TERATOGENIC POTENTIAL OF A PESTICIDE TO INDUCE CLEFT PALATE IN RATS

Potencial teratogênico de um pesticida em induzir fenda palatina em ratos

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Abstract

OBJECTIVES: The aim of this research was to evaluate the teratogenic potential of a pesticide to cause cleft palate in rats.
MATHERIALS AND METHODS: Two groups of females were exposed to Folidol ® (methyl parathion) twice a day, Group 1 was exposed before gestation, and Group 2 during the gestational period. The offspring of females from both groups were submitted to macroscopic evaluation of the palate, using a Leica Stereo Microscope ZOOM 2.000, with 45x magnification.
RESULTS: No presence of cleft palate was verified in the studied specimens. *CONCLUSION:* The studied pesticide did not induce cleft palate in rats fetuses, under the conditions of the present study.
Keywords: Pesticides; Methyl Parathion; Cleft palate; Rats.

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Resumo

OBJETIVO: Esta pesquisa teve como objetivo avaliar o potencial teratogênico de um pesticida do grupo dos organofosforados em causar o aparecimento de fenda palatina em ratos.
MATERIAL E MÉTODOS: Dois grupos de fêmeas foram expostos duas vezes ao dia com aplicação tópica de um pesticida, sendo o Grupo 1 previamente à gestação e o Grupo 2 durante o período gestacional. A prole das fêmeas dos dois grupos foram submetidas à avaliação macroscópica do palato por meio de lupa estereoscópica, com aumento de até 45x.
RESULTADO: Não foi verificada a presença de fenda palatina em nenhum dos espécimes da prole dos dois grupos.
CONCLUSÃO: O pesticida estudado não induziu fendas palatinas em ratos, nas condições do presente trabalho.
Palavras-chave: Pesticidas; Parathion Metílico; Fenda Palatina; Ratos.

Introduction

Orofacial clefting is the commonest malformation of he craniofacial complex, with unfavorable repercussion on eating, phonation, audition and even the dentition of individuals sith these deformities (1). In addition, there may be alterations to facial growth and development, as well as impaired facial esthetic appearance and psychological damage.

Among the etiologic factors of these malformations, those of a genetic nature may be found in around 30 to 35% of cases. Although the significant role of hereditariness in the appearance of such problems is recognized, it is not yet known for certain how this anomaly is transmitted from one generation to another (2). According to Scott (3), some cases may be attributed to direct transmission of abnormal genes from one or the other progenitor or due to a genetic mutation in individual cases. Furthermore, it is known that parents' advanced age may be related to lip and palate malformation (4), since children of mothers over the age of 40 are three times more susceptible to these developmental abnormalities than children whose mothers are under the age of 30 years (3).

Considering non genetic etiologic factors an enormous range of possibilities opens up, among which there are some infectious diseases, toxoplasmosis, diabetes and a diet deficient in nutrients (4) such as folic acid, vitamin A (3) or pyridoxin (5). In addition, lack of oxygen, anemias, exposure to radiation (3), alcohol consumption (6), smoking (7) and use of anticonvulsant medications, salicylates, opiates, benzodiazepines (4), hadacidin antibiotic (8), may also be related to malformations. Some studies observed the development of cleft palate in rats and rabbits after administration of cortisone (9, 10), vitamin A (10) and dexamethasone (10, 11). Regarding the application of anti-inflammatory drugs, Walker (12) observed that triamcinolone, betamethasone and dexamethasone produced cleft palate with significant frequency. The use of methylprednisolone, prednisolone and cortisone, however, did not lead to induction of this malformation.

In addition to each of these factors, genetic and environmental factors may be associated, contributing to the formation of approximately 80% of cleft palates. However, little is known about the mechanism of interaction of genetic and environmental factors in producing development anomalies. The genes would appear to produce substances that play a vital role in the regulation and rhythm of the growth process, but these in turn, depend on the presence of a favorable internal and external (maternal) environment. There is also the possibility of somatic mutations among groups of cells in vital periods of development (3).

Bearing in mind the extensive variety of teratogenic factors, it is difficult to make an exact correlation between exposure to one or more of these factors and the presence of the deformity. From an embryologic point of view a complicating factor is that the formation of the face starts in approximately the 5th week of intra-uterine life, and palatogenesis is completed around the 12th week of gestation. Exposure to one of these factors may occur without one knowing about the gestation in progress.

Perhaps for this reason, medical centers attending patients with congenital facial deformities have associated this malformation with domicile in the rural area and to agricultural crop activities. It is therefore, suspected that chemical substances used in agriculture may be related to the manifestation of cleft lip and palate.

