

Effect of Isotretinoin on Tooth Germ and Palate Development in Mouse Embryos

Eleny BALDUCCI-ROSLINDO
Karina Gonzales SILVÉRIO
Maria Augusta JORGE
Heron Fernando de Sousa GONZAGA

Discipline of Histology, Faculty of Dentistry of Araraquara, UNESP, Araraquara, SP, Brazil

Vitamin A and its derivatives, retinoic acid, tretinoin and isotretinoin, are currently used in dermatological treatments. The administration of high doses of this vitamin provokes congenital malformations in mice: cleft palate, maxillary and mandibular hypoplasia and total or partial fusion of the maxillary incisors. This study compares the tooth germs of the first maxillary and mandibular molars of fetal mice submitted to isotretinoin during organogenesis. Twelve 60-day-old female *Mus musculus* were divided into two groups on the 7th day of pregnancy: treated group - 1 mg isotretinoin per kg body weight, dissolved in vegetable oil, was administered from the 7th to the 13th day of pregnancy; control group - vegetable oil in equivalent volume was administered orally for the same period. On the 16th day of pregnancy, the females were sacrificed, the fetuses were removed and their heads amputated. After standard laboratory procedures, 6- μ m thick serial slices were stained with hematoxylin and eosin for optical microscopy examination. The results showed that both groups had closed palates with no reminescence of epithelial cells; however, the first molar germs of the isotretinoin-treated animals showed delayed development compared to the control animals.

Key Words: isotretinoin, tooth germ, development, palate.

INTRODUCTION

Vitamin A and its derivatives, retinoic acid, isotretinoin and tretinoin, are commonly used systemically and topically in dermatological treatments. The retinoids are a group of medicines in which vitamin A and its natural derivatives, as well as synthetic structural analogs, are included. The latter includes isotretinoin (13-cis-retinoic acid) and tretinoin (all-trans-retinoic acid) that are different just in the acid tail direction (1).

Although it is clinically effective, isotretinoin presented important secondary side effects such as teratogenicity (2), a factor limiting its application. Some studies have reported that the use of high levels of vitamin A is responsible for multiple lesions. Among these, congenital malformation is the most important. Knudsen (3,4), studying exencephalic mouse embryos induced by hypervitaminosis A, observed total or partial fusion of the maxillary incisors and mandibular

molars, bilateral agenesis of the maxillary and mandibular incisors and temporomandibular joint absence. Lorente and Miller (1978), using high levels of retinoic acid and retinil acetate in pregnant mice for 13-15 days, observed that they both produced cleft palate in 90% of the experimental animals. Hendrickx (6) observed that the Rhesus monkey treated by tretinoin presented numerous malformations: hydrocephaly, maxilla and mandible hypoplasia, cleft palate and small deformed ears with low implantation. Many authors have observed the effect of drugs or dental materials on the tooth germ development by *in vivo* and *in vitro* techniques (7-10).

Beeman and Kronmiller (11) verified the presence of endogenous retinoids in embryonic mouse mandible as well as its importance in the formation of the dental lamina at the beginning of odontogenesis and in defining the morphology of incisors and molars. Kronmiller (12,13) performed an assessment in which exogenous all-trans-retinoic acid was used before the

dental lamina formation. They observed the presence of diastemas in the incisor region, supernumerary teeth and incisors ectopically positioned in the molar region.

Because drugs used systemically for therapeutic reasons can affect developing structures, this investigation studied palate closure and the development of the tooth germ of the first maxillary and mandibular molars of fetal mice submitted to isotretinoin during organogenesis.

MATERIAL AND METHODS

Twelve 60-day-old female *Mus musculus* (albino Swiss variation) primiparous mice were used. The animals were fed granular ration and water ad libitum. The gestation period was determined by identifying the vaginal plug as day "zero" of pregnancy after mating during the night, in the proportion of two females for each male of the same specie.

