Effect of Sodium Cyclamate on the Rat Fetal Exocrine Pancreas: a Karyometric and Stereological Study

Efectos del Ciclamato de Sodio en el Páncreas Exocrino Fetal de Ratas:
Estudio Cariométrico y Estereológico

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SUMMARY: The cyclamate, a sweetener substance derived from N-cyclo-hexyl-sulfamic acid, is largely utilized as a non-caloric artificial edulcorant in foods and beverages as well as in the pharmaceutical industry. The objective of this study was to evaluate karyometric and stereological alterations in the rat fetal pancreas resulting from the intraperitoneal administration of sodium cyclamate. The exocrine pancreas of ten fetuses of rats were evaluated, five treated and five controls chosen at random, in which five rats that received from the 10th to 14th days of pregnancy an intraperitoneal daily injection of sodium cyclamate at 60 mg/Kg of body weight during 5 days. At the 20th day of gestation, the animals were removed and weighed, as were their placentas; the length of the umbilical cords also were measured. After the laboratory processing, semi-seriated 6mm cuts stained with haematoxyline and eosine were performed. In seven karyometric parameters (major, minor, and medium diameters, volume, area, perimeter, and volume-area ratio), the increase was statistically significant in the treated group when compared with control group. Stereological parameters showed in the treated group a significant increase in the cellular volume and a significant reduction in the numerical cellular density. These results showed that the sodium cyclamate in pregnant rats led to retardation of fetal development and hypertrophy in the exocrine pancreas of the rat fetuses.

KEY WORDS: Sodium cyclamate; Exocrine pancreas; Karyometry; Stereology.

INTRODUCTION

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

Cyclamate was discovered in 1937 at the University of Illinois, USA, by the graduate student Michael Sveda who accidentally perceived its sweet taste, 30 times sweeter than saccharine but without its bitter flavor. Beginning in 1959, the Food and Drug Administration (FDA) added cyclamate

to the list of safe substances, thus permitting its use as a non-caloric artificial edulcorant for diabetics (Audreith & Sveda, 1944; Ahmed & Thomas, 1992).

During the 1960s, the mixture of cyclamate and saccharine in a 10:1 proportion, drove an increase in the consumption of this substance in the USA to a level approaching 8.943 tons of cyclamate in 1969 (Burbank & Fraumeni, 1970).

In the following year, Price *et al.* (1970) evaluated the development of tumors in the bladder of rats treated with high doses of cyclamate, which was interpreted by the Food and Drug Administration (FDA) as a substance that possibly

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induces cancer. Subsequent to this study, the U.S. Department of Health, Education and Welfare (HEW) concluded that cyclamate did not presented any value in the treatment of obesity or diabetes and its utilization became prohibited in the USA, remaining so to this day (Egeberg *et al.*, 1970).

However, the World Health Organization's Joint Expert Committee on food Additives approved the use of sodium cyclamate in 1977, as an alimentary additive in more than forty countries including Brazil (Boop *et al.*, 1986), although several experimental results from that era presented reasons for its non-utilization (Oser *et al.*, 1968; Pitkin *et al.*, 1969; Pitkin *et al.*, 1970; Kroes *et al.*, 1977).

Despite the affirmation of Assunção *et al.* (1994) that in Brazilian diabetics the consumption of this additive is less than 11 mg/Kg of body weight in relation to acceptable daily ingestion, the group which is expected to present elevated utilization of edulcorants, it is known that the sucrose substitution is growing, and can affect pregnant women, which represents a great risk, because according to Pitkin *et al.* (1969, 1970), sodium cyclamate can cross the placental barrier and approach a fetal concentration one fourth that of maternal one.

The objective of this study was to evaluate the karyometric and stereological alterations of fetal exocrine pancreas of rats resulting from the intraperitoneal administration of sodium cyclamate, from the tenth to the fourteenth day of pregnancy, subdivided into the following items:

- Evaluation of the intrauterine growth of the fetus by means of the fetal and placental weights and the length of the umbilical cord.
- Karyometric and stereological evaluation of pancreatic acinar cells of rat fetuses.

MATERIAL AND METHOD

In this study, the pancreas of ten fetuses rats were evaluated, five treated and five controls chosen at random, in which five rats that received from the 10th to 14th pregnancy day an intraperitoneal injection of sodium cyclamate at 60 mg/kg of body weight (treated group) and five that received the same via saline solution 0.9% (control group). On the 20th pregnancy day, the animals of both groups were weighed, as were their placentas, on a precision balance; the length of the umbilical cords also were measured. After the laboratory processing were obtained semi-seriated 6µm cuts stained with hematoxilin and eosine.

From each animal selected, 50 pancreatic acinar cells nuclei were evaluated with an optical microscope with camera lucida (H500 hund Wetzlar) and final magnification of 1240 times which contoured the elliptical structures on white sulfite paper with a black number 2 pencil. For the determination of the major (D) and minor (d) diameters with a millimeter ruler, the following karyometric parameters were obtained: mean geometric diameter, ratio of longest to shortest axis, perimeter, area, volume, ratio of volume to areas, eccentricity, shape factor, and contour index.

