International Journal of Morphology
On-line version ISSN 0717-9502

Int. J. Morphol. vol.22 no.2 Temuco 2004

http://dx.doi.org/10.4067/S0717-95022004000200009

Int. J. Morphol., 22(2):149-154, 2004.

PHENYTOIN ACTION IN THE LINGUAL MUCOSA OF RAT FETUSES: MORPHOMETRIC STUDY

ACCIÓN DE LA FENITOINA EN LA MUCOSA LINGUAL DE FETOS DE RATA: ESTUDIO MORFOMÉTRICO

***,**Selma Siéssere; *Marisa Semprini; *Ruberval A. Lopes; ***Reinaldo Azoubel; *Miguel A. Sala; *Maria G. C. Mattos; *Simone Cecílio Hallak Regalo**

* College of Dentistry of Ribeirão Preto / São Paulo University, Ribeirão Preto, SP, Brazil.
** College of Dentistry of Uberaba / Uberaba University, Uberaba, MG, Brazil.
*** College of Medicine of São José do Rio Preto, São José do Rio Preto, SP, Brazil.

Correspondence to:

SUMMARY: Phenytoin (diphenylhydantoin, dilantin) has been the chosen anti-convulsive for the treatment of sudden disorders since the late 1930's. Several epidemiological and clinical studies in human beings showed an increase in malformations in descendants of which mothers took phenytoin during gestation, such as cranio-facial development and growth abnormalities, distal phalangeal hypoplasia, and light or moderate mental deficiency. This work had the goal to study the lingual mucosa, under histopathological and morphometric standpoints, in rat fetuses which were given an intraperitonial injection, consisting of a unique dose of phenytoin (70 milligrams/kilogram of their weight). Being that, we included the examined

material in paraffin, 6 micrometers thick, and also colored these incisions using hematoxylin and eosin. The morphometric parameters were estimated using drawings (50 sketches) made on paper and then measured using a millimetric scale, obtained with the aid of a light camera JENA, with final magnification 1000X. The statistical confrontation was performed employing Mann-Whitney non-parametric test. The results showed that the average body weight of treated animals decreased. Histopathological and morphometric changes at all lingual regions were observed.

KEY WORDS. 1. Phenytoin; 2. Lingual mucosa; 3. Rat; 4. Morphometry.

RESUMEN: Desde 1930 la fenitoina (difenilhidantoina) ha sido el anticonvulsivante elegido en el tratamiento de desórdenes súbitos. Numerosos estudios epidemiológicos y clínicos en humanos mostraron un incremento en malformaciones en descendientes de madres que ingirieron fenitoina durante la gestación, tales como: desarrollo cráneofacial y crecimiento anormales, hipoplasia falángica distal, y alta o moderada deficiencia mental. Este trabajo tiene como objetivo estudiar la mucosa lingual, analizando los aspectos histopatológico y morfométrico, en fetos de ratas a los cuales se colocó una dosis única de fenitoina (70 mg/kg de peso) vía intraperitoneal. El material examinado fue incluido en parafina y cortada a 6 µm y luego teñido con HE. Los parámetros morfométricos fueron estimados, usando dibujos (50 bosquejos) hechos en papel y medidos usando una escala milimétrica, obtenido con la ayuda de una cámara de luz JENA, con un aumento final de 1000X. El análisis estadístico fue realizado empleando el test no paramétrico Mann-Whitney. Los resultados mostraron que el peso promedio de los animales tratados decreció. Además, fueron observados cambios histopatológicos y morfométricos en todas las regiones linguales.

PALABRAS CLAVE. 1. Fenitoina; 2. Mucosa lingual; 3. rata; 4. Morfometría.

