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Morphological studies of rat foetus skeletons: test for teratogenicity of nootropic drug pyramem.

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The teratogenic effect of the nootropic drug Pyramem on the skeleton and internal organs of rats was investigated. Pregnant Wistar rats were treated orally by 200 mg/kg b. m. Pyramem and 200 mg/kg b. m. during the period of organogenesis since the 7th until 15th day of pregnancy. The effect of Pyramem on the fetuses in terms of malformations, skeletal fragility and abnormalities in the internal organs (lungs, liver, spleen and kidneys) close to delivery (day 20-21) was analyzed. The nootropic Pyramem failed to prove to be embryotoxic agents at all.

Key words: toxicology, teratogenicity, "Double staining method", "Wilson section method", nootropic drugs.

Introduction

Nootropics are a relatively new class of drugs developed actively in recent years in the hope that they will assist in the impaired nerve cell regeneration, enhance the intellectual and memory capacities as well strengthen the adaptive possibilities of the CNS towards extreme requirements because of their antioxidant effect (3). This study aims at investigating the nootropic drug „Pyramem" for evaluation its teratogenic effect and its skeletal and organ toxicity, in particular.

Material and methods

Experimental model for evaluation of the teratogenicity in rats.

The study of teratogenicity required application of the studied substances on pregnant female Wistar rats (n=30) during the organogenesis (day 7-17) according to the International Conference on Harmonisation (2) (Fig. 1).

Pregnant Wistar rats (15 per group) were dosed by intraperitoneal application with Pyramem 200 mg/kg b. m. and physiological solution 10 ml/kg b.m. during the period of organogenesis (days 7-15), taking day one when the female was found to be sperm

+...Dose application...+

mating ↓↓↓↓↓↓↓↓↓↓↓↓

0.....7.....17.....20 (21) – euthanasia

..... days after coitus ..... examination of the fetuses fixation of the internal organs and the skeleton

Fig. 1. Teratologic studies in rats (according to EEC, FDA, OECD, etc.).

positive. On day 21 after conception were extracted via caesarian section and were explored for skeletal toxicity, using the method of "Double skeleton staining" with Alcian blue and Alizarin red (4,5) (Fig. 2). The fetuses were studied microscopically to detect any internal organ abnormalities (lung, liver, spleen etc.), using 20 body slices with thickness 1 mm. (Wilson Section Method) (Fig. 3).

Fig. 2. Double skeleton staining method.