In Brazil, up to 1986, substances with the so-called organic-chlorides - toxic substances that had long been prohibited in various countries in the world - were allowed to be used in agriculture. In spite of this prohibition, pesticides with a chemical composition that is highly toxic to the human being, and with have a large residual effect, are still in use. Among them are those of the organophosphate type, such as Folidol ®, whose active substance is methyl parathion, commonly used because it is useful against variety of agricultural pests.

Various authors (13, 14, 15, 16, 17,) have related that methyl parathion is a teratogenic agent. Tian et al. (18) observed skeletal malformations and cleft palates when an organophosphate was applied in rats. According to Varnagy and Deli (16), however, small doses of methyl parathion, at concentrations used in agriculture, do not present a teratogenic or lethal effect, and in accordance with the Extension Toxicology Network (19) the available evidences indicate that this substance does not cause malformations.

Therefore, the aim of this study was to assess the teratogenic potential of a pesticide of the organophosphate group (Folidol ®) to cause the appearance of cleft palate in rats.

Materials and methods

The sample consisted of 42 *Wistar* rats aged 60 days, of which 14 were males and 28 females. The males constituted a single group and remained in the study until the mating stage, while the females were divided into two groups:

- Group 1 (n=14): Specimens exposed to Folidol ® (Bayer, São Paulo, Brazil) daily before gestation, in a period of 30 days of development (from the 61st to the 92nd day).
- Group 2 (n=14): Specimens exposed to Folidol ® (Bayer, São Paulo, Brazil) daily during the gestation period, (from the 100th to the 121st day).

The experimental method used and all the procedures performed were in compliance with the ethical and legal recommendations specified for animal experimentation (20, 21). This research was approved by the Research Ethics Committee of the Universidade Tuiuti do Paraná, registration number CEP-UTP – 20/2003.

The tested chemical agent was the insecticide Folidol ® (Bayer, São Paulo, Brazil). The classification of the product in the Ministry of Agriculture of Brazil is Class I (extremely toxic).

The product was used in a 1/250ml dilution, prepared every three days, in accordance with the manufacturer's guidance. The tip of the paint brush was immersed in the solution and gently pressed against the edge of the glass to run off the excess. Next, the product was applied topically on the dorsal portion of each paw, in a single movement. The four paws of each female were painted with Folidol twice a day, at 9:00AM and at 2:00PM.

The 42 animals were placed in special cages for breeding rats, in the maximum number of 3 animals per cage, males been isolated from females.

When they were 90 days old, the males were put into new cages for mating, so that each male would occupy one cage. They stayed there for two days at the end of which they were returned to their development cages. Without cleaning cages occupied by the males after they were removed, the females were placed in them in the proportion of two to each cage. The purpose of this stage was to obtain the Whitten effect, that is, to induce the females to be in heat (22). The next stage was mating, which lasted for two days (the 98th and 99th days), after which the males were dispensed from the study.

While the females in Group 2 were exposed to Folidol ®, daily during the gestation period, (from the 100th to the 121st day), those in Group 1, during the same period, remained in new cages without any exposure to the tested substance.

Although the normal gestation period is 22 to 23 days, all the females were killed on the 122nd day of the experiment - the 20th day of gestation. Next, laparotomy was performed in each of the pregnant animals, to collect the fetuses. The cephalic extremity of each of the specimens was separated from the remainder of the body, exeresis of the mandible was performed. A 10% formalin solution was used for storing the specimens.

Macroscopic assessment of the palate consisted of observing whether or not there was presence of cleft palate. To do this, a Leica ZOOM 2.000 Stereoscopic Microscope, with a magnification of up to 45x was used.

Results

The results are presented in Tables 1 and 2. Table 1 refers to Group 1, total of 110 fetuses. Cleft palate was not observed in any of the specimens. In the same way, in Group 2 none of the 103 fetuses presented the malformation (Table 2).

TABLE 1 - Number of females, of progeny individuals and of affected individuals, in Group 1 in 2003

Females	1	2	3	4	5	6	7	8	9	10	11	12	13	14
No. of Progeny Individuals	9	9	8	10	9	*	10	10	10	9	*	9	9	9
No. of Affected Individuals	0	0	0	0	0		0	0	0	0		0	0	0
Affected Individuals (%)	0	0	0	0	0		0	0	0	0		0	0	0

Source: PUCPR Vivarium.
Note: * Females not pregnant.

