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POTENTIAL EMBRYOTOXIC EFFECT STUDY OF MINOXIDIL-CONTAINING LOTION IN EXPERIMENT WITH FEMALE RATS

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The purpose of this study was to establish maternal safety of topical preparation containing 5 % minoxidil, as well as safety for fetal development, fetus-maternal functional responses in experiments with laboratory rats using doses and methods of administration recommended by the manufacturer. Based on the results obtained under these experimental conditions, a lotion containing 5 % minoxidil does not cause changes to the reproductive system of rats and does not demonstrate embryotoxic effect.

Key words: minoxidil, androgenetic alopecia, embryotoxic effect.

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ДОСЛІДЖЕННЯ ПОТЕНЦІЙНОГО РИЗИКУ ЕМБРІОТОКСИЧНОСТІ ЛОСЬЙОНУ ІЗ ВМІСТОМ МІНОКСИДИЛУ В ЕКСПЕРИМЕНТІ НА САМИЦЯХ ЩУРІВ

Проведені випробування по встановленню безпеки топічного препарату із вмістом 5 % міноксидилу для материнського організму та розвитку плоду в експериментах на лабораторних щурах в дозах та режимах застосування рекомендованих виробником. На основі отриманих результатів в даних умовах експерименту лосьйон із вмістом 5 % міноксидилу не викликає змін репродуктивної системи щурів та не проявляє ембріотоксичного ефекту.

Ключові слова: міноксидил, андрогенетична алопеція, ембріотоксичний ефект.

The work is a fragment of the research project "Introduction of alternative methods to study toxicity of chemical factors in the development of hygienic regulations and precautions", state registration No. 0120U002145.

Topical preparations containing minoxidil are recommended in modern trichology as the most effective control agents for androgenetic alopecia (AGA) [8]. These products are marketed as over-the-counter medicines within the pharmacy network or via the Internet and positioned by the manufacturers as "skin care products". This leads to an increase in their uncontrolled use by general public. It should be noted that according to EU Directive 1223/2009 [11], cosmetic products should not contain substances of medicinal products and, accordingly, the "skin care products" are outside the legal field. Thus, the products safety is understudied, the dosage and methods of administration are not fully justified.

According to various data, 30–75 % of the population are diagnosed with AGA [6]. This disease is a major medical and social problem associated with a significant impact on the human quality of life, often causing significant psychological problems [4].

The use of topical agents containing minoxidil in the female cohort of patients is subject to some precautions [14]. According to FDA (Food and Drug Administration), topical minoxidil reduced fertility in rodent experiments. However, clinical data on its safety in pregnant patients are limited [12, 15]. Data provided on the experimental effect of minoxidil in pregnant female rats with subcutaneous administration at doses of 0, 1, 11 and 120 mg/kg. The highest dose, 92-fold exceeding the clinically recommended one, caused increased foetal mortality, external developmental pathologies, and skeletal abnormalities. At the same time, this dose caused a decrease in maternal body weight gain, food intake and, accordingly, effects observed were interpreted as minoxidil maternal toxicity [7].

The purpose of the study was to establish maternal safety of topical preparation containing 5 % minoxidil, as well as safety for fetal development, fetus-maternal functional responses in experiments with laboratory rats using doses and methods of administration recommended by the manufacturer.

Material and methods. Experiment included clinically healthy intact outbred female rats of reproductive age, weighing 180–240 g, provided by Lviv National Medical University vivarium. The experimental animals were kept under optimal vivarium conditions in plastic cages with free access to standard granulated feed and drinking water. Animal study was carried out in compliance with bioethics principles, legal regulations and requirements in accordance with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" [3].

Medicinal product "MinoX 5" (Minox Technology Trading LLC, Ukraine) was used in the study. The active pharmaceutical ingredient of the drug – Minoxidil, according to chemical nature, refers to pyrimidine derivatives. Chemical name – 6-(Piperidinyl)-2,4-pyrimidinediamine-3-oxide.

