ARTIGO ORIGINAL

Efeitos do Ciclamato de sódio e do Aspartame na Placenta de Ratas - Estudo morfométrico

Effects of Sodium Cyclamate and Aspartame on the Rat Placenta A Morphometric Study

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ABSTRACT

Objective: To evaluate the effects on the placenta of the administration to rats during embryogenesis, of sodium cyclamate or aspartame. **Method**: Administration of respectively, 14 mg/kg of aspartame via an orogastric sound to a group of rats during their tenth to fourteenth day of pregnancy, of 60 mg/kg of sodium cyclamate intraperitonially to another group, and of equivalent volumes of saline by the same routes to controls. On the twentieth day of pregnancy five foetuses of each group were aleatorily selected for study. Kariometry was used to evaluate nuclear parameters of the decidua, spongy layers and chorionic villi of the placenta. **Results**: Weights of foetuses and placentas, as well as lengths of cords were lower in treated rats, compared to control. While no changes were observed in the decidual layer of the cyclamate-treated group, this layer was altered by aspartame treatment. Nuclear parameters in the spongy layer and chorionic villi were altered in both, cyclamate and aspartame-treated groups. **Conclusions**: The study numerically demonstrated placenta intoxication by sodium cyclamate or aspartame and consequent repercussion on foetuses of the use of these substances during pregnancy.

Key words: Cyclamate; aspartame; placenta; karyometry.

INTRODUCTION

Cyclamate, a derivative of N-cyclo-hexyl-sulfamic acid (CHS), is widely utilized as an artificial non-caloric sweetener of foods and drinks (YAMAMURA *et al*. 1968, SUENAGA *et al.* 1983), and in the pharmaceutical industry (BARLATTANI, 1970). It is odourless, and soluble in water, alcohol and propyleneglicol (SAIN & BERMAN, 1984), more stable than aspartame in supporting temperature variations (BARLATTANI, 1970).

Cyclamate`s sweetening taste, thirty times higher a sweetener than that of saccharose, and free of the bitterness of saccharine (three hundred times higher a sweetener than saccharose), was discovered in 1937 at the University off Illinois, EUA (EHHP), by Michael Sveda, who accidentally noticed its sweet taste (AUDREITH & SVEDA, 1944). In the beginnings of 1959, the Food and Drug Administration (FDA), added cyclamate to its list of safe substances (AHMED & THOMAS, 1992), permitting its use mixed with saccharin, as an artificial sweetener for diabetics (first qeneration sweeteners).

Next year, Price *et al*. (1970), observed the development of bladder tumors (PRICE *et al*.,1970; BRYAN & ERTÜRK, 1970), in rats submitted to high doses of cyclamate, a finding interpreted by the FDA, as being a possible carcinogenic substance (EHHP, 2000; NCI, 2003). The North -American Department of Health and Education, concluded that cyclamate did not present any value for diabetic or obesity treatment (EGEBERG *et al*., 1970), and prohibited its use in the USA; as it remains until now (EHHP, .2000). In 1977, the Committee of Alimentary Additives of the World Health Organization (WHO) however, approved the use of sodium cyclamate in over 40 countries (BOOP *et al*., 1986), including Brazil (AHMED & THOMAS, 1992).

It is known that saccharose substitutes are increasingly employed and a high risk to pregnant women, since according to Pitkin *et al.* (1970), sodium cyclamate crosses the placental barrier reaching a foetal concentration equivalent to one-fourth of the maternal level.

Aspartame in turn, was accidentally discovered in 1965 by the chemist James Schlatter who, when trying a tetrapeptide active against ulcers, found a white powder, having an intense sweet taste, the N-alpha-aspartyl-phenylalanyl-1-methyl ester, aspartame, a second generation sweetener (EHHP, 2000; PACHIONE, 2003).

Aspartame was the first sweetener close to sugar in flavour, and after its entrance into the market in 1981 (EHHP, 2000; NCI, 2003) ended the hegemony of the cyclamate-saccharine sweetening mixture (PACHIONE, 2003).

A synthetic compound, aspartame contains two aminoacids, and technically is considered caloric; however, thanks to its 200 times higher sweetening power than sugar (CALORIC CONTROL COUNCIL, 2004) its energetic value is insignificant (EHHP, 2000; PACHIONE, 2003). Composed of 50% phenylalanine, 40.% aspartate, and 10% methanol, aspartame is metabolised in the gastrointestinal tract into its three components (BUTCHKO *et al*., 2002), and its present use is inconvenient by phenylacetonuria patients (EHHP, 2000; PACHIONE, 2003; CALORIE CONTROL COUNCIL, 2004). Aspartame consumption may cause damages due to its contribution to plasma formaldehyde formation (TROCHO *et al*., 1998). Allergic reactions have also been described to it (ROBERTS, 1996)

Aspartame releases methanol, a substance that is changed into formaldehyde, highly toxic to hepatic function and of ocular toxicity and capable of evoking severe alterations, including cancer (TROCHO *et al*., 1998).