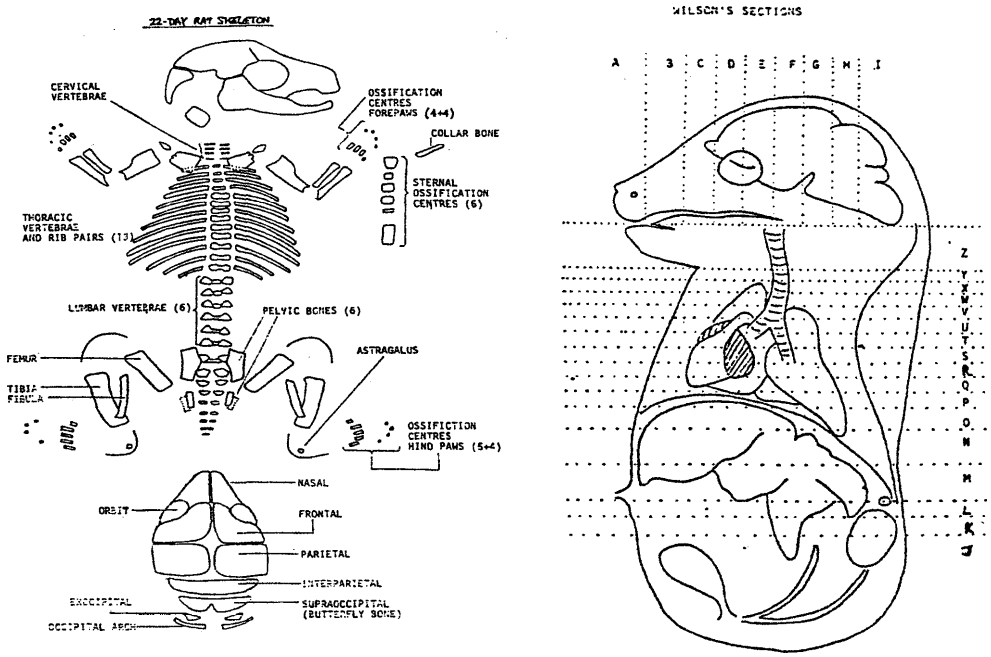

Fig. 3. "Wilson Section Method

"Double staining" method for skeletal examination

Staining of the skeleton was made with 0,15 % of Alcian blue and 0,005 % of Alizarin red S. All the cartilages were stained in blue but the ossified bones in red. The entire fetal skeleton was examined and all the abnonnalities, variations in degrees of ossification and lack of cartilage were recorded. Each bone was assessed for its size, shape, relative position and number of bones, and ribs and data were compared with the controls (Fig. 2).

Bouin's fixation for internal examination using the Wilson section method

Fetuses for visceral examination were processed using Bouin's solution to allow examination of the internal organs. Sections were then examined using a microscope to find out any abnormalities in the internal organs such as liver, lungs, kidneys and spleen (Fig. 3). Data were statistically processed by using the variation analysis and Student's /-test.

Results

Pyramem showed no evidence of teratogenicity. The body weight and the length of the fetuses, the weight of the placentas and livers of adult rats treated with Pyramem showed no statistically significant differences from controls with physiological solution (Table 1).

Table 1. Weight and length of fetuses, weight of placentas and livers.

	Weight fetuses (g)	Length fetuses (mm)	Weight placentas (g)	Weight livers (g)	n
Controls (phys. sol.)	2.81 ± 0.24	33.48 ± 1.21	0.45 ± 0.07	0.2 ± 0.017	29
Pyramem	2.72 ± 0.28	33.69 ± 1.02	0.46 ± 0.08	0.18 ± 0.01	38
	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	

On inspection of the fetuses from the group with Pyramem no anomalies were seen regarding the skeleton: head, body, extremities. Lack of teratogenic effect was detected, using following indices: Gross appearance – external: Coloration of the fetus; Subcutaneous hemorrhages; Gross abnormalities as: spina bifida, anencephaly, exencephaly, arhinencephaly; cebocephaly; Head: eyes, ears, nostrils, tongue, palate, mouth. Extremities: anterior, posterior (number of fingers, syndactyly, micromelia). Rear part of the body: anus (abnormalities) – atresia ani; tail (deformities, lack of tail; genitals (2 mm for male gender, 1 mm for female gender).

Test for skeletal toxicity

When the nootropic drug Pyramem was tested for skeletal toxicity with the "double staining method" all cartilages stained in blue, and the ossified bones in red. The examination of the whole skeleton for variations in the level of ossification, lack of cartilages and any possible abnormalities no deviations from the norm were found. For each bone the dimensions, type, relative position, number of bones and ribs were denoted and compared to controls. The nootropic drug Pyramem that was tested did not induce skeletal toxicity.

Microscopy

On microscopy examination of the fetuses for abnormalities of the internal organs (lung, liver, spleen, kidneys etc.) using the Wilson section method no anomalies were found in any of the explored organs.

Discussion

In the current experiment a test for fetal and maternal toxicity of the Pyramem was done. The body weight, and the length of the fetuses, the weight of the placentas, and livers of rats treated with Pyramem, did not show statistically significant difference compared to parameters with controls. The inspection of the fetuses showed no abnormalities.

The nootropic drug Pyramem was tested for skeletal toxicity using double skeleton staining method with alizarin red and alcian blue (1,4,5). All cartilages stained in blue, and the ossified bones in red. Thus the cartilaginous and the bone part of the skeleton were examined, and any possible abnormalities could be detected. The animals treated with Pyramem showed no difference compared to the controls with physiological solution.

The Wilson section method, which is standard teratological method and still represents the most utilized technique to examine the visceral organs, was used to explore the soft tissues. No impairment was found in any of the examined internal organs in animals treated with Pyramem.

Conclusion

This experiment convincingly demonstrates that nootropic drug Pyramem does not manifest data indicative for teratogenicity.

The double staining method used in the current study is a fundamental part of the teratological studies for assessment of the toxicity of the xenobiotics and non-chemical factors for the individual development.

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