The study tested a therapeutic dose of 5 % product 0.1 ml/kg, applied to a previously depilated body area at the same time of a day for 20 days of gestation. The control group received carrier– 30 % ethyl alcohol.

Animals with stable oestrous cycle were selected based on cytological analysis of vagina. Female rats in proestrus and mating oestrus phases were fitted at a 2:1 ratio to male rats overnight. The day sperm cells were identified in the vaginal smear was considered the first day of gestation. The animals were divided into two groups by "blind ranging" method –10 per each – experimental and control one. In randomization, body weight served as the primary criterion.

The experimental female rats were examined every day of gestation for clinical signs of toxicity; changes in body weight were also monitored. Individual water and feed intake rates were determined on gestation days: 0, 2, 4, 6, 8, 10, 12, 14, 16, and 18.

Female rats were sacrificed on Gestation Day 20 with subsequent laparotomy and isolation of the uterine horns with ovaries. Ovaries examined to determine the corpora lutea count, horns of the uterus – the number of live, dead and resorbed foetuses, crown-rump length and fetal weight, as well as placenta size and weight; calculated body weight index and fetal-placental index.

Based on these experiments, the following parameters were calculated: total embryonic mortality; pre-implantation mortality; post-implantation mortality; and intrauterine survival rate. Total embryonic mortality was calculated using the difference between the number of corpora lutea during pregnancy and the number of live foetuses. Then, the resulting value was identified as the percentage share of the corpora lutea during pregnancy. Pre-implantation mortality was determined using the difference between the number of corpora lutea and the number of implantation sites and resulting value was identified as the percentage share of the corpora lutea. Post-implantation mortality was determined using the difference between the number of implantation sites and the number of live foetuses and the resulting value was identified as the percentage share of the number of implantations. The intrauterine survival rate was determined based on the ratio of live foetuses to corpora lutea.

The autopsy data obtained from one female rat and the mean of one litter were used as an independent variable per observation unit.

Microsoft Office Excel 2016 spreadsheets were used for accumulation, correction, systematization of baseline information and visualization of results obtained. The Kolmogorov-Smirnov test was performed to verify the normality of distribution. The parametric data are described in terms of mean (M) and standard deviations (SD), while the nonparametric data are described in terms of median (Me) and quartiles. Given the compliance with the normality of distribution, the significance of differences obtained for comparable values was accessed using Student's t-test and Mann-Whitney U-test in the cases of deviation in the Gaussian distribution law from normal. Significance level in testing statistical hypotheses was taken as $p \leq 0.05$.

Results of the study and their discussion. During gestation, no symptomatic signs of systemic toxicity, such as ataxia, sedation, diarrhea, cyanosis and hair loss, were observed in experimental animals; no mortality was reported, as well as significant differences in water (fig. 1) and food (fig. 2) consumption compared to controls. There were no statistically significant differences in the weight gain of dams compared with the control group. Minoxidil application does not affect the duration of pregnancy.

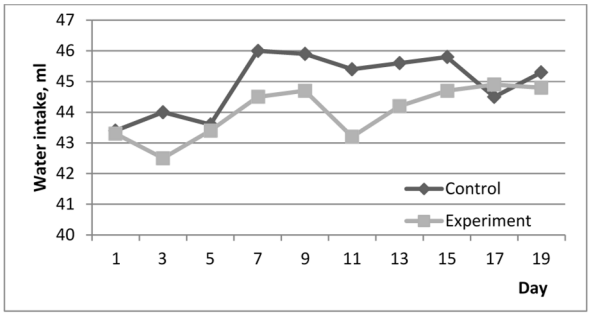

Fig. 1: Water consumption by female rats receiving minoxidil topical lotion during gestation. Given are mean values compared to controls.

