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Effects of Sodium Cyclamate on the Rat Placenta: A Morphometric Study

Efectos del Ciclamato de Sodio en la Placenta de Rata: Estudio Morfométrico

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SUMMARY: To evaluate the effects of sodium cyclamate on the rat placenta by its administration in the period of embryogenesis. The sodium cyclamate was administered by intraperitoneal route in rats of the treated group at the dose of 60 mg/Kg, from the tenth to fourteenth day of gestation, while the equivalent volume of saline solution was given to the control group, by the same route. On the twentieth day of pregnancy, 10 fetuses (5 from each group) were chosen at random for study. The technique of cariometry was utilized for evaluation of nuclear parameters of cells in deciduous and spongy layers, and of chorionic villi in the rat placenta.

The weights of treated fetuses and their placentas were less than those of the control group, while umbilical-cord length in the treated group was shorter than that in control fetuses. There were no alterations in the deciduous layer. In the placental spongy layer were found alterations of the following parameters: major diameter, mean diameter, perimeter, area, volume, the volume/area ratio and eccentricity. The altered parameters in chorionic villi were the following: mean diameter, perimeter, area, volume and volume/area ratio.

This study demonstrated placental alteration with the use of cyclamate by the pregnant rat, and its repercussion in fetal weight and umbilical-cord length.

KEY WORDS: Sodium cyclamate; Placenta; Cariometry.

INTRODUCTION

Cyclamate, derived from the acid N - cyclo – hexyl – sulfamic (CHS), is amply utilized as a non-caloric artificial sweetener in foods and beverages (Suenaga *et al.,* 1983; Yamamura *et al.,* 1968) and in the pharmaceutical industry (Barlattani, 1970). Cyclamate is odorless and soluble in water, alcohol and glycol propylene (Sain & Berman, 1984), and is more stable than aspartame and saccharine, and supports temperature variations (Barlattani).

Cyclamate was discovered in 1937, at the University of Illinois, USA (EHHP, 2000), by Michael Sveda, who accidentally discovered its sweet taste, 30 times sweeter than that of sucrose but without the bitter flavor of saccharine (Audreith & Sveda, 1944), which is 300 times sweeter than sucrose. At the beginning of 1959, the Food and Drug Administration (FDA) added cyclamate to its list of safe substances (Ahmed & Thomas, 1992), permitting its use as an artificial sweetener for diabetics. A mixture of cyclamate and saccharine (first-generation sweeteners) at the proportion of 10:1 presented an increase in consumption in the USA to a level of approximately 8,943 tons of cyclamate in 1969 (Burbank & Fraumeni Jr., 1970).

In the following year (1970), Price *et al*. and Bryan & Ertürk observed the development of bladder tumors in rats submitted to high doses of cyclamate, which was interpreted by the Food and Drugs Administration (FDA) as a substance possibly cancerigenic (EHHP, 2000; NCI, 2003). Subsequent to this study the North American Department of Health and Education concluded that cyclamate did not present any value for treatment of diabetes and obesity (Egeberg *et al*., 1970), and its use was prohibited in the USA, and remains so until today (EHHP). However, in 1977, the Committee on Food Additives at the World Health Organization (WHO), approved the use of sodium cyclamate as a food additive in more than 40 countries (Boop *et al.*,

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1986) including Brazil (Ahmed & Thomas), although experimental results present reasons for its non-utilization.

Despite the affirmation of Assunção *et al.* that the consumption of this substance by Brazilian diabetics is less than 50 mg/Kg of body weight (standardized dose as acceptable daily intake), it is known that the substitution of sucrose is growing, and in pregnant women there is a great risk, as in accord with Pitkin *et al.* (1970), sodium cyclamate crosses the placental barrier approaching a fetal concentration of this substance equivalent to one quarter of the existent maternal concentration.

More recent studies have failed to demonstrate that cyclamate is carcinogenic or co-carcinogenic. However, other studies will need to be completed before cyclamate might be approved for commercial use as a food additive in the United States (NCI).

It should be emphasized that studies with laboratory animals permit in a short time and under controlled conditions, the obtainment of information on toxic potential of chemical substances in organisms during development, according to Arruda *et al. (2003).* Yet it is important to note that most publications on the effects of sodium cyclamate occurred in the 1960s and 1970s, and that this output has reduced afterwards mostly due to prohibition of the use of this substance by the FDA in the United States of America, in 1969 (Egeberg *et al.*).

The objective of the present study will be to evaluate placental morphometric alterations detected in rats submitted to intraperitoneal administration of sodium cyclamate from the tenth to fourteenth day of pregnancy, in the following manner:

- Evaluation of intrauterine fetal growth through weights of the fetus and placenta, and umbilical-cord length;

- Morphometric evaluation of the nucleus of rat placental cells.