On the 7th day of pregnancy, the females were divided into two groups: treated group - 1 mg of isotretinoin per kg body weight, dissolved in vegetable oil, was administered orally from the 7th to the 13th day

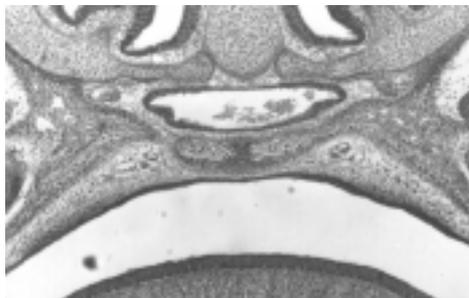


Figure 1. Control group: Closed palatal processes in the midline and formation of osseous trabeculae. H&E. Magnification: 100X.



Figure 2. Treated group: Closed palatal processes in the midline and no formation of osseous trabeculae. H&E. Magnification: 100X.

of pregnancy; control group - vegetable oil in an equivalent volume was administered orally for the same period.

After 16 days of pregnancy, the females of both groups were sacrificed by ip injection of 10% chloral hydrate (4 ml/100 g body weight). Following an abdominal incision, the uterus was removed and placed on a Petri plate containing saline solution and the fetuses were removed and weighed separately. They were examined macroscopically for morphologic alterations.

Six fetuses of each female mouse had their heads removed, fixed in Bouin solution, decalcified in 20% sodium citrate and 50% formic acid solution in equal parts (14). To histologically analyze the palate and first mandibular and maxillary molar germ development the heads were included in paraffin, and serial 6- μ m thick slices were stained with hematoxylin and eosin. The sections were analyzed with an optic microscope.

RESULTS

The macroscopic evaluation of the sacrificed fetuses did not show malformation or resorption. The average body weight of the treated fetuses was 0.92 g and the control fetuses 1.2 g.

The palates of both animal groups at 16 days of fetal life were completely closed with no reminescence of epithelial cells in the fusion line of palatal processes with each other and with the nasal septum. In the control group, the formation of thin osseous trabeculae within the palatal processes that were growing toward the midline was observed. Within the osseous trabeculae there were osteoblasts at the periphery with voluminous and central nuclei while the osteocytes in the lacunas could be seen in the central portion (Figure 1). The treated animals had no formation of these osseous trabeculae (Figure 2).

The first maxillary and mandibular molar germs of the control group were at the late cap stage phase and it was possible to distinguish all formative components of the teeth: enamel organ, dental papilla and dental follicle (Figure 3A). The external epithelium of the enamel organ was constituted by cuboid cells with rounded nuclei layers; the stellate reticulum, formed by polygonal cells linked by cytoplasmic extensions and separated by different sized spaces, was thicker in the intercuspid areas; the intermediary space was constituted of 2-3 layers of elongated cells with ovoid nuclei

and dense chromatin. Adjacent, the inner epithelium in cuspid areas was formed by highly cylindrical cells with basophilic cytoplasm and elongated basal nuclei, in palisade form, originating the pre-ameloblasts. In the other areas, the cells were short and cylindrical and the nuclei were located in many positions (Figures 3B and 3C).

The enamel organ concavity had a condensation of cells that established the dental papilla. The peripheral cells of the dental papilla, especially in cuspid regions, were elongated, of variable sizes, with basal nuclei, and cytoplasmic elongation slightly eosinophilous, which are characteristics of odontoblasts. The central region showed ectomesenchymal cells and blood vessels of different diameters (Figures 3B and 3C).

When the odontoblasts and pre-ameloblasts were

separated, a thin eosinophilous zone was shown, which represented the future enamel-dentin limit.

The first maxillary and mandibular molar germs of the treated group were at different stages of development: cap or bell-shaped phases (Figure 4A). The first molar germs in the cap phase had enamel organs formed by three different layers of cells: the outer layer with cuboid cells formed the external epithelium; the inner layer or the internal epithelium had columnar cells with basal nuclei in the cuspid region and in the other areas the cells were cuboid; in the central portion, there were rounded to polygonal cells, separated by the presence of extracellular liquid, which is the stellate reticulum (Figure 4C).

