Study of the Occurance of Labio-Palatine Cleft Embryogenic Disorders of the Stomatognated System by Using Lots of Wistar Rats Females

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Labio-maxilo-palatine clefts are embryogenesis disorders of the stomatognathic system that occur due to the action of genetic or non-genetic factors in weeks 5-6 of intrauterine life. The clefts are part of the group of congenital malformations of the face. They appear from birth as a slit in the upper lip, hard palate and/or soft palate.Labio-maxilo-palatine cleafts are a current medical problem, especially regarding surgical recovery; they are congenital malformations of the face characterized by the absence of substance in the upper lip and/or in the palatine vault caused by the lack of fusion of labial and / or palatine buds during embryonic development.Simple labial cleft concerns only the lip, unlike the complete labial cleft which affects also the alveolar maxillary arch. In the case of this malformation, more serious than labial cleft, the cleft also affects the bone structures (the alveolar maxillary arche, the buccal palate) so that the oral cavity communicates with the nasal cavity and prevents sucking in infants. An early surgical recovery restores the integrity of anatomical structures, reestablishes disturbed functions, creates favorable conditions for speech education and social adaptation of the child according to age.The procedure is applied prior to the primary suture of the lip and of the anterior palate in order to minimize the dislocation of the segments of the maxillary arch. The correction of the malformation of the jaw arch in the case of bilateral clefts is much more difficult, but it has a greater importance due to the difficulty of reconstruction of the muscular plan and severe deformations, what will persist if the segments can not be aligned.In the bilateral clefts, the lateral segments are displaced medially, and the medial segment is protruding. The purpose of preoperative orthodontic treatment is to reposition the lateral segments so that the medial segment can be coaxed between them together as a vault key of the maxillary arch. In order to study the occurrence of labio-palatine cleft, we used eight groups of Wistar females rat, weighing between 200 and 250 grams. Each sample consists of 10 females rats. Most of the labial and palatine clefts are the result of multiple, genetic and non-genetic factors, each producing a minor developmental defect, called multifactorial heritage;It can be represented by a model in which responsibility for a disease is a variable caused by a combination of genetic and environmental factors. The therapeutic outcome depends on the complexity of the malformation, on the moment of surgery and on the selection of the most modern techniques and equipment suitable for the patient.

Keywords: facial malformations, labial cleft, surgical recovery, orthodontic treatment, stomatognated system.

Facial malformations and, especially labio-palatine clefts have always prompted a great interest both in hereditary transmission problems [1], transmission mechanisms, teratogenic factors, and especially on facial aesthetics and treatment issues [2] is a public health problem due to the significant increase of incidence, but above all through early diagnosis and treatment issues raised [3].

Surgical treatment is the main therapeutic approach [4], but due to the multidisciplinary approach it can be achieved a significant increase in optimal results obtained both in cosmetic and orthodontic [5]. In practice, there are three situations in which preoperative orthodontic treatment is extremely necessary: complete unilateral collapsed labial-palatine clefts with collapse of the small maxillary segment; unilateral labial-maxillary clefts with obliquity of the small segment; complete bilateral clefts with collapses of lateral segments and moderate protrusions of the medial segment [6].

Labio-palatine clefts can coexist in craniofacial malformations such as Pierre-Robin syndrome, Down syndrome (trisomy 21), Patau syndrome (trisomy 13-15); Optis syndrome; *cri du chat* syndrome, Edwards syndrome (trisomy 17-18) [7].

The mandibular and maxillary buds are derived from the first pharyngeal arch, that, during the embryonic development will form [8]: bone structures: upper jaw, mandible, hard palate, internal ear bones (hammer and anvil), a part of the sphenoid and temporal bone; muscles structures, including: masticatory muscles (temporal, masseter, pterigoids), soft palate tensor muscles and tympanic tensor muscle [9]. 50% from the case of familial labial and palatine cleft have autosomal dominant transmission [10].

The study aims to impose the use of several methods and the use of vast and diversified material.

Experimental part

Matherial and method

In order to study the occurrence of labio-palatine cleft, we used eight groups of Wistar females rat, weighing between 200 and 250 g. Each sample consists of 10 females rats [11].

The animals were kept in standard laboratory conditions and received the same diet, to ensure their caloric support, vitamins and minerals. Each rat was numbered in each lot from 1 to 10, respectively from 1 to 12.

Female rats were accommodated in the same container with normal adult males who did not receive any substance, one night after which the males were removed.