INTRODUCTION

Phenytoin (diphenylhydantoin, dilantin) has been the chosen anti-convulsive for the treatment of sudden disorders (attack, epilepsy single episode) since the late 30's (Merrit & Putnam, 1938). Several epidemiological and clinical studies in human beings (Mirkin, 1971) showed an increase in malformations in descendants of which mothers took phenytoin during gestation, such as cranio-facial development and growth abnormalities, distal phalangeal hypoplasia, and light or moderate mental deficiency (Fetal Hydantoinic Syndrome SHF), described by Hanson & Smith (1975). Phenytoin has shown to be teratogenic in mice (Harbison & Becker, 1972; Millicovsky & Johnston, 1981) and in rats (Harbison & Becker). Although those studies on animals have been aimed the fissured palate, Lorente *et al.* (1981) developed a new model that reproduces other common malformations. However, phenytoin acts in the stratified squamous epithelium along the several areas of the rat fetus' body in a very similar way, and turns into slender epithelium, with smaller size cells and nucleus and greater number of cells by mm^3, as observed in the mouth flooring (Gonzaga *et al.*, 2002).

In this work, Wistar female rats were employed in order to check the phenytoin action in the lingual epithelium as well as in the tongue muscles. Therefore, we aim to study the lingual mucosa, under histopathological and morphometric standpoints, in rat fetuses which were given an intraperitonial injection, consisting of a unique dose of phenytoin (70 milligrams/kilogram).

MATERIAL AND METHOD

Wistar albino female rats, average weight 170 grams, were employed and divided into two groups.

Group 1. On the tenth pregnancy day, female rats received an intraperitonial injection of a unique dose of phenytoin, 70 milligrams/kilogram.

Group 2. Control animals received saline at same conditions.

After sacrifice with anesthetic ether on the twentieth pregnancy day, the rats were submitted to a wide incision of the uterus and abdomen, and the fetuses were collected and immediately immersed in alcohol fixing solution 85 milliliters; formalin - 10 milliliters and acetic acid 5 milliliters, for as long as 24 hours. All fetuses, after fixing, were weighed on a Mettler precision scale, washed off, and immersed in 80% alcohol.

The heads were separated from the bodies, and then sectioned longitudinally. Afterwards, the heads were dehydrated, diaphanized, included in paraffin, which was then sectioned in slices (6 micrometers thick), then colored in hematoxylin and eosin.

The karyometric parameters for the lingual mucosa were: mean diameter, perimeter, relationship between the largest and the smallest diameters, volume, area, relationship between volume and area, form coefficient, contour index and eccentricity.

The largest (D) and smallest (d) diameters of the nucleus were estimated over drawings (50 sketched) on paper, obtained with the aid of a light camera, with final magnification 1000X. The statistical confrontation

was performed employing Mann-Whitney non-parametric test.

RESULTS

Body weight: The average body weight of all treated animals was 4.19 grams, and 1.42 grams for the control group.

Histopathology: The epithelium of the ventral tongue region of control animals showed to be thicker than that of treated animals (Fig. 1).

In the anterior dorsal region of the tongue, the epithelium showed to be less thick, with no papilla; the cells from the prickle-cell and basal layer showed to be more rounded and morphologically very alike, and their nuclei showed loose chromatin, evident nucleolus, and two-nucleolus nuclei were often observed. The prickle-cell and basal layer showed to be disorganized; the granular layer was absent as well as the horny layer, consequently (Fig.2).

The posterior dorsal region of the tongue showed to be less thick, comparing to the anterior region, however, with cell morphological characteristics very similar to the anterior region and, also showed the absence of granular and horny layers (Fig.3).

