Effects of Aspartame on Maternal-Fetal and Placental Weights, Length of Umbilical Cord and Fetal Liver: A Kariometric Experimental Study

Efectos del Aspartame sobre el Peso Materno-Fetal y Placentario, Largo del Cordón Umbilical e Hígado Fetal: Un Estudio Cariométrico Experimental

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SUMMARY: Aspartame is a synthetic sweetener consumed by more than half the adult population in 75 countries. Their metabolites can be toxic, principally to the liver and retina, and there are few studies on the use of aspartame in gestation.

Twenty pregnant rats were weighed and allocated randomly (n=5 per group) to receive 14 mg/kg aspartame or water by oral-gastric drip. Treated T1: aspartame diluted in water at room temperature; Treated T2: aspartame diluted in water heated to 40° C; control C1: water at room temperature; and control C2: water heated to 40° C. Placentas were weighed, umbilical cords measured and 1000 nuclei of fetal hepatocytes (250 from each group) were analyzed morphometrically utilizing the technique of kariometry, with application of the Mann-Whitney U-Test.

There were reductions in mean placental and maternal-fetal weights, in umbilical-cord length, and the majority of kariometric parameters of the hepatocytes in the group treated with aspartame diluted in distilled water at room temperature. Reduction of placental and maternal-fetal weights occurred, shortening of the umbilical cord, and decrease in kariometric parameters in fetal hepatocyte nuclei after administration of aspartame diluted in distilled water at 40°C temperature. The use of aspartame during gestation can be prejudicial to the fetus.

KEY WORDS: Aspartam; Fetus; Gestation; Liver; Kariometry.

INTRODUCTION

Aspartame (NL-alpha-Aspartyl-L-phenylalanine 1-methyl ester) (Araújo *et al.*, 1990) is a synthetic sweetener of low caloric value formed from the union of two amino-acids, aspartic acid and phenylalanine, with sweetening power 180 to 200 times greater than that of saccharose. It is consumed by half the adult population, in 75 countries, in the form of approximately 6000 products, such as soft drinks, chewing gum, fruit juices, gelatins and jellies (Grenby, 1991; Sanyudes, 1990).

Aspartame is metabolized in the gastrointestinal tract in aspartic acid, methanol and phenylalanine (Camfield *et*

al., 1992), being that 10% of this metabolism results in methanol, which is oxidated in formaldehyde and formate in many tissues. Formic acid is considered the principal metabolite responsible for deleterious effects of acute intoxication by methanol in humans and animals (Butchko *et al.*, 2002), able to cause blindness and loss of hepatic function since the liver and retina concentrate the greatest quantity of bioproducts of aspartame during intoxications (Trocho *et al.*, 1988). Other symptoms may include allergic reactions in up to 20% of cases (urticaria, respiratory symptoms and edema of the lips, tongue, throat or salivary glands) or convulsive crises (Roberts *et al.*; Trocho *et al.*).

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Since the prevalence of acute intoxication by aspartame tends to be low, there has been great acceptance of the product as a potent hypocaloric and trustworthy substitute of sugar (Butchko *et al.*). Considering the scarcity of literature in relation to the use of aspartame during gestation as well as its analysis at elevated temperatures, the objective of the present study was to evaluate experimentally, by means of kariometry, possible alterations in maternal-fetal body weight, placental weight, length of the umbilical cord and in nuclei of fetal hepatocytes, after administration of aspartame diluted at room temperature or heated to 40° C.

MATERIAL AND METHOD

After ethical approval, twenty virgin rats were utilized (*Rattus Norvergicus albinus*, Wistar variety) (Kalter, 1974), weighing between 213 and 272 g, and conditioned at night in communal cages, at the proportion of four females per one male. On the 9th, 10th and 11th days of pregnancy (detected by vaginal smear), they were randomly placed into four experimental groups, with receipt of 14 mg/kg of aspartame by gavage. Treated group T1: 5 rats using aspartame diluted in water at room temperature; treated group T2: 5 rats using aspartame diluted in water heated to 40° C; control group C1: 5 rats administered water at room temperature, and control group C2: 5 rats using water heated to 40° C.

On the 20th day, all the rats were sacrificed by inhalation of sulfuric ether with extraction of fetuses, immediately immersed in a solution of Alfac for 24 hours. All fetuses were weighed on a precision balance and conserved in alcohol 80% for extraction of their respective livers, which were dehydrated, cleared and enclosed in paraffin in microtome section (6 µm thickness 45 µm space between cuts) and coloration by hematoxylin-eosin.

The kariometric technique was utilized for morphometric study (Harkema, 1997), with random selection of five fetuses from each group. The cuts were focalized and analyzed under light microscope (H 500 hund Wetzlar ®) with the objective of immersion (augmented 100 times) and clear chamber (Leitzwetzlar – Germany ®). Nuclei of hepatocytes were projected onto paper with final augmentation of 1240 times. In the nuclear images obtained (50 images for each animal) outlined with a black number two pencil, were measured major (D) and minor (d) axes with millimeter ruler.