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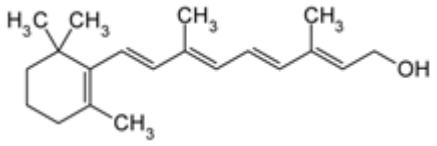
We mention that the rate of spontaneous occurrence of labio-palatine clefts for Wistar rats is 4.5%.

Lot A of females rats was the control group and did not receive any substance. Lot B of female rats received retinoic acid derived from vitamin A, known as teratogen [12] which determine labio-palatine cleft inrodent. Each animal received 50mg of retinoic acid / kg body weight per day, intraperitoneal, single dose per day until pregnant females gave birth.



Retinoic acid-belongs to the family of chemical compounds called retinoids

Retinol is derived from isoprene and has a hydroxide as functional group.The first complete synthesis of this compound was found by David Adriaan van Dorp and Josef Ferdinant Arens in 1947. George Wald won the 1967 Nobel Prize for Physiology and Medicine for his work with retinal pigments (also called visual pigments), which led to the understanding of the role of vitamin A in vision. Many of the non-visual functions of vitamin A are mediated by retinoic acid, which regulates the appearance of genes by activating intracellular retinoic acid receptors [13].

The lot C of females rets received retinoic acid, the same dose and the same mode of administration, plus complex of B vitamins (B1, B2 and B6), 50 mg B complex / kg body weight / day, intraperitoneal administration, single daily dose, complex B being administered one hour before the administration of retinoic acid. The lot D of females rats received retinoic acid, the same dose and the same route of administration plus magnesium acemamate, 0.5mEq / kg body weight / day, intraperitoneal dose, single dose per day, magnesium acemamate being administered one hour before administration of retinoic acid.

The rat group E received retinoic acid, the same dose and the same administration as group B plus nickel chloride, 0.5mEq/kg body weight/day, intraperitoneal administration, single dose per day, nickel chloride being administered with a hour before retinoic acid administration. The rat F lot received retinoic acid. Each animal received 50mg of retinoic acid/kg body weight/ day, intraperitoneal administration, single dose per day, only week of gestation

Rat G lot received retinoic acid. Each animal received 50 mg retinoic acid/kg body weight/day, intraperitoneal administration, single daily dose, only in the second week of gestation. Rat group H received retinoic acid/kg body weight/day, intraperitoneal administration, single dose per day, only the last week of gestation.

To each lot was noted the number of females who developed gestation, the number of newborns to each pregnant female and the malformations presented at the head of each of them [14]. Also, it has been observed up to a month the number of newborns that survived in each female. One month after the birth all animals (both females with newborns and non-pregnant females) were anesthetized with Pentotal and sacrificed for carotid sections. There was a final examination of the animal's head. From each sacrificed animal, were extracted lungs, kidneys, gonads, spleen and liver, in order to perform histopathological examination of these organs. The harvested organs were fixed in 15% formol contained in paraffin, then longitudinal serial sections were made at distances of 50 microns, sections of 4 microns thickness.

The sections were stained with the following methods: hematoxylin-eosin; [15] Van Gieson's tricromatic staining; PAS staining (with Schiff's acid); Gordon Sweat staining for reticulin; Scharlach red staining for fat; Szekely's tricromatic staining.

The colored lamellae were then examined with the optical microscope.

Results and discussions

Lot A (witness) who did not receive any substance showed the following results: 7 out of 10 rats remained pregnant. Each of these pregnant rats gave birth to live newborns, of which only two (one from the 4 and 7 rats) died after birth, but because of their small birth weight.It should be noted that no newborn presented any malformation in the skull.

Histopathological examination of the organs taken from both the mother and the baby presented a normal pattern of the organs architecture.

In the case of the lung in the control group - the normal structure of the alveoli and the bronchiole is noted. Liver-control group with normal structure of the liver lobe. Kidney - control lot with normal structure of the urinary tubes. Spleen -control group, normal structure of lymphoid follicles.Test-control group- normal structure of seminiferous tubes.Ovary – control lot - normal ovary structure.

Lot B-It is noted from the table that several rats (four) have not remained pregnant, presumably retinoic acid also affects fertility in rats.

What is important to note is that, three of these rats gave birth to a baby with a malformation at the head. In the case of the rat 1 of this lot, the newborn with malformation presented only a slight labial cleft, which did not affect its feeding capacity (it survived one month after birth and showed a normal development). The newborn with labia cleft was male.