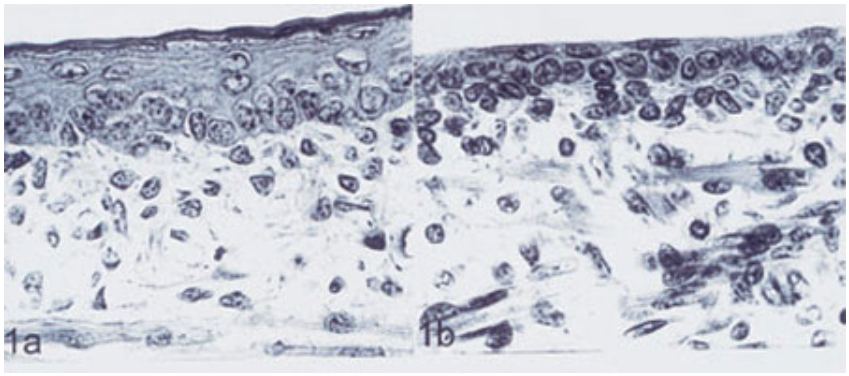

Fig. 1. Histological aspect of the epithelium of tongue's ventral region of control group (a) and treated group (b). Decreased epithelium thickness and a smaller cell volume in the treated group can be observed. H. E. Magnification 340X.

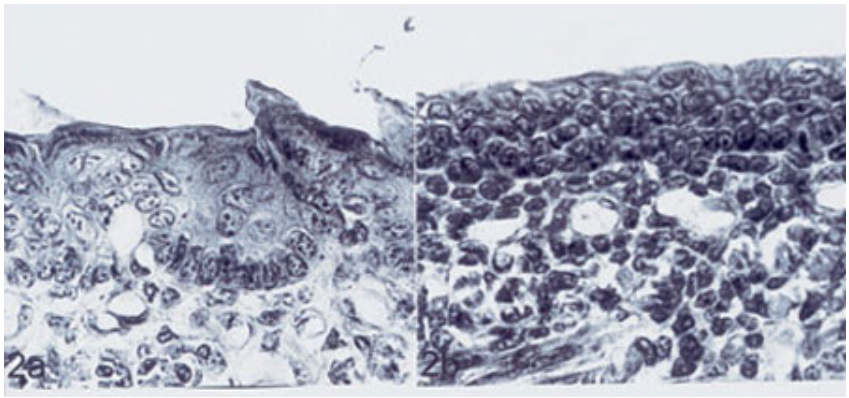

Fig. 2. Histological aspects of the epithelium of tongue's anterior dorsal region of control group (a) and treated group (b). Decreased epithelium thickness and papilla absence in the treated group can be observed. H. E. Magnification 340X.

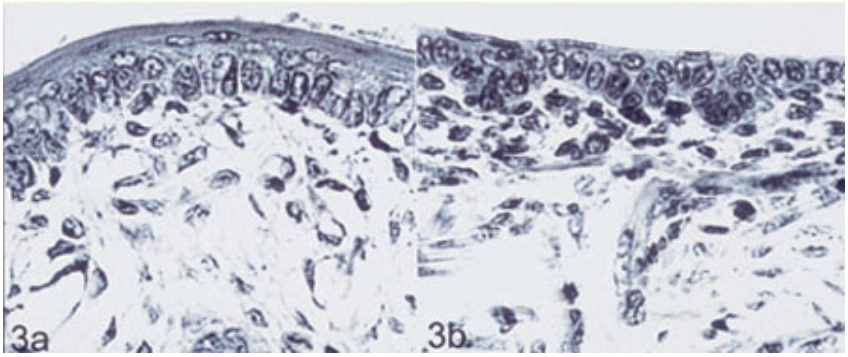

Fig. 3. Histological aspects of the epithelium of tongue's posterior region of control group (a) and phenytoin treated group (b). Decreased epithelium thickness and a greater number of cells in the treated group can be observed. H. E. Magnification 340X.

Morphometric Results: Histometric data provided quantitative results confirming the histological findings (Tables I and II).

Table I. Karyometric parameters average values of the epithelium basal layer cells nucleus from anterior dorsal, posterior dorsal and ventral regions of the tongue of control (C) and treated (T) fetuses. Mann-Whitney test.