TABLE 2 - Number of females, of progeny individuals and of affected individuals, in Group 2 in 2003

Females	1	2	3	4	5	6	7	8	9	10	11	12	13	14
No. of Progeny Individuals	8	10	*	*	*	9	10	9	9	10	10	8	10	10
No. of Affected Individuals	0	0				0	0	0	0	0	0	0	0	0
Affected Individuals (%)	0	0				0	0	0	0	0	0	0	0	0

Source: PUCPR Vivarium.
Note: * Females not pregnant.

Discussion

It is part of Brazilian tradition that children and women, even during gestation, are present in agricultural activity. Thus, it was raised the hypothesis of a possible correlation between cleft lip and palate and the use of chemical substances in agriculture. The scarcity of data in the literature has motivated this research.

Folidol ® is a broad spectrum pesticide that is effective for use in different types of crops. Its active substance belongs to the organophosphate group and its sale and use is prohibited in many 1st world countries, because of its high toxicity and elevated residual effect on the environment.

The teratogenicity of Folidol ® for inducing cleft palate was tested in two ways, by the residual effect of the drug (Group 1), due to the

recognized residual effect of the product on the environment, and secondly by direct exposure during the gestational period (Group 2).

The *Wistar* rat was chosen for this experimental study because the species presents a pathogenesis model similar to that of the human species.

Wistar rats does not appear to have its reproductive cycle altered by contact with the operator during manipulation (23). This justifies the choice of this species of rat, since part of the method involves daily manipulation of the females in order to paint their paws with the solution. Eventual stress or anxiety generated by this manipulation appeared to be of no significance. This factor can be reinforced by the fertilization rate presented. Only two females in Group 1 and three in Group 2 did not have their gestations taken to full term. The reason for laparotomy being performed one or two days before the date forecast for normal birth was because the females tend to eat their smaller or malformed fetuses, and this would have compromised the results of the observation.

The use of drugs that could potentially induce the formation of cleft palate in rats was well described by Nanda et al. (10). The authors obtained a positive correlation between the use of cortisone, dexamethasone and vitamin A and this malformation. This positive correlation was also observed when cortisone administered subcutaneously and dexamethasone administered intramuscularly were assessed (9, 11).

In the present study, however, exposure to the potential teratogenic agent was external, by contact, simulating what could happen when someone handled the drug. The drug presents high liposolubility and is easily absorbed by the skin, lungs and gastrointestinal tract.

In the present study, no presence of cleft palate was verified in any individual offspring. However, this results alone cannot fully exclude the potential teratogenicity of the tested substance. The mechanism of interaction between genetic and environmental factors in the production of development anomalies is complex (3). Furthermore, a single genetic constitution, that is, a single animal species is not sufficient to assess the teratogenicity of a drug, and testing the component in various species is indicated, before making affirmations about its teratogenic potential.

The effects of cortisone, for example, are very variable, depending on the genetic constitution of the specimen the sample is composed of (12). A difference in tolerance to cortisone between different species of mice was observed, resulting in a large variation in the frequency of cleft palate. The authors suggested that this difference is related to the small number of genes (24).

Tian et al. (18) found a different result. When they applied an intraperitoneal injection of 80mg/Kg of the organophosphate pesticide Chlorpyrifos in mice, they observed an increase in the frequency of cleft palates (5.97%) when compared with the Control Group (0.97%). It is possible that the dosage of the drug used in this research was insufficient to cause disturbances in the palatogenesis, corroborating the findings of Varnagy; Deli (16), who affirmed that small doses of the insecticide Wofatox 50 EC (methyl parathion), such as those used in agriculture, do not present a teratogenic or lethal effect on the embryos of chickens and pheasants. Furthermore, there are evidences that methyl parathion does not have a teratogenic effect (19).

Other manifestations of the teratogenic potential of Folidol were reported such as: malformations of the spinal column, tail and bone metabolism alteration during the larval development of *Rana perezi* (13); delayed growth in chicken embryos (15); various teratogenic effects, such as lordosis and cervical scoliosis, spasmodic eyelid contraction and sporadic abdominal fissure with the use of the insecticide Wofatox 50 EC (14); diminished body mass, high incidence of malformation development and deaths with the use of high doses (16); and lastly, atrophy of the cervical column (17).

Another factor to consider from these results is that the males were not exposed to the potentially teratogenic agent. One of the factors regarding to the appearance of cleft palate is related to the presence of a genetic mutation, and this could occur in one of the progenitors or in both of them, although evidences suggest that methyl parathion is characterized as being non-mutagenic (19).

Conclusion

Considering the conditions and limitations of the present study, Folidol ® did not induce palate malformation in rats fetuses.

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