Rat female and weight at start of experiment	Gestation	Number of newborns	Number of newborns with head malformations	Number of newborns who survived one month after birth
1.250	yes	5	0	5
2.210	No	-	-	-
3.225	Yes	4	0	4
4.200	Yes	3	0	2
5.240	No	-	-	-
6.230	Yes	3	0	3
7.250	Yes	6	0	5
8.220	No	-	-	-
9.235	Yes	2	0	2
10.215	yes	3	0	3

Table 1

Rat female and weight at start of experiment	Gestation	Number of live newborns	Number of newborns with malformations of the head	Number of newborns who survived one month after birth
1.240	Yes	4	1	4
2.220	Yes	3	0	3
3.230	No	-	-	-
4.215	Yes	3	1	2
5.250	Yes	4	0	4
6.210	No	-	-	-
7.245	Yes	5	1	4
8.200	No	-	-	-
9.245	Yes	3	0	3
10.235	no	-	-	-

Table 2

Rats females 4 and 7 also gave birth, but their newborns had a more serious malformation, palatine cleft, which affected their feeding potential, and the two pups died about two weeks after birth. Both newbornswith palatine clefts were female.

What is interesting to note in group B of the rats is that all the rats females presented more or less significant changes in the studied organs. Thus, in the lungs there is a dilatation of the capillary vessels, as well as the thickening of the alveolar wall [16] with the accumulation in the alveoli of an eosinophilic material and inflammatory cells (especially lymphocytes). In some animals there are accumulations of lymphoid elements around the bronchiole and around the dilated vessels, suggesting a pro-inflammatory potential of retinoic acid. These elements are also highlighted by special stains.

Histopathological changes are also seen in the liver of animals in group B that received retinoic acid. The first manifestation that can be seen in all the sacrificed rats in the B group is the expansion of all capillary vessels from the periphery of the liver lobe and sometimes also of the centrolobular veins.

The kidney of the rats from lot B also presents histopathological changes. First, it can be seen a slight retraction of the glomeruli in the Bowman capsule. In the urinary tract, there is a slight dilation of the capillaries and a slight decrease in the lumen of the urinary tubes.

Of all the studied organs, the spleen showed the fewest changes under the influence of retinoic acid. As for the gonads, they also presented histopathological changes, especially female gonads in mothers. It should be noted that the organs of the newborns did not show the same histopathological changes as the mothers.

To conclude the effects of administration of retinoic acid in the first week of pregnancy, we find that it increases the incidence of malformations in the skull but also affects the organs that we studied: it produces vasodilation, sometimes marked, and in some organs (lung, liver, kidney, ovaries) causes obvious histopathological changes: thickening of alveolar walls, sometimes with their destruction, alteration of hepatocytes in area I (from the periphery of the classical hepatic lobe), glomerular retraction and urinary tract alterations, and alteration of ovarian follicles.

In group C we monitored the newborns and possible malformations in the skull, obtaining the following results (table 3).

From the analysis of this table it is observed that unlike lot B, which received only retinoic acid, group C that received retinoic acid and vitamin B showed a decrease in the number of newborns with malformations in the skull [17].

Only one newborn showed cranial malformation, but this was a minor malformation (small lip cleft in one male newborn), which allowed the chicken to survive at 1 month. From the experiment on a small number of rats females, the table allows us to conclude that vitamin B has a beneficial effect in labio-palatal clefts induced by retinoic acid: the number of newborns with these malformations is much smaller and the malformation is minor; compared to lot B, more female rats were pregnant [18].

From the analysis of the organs collected from the group C of female rats, it can be seen that the the harmful effects of retinoic acid have been diminished. Thus, in the lung, although there is the same vasodilatation in the pulmonary capillaries, no thickening of the capillary walls or their destruction is observed. The liver of the rats in group C also exhibit the same changes induced by retinoic acid like in the B lot but more diminished. The kidney keeps exactly

Rat female and weight at start of experiment	Gestation	Number of newborns	Number of newborns with malformations of the head	Number of newborns who survived one month after birth
1.210	Yes	2	0	2
2.250	Yes	3	1	3
3.200	No	-	-	-
4.220	No	-	-	-
5.240	Yes	4	0	4
6.230	Yes	1	0	1
7.235	Yes	3	0	3
8.205	No	-	-	-
9.225	Yes	2	0	2
10.245	yes	2	0	2

Table 3

the same changes as in B lot; there is a slight retraction of the glomeruli at the Bowman capsule.The spleen retains its normal architecture. The collected rat testicles show the same vasodilation as in group B but without affecting the normal testicular architecture.

In the case of the ovaries, the microscopic examination revealed dilation of the capillary vessels and a beginning of ovarian follicular cystic dilation, highlighted only at great magnifications, without changing the normal ovarian architecture.