In the enamel organ concavity there were some condensed ectomesenchymal cells forming the dental papilla (Figure 4C). The cuspid regions were slightly

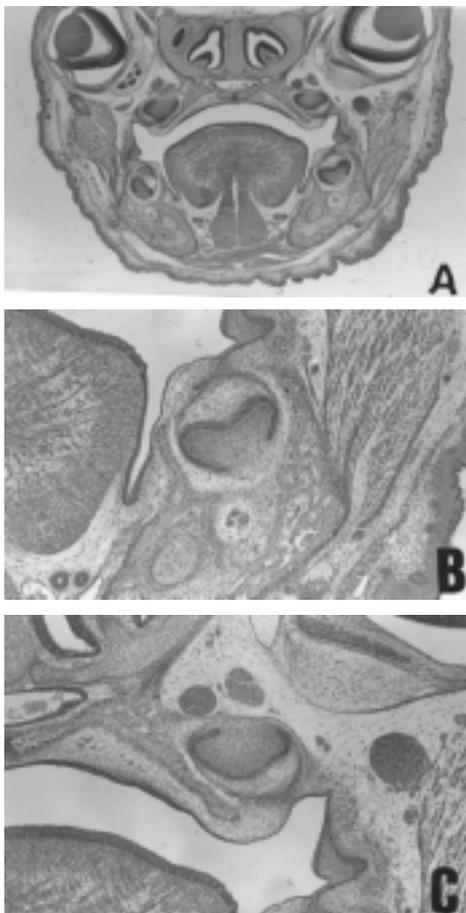


Figure 3. Control group. A: View of the first molar germs at 16 days of fetal life. H&E. Magnification: 32X. B: First mandibular molar germ. H&E. Magnification: 100X. C: First maxillary molar germ. H&E. Magnification: 100X.

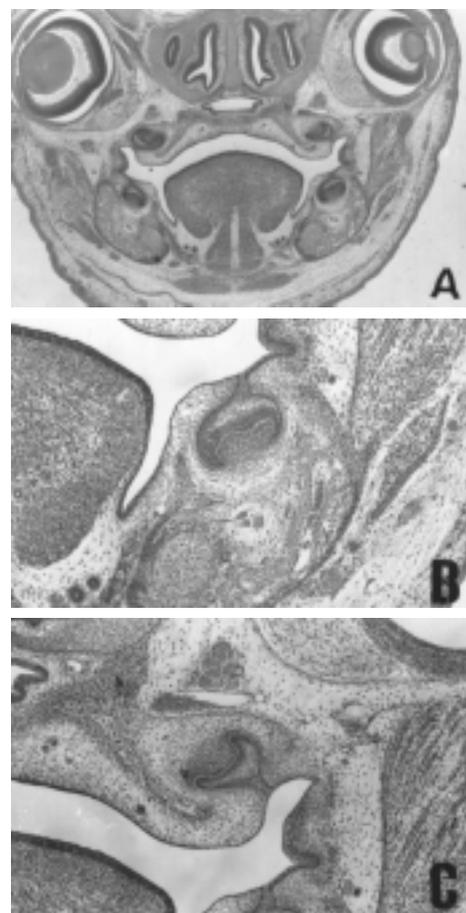


Figure 4. Treated group. A: View of the first molar germs at 16 days of fetal life. H&E. Magnification: 32X. B: First mandibular molar germ. H&E. Magnification: 100X. C: First maxillary molar germ. H&E. Magnification: 100X.

condensed, with nuclei situated in many positions and with slightly basophilic cytoplasm. These are characteristics of pre-odontoblasts. In other peripheral areas, the cells were cuboid with central nuclei. A cell agglomerate of ectomesenchymal cells and blood vessels occupied the central portion of the dental papilla (Figure 4B). Around the tooth germs in the cap phase there could be seen 2-3 layers of cells, forming the dental follicles (Figure 4C). During the initial bell-shaped phase, the first molar germs had defined formative elements (Figure 4A).

In the enamel organ, the outer epithelium was constituted by cuboid cells with central nuclei; the stellate reticulum had greater quantity of extracellular liquid than the ones of the late cap stage phase. Polygonal cells linked by cytoplasmic elongation in the intercuspid areas could also be seen; in the other areas, they were condensed. Adjacent, one or two layers of elongated cells with ovoid nuclei formed the intermediary stratus; the inner epithelium was constituted by columnar cells with differently positioned nuclei in the cuspid regions and, in the other areas, they were low columnar with basal nuclei (Figure 4B).