Formates, derived from it if accumulated, can be responsible for metabolic acidosis (TEPHLY, 1999). Aspartates may participate in the destruction of hypothalamic neurones (SMITH *et al*., 2001).

It is worth pointing out that research using laboratory animals enables the acquisition at a short notice and under controlled conditions of information about the toxic potential to the developing organism of chemical substances (ARRUDA *et al.*, 2003). It is important also to emphasize that most publications concerning research on sodium cyclamate, were reported in the sixties and seventies, but subsequently decreased, largely due to the prohibition by the FDA of its utilization in the United States in 1969 (EGEBERG *et al.,* 1970).

To sum up despite the importance of the placenta, there prevails a scarcity of studies on the effects on this organ of artificial sweeteners in general, and of sodium cyclamate and aspartame in particular.

It was the objective of the present study to evaluate in the following manners, of morphometric alterations of the placenta of rats submitted to the separate administration, of sodium cyclamate or aspartame, during the gestation period extending from the tenth to the fourteenth day of pregnancy:

- evaluation of intrauterine foetal growth by measurement of foetal and placental weights and length of the umbilical cord;
- rnorphometric evaluation of the nuclei of rat placental cells.

MATERIAL AND METHODS

The present study evaluated placentas of 15 albino, Wistar rats of average 50 days of age and 240 gm weight; five rats randomly selected were intraperitoneally given 60 mg/kg of sodium cyclamate, five received 14 mg/kg of aspartame by the orogastric route, all from the tenth to the fourteenth day of gestation; five control rats received by the same routes, an equivalent volume of 0,9% saline.

On the twentieth day of pregnancy, all animals were sacrificed, and foetuses were randomly chosen and the placenta from each rat, were weighed on a precision balance. Following conventional histological excision, semi-serial, 6 m sections were obtained and stained with hematoxilin-eosin; umbilical cords were measured to the same precision. Placental morphometry of placenta decidua, spongy layers and chorionic villi, were karyometrically evaluated with an optical microscope with one clear chamber (LEICA) adapted with a final magnification of 1240 times. Elliptical image contours were drawn on white sulphite paper with a black nº 2 pencil; the largest (D) and smallest (d) diameters in mm of each structure were determined, and the following parameters calculated:

1. Mean diameters: M = (D,d) ½
2. Ratio between the largest and smallest diameters: D/d
3. Perimeters: P= (/2) [1.5 x (D+d) - M]
4. Areas: A= M^2 / 4
5. Volumes: V= / 6 M^3
6. Relations between volume and area: 3/2 M
7. Eccentricity: E = (D + d) ½ (D – d) ½ / D
8. Form coefficients: F= 4 A/P ½
9. Contour indexes: I = P / (A) ½

Diameters measured were submitted to a computer program - NUC - developed in the Stomatology Department of the School of Odontology of Ribeirão Preto, University of Sao Paulo. The non-parametric Mann-Whitney test was utilised for statistical data analysis.

RESULTS AND DISCUSSION

Many individuals nowadays, wish to avoid the ingestion of excessive calories in their food to slenderize, or have to limit it for medical reasons (EHHP, 2000). Artificial sweeteners act to this end by substituting natural sugar

(PACHIONE, 2003) causing a huge increase in their employment, pioneered by sodium cyclamate.

However, scientific data have limited the use of these sweeteners by demonstrating their capacity to induce or at least act as co-factors in the generation of bladder cancer (EHHP, 2000; Price *et al*. 1970; BRYAN & ERTÜRK, 1970; NCI, 2003). Aspartame has been associated with headache and nausea (KOEHLER & GLARUS, 1988; ROBERTS, 1995, 1997) and allergy (ROBERTS, 1996).

In order to identify further damaging effects of sodium cyclamate and of aspartame the present study accessed three rat placentary structures (decidua, spongy layer and chorionic villi), following the administration of 60 mg/Kg body weight of sodium cyclamate or of 14 mg/Kg body weight of aspartame to groups of rats during their tenth to fourteenth day of gestation.

These dosages were used sweeteners because the maximum amount for use in humans of sodium cyclamate, according to the FDA, was 50 mg / kg body weight for many years (OGA 1996) and currently, in relation to aspartame, ADI (acceptable daily index) is 34 mg / kg body weight, but it was 20 mg / kg (SCHIEBLER & KNOOP, 1959).

The treatment period was from the tenth to fourteenth day of gestation because it is the biggest stage of embryogenesis in these animals. Furthermore, it is the time when the largest number the decidual cells present, because they predominate in the placenta on day 6 of pregnancy in rats, increase in number until the 10th day and decreased on day 14, according to Iguchi *et al.* (1993) *apud* Brandini (2000).

