Full Length Research Paper

The Effect of Aspartame on the Activity of Glutathione S-Transferase in Tissues of Brain, Liver, Kidney And Lung of Rats

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Abstract

In this study, the changes in GST activities at 12 and 24 h after treatment with aspartame intraperitoneally at the doses of 50, 100 and 200 mg/kg were examined in tissues of liver, lung, kidney and brain of rats. GST activities were found to be significantly higher in liver tissue, whereas there was no significant difference in lung and kidney tissues. In brain tissue, it was found that the inhibition at 12 h after the aspartame dose of 100 mg/kg was statistically significant, but values for the other doses and periods were similar to that of control.

Keywords: Glutathione S-transferase, Aspartame, Rat.

INTRODUCTION

Aspartame, which is widely used as artificial low calorie sweetener, is added to various products such as foods, beverages, drugs and hygiene and the fact that it has been used within health-conscious communities is striking (Simintzi et al., 2007; Rashidian and Fattahi, 2009; Ogino et al., 2010; Alleva et al., 2011; Chenga and Wu, 2011). Aspartame, which was discovered by James Schatter in 1965 (Iyyaswamy and Rathinasamy, 2012), was approved for use in solid foods by the FDA in 1981 (Tsakiris et al., 2006; Alleva et al., 2011; Chenga and Wu, 2011). Aspartame was discovered by James Schatter in 1965 (Iyyaswamy and Rathinasamy, 2012), was approved for use in solid foods by the FDA in 1981 (Tsakiris et al., 2006; Alleva et al., 2011). It was also approved for use in beverages in 1983. Thus its usage area was expanded. At the present time, the acceptable daily intake of aspartame for both children and adults is 50 mg/kg in the USA and 40 mg/kg in Europe (Alleva et al., 2011; Iyyaswamy and Rathinasamy, 2012; Chenga and Wu, 2011; Marinovich et al., 2013; Gilli et al., 2008). As for the brain, the inhibition regarded as significant when administered in the periods of 12 hours and at the doses of 100 mg has been found at the same level with the control group in other periods and doses.

Aspartame is 180 times sweeter than sugar (Rashidian and Fattahi, 2009; Iyyaswamy and Rathinasamy, 2012; Fernstrom, 2009), 200 times sweeter than sucrose (Cantarelli et al., 2009) and has 4 kcal/gr (Marinovich et al., 2013). Approximately 16000 tons of aspartame are produced and consumed worldwide each year (Rashidian and Fattahi, 2009). It is used in more than 6,000 products including chewing gum, beverages, frozen foods, cereals, pudding, yogurt, vitamins and cosmetics (Chenga and Wu, 2011; Marinovich et al., 2013; Abhilash et al., 2011; Butchko et al., 2002; Kashanian et al., 2013). Aspartame is quickly absorbed into abdominal lumen after oral intake and converted into 3 metabolites in gastrointestinal tract; aspartate (asp) (%40), phenylalanine...
(Phe) (%50) and metanol (MeOH) (%10) (Simintzi et al., 2007; Alleva et al., 2011; Iyyaswamy and Rathnasamy, 2012; Chenga and Wu, 2011; Marinovich et al., 2013; Gilli et al., 2008; Butchko et al., 2002; Kashanian et al., 2013; Simintzi et al., 2007). Aspartame is converted into alanine and oxaloacetate, Phe is converted into tyrosine plus small amounts of phenylethylalanine, and MeOH is also converted into formaldehyde and then formic acid (Simintzi et al., 2007; Alleva et al., 2011; Gilli et al., 2008; Troche et al., 1998; Mourad and Noor, 2011).

Aspartic acid, metabolite of aspartame, is one of the stimulant aminoacids which is normally present in high concentrations in brain. This level is controlled by blood-brain barrier so that brain is protected against great fluctuations of plasma aspartate (Iyyaswamy and Rathnasamy, 2012). As an essential amino acid found in brain, phenylalanine is a product of monoamines and found in almost all foods with protein. On the other hand, elevation of blood phenylalanine which results from the consumption of aspartame can cause brain damage (Iyyaswamy and Rathnasamy, 2012). It has been showed that the levels of brain amino acids are increased after aspartame consumption (Iyyaswamy and Rathnasamy, 2012). Although methanol forms only 10 % of aspartame, it is released after the aspartame consumption. Methanol is a toxic agent leading to systemic toxicity among metabolites (Iyyaswamy and Rathnasamy, 2012). Clinical studies show that excessive aspartame consumption leads to various health problems such as headache, migraine, memory loss, hypersensitivity reactions and cholinergic symptoms (Simintzi et al., 2007; 2008; Rashidian and Fattahi, 2009; Tsakiris et al., 2006; Iyyaswamy and Rathnasamy, 2012; Kim et al., 2011; Cantarelli et al., 2009). However, short-period studies indicate that there is no relationship between memory loss and aspartame consumption (Tsakiris et al., 2006).

