Isotretinoin: action on mice tooth germs and palate

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Abstract
Vitamin A and its derivates, acid retinoic, tretinoin and isotretinoin, are currently used in dermatological treatments. The administration of high doses of this vitamin provokes malformation in the following systems: central nervous, cardiovascular, skeletal, brain and face. This study compares the tooth germs of the first maxillary and mandibular molars and the palate 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 – 2 mg isotretinoin per kg body wight, dissolved in vegetable oil, was administered orally from the 7th to the 13th day of pregnancy; control group – vegetable oil in an 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 were amputted. After standard laboratory procedures, 6-mm-thick serial sections were stained with hematoxylin and eosin for light microscopy examination. The results showed that the control group had closed palates with no traces of epithelial cells and two fetuses of treated group had insufficient development in the lateral palate processes, having lack of fusion in the midline. The first molar germs of the isotretinoin-treated animals showed delayed development compared to the control animals. In conclusion, isotretinoin was shown to be toxic causing retardation of tooth germs and palate development.

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

Introduction

Vitamin A acts on vertebrate organisms during ontogenesis and pre- and post-birth cellular differentiation process, reproduction, normal growth, maintenance of visual function, regulation and proliferation of tissue epithelial structures[1]. Besides this, derivates of vitamin A as retinoids were observed at the endogenous form in embryonic mouse mandible being important in the formation of the dental lamina at the beginning of odontogenesis and in defining the morphology of incisors and molars[6].

Among the synthetic derivates of vitamin A, we may find isotretinoin (13-cis-retinoic acid), currently indicated in the treatment of ichthyosis, illness of Darrier, psoriasis, acne, carcinoma of basal cells and in the prevention of carcinogenesis. In spite of its clinical effectiveness, it may cause some undesirable side effects such as: depression, psychotic behaviors, hydrocephaly and facial malformation. Teratologic effects of vitamin A include malformation of the central nervous, cardiovascular, skeletal, and brain systems and face deformation[2]. The use of isotretinoin by women during the first quarter of pregnancy caused defects in the members as well as gastric and hepatic systems of children[3]. It was also noticed that the critical period was between the 2nd and 5th weeks after conception.

In the rats, mice and monkeys, the placental transference of 13-cis-retinoic acid during the pregnancy period was observed when the clorio-allantoic placenta was well developed[7]. When another derivate of vitamin A, isotretinoin, was systemically administered at the concentration of 1 mg per kg body weight to pregnant mice, the studies[8,9] verified that this drug produced structural alterations in the fetus as alterations at the development of the tooth germ and in the palate closure. Besides these alterations, studies[4,5] observed total and partial fusion of the maxilary incisors and the mandibular molars, agenesis of the incisors, formation of diastema in the incisors region, supernumerary teeth and absence of temporomandibular articulation in mice fetuses. However, as metabolism in rodents is more active than in humans, it is necessary to correlate the exposition period of the embryo to the administered drug doses and its concentration in order to obtain the results that would confirm the described alterations in the literature.

Considering the lack of information on isotretinoin effect in human organism, the aim of the present work was to study the palate closure and tooth germ development of the maxillary and mandibular first molars in mice fetuses submitted to isotretinoin during the organogenesis in the concentration of 2 mg per kg.

Material and Methods

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

On the 7th day of pregnancy, the females were divided into two groups: treated group – 2 mg of isotretinoin per kg body weight, dissolved in vegetable oil, was administered orally, once a day, from the 7th to the 13th day 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/100g body weight). Following an abdominal incision, the uterus was removed and placed on a Petri dish containing saline solution. Fetuses were removed, weigh and measured in the brain tail direction and examined macroscopically for the identification of possible morphologic alteration. After macroscopic analyses, fetuses had their heads removed and fixed in Bouin solution, decalcified by Morse[10] solution for histological analyses of palate and first mandibular and maxillary molar germ development. The heads were embedded in paraffin, and serial 6-mm-thick slices were stained with hematoxylin and eosin. The slides were analyzed by light microscopy.

Results

MACROSCOPIC FINDINGS

All specimens in number of 6 fetuses for each female mouse were histologically analyzed using serial 6um frontal incisions of similar depth and thickness (Figures 1 and 6).