Parameters	Anterior dorsal		Posterior dorsal		Ventral	
	C	**T**	**C**	**T**	**C**	**T**
Largest diameter (μm)	7.20	6.08**	7.27	6.29**	6.74	5.44
Smallest diameter (μm)	4.63	3.74	4.81	3.70**	4.85	4.04**
Mean diameter (μm)	5.98	2.91*	6.44	2.23*	5.89	3.08*
Relationship D/d	1.55	0.85ns	1.52	0.52*	1.39	0.49*
Perimeter (μm)	18.80	9.03*	19.18	9.62*	18.33	9.85*
Area (μm^2)	26.20	16.98*	27.56	21.66	25.74	15.82*
Volume (μm^3)	101.12	83.91**	109.28	79.37**	98.61	81.03
Form coefficient	0.93	0.65*	0.94	0.59*	0.96	0.58*
Contour index	3.68	2.52*	3.66	2.16*	3.62	2.06*
Eccentricity	0.77	0.44	0.75	0.32*	0.69	0.44*
Relationship V/A	3.85	2.20	3.94	1.75*	3.82	2.40*

* Statistically different from the respective control (α=0,01) ** Statistically different from the respective control (α=0,05)

Table II. Karyometric parameters average values of the epithelium thorny layer cells nucleus from anterior dorsal, posterior dorsal and ventral regions of tongue of control (C) and treated (T) fetuses. Mann-Whitney test.

Parameters	Anterior dorsal		Posterior dorsal		Ventral	
	C	**T**	**C**	**T**	**C**	**T**
Largest diameter (μm)	7.61	7.46	7.60	5.14*	7.44	4.70*
Smallest diameter (μm)	4.93	4.62	4.77	4.10	4.55	4.17
Mean diameter (μm)	6.41	3.69**	6.62	3.51*	6.68	2.89*
Relationship D/d	1.55	0.64*	1.60	0.99	1.66	1.90*
Perimeter (μm)	19.92	9.08*	19.69	9.51*	19.13	8.80*
Area (μm^2)	26.61	27.35	28.49	26.07	26.57	24.74
Volume (μm^3)	122.22	88.67**	114.65	88.68*	103.40	85.88*
Form coefficient	0.93	0.91**	0.92	0.81*	0.91	0.86**
Contour index	3.67	2.72	3.69	1.98*	3.71	3.84*
Eccentricity	0.76	0.56*	0.78	0.64*	0.79	0.53*
Relationship V/A	4.08	3.89	4.02	3.79	3.87	3.69*

* Statistically different from the respective control (α =0,01) ** Statistically different from the respective control (α =0,05)

DISCUSSION

Female rats under the 70 mg/kg phenytoin treatment had fetuses with a smaller body weight than those of the control group, a fact also observed by Lorente *et al.* and Elmazar & Sullivan (1981); such difference remained from the post-birth life until 90 days of pregnancy (Elmazar & Sullivan). Weight loss in rats was also observed by Mullenix et al. (1983), and in mice by Harbison & Becker. For Elmazar & Sullivan, one cannot exclude the possibility of some sudden effect in absorption or usage of food, which would lead to undernourishment. One should remember that phenytoin acts in the fetus of rats and mice, decreasing DNA synthesis, as well as protein synthesis (Netzloff *et al.*, 1979; Tassinari *et al.*, 1981).

The decreased body weight of the treated fetus observed in this work reflected in the macroscopic morphological results with decreased values. Such facts point to more immature fetuses.

The tongue mucosa of the fetuses, of treated mothers, showed changes in the three analyzed regions.

It could also be observed that phenytoin acts in the stratified squamous epithelium along the several areas of the rat fetus' body in a very similar way, and turns into slender epithelium, with smaller size cells and nucleus and greater number of cells by μm^3, as observed in the mouth flooring (Gonzaga *et al.*, 2002).