Table 1 shows foetal and placenta weights, and umbilical cord lengths of respectively, control, sodium cyclamate and aspartame-treated rats, as well as their statistical analysis. Mean body weights (2.31 g) of cyclamate-treated animals and of aspartame-treated animals (1.79 g), were significantly lower than those of control rats (2.94 g). Averaged placental weights of treated animals (0.29 g and 0.25 g respectively) were lower than those of controls (0.44 g). Averaged umbilical cord lengths of treated animals (1.93 and 1.62 cm, respectively), were also significantly lower when compared to controls (2.21 cm).

INSERT TABLE 1

On table 1 it can be observed that foetal body weight of the offspring of treated animals was significantly lower than that of controls, indicating an effect of cyclamate and aspartame on rat foetal development.

According to Pitkin *et al*. (1970) following administration sodium cyclamate crosses the placental barrier raising its amniotic fluid levels to one/fourth of those present in the maternal blood.

Brandini (2000) using the same methods studying lead placental effects corroborated our results by demonstrating an interference of morphometry alterations (karyometry and stereology) of the same structures, e.g. the decidua and the spongy layers, showing placenta weight decrease leading to decreased intrauterine foetal development. In another set of data she showed decreased umbilical cord length in treated groups possibly caused by decreased foetal movement, considered by this author, as one of the factors related to general foetal growth in response to tensile forces dependent on foetal movement in the intrauterine space during development. Therefore, reduced umbilical cord length observed in the present study suggests lessened foetal movement during gestation.

Foetal development dependent on nutrients coming from maternal blood via the placenta, if compromised by the presently studied harmful substances, can cause decreased transport of nutrients, oxygen and other elements of foetal circulation, leading to deficient growth and weight.

In the present work, body weights of 2.31 g and 1. 79 g respectively of foetuses exposed to sodium cyclamate or aspartame, were considerable lower than body weights of the controls (2.94 g). This effect was extended to the placentas of the treated groups (0.29 g and 0.25 g, respectively), significantly lower than those of the controls (0.44 g), indicating the toxicity of the artificial sweeteners employed.

Umbilical cord values of cyclamate or aspartame-treated groups (1.93 and 1.62 cm, respectively) also differed in a statistically significant manner from those of controls (2.12 cm).

Studies by Arruda *et al*. (2003), using sodium cyclamate, showed similar results.: the treatment lowered placenta and foetal rat weights, and foetal liver weights; they also evidenced karyometric alterations in these tissues. Studies on aspartame by Portela (2004) and Leme & Azoubel (2006), showed foetal and placenta weight decreases as well as karyometric changes in liver and pancreas, respectively. In studies on cyclamate, Schechter and Roth (1971) observed that besides passing through the placental barrier (confirming studies by Pitkin *et al.*, 1970), cyclamate remained in foetal organs while disappearing from maternal organs.

These findings support present results, by showing no significant changes in karyometric parameters in the decidua (maternal placenta) due to cyclamate, as we indicated in Table 2. In contrast, aspartame treatment caused karyometric parameters of that layer to be significantly altered.

INSERT TABLE 2

Karyometric parameters of the **placentary decidua** of control rats did not show a statistically significant difference, compared to those of cyclamate-treated animals In contrast, these parameters when coming from controls or aspartame-treated rats respectively, showed statistically significant differences.

Karyometric parameters of the **placental spongy layer** of sodium cyclamate or aspartame-treated animals, showed statistically significant alterations when compared to control rats (Table 3).

INSERT TABLE 3

Karyometric analysis of the spongy layer (foetal placenta) both of cyclamate or aspartame groups, showed statistically significant alterations in nuclear sizes, suggesting that the nucleus was shortened and decreased in size and that altered eccentricity related to nuclear form, indicated presence of inadequate cell metabolism.

Table 4 shows karyometric parameters and their statistical analysis of **placental chorionic villi** from sodium cyclamate or aspartame-treated rats. Those from the latter two groups showed statistically significant differences when compared to controls.

INSERT TABLE 4

Table 4 reveals karyometric alterations of chorionic villi (also parts of the foetal placenta) in the cyclamate group (showing decreased nuclear size), while the aspartame-treated group showed increased nuclear size. These changes again suggest inadequate cell metabolism, corroborating cited literature data.

CONCLUSIONS

The results of this study indicate that administration to rats, of either 60 mg/Kg of sodium cyclamate or of 14 mg/Kg of aspartame, form the tenth to the fourteenth day of pregnancy, led to the following changes:

- decreased placenta and foetal weights.
- decreased length of the umbilical cord.
- alterations due to sodium cyclamate of karyometric parameters related to nuclear size in the spongy layer and the chorionic villi, and the eccentricity of the placenta spongy layer.
- alterations by aspartame of karyometric parameters related to nuclear size of the decidua, spongy layer and the chorionic villi of the pacenta, and of karyometric parameters related to nuclear shape of the placentary chorionic villi.
- These results numerically express placentary intoxication due to sodium cyclamate and aspartame and demonstrate the effects on the foetus of the use of these substances during pregnancy.

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