Effect of Sodium Cyclamate on the Rat Fetal Exocrine Pancreas: a Karyometric and Stereological Study
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Efectos del Ciclamato de Sodio en el Pancreas 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...
induces cancer. Subsequent to this study, the U.S. Department of Health, Education and Welfare (HEW) concluded that cyclamate did not present 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.

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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 (mm$^3$) and the nucleus / cytoplasm ratio do not show a statistically significant difference in comparison groups, unlike the cellular volume (mm$^3$) and Numerical Cellular Density ($n$/mm$^3$), 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 (α<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 (α<0.05). These data allow the perception that in the animals from treated group there is an increase volume of acinar cells (α<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 (α<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 Mercou 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.
exocrino de diez de los fetos de rata fueron evaluados, cinco trata-
dos 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 placenta. Asimismo,
se midió la longitud de los cordones umbilicales. Después del pro-
cesamiento de laboratorio, cortes semi-seriados de 6µm, se tiñeron
con hematoxilina-eosina. En siete parámetros cromatomeínicos (día-
metros mayor, menor y medio, volumen, área, perímetro y rela-
ción 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 signifi-
cativo del volumen celular y una reducción significativa en la den-
sidad numérica celular. Estos resultados mostraron que el uso del
ciclamato de sodio en las ratas preñadas causa retardo en el desa-
rrollo fetal e hipertrofia en el páncreas exocrino de los fetos de
rata.

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

REFERENCES

Ahmed, F. E. & Thomas, D. B. Assessment of the
carcinogenicity of the nonnutritive sweetener cyclamate. 

N. & Azoubel, R. Effects of Sodium Cyclamate in
Kidneys of Rats Fetuses: a Morphometric Study. Int. J. 


Audreith, L. F. & Sveda, M. Preparation and properties of
some N-substituted sulphamic acids. J. Org. Chem., 
9:89-101, 1944.

Barlattani, M. Rassegne sintetiche di terapia. Il problema

Boop, B. A.; Sonders, R. C. & Kesterson, J. W. Toxicological

Brantom, P. G.; Gaunt, I. F. & Grasso, P. Long term toxicity
of sodium cyclamate in mice food cosmet. Toxicol., 

Burbank, F. & Fraumeni, J. F. Jr. Synthetic sweetener
consumption and bladder cancer trends in the United

Cattanach, B. M. The mutagenicity of cyclamates and their

Egeberg, R. O.; Steinfield, J. L.; Frantz I.; Griffith, G. C.;
Knowles, R. H. Jr.; Rosenow, E.; Sebrell, H. & Van
Itallie, T. Report to the secretary of HEW from the
Medical Advisory Group on cyclamates. JAMA, 

Fitzhugh, O. G.; Nelson, A. A. & Frawley, J. P. A comparison
of the chronic toxicities of synthetic sweetening agents.

histological findings and further experimental data on
the question of cyclamate tolerance in the guinea pig.

Kroes, R.; Peters, P. W. J.; Berkvens, J. M.; Verschuuren, H.
G.; De Vries, T. H. & Van Esch, G. J. Long-term toxicity and
reproduction study (including a teratogenicity study)
with cyclamate, saccharin and cyclohexylamine. 

Martins, A. T.; Azoubel, R.; Lopes, R. A.; Di Matteo, M. A.
S. & Arruda, J. G. F. Effect of Sodium Cyclamate on the 
Rat Fetal Liver: A Karyometric and Stereological Study. 

Merz, W. A. Streekmessung na gerichteten strukturen im
mikroskop und ihre Anwendung Zur Bestimmung von
oberflachen Volumen relationen in knochengewebe. 

Moessinger, A. C.; Blanc, W. A.; Marone, P. A. & Polsen, 
D. C. Umbilical cord length as an index of fetal activity:

Oser, B. L.; Carson, S. & Vogin, E. E. Growth and
reproduction studies with cyclamate-saccharin (10:1) in

Pitkin, R. M.; Reynolds, W. A. & Filer, L. J. Cyclamate and
cyclohexylamine: transfer across the hemochorial

Pitkin, R. M.; Reynolds, W. A. & Filer, L. J. Placental transmission
and fetal distribution of cyclamate in early human


Torres de Mercau, G; Riera de Martinez Villa, N; Mercau, G A; Martinez Riera, N; Soria de Santos, N & Vitalone, H. Alteraciones en la citomembrana y celulas de superficie del intestino grueso por accion de los edulcorantes. *Acta Gastroenterol. Latinoam., 25(1):*35-9, 1995.