The exact mechanism in which the phenytoin acts in the lingual mucosa of rat fetus is not yet known. The drug passes through the placenta, concentrates in fetal liver, and is then expelled (Mirkin). Phenytoin may also be expelled by lactation (Mirkin). Several mechanisms have been described, with the attempt to explain the changes observed here and also those reported in literature. They may be summarized as follows:

1.Interference on the conjunctive tissue metabolism (Harbison & Becker); DNA synthesis inhibition, which leads to cellular proliferation inhibition (Netzloff *et al*.) and protein synthesis decrease (Tassinari *et al*.); disturbances in the folate metabolism (Wilson & Fradkin, 1967); changes in the vitamin K metabolism (Howe *et al.*, 1995).

2. Phenytoin bio-activation into an intermediate toxicant reagent (epoxide) by the P450 cytochrome (Finnell & Dansky, 1991).

3.The changes (malformations) mainly begin by the adverse pharmacological action at the embryonic heart during a sensible period, leading to embryonic hypoxia/ischemia. Maternal hemodynamic changes may contribute to embryonic hypoxia; however, these changes are not of such magnitude they could explain the hypoxia-related malformations by themselves (Danielsson *et al*., 1992). The tissue necrosis, showed, as malformations in term fetuses, may be an immediate consequence of the hypoxia and/or the generation of types of reactive oxygen radicals during the re-oxygenation. In adult tissues, the generation of types of reactive oxygen radicals has been related to tissue damage during the ischemical heart re-oxygenation (after myocardium infarct), and the central nervous system after brain hemorrhage (Gutteridge, 1993). Evidences came up lately, at embryo culture, pointing that oxygen free radicals are generated within the embryo as result of the re-oxygenation after hypoxia/ischemia transitory episodes (Azarbayjani *et al.*, 2001).

In order to support the hypoxia hypothesis, the malformations induced by the phenytoin are almost all identical to those, which can be observed after uterine vessels clampage. For both cases, the malformations are preceded by the same precocious pathological changes (edema, vascular breach, hemorrhage and at last tissue necrosis) during the same pregnancy phase. Short periods of clampage (30 minutes) and phenytoin low teratogenic doses at the same pregnancy phase induce mainly distal digital hypoplasia. Longer periods of clampage (45-90 minutes) and higher phenytoin doses induce more serious malformations, as example: central nervous system defects and members' amputation serious defects (clampage: Webster; 1996; phenytoin: Danielsson *et al.*). Further on, in order to support the hypoxia theory, the maternal hyperoxia has shown great reduction on the incidence of malformation induced by phenytoin (Millicovsky & Johnston).

The present work demonstrated that the phenytoin treatment caused morphologic and morphometric alterations in the rats' lingual mucosa. The epithelium presented hypotrophied, which was demonstrated by histometric data statistically different from the respective control.

ACKNOWLEDGMENTS

We would like to thank Luisa Caliri Juzzo for translating this paper to the English Language.

REFERENCES

Azarbayjani, F. & Danielsson, B. R. G. Phenytoin - induced cleft palate: evidence for embryonic cardiac bradyarrhythmia due to inhibition of delayed rectifier K+ channels resulting in hypoxia-reoxygenation damage. *Teratology, 63(3)*:152-60, 2001. [Links]

Danielsson, B. R.; Danielson, M.; Rundqvist, E. & Reiland S. Identical phalangeal defects induced by phenytoin and nifedipine suggest fetal hypoxia and vascular disruption behind phenytoin teratogenicity. *Teratology, 45(3)*:247-58, 1992. [Links]

Elmazar, M. M. & Sullivan, F. M. Effect of prenatal phenytoin administration on postnatal development of the rat: a behavioral teratology study. *Teratology, 24(2)*:115-24, 1981. [Links]

Finnell, R. H. & Dansky, L. V. Parental epilepsy, anticonvulsant drugs, and reproductive outcome: epidemiologic and experimental findings spanning three decades, 1: Animal studies. *Reprod. Toxicol., 5(4)*:281-99, 1991. [Links]