DISCUSSION

The present study verified the effect of the systemic use of isotretinoin on the first maxillary and mandibular molar germs and closure of palate of mouse embryos that received this drug from the 7th to the 13th day of fetal life. This animal was used because Cohn (15) reported that the molar germs of these animals were more adequate for dental investigations.

Isotretinoin is a drug used in the treatment of cystic acne and has a teratogenic effect in humans, causing multiple lesions as congenital malformations (16,17).

According to Kocchar (18) and Kocchar and Penner (2) this drug has lower toxicity in pregnant mice, probably because of its short half-life and limited placental transference in these animals. They also concluded that continuous use of this drug at a greater time interval induces a lower frequency of defects in organs and cleft palate. On the other hand, these defects and the absence of palate closure were greater when used at shorter intervals.

Studies performed by Ritchie and Webster (19) and by Webster et al. (20) aimed to determine the

teratogenicity of 500 ng/ml of isotretinoin in mice and reported that this drug in vitro or in vivo administered 6 h before cell migration from the neural crest was sufficient to induce severe defects in the second branchial arch in the great majority of the exposed embryos.

Studies performed by many authors (3,5,12,13) confirmed the alterations caused by vitamin A and its derivatives: supernumerary teeth, incisor diastemas, total or partial fusion of the maxillary incisors and mandibular molars, cleft palate and absence of incisors in animals.

Odontogenesis is characterized as a consequence of many complex inductive interactions between two embryonic tissues, the epithelium of the first branchial arch and the mesenchyme, derivative of the cranial neural crest cells (11). The first sign of odontogenesis is the stratification of the oral epithelium in the free maxillary borders, in specific sites to form the dental lamina (14,21).

In the present study, the drug was administered before and during the first molar germ formation allowing us to observe the action of isotretinoin during the development and closure of the palate. The palate from both groups fused completely with no reminiscence of epithelial cells in the fusion line between the palatal processes and between them and the nasal septum. The results of the control group showed that the first molar germs were in the late cap stage phase and all the formative components of the teeth could be recognized. In the treated group, the first molar germs were in different stages of development, late cap stage phases. Slower growth and development of its structures compared to the control group was evident.

The literature reviewed confirmed the cytotoxicity of vitamin A, its natural derivatives and synthetic structural analogs. Our results also showed the toxicity of isotretinoin with a retardation of tooth germs and palate development. More research is necessary to determine the exact action of vitamin A and its derivatives.

ACKNOWLEDGMENTS

The authors would like to thank the financial support provided by FAPESP (nº. 96/06730-3).

RESUMO

Balducci-Roslindo E, Silvério KG, Jorge MA, Gonzaga HFS.

Efeito da isotretinoína no germe dentário e no desenvolvimento do palato em embriões de camundongos. *Braz Dent J* 2001;12(2):115-119.

A vitamina A e seus derivados, ácido retinóico, tretinoína e isotretinoína, são diariamente utilizados em tratamentos dermatológicos. A administração de altas doses desta vitamina provoca malformações congênitas em ratos: fendas palatinas, hipoplasia maxilar e mandibular e fusão parcial ou total de incisivos superiores. Este estudo comparou os germes dentais de primeiros molares superiores e inferiores de fetos de camundongos submetidos à isotretinoína durante a organogênese. Doze fêmeas *Mus musculus* com 60 dias de idade foram divididas em dois grupos no 7º dia de prenhez: grupo tratado - 1 mg de isotretinoína por kg de peso corporal dissolvida em óleo vegetal, foi administrada do 7º ao 13º dia de prenhez; grupo controle - óleo vegetal em volume equivalente foi administrado oralmente durante o mesmo período. No 16º dia de prenhez, as fêmeas foram sacrificadas, os fetos foram removidos e suas cabeças amputadas. Após o processamento laboratorial, cortes seriados de 6-µm de espessura foram corados com hematoxilina e eosina para análise no microscópio óptico. Os resultados mostraram que ambos os grupos tiveram os seus palatos fechados sem células epiteliais remanescentes; entretanto, os germes dos primeiros molares dos animais tratados com a isotretinoína mostraram desenvolvimento retardado comparado aos animais controles.