Glutathione S-transferases (GSTs) are a family of Phase II detoxification enzymes that play role in cellular defense against xenobiotics and carcinogens, convert them into more resolvable and more degradable molecules by conjugating with both reduced Glutathion (GSH) and an electrophilic center (Marinou et al., 2005; Ari and Dere, 2003; Koç, 2008; Yelkovan et al., 2001; Akbaş et al., 2012; Tozkoparan and Aytaç, 2007; Parıldar et al., 2003; Karkucak et al., 2012; Göze et al., 2001; Orhan and Şahin, 1995; Herkbert et al., 1992; Howie et al., 1998; Lewis et al., 1989; Ommen et al., 1990; Mourad and Noor, 2011; Vural et al., 2010). GSTs play an important role in, transporting amino acids and synthesizing protein and DNAAs as well as protecting SH-containing proteins and serving as a coenzyme in many reactions (Konukoğlu and Akçay, 1995). This enzyme family is known as both detoxification constructor and intracellular binding and transporter. These enzymes play important role in the biotransformation of xenobiotics. GSTs catalyze the conjugation of glutathione with many electrophilic compounds (Koç, 2008; Yelkovan et al., 2001; Göze et al., 2001). The compounds formed after conjugation are either removed from organism or converted into mercapturic acids via the classical removal pathway (Yelkovan et al., 2001; Akbaş et al., 2012).

GSTs are found in mammals, insects, fish, birds, snails, sharks and many microorganisms (Ari and Dere, 2003; Koç, 2008; Yelkovan et al., 2001; Ommen et al., 1990). They are frequently observed especially in liver, in cytosol and membrane of many organs such as small and large intestine, kidneys, lungs, breast, spleen, testis and placenta. GSTs are involved in the elimination of toxic agents taken via foods and the transport of nonsubstrate ligands conjugated with GSH including heme, bilirubin, bile salt and fatty acid, as well as the isomerization of prostaglandins. Furthermore, they can also connect similar types of compounds to each other with covalent bonds to prevent body damage from reactive electrophilic compounds (Ari and Dere, 2003; Koç, 2008; Yelkovan et al., 2001). GSTs play role in the metabolism of a broad range of electrophilic substrate group including antibiotic, vasodilator, analgesic, anticaner drugs, herbicide, insecticide, carcinogenes (Parıldar et al., 2003; Karkucak et al., 2012).

In this study, we aimed to contribute to the studies on aspartame by investigating the effect of it on GST activities in liver, kidney, lung and brain tissues in rats.

**MATERIAL AND METHODS**

**Animals, Experimental design and doses**

In this study, the food preservative aspartame (CAT No: A5139) was used as the test substance. 8-10 week old male Wistar albino rats, weighing 180-200 g were used for the experiment. The rats were divided into 7 different groups of 6 animals each. The rats were obtained from the Experimental Medical Research Unit at Kocaeli University, Turkey. The rats were randomly selected and housed in polycarbonate cages with free access to tap water and rat chow with a 12 h dark/light cycle. The temperature value of the animal laboratory was 22±2 °C and the relative humidity was 50-70%. All procedures on animals were performed in accordance with the guidelines of the Animal Ethics Committee of the Kocaeli University School of Medicine (KOU HADYEK 1/2-2012). There were six treatment groups and one control group. Aspartame was dissolved in distilled water. The rats were administered a single dose of aspartame intraperitoneally at concentrations of 50, 100, and 200 mg/kg b.w. for 12 and 24 h before sacrifice. The distilled water which was administered to the control group was referred for the solvent control. The rats were sacrificed by cervical dislocation after aspartame treatment and the necessary studies were commenced.
**Extraction of Tissues**