There were analyzed, in total, 1000 hepatocyte nuclei (250 for each group) utilizing the following morphometric parameters (Sala *et al.*, 1994): major diameter, minor diameter, mean diameter, ratio between major and minor diameter,

perimeter, volume, area, volume-to-area ratio, coefficient of form, index of contour and eccentricity. Diameters and perimeters were measured in micrometers (µm), volumes in cubic micrometers (µm³) and areas in square micrometers (µ²). For mathematical calculations of morphometric studies, a computer program in Advanced Basic language was utilized. The non-parametric Mann-Whitney U-test was used for statistical analysis of data (Conover, 1999; Siegel, 1975) with values considered statistically significant for $U \leq 4$, and adoption of alpha error of 5%.

RESULTS

1. Maternal-fetal and placental weights and measurement of the umbilical cord.

There was a statistically significant reduction of arithmetic means of final body weights and mean of differences between initial weight and final weight, in the group treated with aspartame diluted in distilled water at room temperature, in relation to the control group (Table I). As to arithmetic means of body weights, a significant reduction occurred only for the mean difference between initial and final weights in the group treated with aspartame diluted in distilled water heated to 40° C, compared with the control group (Table I).

With regard to arithmetic means of body weights and placental weights, there was a statistically significant reduction for the treated groups (in both aspartame diluted in distilled water at room temperature and in water heated to 40° C) relative to the control group (Table II). There was also a reduction in mean measurements of umbilical cords only in the group treated with aspartame diluted in distilled water at room temperature (Table II).

2. Morphometric results (kariometry).

A statistically significant reduction was demonstrated for the major, minor and mean diameters; for volume, area, perimeter, volume-to-area ratio and index of contour (Table III) of hepatocyte nuclei in fetuses of rats treated with aspartame diluted in distilled water at room temperature, in relation to the control group.

There was no statistical difference between treated and control group for D/d ratio, eccentricity and coefficient of form in hepatocyte nuclei of rat fetuses treated with aspartame diluted in distilled water at room temperature (Table III). For the group treated with aspartame diluted in distilled water heated to 40° C, there was no statistical difference for any kariometric parameter relative to the control group (Table III).

Table I. Mean values of initial corporal weight, final corporal weight, difference between final and initial corporal weight and number of fetuses in control groups (C1 and C2) and treated groups (T1 and T2). Mann-Whitney test (U) and p-value (p).

Parameter	Aspartame diluted in distilled water at room temperature				Aspartame diluted in distilled water heated to 40° C			
	C1	T1	U	p[U]	C2	T2	U	p[U]
Initial weight (g)	247.8	229.8	4	0.048*	240.7	245.5	9	0.274
Final weight (g)	333.2	286.4	0	0.004*	346.1	338.9	11	0.421
Difference between final and initial corporal weight (g)	89.2	56.6	0	0.004*	107.4	93.4	0	0.004*
Number of fetuses	09	10	7	0.155	11	10.4	7	0.155

* Significance difference p=0,05

Table II. Mean values of corporal weight, placental weight and umbilical cord length in control groups (C1 and C2) and treated groups (T1 and T2). Mann-Whitney test (U) and p-value (p).

Parameter	Aspartame diluted in distilled water at room temperature				Aspartame diluted in distilled water heated to 40° C			
	C1	T1	U	p[U]	C2	T2	U	p[U]
Corporal weight (g)	2.503	1.557	1	0.008*	2.685	1.720	0	0.004*
Placental weight (g)	0.398	0.247	2	0.016*	0.339	0.263	3	0.028*
Umbilical cord length (cm)	1.8	1.5	2	0.016*	1.8	1.7	6	0.111

* Significance difference p=0,05

Table III. Mean values of kariometric parameters of hepatocyte nuclei in control groups (C1 and C2) and treated groups (T1 and T2). Mann-Whitney test (U) and p-value (p).

Parameter	Aspartame diluted in distilled water at room temperature				Aspartame diluted in distilled water heated to 40° C			
	C1	T1	U	p[U]	C2	T2	U	p[U]
Greatest nuclear diameter	1.49	1.30	0	0.004*	1.44	1.44	11	0.421
Smallest nuclear diameter	1.11	0.94	0	0.004*	1.03	1.03	10	0.345
Mean diameter	1.28	1.11	0	0.004*	1.22	1.21	11	0.421
Greatest/smallest diameter ratio	1.36	1.39	6	0.111	1.40	1.41	12	0.500
Nuclear volume	1.15	0.74	0	0.004*	0.96	0.97	11	0.421
Nuclear area	1.31	0.97	0	0.004*	1.18	1.17	10	0.345
Nuclear perimeter	4.11	3.55	0	0.004*	3.92	3.92	11	0.421
Volume/area ratio	0.85	0.73	0	0.004*	0.81	0.81	8	0.210
Eccentricity	0.64	0.66	7	0.155	0.68	0.68	11	0.421
Nuclear shape coefficient	0.95	0.95	8	0.210	0.95	0.95	10	0.345
Contour index	3.61	3.62	3	0.028*	3.62	3.63	10	0.345