The macroscopic evaluation of the sacrificed fetuses on the 16th day of fetal life did not show external malformation, re-absorption or dead born mice. However, it was verified that the isotretinoin-treated fetuses presented average body size and weigth (1,13 g) inferior to the control fetuses (1,42 g).

MICROSCOPIC FINDINGS

Control Group

The palate was totally fused without epithelial cells remaining in the fusion line of the palate process with the nasal septum (Figure 1). Bone trabeculae with variable size and thickness growing toward the midline, containing in its surface osteoblasts was also observed (Figure 2). Tooth germs in cap stage were connected to oral epithelium through the dental lamina. Figures 3 and 4, demonstrate that the enamel organ was constituted by outer epithelium formed with a layer of cubic cells and the stellate reticulum formed by polygonal cells. The intermediary stratum was constitued by elongated cells. Adjacently, the internal ephitelium in the cusp area was formed by highly cylindrical cells arranged perpendicularly to the dental papilla forming the pre-ameloblasts, and in the other areas the cells were short and cylindrical. In the peripheral cells of the dental papilla,

especially in cusp regions, short cylindrical cells were identified, the future odontoblasts arranged in parallel exhibiting central rounded nuclei. In the central region, it was possible to observe a loose connective tissue, highly cellularized, containing ectomesenchymal cells, fibroblasts, and blood vessels of small diameter. The dental follicles involving the whole embryo were constituted by loose connective tissue rich in cells arranged in parallel rows with dental organ surface. In the lateral areas of the dental organs, neoformed thin immature bone trabeculae was noticed, with large medullar space, showing osteoblasts in their periphery and osteocytes identified in the interior of mature trabeculae.

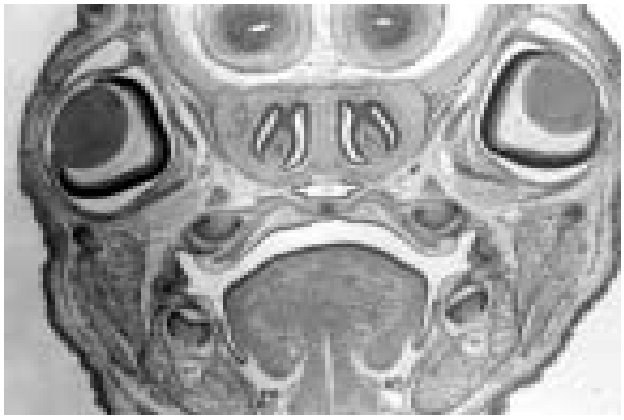

Figure 1 – Control Group – Frontal slice of the head of embryos. H&E. Magnification: 32X.

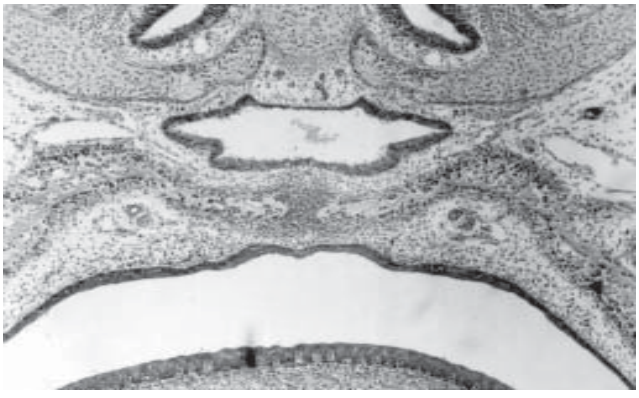

Figure 2 – Control Group – Close palatal processes in the midline and formation of bone trabeculae. H&E. Magnification: 125X.