The same material was submitted to stereology, also by means of an optical microscope with a camera lucida and a preconized grade application by Merz (1967). A projection was completed in 20 different fields of 100 points each for a total of 2000 points for each element from the groups. As a consequence of this technique, the parameters obtained were: cytoplasmic volume, cellular volume, ratio of nucleus to cytoplasm and numerical cellular density.

For statistical comparison of the morphometric results obtained in the treated and control groups, the non-parametric Mann-Whitney test was utilized.

RESULTS

The quantitative parameters for fetal weight, placenta weight and umbilical-cord length in both control rat fetuses (C) and those treated with sodium cyclamate (T) can be see in Table I as well as the statistical analysis.

Examination of Table I enables verification that all quantitative parameters presented significant statistical differences between groups. Treated animals present reduction in the three measures.

In Table II, mean differences in nuclear parameters are demonstrated for control (C) and treated (T) groups as well as the statistical analysis.

It can be observed that, of all the karyometric parameters presented, the seven which were statistically different from the control group were: major, minor and mean diameters (µm), volume (µm³), area (µm²), perimeter (µm) and the volume-area ratio. Conversely, the four data that did not show statistically significant differences were: major-minor diameter ratio, eccentricity, coefficient of form and contour index.

In Table II, mean differences in stereological parameters are demonstrated for control (C) and treated (T) groups as well as the statistical analysis.

Table I. Mean values for fetal weight and placental weight (g); umbilical-cord length (cm) of control fetuses (C) and treated with sodium cyclamate (T). Mann-Whitney test.

Parameter	Control	Treated	U	P [U]
Fetal weight	2.94	2.31	0*	0.004
Placental weight	0.44	0.29	0*	0.004
Umbilical-cord length	2.12	1.93	0*	0.004

*a < 0.01.

Table II. Mean values for major, minor and mean diameters (mm), and the ratio between major and minor diameter, volume(mm³), area(mm²), perimeter(mm), volume-area ratio, eccentricity, shape factor and contour index of the nuclei of pancreatic acinar cells of control rat fetuses (C) and treated with sodium cyclamate (T). Mann-Whitney test.

Parameter	Control	Treated	U	P [U]
Major diameter	11.48	12.63	0*	0.004
Minor diameter	7.84	8.87	0*	0.004
Mean diameter	9.37	10.54	0*	0.004
Major-minor diameter ratio	1.37	1.34	6 ^{ns}	0.111
Volume	462.74	635.61	0*	0.004
Area	71.24	88.33	0*	0.004
Perimeter	30.68	34.08	0*	0.004
Volume-area ratio	6.30	7.03	0*	0.004
Eccentricity	0.70	0.68	6 ^{ns}	0.111
Shape factor	0.94	0.95	8 ^{ns}	0.210
Contour index	3.67	3.65	6 ^{ns}	0.111

*a < 0.01; ns= not significant.

Table III. Mean values of nucleus-cytoplasm ratio, cytoplasm volume (mm³), cellular volume (mm³), numerical cellular density (n°/mm³) of the exocrine pancreas of control rat fetuses (C) and treated with sodium cyclamate (T). Mann-Whitney test.

Parameter	Control	Treated	U	P [U]
Nucleus-cytoplasm ratio	0.2491	0.2749	12 ^{ns}	0.500
Cytoplasm volume	1847.04	2251.62	6 ^{ns}	0.111
Cellular volume	2309.78	2887.24	4*	0.048
Numerical cellular density	438076.86	351695.64	4*	0.048

*a < 0.05; ns= not significant.

A stereologic analysis of fetal pancreatic acini of control rats (C) and treated with sodium cyclamate (T), shown in Table 3, revealed that the cytoplasm volume (mm³) and the nucleus / cytoplasm ratio do not show a statistically significant difference in comparison groups, unlike the cellular volume (mm³) and Numerical Cellular Density (n° cells/mm³), when the Mann-Whitney test is applied

DISCUSSION

The effect of cyclamate in the pancreas was evaluated initially by Hagmüller *et al.* (1969), when they found the sweetener effect on blood glucose and the increase in alpha cells of the Islets of Langerhans from animals that received

through the drinking water at a dose of 0,5 and 2%. Although Hagmüller *et al.* have detected pancreatic alterations induced by the sweetener, many authors stated that no harmful effects on the structure and functioning of the endocrine pancreas in mice, rats and dogs (Fitzhugh *et al.*, 1951; Hagmüller *et al.*; Brantom *et al.*, 1973). Thus, the present study, using morphometric methods, demonstrates, as never before, the changes determined by sodium cyclamate on the pancreatic acini of rat fetuses.