Summarizing the changes found in organs collected from animals in group C, we can conclude that vitamin B positively modulates the harmful action of retinoic acid, both on the cranial malformation and on the studied organs.

Vitamin B is known to have prophylactic action on the appearance of labio-palatine clefts. It can be speculated that retinoic acid is less harmful in the presence of vitamin B due to the fact that it modulates the harmful action of retinoic acid on the studied organs.

The lot D of females rats has the results presented in table 4.

Newborns that showed malformations in the skull showed only minor malformations that were found to be lip cleft [19] in one case, and micrognation in other case, both malformations endangering the life of the newborns, both surviving one month after birth.

After harvesting more organs from mothers and babies, we found an improvement in anatomo-pathological changes [20].

Summarizing the changes observed in the harvested organs from the animals in group D, we can conclude that magnesium acemamate (magnesium sulphate is a solid, crystalline substance, white, odorless, hygroscopic, bitter; there are several hydrated forms of which the most important is magnesium sulphate heptahydrate $MgSO4 \cdot 7H2O$ *called epsomit*e or *bitter salt*) positively modulates the harmful action of retinoic acid on both the malformations of the skull and on the studied organs.

It should be noted that magnesium acemamate, although having a somewhat protective action against the harmful effects of retinoic acid, its action on some of the studied organs is not as strong as that of vitamin B complex but comparable to it.

Lot E - and at this lot we monitored newborns and possible malformations in the skull; there was an increase in the number of newborns with malformations in the skull, as well as an increase in the number of newborns that did not survive at one month. Female 3 gave birth to a baby with a palatine cleft (female) that survived one month after birth.

Rat female 5 gave birth to two malformed newborns, of which one with a minor lip cleft, which survived at birth and one with a palatine cleft that died three weeks after birth. Both newborns were male.

Rat female 7 gave birth to a single malformed newborn, with micrognathia (female), which allowed one month's survival. Female 10 gave birth to a single newborn, with palatine cleft (female) that died 18 days after birth.

It is found that nickel potentiates congenital defects induced by retinoic acid, with an increase in the number of newborns, which presents either lip cleft or palatine clefts.

After the anatomopathological examination of the organs harvested from the group E rats, it reveals a worsening of the effects induced by retinoic acid. Thus in the lungs there is a more pronounced thickening of the alveolar walls and the intense dilatation of the pulmonary capillary vessels. Around some of them is an accumulation of inflammatory elements.

Extremely interesting histopathological changes are seen in the liver of animals in group E that received retinoic acid and nickel chloride, data corresponding to those in the literature.

The first manifestation that can be seen in all slaughtered rats in group E is the expansion of all capillary vessels at the periphery of the classical hepatic lobe and of the centralobular veins, more obvious effects compared to group B.

And in the kidney, changes induced by retinoic acid are accentuated by nickel. First of all, glomerular retraction is accentuated, sometimes accompanied by micro-hemorrhagia. As for urinary tubes, vasodilatation is accentuated, and narrowing of lumen of urinary tubes is almost complete. Some tubes have ruptured tubular epithelium and their lumen is occupied by eosinophilic material and red blood cells [21].

These elements could lead to the idea that this combination of retinoic acid and nickel chloride, because of its elimination through the kidneys would have a toxic effect on the tubular epithelium or, from the combination of retinoic acid with nickel chloride would result a metabolite with urinary excretion with the same toxic effect on the tubular epithelium.

Also in the spleen collected from animals of group E, there is a slight change of the normal splenic architecture, in the sense of the presence of a slight vasodilatation and a moderate dispersion of the lymphatic follicles. At the level of the testicles harvested from the males newborns, there is a accentuated vasodilatation at the level of the seminiferous tubes, but with the preservation of the testicular architecture and the spermatogenesis. The adult rat ovarie in group E macroscopically presents the same changes as in group B, but with a more pronounced reddish tint. Microscopically, there is an increased capillary vasodilatation as well as a much larger number of cystic degenerated ovarian follicles.

Rat female and weight at start of experiment	Gestation	Number of newborns	Number of newborns with malformation of the head	Number of newborns who survived one month after birth
1.230	No	-	-	-
2.220	Yes	2	0	1
3.200	Yes	1	0	1
4.210	No	-	-	-
5.250	Yes	5	1	5
6.215	Yes	2	0	2
7.230	Yes	4	0	4
8.205	Yes	2	0	2
9.220	No	-	-	-
10.240	yes	3	1	3

Table 4

Summarizing the pathological changes from the studied organs we can conclude that nickel in its form of administration (nickel chloride) potentiates the negative effects of retinoic acid, emphasizing in particular the vasodilation and proinflammatory effects at all the studied organs and inducing severe negative changes related or not to this vasodilatation. Nickel is otherwise known by its negative effects on various segments of the body.