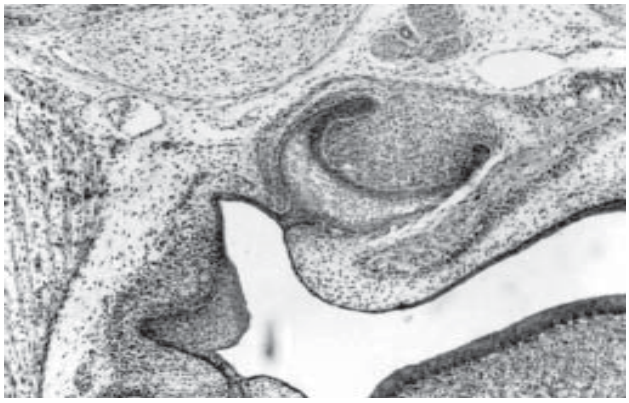

Figure 3 – Control Group – First maxillary molar germs. Cap Phase. H&E. Magnification: 125X.

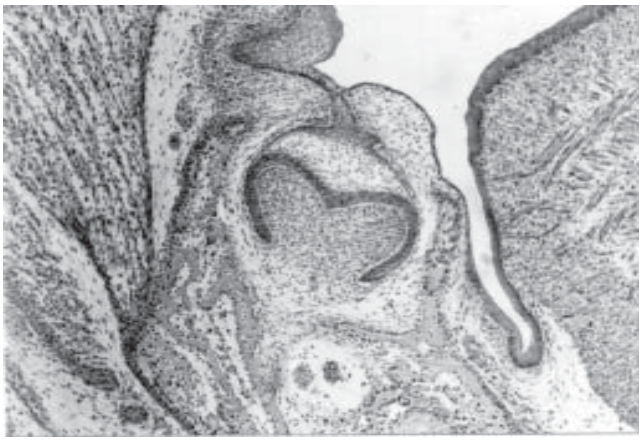

Figure 4 – Control Group – First mandibular molar germs. Cap Phase. H&E. Magnification: 125X.

Treated Group

The microscopic analyses revealed that the majority of the specimens had fused palates however, two fetuses presented insufficient fusion in the lateral palate processes, having lack of fusion in the midline (Figure 5). The fetuses with fused palates showed an absence of bone trabeculae with cellular differentiation and multiplication mainly around the blood vessels characteristics of the beginning and end of the ossification process (Figure 7).

The tooth germs of the first molars were found in different development stages including the cap or initial bell-shaped phase for some inferior embryos (Figures 8, 9, and 10). In the cap phase (Figure 9), three different layers of cells were observed. The external layer was constituted by cuboid cells forming the external epithelium. The inner layer, or inner epithelium, was constituted by short cylindrical cells in the cusp regions, and in the others regions by cuboid cells. On the central layer, an agglomeration of rounded and indifferentiated cells that would form the stellate reticulum and the intermediary stratum with discrete deposition of intracellular substance between stellate reticulum cells beginning a differentiation of enamel organ cell layers was noticed.

Figures 8, 9 and 10, demonstrate a higher concentration of ectomesenchymal cells and small diameter vessels in the concavity of the enamel organ. Beyond the dental follicle around the dental germ was constituted by connective loose tissue highly cellularized with fibroblasts arranged in parallel rows, except in the region of dental lamina. Adjacent to the dental organ, in the majority of specimens, thin irregular immature bone trabeculae with wide medullar spaces, showing osteoblasts with spherical nuclei located on its surface was noticed. Osteocytes in small number were seen in the interior of mature bone trabeculae.

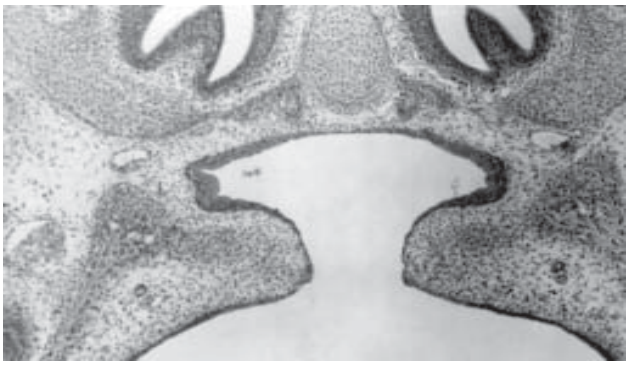

Figure 5 – Treated Group – Palatal processes having lack of fusion in the midline. H&E. Magnification: 125X.