MATERIAL AND METHOD

In the present study the placentas of 10 rats were evaluated, divided into 5 treated rats and 5 control rats, chosen at random; the treated rats received 60 mg/Kg of body weight sodium cyclamate intraperitoneally from the 10th to 14th day of gestation, and the 5 rats from the control group received by the same route, the equivalent volume of saline solution 0.9%.

On the 20th day of pregnancy, animals of the treated and control groups were weighed, as well as one randomly chosen fetus from each rat and its placenta, on a precision balance; posterior conventional histological technique was realized, obtaining semi-serrated cuts of 6 µm that were colored by hematoxylin-eosin; the umbilical cords were measured to the same precision. The placenta was then evaluated by morphometry though cariometry. The following placental elements were evaluated: decidua, spongy layer and placental chorionic villi.

Cariometric parameters were obtained with an optical microscope with one clear chamber (LEICA) adapted with a final magnification of 1240 times, with only elliptical images being drawn with contour using a dark number 2 pencil on a white sulfite piece of paper, determining the major and minor diameters of each structure in millimeters, and calculated:

1. Mean diameter: M = (D,d) 1⁄2
2. Ratio between diameter major and minor diameters: D/d
3. Perimeter: P = (p/2) [1.5 x (D + d) – M]
4. Area: A= p M2 / 4
5. Volume: V = p / 6 M3
6. Ratio between volume and area: 3/2 M
7. Eccentricity: E = (D + d) 1⁄2 (D – d) 1⁄2 / D
8. Coefficient of form: F= 4 p A/P 1⁄2
9. Index of contour: I = P / (A) 1⁄2

To obtain the data cited, the measured diameters were submitted to a computational program – NUC – developed in the Department of Stomatology of the School of Odontology, at the University of Sao Paulo, at Ribeirão Preto. For evaluation the statistical parameters obtained, the non-parametric Mann-Whitney test was utilized.

RESULTS

The quantitative parameters of fetal weight, placental weight and umbilical-cord length, for both controls and the group treated with sodium cyclamate, as well as its statistical analysis, can be seen in Table I. It is verified that the mean corporal weight, in grams, of treated animals (2.31g) was diminished in relation to controls (2.94 g), showing a statistically significant difference between the groups; in the same way, the mean placental weight in grams (0.29 g), was less than in the controls (0.44 g) and the umbilical-cord length in centimeters of the treated animals (1.93 cm) was reduced compared to the controls (2.12 cm), also showing, for the latter two parameters, a statistically significant difference between groups.

Measures	Control	Treated	Value of p
Fetal weight	2.94	2.31	p [U]: 0.004 *
Placental weight	0.44	0.29	p [U]: 0.004 *
Umbilical-cord length	2.12	1.93	p [U]: 0.048 **

* statistically significant for a < 0.01
** statistically significant for a < 0.05

Table I. Mean values of fetal and placenta weights, and umbilical-cord length of control rats and those treated with sodium cyclamate. Mann-Whitney Test.

The cariometric parameters of the placenta decidua in rats from the control group and those treated with sodium cyclamate, as well as their statistical analysis, can be seen in Table II, where it can be observed that cariometric parameters presented no statistically significant difference between animals of the control and treated groups.

Table II. Mean values of Major Diameter, Minor Diameter, Mean Diameter, D/d Ratio, Perimeter, Nuclear area, Nuclear volume, Volume/area ratio, Eccentricity, Coefficient of form and Index of contour of cell nuclei of placenta decidua (in millimeters) of control rats and those treated with sodium cyclamate. Mann-Whitney Test.

Measures	Control	Treated	Value of p
Major Diameter	19.43	19.43	p[U] : 0.500 ***
Minor Diameter	13.75	13.37	p[U] : 0.345 ***
Mean Diameter	16.26	16.03	p[U] : 0.421 ***
Ratio between major diameter and minor diameter	1.47	1.51	p[U] : 0.155 ***
Perimeter	52.65	52.13	p[U] : 0.421 ***
Area	220.40	209.85	p[U] : 0.345 ***
Volume	2689.40	2423.86	p[U] : 0.345 ***
Ratio between volume and area	10.84	10.69	p[U] : 0.421 ***
Eccentricity	0.66	0.69	p[U] : 0.111 ***
Coefficient of form	0.94	0.93	p[U] : 0.345 ***
Index of contour	3.66	3.68	p[U] : 0.155 ***

*** not statistically significant

The cariometric parameters of the placental spongy layer in rats from the control group and those treated with sodium cyclamate, as well as their statistical analysis, can be seen in Table III, where it can be observed that cariometric parameters of treated animals were diminished in a statistically significant manner in relation to controls, in values for major and mean diameters, perimeter, nuclear area, nuclear volume and volume/area ratio and eccentricity.