Gonzaga, R. F.; Lopes R. A.; Souza, R. A.; Mattos, M. G. C. & Semprini, M. *Ação da fenitoína na mucosa do assoalho da boca de fetos de rato: estudo morfológico, morfométrico e estereológico.* Resúmenes del II Congreso Interno de Investigación de la Facultad de Odontología Pierre Fauchard de la Universidad Autónoma del Paraguay, 1:22, 2002. [Links]

Gutteridge, J. M. C. Free radicals in disease processes: a compilation of cause and consequence. *Free Radic. Res. Commun., 19(3)*:141-58, 1993. [Links]

Hanson, J. W. & Smith, D. W. The fetal hydantoin syndrome. *J. Pediatr., 87(2)*:285-90, 1975. [Links]

Harbison, R. D. & Becker, B. A. Diphenylhydantoin teratogenicity in rats. *Toxicol. Appl. Pharmacol., 22(2)*:193-200, 1972. [Links]

Howe, A. M.; Lipson, A. H.; Sheffield, L. J.; Haan, E. A.; Halliday, J. L.; Jenson, F.; David, D. J. & Webster, W. S. Prenatal exposure to phenytoin, facial development and, a possible role for vitamin K. *Am. J. Med. Genet., 58(3)*:238-44, 1995. [Links]

Lorente, C. A.; Tassinari, M. S. & Keith, D. A. The effects of phenytoin on rat development: an animal model system for fetal hydantoin syndrome. *Teratology, 24(2)*:169-80, 1981. [Links]

Merrit, H. H. & Putnam, T. J. Sodium diphenylhydantoinate in treatment of convulsive disorders. *J. Am. Med. Assoc., 111(12)*:1068-73, 1938. [Links]

Millicovsky, G. & Johnston, M. C. Maternal hyperoxia greatly reduces the incidence of phenytoin-induced cleft lip and palate in A/J mice. *Science, 212(4495)*:671-2, 1981. [Links]

Mirkin, B. L. Diphenylhydantoin: placental transport, fetal localization, neonatal metabolism and possible teratogenic effects. *J. Pediatr., 78(2)*:329-37, 1971. [Links]

Mullenix, P.; Tassinari, M. S. & Keith, D. A. Behavioral outcome after prenatal exposure to phenytoin in rats. *Teratology, 27(2)*:149-57, 1983. [Links]

Netzloff, M. L.; Streiff, R. R.; Frias, J. L. & Rennert, O. M. Folate antagonism following teratogenic exposure to diphenylhydantoin. *Teratology, 19(1)*:45-9, 1979. [Links]

Tassinari, M. S.; Lorente, C. A. & Keith, D. A. The effects of prenatal phenytoin exposure on tissue protein and DNA levels in the rat. *J. Craniofacial. Genet. Dev. Biol., 1(3):*315-30, 1981. [Links]

Webster, W. S.; Brown-Woodman, P. D.; Snow, M. D. & Danielsson, B. R. G. Teratogenic potential of almokalant, dofetilide, and d-sotalol: drugs with potassium channel blocking activity. *Teratology, 53(3)*:168-75, 1996. [Links]

Wilson, J. G. & Fradkin, R. Interrelations of mortality and malformations in rats. *Teratology, 7(1)*:57-8, 1967. [Links]

Correspondence to:

Prof. Dra. Marisa Semprini
Faculdade de Odontologia de Ribeirão Preto USP
Departamento de Morfologia, Estomatologia e Fisiologia
Avenida do Café, s/n Bairro: Monte Alegre
CEP: 14040-904
Ribeirão Preto SP
BRASIL

e-mail: msemprin@forp.usp.br

Received : 27-02-2004
Accepted: 29-04-2004

Casilla 54-D

Temuco - Chile

Tel.: (56-45) 232 5571

Fax: (56-45) 232 5600

e-Mail
ijmorpho@ufrontera.cl