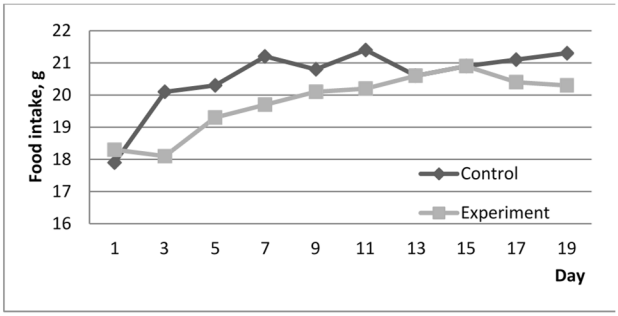

Fig. 2: Feed intake by female rats receiving minoxidil topical lotion during gestation. Given are mean values compared to controls.

Following amniotomy and cord cutting fetal respiration became autonomous; the skin cover was pink with no pigmentation. The skin surface had a wrinkled, coarse-grained appearance. Gross examination of embryos from control and experimental groups showed no anatomical abnormality. The skull was within normal, of oval-oblong shape. The auricle and eyelids closed. The anterior abdominal wall was

imperforated with no signs of umbilical hernia. The tail was of regular length. The limbs had a well-developed shoulder, forearm, bone, thigh, lower leg and foot. The position, shape of the limbs, the number of digitus and their size in the embryos from experimental and control groups were within the normal range.

Topical application of the drug did not cause changes in the parameters characteristic of embryogenesis: total number of foetuses, number of dead embryos and number of live foetuses per dam vs control (table 1). Organometric analysis of placental development in the experimental group receiving 5 % minoxidil (table 1) shows that the mean weight and diameter of placenta does not differ significantly from those of the control group. Morphometric parameters of feti in the experimental group had statistically significant increase in craniocaudal dimension and a tendency, but statistically insignificant, to feti weight increase in the control group. That is, the effect of topical minoxidil during gestation in our experiment caused fetal macrosomia.

Table 1

Caesarean section data of dams treated with topical minoxidil lotion from Gestation Day 1 to Gestation Day 19

Parameter	Control group	Experimental group
Fetal weight, g (M±SD)	2.75±0.65	3.49±0.9
Crown-Rump Length, mm (Me [Q1;Q3])	Me 31 [25 % -28.5; 75 % - 33]	Me 34 [25 % -31.5; 75 % - 37]*
Placenta weight, g (M±SD)	0.54±0.17	0.59±0.1
Placenta size, mm (M±SD)	12.55±1.7	13.67±1.1
Yellow bodies count per 1 dam, units (M±SD)	10.29±2.8	8.40±1.5
Number of implantation sites per 1 dam, units (M±SD)	7.29±3.5	7.00±2.5
Number of live foetuses per 1 dam, units (M±SD)	7.29±3.5	7.00±2.5
Number of resorption sites per 1 dam, units (Me [Q1;Q3])	0	Me 0 [25 % -0; 75 % - 1]
Fetal-placental index (M±SD)	0.21±0.1	0.19±0.07
Body weight index (M±SD)	0.1±0.019	0.09±0.015

* – statistically significant differences vs control group ($p<0.05$)

Based on the above data (Tables 1, 2), such parameters for assessing embryotoxic effect, as the corpora lutea count, number of implantation sites, and, accordingly, pre-implantation death, were not statistically different in the experimental and control groups. Post-implantation and total mortality also did not show a negative tendency in the experimental group.