Table III. Mean values of Major Diameter, Minor Diameter, Mean Diameter, D/d Ratio, Perimeter, Nuclear area, Nuclear volume, Volume/area ratio, Eccentricity, Coefficient of form and Index of contour of cell nuclei of placental spongy layer (in millimeters) of control rats and those treated with sodium cyclamate. Mann-Whitney Test.

Measures	Control	Treated	Value of p
Major Diameter	17.08	15.54	p[U] : 0.016 **
Minor Diameter	11.83	11.00	p[U] : 0.075 ***
Mean Diameter	14.15	13.01	p[U] : 0.048 **
Ratio between major diameter and minor diameter	1.48	1.44	p[U] : 0.274 ***
Perimeter	45.90	42.11	p[U] : 0.028 **
Area	171.45	141.73	p[U] : 0.028 **
Volume	1921.11	1398.07	p[U] : 0.028 **
Ratio between volume and area	9.43	8.67	p[U] : 0.048 **
Eccentricity	0.68	0.65	p[U] : 0.048 **
Coefficient of form	0.94	0.94	p[U] : 0.345 ***
Index of contour	3.66	3.65	p[U] : 0.210 ***

** Statistically significant for a < 0.05
*** Not statistically significant

The cariometric parameters of placental chorionic villi in rats from the control group and those treated with sodium cyclamate, as well as their statistical analysis, can be seen in Table IV. It can be observed that cariometric parameters of treated animals were diminished in a statistically significant manner in relation to controls, in values for mean diameter, perimeter, nuclear area, nuclear volume and volume/area ratio.

Table IV. Mean values of Major Diameter, Minor Diameter, Mean Diameter, D/d Ratio, Perimeter, Nuclear area, Nuclear volume, Volume/area ratio, Eccentricity, Coefficient of form and Index of contour of cell nuclei of placental chorionic villi (in millimeters) for control rats and those treated with sodium cyclamate. Mann-Whitney Test.

Measures	Control	Treated	Value of p
Major Diameter	12.21	11.46	p[U] : 0.075 ***
Minor Diameter	8.30	7.82	p[U] : 0.111 ***
Mean Diameter	10.02	9.41	p[U] : 0.048 **
Ratio between major diameter and minor diameter	1.50	1.51	p[U] : 0.345 ***
Perimeter	32.60	30.63	p[U] : 0.048 **
Area	80.63	71.16	p[U] : 0.048 **
Volume	563.00	466.51	p[U] : 0.048 **
Ratio between volume and area	6.67	6.27	p[U] : 0.048 **
Eccentricity	0.69	0.69	p[U] : 0.500 ***
Coefficient of form	0.93	0.93	p[U] : 0.500 ***
Index of contour	3.67	3.67	p[U] : 0.500 ***

** statistically significant for a < 0.05
*** not statistically significant

DISCUSSION

Diet and/or light products bring with them the idea of attaining a beautiful and, most of all, a healthy body (Pachione, 2003). The diet / light market is shown each year to be more robust. Some persons want to avoid ingesting excess calories, or they may necessitate limitation of sugar intake for medical reasons (EHHP). The growing search for healthy diets and the squalid standards of beauty has prompted sales to fatten by more than 10% per year (data from ABIAD – Brazilian Dietetic Food Industry Association (Pachione).

The principle of sweeteners always remains the same: to confer sweet flavor as a sugar substitute (Pachione). The preoccupation with its use is due to great growth in classes of sweeteners, with the cyclamate class being the pioneer to appear at the market.

With stronger or less clear evidence of carcinogenicity of edulcorante cyclamate sweetener or of cyclamate / saccharine mixture, its limitations rest on the scientific data as inductors or, at least, co-factors in human bladder cancer, which if not proven absolutely, at minimum leave doubt, fright and discomfort (EHHP; Price *et al.*; Bryan & Ertürk; NCI).

With the objective of identifying possible damaging effects from the use of cyclamate, this study evaluated the placental tissue of rats though three structures (deciduous and spongy layer, and chorionic villi), after the administration of 60 mg/Kg of body weight sodium cyclamate, from the tenth to fourteenth day of gestation.

Through analysis Table I, it is observed that the fetal weight of the treated group was less than that of the controls in a statistically significant manner, suggesting action of cyclamate substance on fetal development.

According to Pitkin *et al.*, sodium cyclamate crosses the placental barrier, approaching the amniotic fluid at a proportion of one fourth of maternal blood concentration, and thus reaching the fetal tissues.

Brandini (2000), in his study of the placenta and lead, corroborates our results identifying interference of the substance in the placenta through alterations of placental morphometry (cariometry and stereology), by the same method in the structures that were also evaluated in the present work (cariometry of deciduous and spongy

layers). Yet it shows the consequent diminution of placental and fetal weights in the treated group, suggesting retardation in development of the placenta and restriction of intrauterine fetal growth; an additional datum is diminution of umbilical-cord length in this group, by possible diminution of fetal movements; umbilical-cord length is one of the factors related to fetal growth in general. It grows in response to tensor forces that depend on fetal movement and on intrauterine space during development.