According to Pitkin *et al.* (1970), the fetal concentration of sodium cyclamate in pregnant women may reach 1/4 of the maternal concentration as a function of this capacity to permeate the placental barrier. In this manner the quantitative alterations verified in this work are in accord with those of the latter author and yet in accord with Roby & Soares (1993) who found that placental morphology, position and function can be utilized as a maturation index of the placenta; and Moessinger *et al.* (1982) who found that umbilical-cord length is one of the factors related to fetal growth. Thus reduction of its length could indicate diminished fetal movement in the gestation period on account of sodium cyclamate.

The karyometric study of acinar cells of the fetus showed a statistically significant increase ($\alpha < 0,01$) of Major, Minor and Mean Diameters, Volume, Area, Perimeter and Volume-Area Ratio in the treated group. There is, therefore, that the cell nuclei of this region in fetuses belonging to the treated group presents significantly higher than those of the constituents of the control group.

However, the parameters major-minor diameter ratio, eccentricity, shape factor and contour index are not different when treated and controls are compared, allowing thus the perception that nuclei that have higher size in the group treated do not have changes in their forms.

Stereological results of the present study ratify the propositions cited since, because of the four parameters obtained from the application of stereological techniques, two - cellular volume and cellular numerical density- were statistically different in comparisons between the control and treated groups ($\alpha < 0,05$). These data allow the perception that in the animals from treated group there is an increase volume of acinar cells ($\alpha < 0,05$) without, however, the increase of the cytoplasm and changes in the proportion between this and the nucleus. These cells in treated fetuses are few in number ($\alpha < 0,05$), which is characterized by Cellular Numerical Density parameter that defines the number of cells per cubic millimeter of tissue. It is noted, then, that although the cytoplasm of the aforementioned is not increased, the pancreatic acinar cells are well, even with a reduction of their number per mm³ of tissue.

Martins *et al.* (2005) using histometric techniques, analyzed the effects of sodium cyclamate on the rat fetal liver. They found large hepatocytes and significant nuclear changes similar to those observed in this study, suggesting hepatotoxicity by the sweetener. Similarly, Arruda *et al.* (2004) found extensive changes in glomeruli and in nuclei of cells of proximal convoluted tubule of fetuses of rats given the sweetener. The authors concluded that these changes suggest fetal nephrotoxicity.

Although some works affirm an absence of toxic effects from sodium cyclamate on organisms (Oser *et al.*; Takayama *et al.*, 2002), many studies clearly demonstrate an injurious effect of this substance on cellular activity (Cattanach, 1976; Sasaki *et al.*, 2002) these studies show an alteration in the synthesis of cellular RNA and DNA, confirming what was initially proposed by Pitkin *et al.* (1969).

Thus, increasing the size of nuclei and pancreatic acinar cells, with consequent reduction of their number, featuring a hypertrophy detected by this study, could be attributed to, by activity of sodium cyclamate, DNA RNA inadequate synthesis and/or proteins that maintain the normal cellular process.

This hypothesis also finds support in the study of Torres de Mercau *et al.* (1995) that, when administering mixture of cyclamate and saccharin to rats, in equal parts for 90 days, found the occurrence of hypertrophy of the large intestine. The authors attributed this result to the structural changes of the cell membrane and nuclear DNA promoted by sodium cyclamate.

Thus can be suggested that sodium cyclamate administered intraperitoneally from the 10th to the 14th day of pregnancy causes: a. diminished fetal weight, placental weight and umbilical-cord length compared to the control group, suggesting retardation of fetal development, and b. hypertrophy of acinar cells of fetal rats.

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RESUMEN: El ciclamato, es una sustancia derivada del ácido N-ciclo-hexil-sulfámico, bastante usada como edulcorante no calórico en los alimentos y bebidas, así como en la industria farmacéutica. El objetivo de este estudio fue evaluar las alteraciones cariométricas y estereológicas en páncreas fetal de rata tras la administración intraperitoneal de ciclamato de sodio. El páncreas

exocrino de diez de los fetos de rata fueron evaluados, cinco tratados y cinco controles seleccionados al azar, en el que cinco ratas recibieron del día 10 al día 14 de preñez una inyección intraperitoneal diaria de ciclamato de sodio a 60 mg/Kg de peso corporal durante 5 días. En el día 20 de gestación, los animales fueron retirados y pesados, al igual que sus placentas. Asimismo, se midió la longitud de los cordones umbilicales. Después del procesamiento de laboratorio, cortes semi-seriados de 6µm, se tiñeron con hematoxilina-eosina. En siete parámetros cariométricos (diámetros mayor, menor y medio, volumen, área, perímetro y relación área/volumen). El aumento fue estadísticamente significativo en el grupo tratado comparado con el grupo control. Los parámetros estereológicos mostraron en el grupo tratado un aumento significativo del volumen celular y una reducción significativa en la densidad numérica celular. Estos resultados mostraron que el uso del ciclamato de sodio en las ratas preñadas causa retardo en el desarrollo fetal e hipertrofia en el páncreas exocrino de los fetos de rata.

PALABRAS CLAVE: Ciclamato de Sódio; Páncreas exocrino; Cariometría; Estereología.

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