Liver, lung, kidney and brain tissues were rapidly removed from sacrificed rats and rinsed with ice-cold 0.15 M KCl. After wiped with a tissue paper, tissues were weighed on a microbalance. Tissues were transferred into 3 volumes (w/v) of ice-cold 0.15 KCl (0.5 g tissue into 1.5 mL) and homogenized at 1500 r/min stirring speed with 4 pulse time on T-line laboratory stirrer-type homogenizer (model No:136-2). Every homogenate was centrifuged at 48000xg for 30 minutes in a Sorval RC-5 superspeed refrigerated centrifuge (Dupont Instruments). It was paid attention that processes of homogenization and centrifugation were carried out at +4°C.

**Specific Activity Analysis**

10 mM potassium phosphate buffer (pH: 6.5), 5 mM. GSH (prepared from potassium phosphate buffer with). CDNB (0.5 M). GST activity was determined after monitoring the thioether bond formation between GSH and CDNB at the 340 nm wavelength by the spectrophotometer (Habig et al., 1974). Bradford assay was used for protein analysis (Bradford, 1976) and bovine serum albumin was used for protein standard.

**Statistical analysis**

The collected data were analyzed by using the statistical program SPSS 13.0. Data for the control and experiment groups were evaluated by using t test. The mean values were determined with ANOVA test. Probability p<0.05 was considered to be significant. Pearson correlation test was used to evaluate relationships between variables (Jerrold, 1984).

**RESULTS**

Time-dependent alterations of GST activities of liver, kidney, lung and brain in rats, which were administered with aspartame, are presented in Table 1. Any statistically significant differences were not observed in GST activities of kidney and lung between the experimental groups and the control group. All doses of aspartame increased GST activities in liver in 12 h after treatment, but only 50 and 200 mg doses caused statistically significant increases compared to the control group. Table 1 shows that increases in activities of GST for all doses in 24 h after treatment are statistically important.

In brain, the dose of 100 mg aspartame in 12 h after treatment significantly reduced the activity of GST compared to control group, whereas enzyme activities at the dose of 200 mg were similar with the control values. GST activity at the other dose and period was almost the same with the control group (Table 1).

**DISCUSSION**

The effect of aspartame on human body is mostly related with its metabolic concentration (Simintzi et al., 2007; Tsakiris et al., 2006). The studies with neonatal rodents show that high-dose intake of aspartic acid causes hypotonic necrosis, but the studies with infant primates show converse results. The finding that high-dose intake of aspartame may cause various diseases is not surprising at all. Among the aspartame metabolites, methanol is a toxic agent that causes systemic toxicity. In some studies, it has been shown that cellular content of glutathione and activities of glutathione related enzymes are reduced in methanol intoxication (Tsakiris et al., 2006).

In the study by Simintzi and co-workers, it was observed that aspartame metabolite administration at the dose of 10 mg/kg did not lead to significant changes in enzyme activity in frontal cortex. On the other hand, increased acetyl-choline esterase activity was observed after administration of sweetener in high concentrations at 34, 150 and 200 mg/kg. The amount of aspartame concentration is usually equal to 34 mg/kg consumption of sweetener and this dose does not affect enzyme activities (Simintzi et al., 2007).

Artificial sweetener safety has been the subject of debate since its discovery. Scientists disagree on the claim that artificial sweetener usage is related to various diseases such as lymphoma, leukemia, bladder and brain cancers, chronis fatigue, parkinson, alzhemier, multiple sclerosis, autism and systemic lupus. Community-based human epidemiological studies and pre-clinical studies on animals have shown that the factors effecting energy balance such as calorie intake, nutrition and exercise may affect cancer development and progression. Several studies have also shown a relationship between angiogenesis and malignant transformation. In a study by Alleva and co-workers, it was reported that angiogenesis was related to aspartame consumption. According to this research group, consuming low dose of aspartame may cause angiogenesis as well as regeneratory cytokine production. Specifically, it may lead to the both erk and p38 activations by inducing the release of soluble receptors in endothelial cells. Thus, new blood vessels are created, which may result in different pathogenesis such as retinopathy, rheumatoid arthritis and cancer (Alleva et al., 2011).