Table 2

Estimated embryotoxicity parameters with topical application of minoxidil to female rats during pregnancy

Parameter	Control group	Experimental group
Total embryonal mortality, % (Me [Q1;Q3])	Me 16.7 [25 % -15.0; 75 % - 47.3]	Me 10.0 [25 % - 0; 75 % - 33.3]
Pre-implantation mortality, % (Me [Q1;Q3])	Me 16.7 [25 % -15.0; 75 % - 47.3]	Me 10.0 [25 % - 0; 75 % - 33.3]
Post-implantation mortality, % (Me [Q1;Q3])	0	0
Intrauterine survival rate, % (Me [Q1;Q3])	Me 83.3 [25 % -52.7; 75 % - 85.0]	Me 90.0 [25 % -66.7; 75 % - 100]

The data obtained are consistent with the results of pharmacokinetics and pharmacodynamics studies with topical minoxidil. The studies showed that minoxidil has insignificant potential of skin permeation. The distribution study of minoxidil labelled with radioactive carbon after topical application of 1 % and 5 % products showed the absence of drug in faeces. At the same time, minoxidil was found in skin washing within the range of 41 % to 45 % of the applied dose. Approximately, 1.4 % of topical minoxidil is absorbed through intact scalp. Minoxidil does not bind to plasma proteins and does not cross the blood-brain barrier. The kidneys excrete approximately 95 % of systemically absorbed drug and its metabolites within 4 days [13]. In rats and monkeys, the exposed skin areas maintained 30–78 % of minoxidil dose used. It is suggested that part of the applied minoxidil binds to skin structural elements and releases slowly into systemic blood. This mechanism and individual sensitivity explain the reports of tachycardia and palpitations with topical application in some patients. It is considered that the extent of absorption of minoxidil when applied topically is insignificant and it does not cross the blood-brain barrier [10].

Regarding fetal macrosomia we discovered, we can hypothetically assume that this effect is caused by ability of minoxidil to stimulate cell proliferation and data on increase in cellular synthesis of DNA under the influence of minoxidil [13]. From the standpoint of modern scientific knowledge, the main mechanisms of pharmacological activity of minoxidil are considered, firstly, as induction of growth factors, secondly, inhibition of cell apoptosis and thirdly, increase blood flow due to arteries dilation [2]. It should be noted that such pathological condition as macrosomia is quite often accompanied by increased risk of

formation of deviations from normal development of organs and systems, in particular cardiovascular, immune, hormonal, in the foetus [1].

Even scarce reports on adverse effects on the fetus by minoxidil topical application during the mother's pregnancy do trigger concern [7]. In addition, it is noted that although no teratogenic effects were detected in rats exposed to minoxidil from the 6th to 15th days of gestation in doses 20 to 70 times higher than therapeutic ones, this exposure regime caused decrease in manure and increase in resorption in rabbits [10]. Uncontrolled use of cosmetics containing minoxidil can trigger violation of application modes, which in turn leads to increased absorption of the substance and side effects [5].

Currently, given potential danger to pregnant women, the pharmaceutical substance minoxidil belongs to group C (clinical data are not available and animal studies are not available, or clinical data are not available, but animal studies show adverse effects to the fetus (45 %) according to US Food and Drug Administration classification. According to Swedish Catalogue of Approved classification, it belongs to group B:3 (reproduction toxicity studies in animals have revealed an increased incidence of fetal damage, the significance of which is considered uncertain in humans; similar to FDA category C (12 %) according to Swedish Catalogue of Approved classification; to group C (drugs which, owing to their pharmacologic effects, have caused or may be suspected of causing, harmful effects on the human fetus or neonate without causing malformations. These effects may be reversible (24 %) according to Drug Evaluation Committee classification [9].

Taking the data obtained in the course of our experiment and based on modern scientific reports into account, we believe that possible teratogenic effects of minoxidil and the substance effect on the 2-nd and 3-rd generation of foetus must be studied.

Conclusion

Regulatory bodies exercising control over medications in a number of countries include minoxidil to drugs likely causing adverse effects on offspring health if used during pregnancy. Topical application of lotion containing 5 % minoxidil in therapeutic dose to experimental rats during gestation does not cause changes to the reproductive system of rats and does not affect embryonic development except for craniocaudal fetal dimension. A promising area of further research is to study minoxidil teratogenicity and its effect on the 2-nd and 3-rd generation of the offspring**.**

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