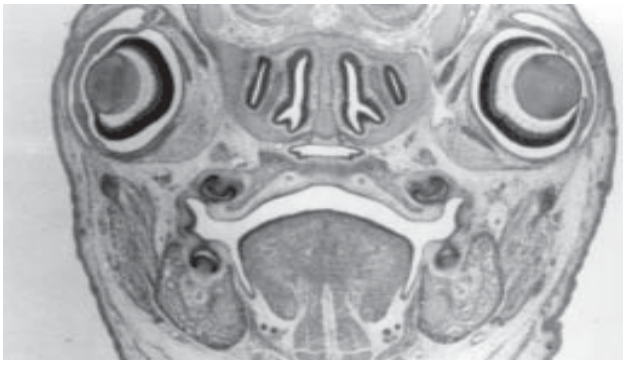

Figure 6 – Treated Group - Frontal slice of the head of embryos. H&E. Magnification: 32X

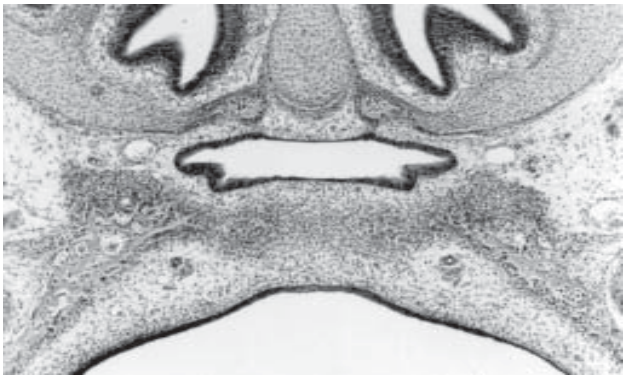

Figure 7 – Treated Group – Closed palatal processes in the midline and no formation of bone trabeculae. H&E. Magnification: 125X.

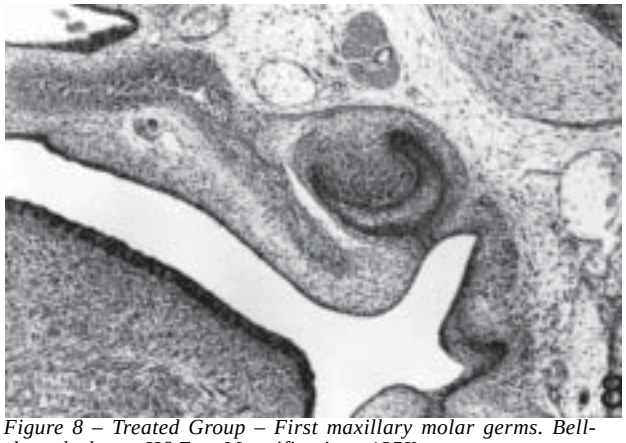

Figure 8 – Treated Group – First maxillary molar germs. Bell-shaped phase. H&E. Magnification: 125X.

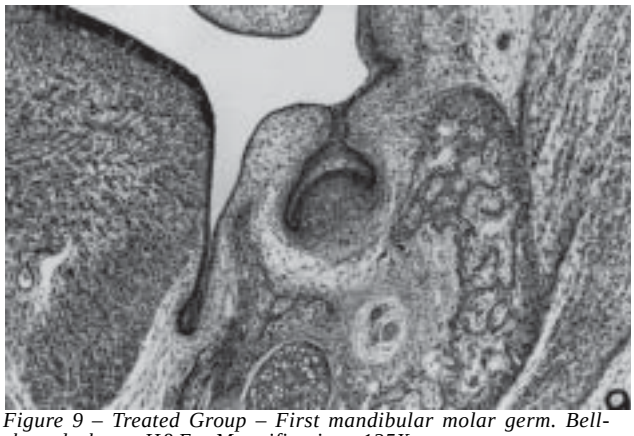

Figure 9 – Treated Group – First mandibular molar germ. Bell-shaped phase. H&E. Magnification: 125X.

