSS for windows statistical package (version 20.0, SPSS Institute Inc., Cary, North Carolina. Statistical significance between the different groups was determined by one-way analysis of variance (ANOVA). When the groups showed significant difference then Tukey's multiple comparison tests was followed and the significance level was fixed at $P < 0.05$.

3. Result

3.1. Effect of Aspartame on membrane-bound enzymes

The data are presented as bar diagram (Figs. 1–3) with mean ± SD. The membrane-bound enzymes (Na^+K^+ ATPase, Mg^{2+} ATPase and Ca^{2+} ATPase) in spleen, thymus and lymph node of folate-deficient animals was similar to control animals. In folate-deficient diet fed rat treated with Aspartame for 15 days, showed a decrease in entire membrane-bound enzymes (Na^+K^+ ATPase, Mg^{2+} ATPase and Ca^{2+} ATPase) level irrespective of the duration of exposure (15 days, 30 days as well as 90 days) when compared to the control as well as folate-deficient groups. There were marked decreases in the ATPase level of 30-days and 90-days Aspartame-treated animals when compared to control, folate-deficient as well as 15-days Aspartame-treated animals. Moreover, this decrease was more marked in 90 days Aspartame exposed animals than the 30-days exposed animals.

3.2. Effect of Aspartame on serum electrolyte level

The data are presented with mean ± SD in Table 1. The electrolytes (Na^+, K^+, Ca^{2+} & Mg^{2+}) in serum of folate-deficient diet