In group F, it is found that the results are similar to those obtained in group B, founding three newborns with malformations in the skull, of which two (both males) with minor lip clefts, who survived one month after birth and only one newborn (female) with major palatine cleft, who did not survive a month.

Regarding the pathological changes encountered at the level of the various organs, the same changes as in the B group were found but slightly attenuated.

In the lot G, no babies with malformations in the skull were born, although pathological changes in the studied organs at one month after birth were similar to those of F lot. One newborn did not survive at one month but from cause of low birth weight.

It is noticed that in the H lot no rat babies were born with malformations in the skull , more than all the newborns survived one month after birth. The histopathological changes found one month after birth were similar to those in lots F and G.

The explanation for the differences between these three groups is given by the time of administration of retinoic acid during gestation. In group F, where malformations are noted, retinoic acid was administered in the first week of gestation, which demonstrates that the critical period for intervention of the teratogenic agents, for skull malformations, in rats, is the first week of gestation. If the teratogenic agent intervenes after the first week of gestation, when the cephalic extremity organogenesis has ended in rats, it produces far less obvious manifestations.

Researches on animals have shown that there is a dose-response relationship for teratogens. However, it is known that the doses used to produce anomalies in animals are much higher than those to which humans are exposed. For this reason, animal data are not applicable to pregnant women. For a drug to be considered teratogenic, it must be observed a dose-response relationship; for example, if exposure during pregnancy is greater, the phenotypic effects will be more severe.

Most of the lip and palatine clefts are the result of multiple, genetic and non-genetic factors, each producing a minor developmental defect, called multifactorial heritage [22]; it can be represented by a model where the "responsibility" for a disease is a variable determined by the combination of genetic and environmental factors.

Labio-palatine clefts in humans are transmitted through multifactorial inheritance. The prevention of these abnormalities [23] can be done by reducing exposure to exogenous factors to zero.

The postnatal assessment of malformations is done through a detailed clinical examination; children [24] with anomalies should be registered in a national or local registry, in order to be treated appropriately and in time, avoiding facial defects in adults [25].

The sagittal growth of the jaw appears to be limited in patients with unoperated palatine cleft (and which becomes more severe with aging) but also to those who are operated [26]. The basis of this jaw growth deficit is not a single factor, as evidence being the studies performed on unoperated patients and those operated for palatine cleft.

Dental anomalies in patients with cleft occur more frequently than in those who did not suffer from this disorder: changes in number - inclusions, dental agenesis, supernumerary teeth, hipodonþii; shape changes, especially in upper lateral incisors.

The size of the teeth was smaller both in the mid-distal and vestibulo-oral ways in all types of clefts compared to the control group. The smallest teeth were found in the palatal cleft, although in earlier studies they were the same size as the control group. The smallest tooth was recorded as the upper lateral incisor on the cleft side, aspect which was also found in other studies. These results show a clear difference between the two types of clefts, which are genetically and embryological distinct.

The treatment of these facial defects depends on the close collaboration between the pediatric surgeon, the oro-maxillo-facial surgeon and the family [27].

Conclusions

Retinoic acid, a teratogenic agent for rats, when given in the first week of gestation (when it takes place the the organogenesis of cephalic extremity, at the studied animals), determine various malformations in the skull. The most common malformations induced by retinoic acid are labio-palatine clefts. In our studies, lip clefts are more common in male newborns and palatine clefts are more frequent in female newborns.

Retinoic acid induces histopathological changes in several organs.

Our study additionally proves that the labio-palatine clefts in humans are transmitted through multifactorial inheritance. The prevention of these anomalies can be done by reducingto zero the exposure to exogenous factors, especially during the first 10 weeks of pregnancy.

The therapeutic outcome depends on the complexity of the malformation, on the moment of surgery and on the selection of the most modern and appropriate techniques and equipment for the patient.

The finding that sometimes the labio-maxilo-palatine clefts are accompanied by both antecedents and collaterals, with malformations of other segments of the body, may prove to be affected by genetic *nodules* with somatic consequences at different levels. Thus, the clefts can be included in some chromosomal anomalies that cause general syndromes such as Treacher-Collins, Pierre-Robin and Klippel-Fiel.

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