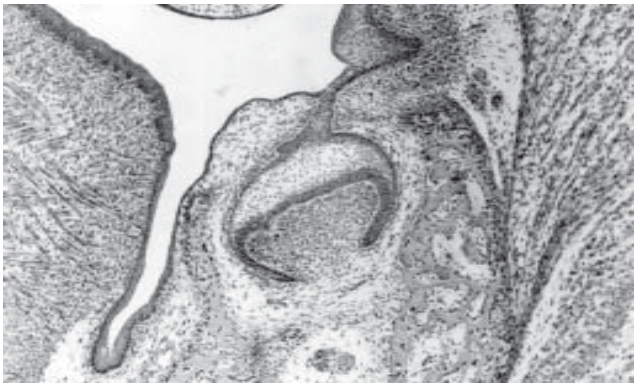

Figure10 – Treated Group – First mandibular molar germ. Cap phase. H&E. Magnification: 125X.

Discussion

Odontogenesis in mice begins at the 9th day of fetal life and the formation of dental bud occurs at the beginning of the 12th day[11,12]. In this period, drugs that have teratogenic potential, such as vitamin A and derivates, may cause the formation of the supernumerary bud and molar substitution by incisors in the molar region, as verified by Kronmiller et al.[5] when they administered all-trans-retinoic acid in the exogenous form in the period previous to the formation of the dental lamina.

Studies[13,14] that administered isotretinoin and its metabolie 4-oxi-isotretinoin to pregnant female mice, showed that these drugs may exert inhibitory effect on the migration of the neural-crested cells, besides promoting reduction of members and palate opening. Ritchie & Webster[15] carried out some studies with the purpose of determining the teratogenicity of isotretinoin and showed *in vitro* that use of this drug in the concentration of 500 ng/ml for a period of a least 6 hours of exposure before the migration of the cells in the neural crest, was enough to induce severe defects on the second visceral arch in most of the exposed embryos.

These results are likely to explain the two fetuses with palate

opening, caused by the insufficient development of lateral palatine processes, when 2 mg of isotretinoin per kg of body weight was administered to pregnant female mice, during the period between the 7th and the 13th day of gestation.

Concerning odontogenesis, we know that tooth development is related to a series of complex inductive interations between two embryonic tissues, the epithelium in the first branchial arch and the ectomesenchyme derived from neural crest cells. In the gastrula phase of embryonic development, the ectoderm and the mesoderm respond more to the excess of vitamin A, and the endoderm responds less[16,17]. During organogenesis, vitamin A produces an interruption in the cephalic ectomesenchyme or alters significantly cell properties[18]. Subsequently, the action of vitamin A has more negative effects on bones, cartilages, and dental organs than positive effects on growth in general.

It is knwon that vitamin A is mobilized in liver stocks and it is taken to the peripheral tissues by means of highly regulated transportation. Takahashi & Smith[19] verified that in rat embryos, during the pregnancy period, the presence of protein connects to retinol (RBP) and that the transportation system works in the intra-uterine life of mammals. The presence of those proteins in fetal organs was also studied by Lorente & Miller[20], who verified that a direct action of vitamin A on tissues and organs is more probable than placental alterations.

In the literature we did not find histological works on placentas showing the systemic action of isotretinoin. However, we observed in this study some placental alterations in the treated group (not shown). These alterations indicated an unfavourable development for their gestational age. Since the development of teeth can be influenced by nutritional status[21,22] the placental alterations would no be responsible for the embryological alterations caused by isotretinoin.

The data obtained from the fetuses from the treated group suggest that isotretinoin, even when used in low dose, 2 mg per kg of body weight, but administered from the 7th to the 13th day of pregnancy may cause insufficient development and growth of the lateral palatine processes, delay in the development of the first mandibular and maxillary tooth germs, and lower size and weight, when compared to the fetuses from the controlled group.

When Balducci-Roslindo[9] et al. administered 1 mg per kg of isotretinoin in female mice during the period from the 7th to the 13th day of pregnancy, they noted the same alterations in fetal development and growth and in their bone formation.

We can conclude that isotretinoin had an adversed effect on the size and weigth of the fetuses examed. Additionaly, this drug may affect the formation of bone tissue in the palatine process and a delay in the development of the first mandibular and maxillary molars.

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