Thus, umbilical-cord diminution as observed in this study suggests diminution of fetal movement during gestation.

Adequate fetal development depends on substances that come from maternal blood through the placenta.This, compromised by the substance in the study, presents diminished blood flow with consequently less arrival of nutrients, oxygen and other elements of fetal circulation, resulting in deficiency of growth and fetal weight.

These findings also were encountered in our study, in which it was observed that the weight of treated fetuses (2.31 g) was less than that of controls (2.94 g), while placental weight in the treated group (0.29 g) also was less than the weight of placentas from the control group (0.44 g), suggesting toxicity from the sweetener utilized.

With respect to umbilical-cord length, it diminished from 2.12 g (control group) to 1.93 g (treated group). All these results were statistically significant, as shown in Table I.

Other studies in the literature involving sodium cyclamate also obtained similar results in relation to lower placental and fetal weights in rats, and shorter umbilical-cord length, including Arruda *et al*., who worked with cyclamate and the fetal kidney and Martins *et al*. (2005), who studied cyclamate and the fetal liver. Besides, these works showed alterations in cariometry of the respective tissues from each study.

In the same manner, Portela & Azoubel (2004) encountered similar findings in relation to and placental weights, and umbilical-cord length, from studying amicacine and fetal kidney, besides the nephrotoxicity of the substance.

In a study with cyclamate, Schechter & Roth (1971) observed that after 7 hours of intravenous administration of cyclohexylamine marked with carbon 14 to pregnant rats, the most important metabolite of cyclamate, radioactivity was not found in maternal organs. In contrast to the maternal organism, the fetus presented intense radioactivity. Thus, besides passage through the placental barrier, confirming the studies of Pitkin *et al.*, the present work supplies valuable data in relation to the presence of cyclamate in fetal organs and its absence in maternal organs.

These results can sustain our findings, given that in our study there was no statistically significant alteration in any cariometric parameter related to the deciduous layer (maternal placenta), as shown in Table II.

Nevertheless, in the cariometric analysis of the spongy layer (fetal placenta) the following altered parameters can be observed with statistical significance, as demonstrated in Table III: major diameter, mean diameter, perimeter, area, volume and relation between volume and area, suggesting nucleus is shortened and diminished, and altered eccentricity, which suggests inadequate cellular metabolism.

In analyzing Table IV, cariometry of chorionic villi (also the fetal placenta), can be observed with the following alterations in a statistically significant manner: mean diameter, perimeter, area, volume and relation between volume and area, also suggesting nuclear diminution.

All of these findings are in agreement and corroborate the scientific literature cited. Our results numerically express the placental intoxication with cyclamate and consequent fetal repercussion from the use of this substance in pregnancy.

CONCLUSIONS

The results of the present work suggest that administration of 60 mg/Kg of body weight of sodium cyclamate from the tenth to fourteenth day of pregnancy in the rat causes:

- Diminution of placental and fetal weights.
- Diminution of umbilical - cord length.
- Alterations of major diameter, mean diameter, perimeter, area, volume and relation between volume and area, and of eccentricity of spongy layer of the placenta.
- Alterations of mean diameter, perimeter, area, volume and relation between volume and area of chorionic villi of the placenta.

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RESUMEN: El objetivo del trabajo fue determinar los efectos del ciclamato de sodio en la placenta de ratas, administrado durante el periodo de la embriogénesis. Fue administrado por vía intraperitoneal, en las ratas del grupo tratado, ciclamato de sodio en una dosis de 60 mg/Kg, desde el 10º al 14º día de gestación, siendo inyectado un volumen equivalente de solución salina en el grupo de ratas control. En el 20º día de preñez, 10 fetos (5 de cada grupo) fueron escogidos al azar para su estudio. Fue utilizada la técnica de cariometría para la evaluación de los parámetros nucleares de las células de las capas decidua, esponjosa y de las vellosidades coriónicas de la placenta de las ratas.

El peso de los fetos tratados y de sus placentas fue menor que el del grupo control, así como también el funículo umbilical del grupo tratado fue más corto que el de los fetos controles. No hubo alteraciones en la capa de la decidua. En la capa esponjosa placentaria ocurrieron alteraciones de los siguientes parámetros: diámetro mayor, diámetro medio, perímetro, área, volumen, relación volumen/área y excentricidad. Los parámetros alterados en las vellosidades coriónicas fueron: diámetro medio, perímetro, área, volumen y relación volumen/área. El estudio demostró alteración placentaria con el uso del ciclamato de sodio en la rata preñada y su repercusión en el peso fetal y largo del funículo umbilical.

PALABRAS CLAVE: Ciclamato de Sodio; Placenta; Cariometría.

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