* Significance difference p=0,05

DISCUSSION

Epidemiological studies of alimentary additives are important in toxicological risk evaluation for humans, though these are difficult to accomplish since the exposure cannot be evaluated precisely and the analysis of risk depends, principally, on toxicological laboratory studies (Sasaki *et al.*, 2002). Cumulative effects derived from chronic administration of aspartame suggest that its ingestion can result in progressive accumulation of formaldehyde products, which could explain the chronic effects that aspartame consumption can induce in sensitive tissues, such as in the brain and liver (Trocho *et al.*).

In rats, gestation lasts twenty-one days and embryogenesis is initiated on the eighth day, and is prolonged until the fourteenth day (Rugh, 1968). During this phase exposure to a teratogenic agent can provoke specifically malformations of diverse organs in development. In this study aspartame was administered on the 9th, 10th and 11th days of gestation, which coincided with the teratogenic period (Rugh). The results presented suggested that aspartame caused damage to the rat fetuses.

One of the deleterious effects of aspartame observed in this study was demonstrated by diminution of fetal weight. The mean body weight of fetuses belonging to the group treated with aspartame diluted in distilled water at room temperature, was observed to be significantly diminished, in relation to the control group fetal weight. There was also a statistically significant difference between initial and final body weights in rats treated with aspartame diluted in distilled water heated to 40° C, compared to that of the control group, which is suggestive of weight reduction caused by the use

of aspartame (Astrup *et al.*, 2002; Kanders *et al.*, 1988, 1992; Toledo & Ioshi, 1995; Tordoff & Alleva, 1990).

The length of the umbilical cord has been considered a trustworthy indicator of fetal movement by being influenced by the frequency of fetal movement and by space available in the uterine cavity. It is known that diminution of space or blockage of fetal movement cause diminution of the extension and length of the umbilical cord (Miller *et al.*, 1981; Moessinger *et al.*, 1982; Yyengar & Rapp, 2001). In this study, placentas from animals of the two treated groups were significantly diminished in relation to the control group, which may suggest reduction in space available in the uterine cavity as consequent shortening of the umbilical cord.

Kariometric studies permit the obtainment of data of both physiological and pathological interest, effect quantitative correlations between normal or altered structure and function and aid in diagnosis of cellular alterations (Chalkley *et al.* 1943,1949; Sala *et al.*, 1980). This kariometric study of hepatocyte nuclei suggest maternal hepatic toxicity from administration of aspartame diluted in distilled water at room temperature, which provokes question as to maturation and degree of hepatic recuperation with the use of aspartame during gestation, necessitating future investigations.

Products with aspartame contain on packaging a warning to carriers of phenylketonuria about the presence of phenylalanine (Grenby). It would be of interest for such products to carry advisories for pregnant women regarding the risk of ingesting aspartame, in the same way it happens with medicaments. This study suggests that consumption of products containing aspartame be avoided during the gestational period.

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RESUMEN: El aspartame es un endulzante sintético consumido por más de la mitad de la población adulta, en 75 países. Sus metabolitos pueden ser tóxicos, principalmente en el hígado y retina y hay algunos estudios sobre el aspartame en el embarazo.

Veinte ratas preñadas fueron pesadas y distribuidas aleatoriamente (n=5 por grupo) y recibieron 14 mg/Kg de aspartame o agua por vía oral- gástrica. Tratamiento 1: aspartame diluido en agua a temperatura ambiente; Tratamiento T2: aspartame diluido en agua tibia a 40 °C; control C1: agua a temperatura ambiente, y control C2: agua tibia a 40° C. Las placentas fueron pesadas, el cordón umbilical medido y 1000 núcleos de hepatocitos fetales (250 de cada grupo) se analizaron morfométricamente utilizando la técnica de cariometría con aplicación del Test U de Mann-Whitney U-Test.

En el grupo tratado con aspartame diluido en agua a temperatura ambiente, hubo reducción en los pesos promedios de la placenta y materno-fetal, largo del cordón umbilical y en la mayoría de los parámetros cariométricos de los hepatocitos. Lo mismo ocurrió en el grupo tratado con aspartame diluido en agua a 40 °C. El uso del aspartame durante las gestación puede ser perjudicial para el feto.

PALABRAS CLAVE: Aspartame; Feto; Gestación; Hígado; Cariometría.

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