Unitermos: isotretinoína, germe dentário, desenvolvimento, palato.

REFERENCES

- Di Giovanna JJ. Retinoids for the future: oncology. *J Acad Dermatol* 1992;27:534-537.
- Kocchar DM, Penner JD. Development effects of isotretinoin and 4-oxo-isotretinoin: The role of metabolism in teratogenicity. *Teratology* 1987;36:65-75.
- Knudsen PA. Fusion of upper incisors at bud or cap stage in mouse embryos with exencephaly induced by hypervitaminose A. *Acta Odont Scand* 1965;23:549-565.
- Knudsen PA. Congenital malformations of lower incisors and molars in exencephalic mouse embryos, induced by hypervitaminose A. *Acta Odont Scand* 1966;24:55-71.
- Lorente CA, Miller SA. Vitamin A induction of cleft palate. *Cleft Palate J* 1978;15:378-385.
- Hendrickx AG, Silverman S, Pellegrini M, Steffek AJ. Teratological and radiocephalometric analysis of craniofacial malformations induced with retinoic acid in "Rhesus monkeys". *Teratology* 1980;22:13-22.
- Roslindo NC, Hetem S, Roslindo EB, Ramalho LTO. Effects of AZT on intra-ocular tooth germ development. *J Dent Res* 1994;73:762 (abstract).
- Roslindo EB, Roslindo NC, Hetem S, Maruyama NT, Vilarinho S. Reorganização do processo condilar da articulação temporomandibular após condilectomia unilateral em camundongos tratados com Ziruduvina (AZT). *Rev Cienc Biom UNESP* 1996;17:55-66.
- Roslindo NC, Hetem S, Roslindo EB, Ramalho LTO, Konischi RN. Effects of glass ionomer materials on tooth germs *in vitro*. *J Dent Res* 1995;73:808 (abstract).
- Hetem S, Roslindo EB, Ramalho LTO, Roslindo NC, Zunfrilli FS. Efeito do metotrexato sobre o desenvolvimento de germes dentais transplantados para a câmara anterior do olho. *Rev Odont UNESP* 1995;24:211-220.
- Beeman CS, Kronmiller JE. Temporal distribution of endogenous retinoids in the embryonic mouse mandible. *Arch Oral Biol* 1994;39:733-739.
- Kronmiller JE, Upholt WB, Kollar GJ. Retinol alters murine odontogenic patterning and prolongs EGFm RNA expression *in vitro*. *Arch Oral Biol* 1992;37:129-138.
- Kronmiller JE, Beeman CS, Nguyen T, Berndt W. Blockade of the initiation of murine odontogenesis *in vitro* by citral, an inhibitor of endogenous retinoic acid synthesis. *Arch Oral Biol* 1993;40:646-652.
- Morse A. Formic acid-sodium citrate decalcification and butyl alcohol dehydration of teeth and bone for sectioning in paraffin. *J Dent Res* 1945;24:143-153.
- Cohn SA. Development of the molar teeth in the albino mouse. *Am J Anat* 1957;101:2-15.
- Geelen JAG. Hypervitaminose A induced teratogenesis. *Crit Rev Toxicol* 1979;6:351-375.
- Shalita AR, Cunningham WJ, Leyden JJ, Pochi PE, Staruss JS. Isotretinoin treatment of acne and related disorders: An update. *J Am Acad Dermatol* 1983;9:629-638.
- Kocchar DM. Teratogenic activity of retinoic acid. *Acta Pathol Microb Scand* 1967;70:398-404.
- Ritchie H, Webster WS. Parameters determining isotretinoin teratogenicity in rats embryo culture. *Teratology* 1991;43:71-81.
- Webster WS, Johnston MC, Lammer GJ, Sulik KK. Isotretinoin embryopathy and cranial neural crest. An *in vivo* and *in vitro* study. *J Craniofac Genet Dev Biol* 1986;6:211-222.
- Hay MF. The development *in vivo* and *in vitro* of the lower incisor and molars of the mouse. *Arch Oral Biol* 1961;3:86-109.

Accepted December 18, 2000