GSTs, which are playing a role in antioxidant defense, are multifunctional detoxification enzymes. GSTs are encoded by polymorphic genes leading to individual differences in removal of toxic intermediates and oxidative
Aspartic acid, a metabolic product of aspartame, is mostly excreted via the lungs in the form of CO₂. In human, generally, after it is received with the oral dose of 34 mg/kg, its plasma level does not change. However, some of the phenylalanine which is formed in the intestine following ingestion of aspartame, is excreted as CO₂. Glutathione reductase plays important role in antioxidant protection catalysing the reduction of GSSG-GSH in the cell. The observed activity in the glutathione reductase may be the reason for the decrease in GSH levels in animals treated with aspartame. Since methanol metabolism is related to the reduced GSH level, a decrease in the reduced GSH levels is observed in aspartame treated groups. GSH is a factor needed for the detoxification of methanol. In a study, the significant decreases in GSH concentration and glutathion activity were reported in the brain of animals treated with aspartame at 100 and 500 mg/kg doses (Ashok and Sheeladevi, 2014). In our study, GST activities in brain tissues in rats treated with aspartame at 100 and 500 mg/kg were increased, but all activities had returned to normal levels by the day 90 (Stanley, 2013).

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GSTs belong to a large enzyme family involving in the detoxification of agents, signals serving as ligand and catalyzing the conjugation of drugs and chemicals. In the present study, there were significant increases in GST activities in liver compared to those in kidney and lungs for the same dose and period (Table 1). Considering the previous studies, we can say that liver tissue is affected more rapidly and it is more sensitive to oxidative stress than kidney, lung and brain. Indeed, in an experimental study by Iman and co-workers, aspartame administered for speficic time periods at three doses were found to cause a significant increase of GST activity in liver tissue.

### Table 1. Changes in the effect of aspartame on GST activity in liver, kidney, lung and brain, in relation to time

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Doses /Hour</th>
<th>12 h mean ± SD</th>
<th>24 h mean ± SD</th>
<th>12 h mean ± SD</th>
<th>24 h mean ± SD</th>
<th>12 h mean ± SD</th>
<th>24 h mean ± SD</th>
<th>12 h mean ± SD</th>
<th>24 h mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>GST</td>
<td>Control</td>
<td>20.554 ± 2.88*</td>
<td>18.722 ± 6.70*</td>
<td>6.833 ± 0.62*</td>
<td>8.424 ± 0.77*</td>
<td>2.170 ± 0.07*</td>
<td>2.163 ± 0.09*</td>
<td>3.272 ± 0.69*</td>
<td>2.687 ± 0.52*</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>27.812 ± 3.01†</td>
<td>25.005 ± 3.76†</td>
<td>8.223 ± 0.35*</td>
<td>7.962 ± 0.56*</td>
<td>2.110 ± 0.45*</td>
<td>2.150 ± 0.71*</td>
<td>2.708 ± 0.04*</td>
<td>2.717 ± 0.46*</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>22.086 ± 1.56*</td>
<td>29.850 ± 1.89‡</td>
<td>7.919 ± 0.66*</td>
<td>7.560 ± 0.70*</td>
<td>2.250 ± 0.15*</td>
<td>2.211 ± 0.30*</td>
<td>2.331 ± 0.57†</td>
<td>2.768 ± 0.56*</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>28.084 ± 2.82†</td>
<td>25.696 ± 0.63‡</td>
<td>7.234 ± 0.35*</td>
<td>8.016 ± 0.66*</td>
<td>2.160 ± 0.35*</td>
<td>2.307 ± 0.06*</td>
<td>2.670 ± 0.64*</td>
<td>3.005 ± 0.43*</td>
</tr>
</tbody>
</table>

* Data shown with the same symbols in the vertical column are not different from each other at 0.05 statistical levels (*, †, ‡)

r: All data in the table showed enzyme activities U. (mg protein)-1

SE: Standart Error.
reported that GST activities are found at increased levels in patients with primer hepatocellular carcinoma, GST measurements are useful in hepatocellular carcinomas and GST is a marker of hepatocellular damage (Aköz et al., 2000). Even if aspartame is considered as an innocent artificial sweetener, phenylalanine, its metabolic product formed following intake of aspartame, may be harmful to the people with metabolic genetic diseases, and toxic effects related with methanol may also occur. Thus, continuous consumption of aspartame, an artificial sweetener used widely in the world, will cause adverse effects in metabolism.

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**REFERENCES**


