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Original Article

Evaluation of the effect of acyclovir inhalation on embryotoxicity parameters in laboratory rats

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ABSTRACT

This paper presents the study results of the embryotoxic effect of the antiviral drug acyclovir in terms of industrial toxicology. Experimental studies were performed on laboratory rats through inhalation modelling of the drug at the concentrations of 0.1 mg/m^3 and 1.0 mg/m^3. Concentration of 1.0 mg/m^3 caused a decrease in placenta size, number of litter and fetal survival with simultaneous increase in crown-rump length.

KEYWORDS: acyclovir, embryotoxicity, pharmaceutical industry, industrial toxicology, inhalation.

INTRODUCTION

The occupational health issues affecting pharmaceutical professionals have been discussed during the last decade in many publications [1-4]. Hazard concerns on occupational exposure to pharmacological substances differ from their therapeutic effects in patients. Employees who are in contact with such substances have to adjust their body functioning. However, while such a modification is desirable in patients, it is unacceptable for pharmaceutical professionals [5]. In addition, there is a difference in exposure routs of pharmaceutical substances under industrial conditions and in clinical practice. Inhalation is one of the most important exposure routes for biologically active compounds at the pharmaceutical enterprises. This aspect can significantly affect the compounds' bioavailability and, hence, toxicity [6].

Since 2004, the National Institute for Occupational Safety and Health (NIOSH) regularly lists drugs hazardous to pharmaceutical professionals. Approximately half of the drugs are classified as anti-cancer drugs, and remaining part includes hormonal agents, immunosuppressants, antiviral drugs, etc. The reproductive system is identified as one of the most vulnerable targets in pharmaceutical professionals [7].

This paper presents study results of the embryotoxic effect of the antiviral drug acyclovir in terms of industrial toxicology.

Nobel laureate Gertrude B. Elion who synthesized acyclovir in 1977 describes in her work [8] the mechanism of action of acyclovir. Subsequently, acyclovir was proved to cross the placental barrier [9], and the embryotoxic and teratogenic effects caused by its subcutaneous administration were identified [10, 11]. At the same time, the experiments with oral acyclovir in the course of reproductive/fertility studies in two generations of mice showed no side effects [12].

The purpose of our study was to evaluate the embryotoxic effects of acyclovir when inhaled, which is a typical exposure route in pharmaceutical enterprises.

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MATERIALS AND METHODS

Animals and reagents

Experimental studies of the embryotoxic effect of the antiviral drug acyclovir (IUPAC Name is 2-amino-9-(2-hydroxyethoxymethyl)-1~{H}-purin-6-one) were conducted in 30 non-pedigree female white rats aged between 3 and 3.5 months with a body weight of 180-230 g (supplier: "Biomodelservice", Kyiv, Ukraine). This work involving experimental animals was performed in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 18.03.1986).

Experimental design

The initial gestational age of rats was established using the vaginal swabbing method [13]. The animals were divided using the blind randomization method into three groups of 10 animals each: two experimental groups and one control group. The body weight was taken as the main randomization criterion.

Experimental animals received, through a probe, intranasal injections of the acyclovir solution (sterile isotonic 0.9% NaCl solution was used as the vehicle) at the doses corresponding to concentrations of 0.1 mg/m^3 and 1.0 mg/m^3 simultaneously for 20 days. The concentrations of the acyclovir substance were taken as the subthreshold and threshold values of the overall toxic effect during inhalation exposure. The animals in the control group received intranasal injections of normal saline.

The condition of pregnant rats was monitored daily to evaluate the embryotoxic effect of the studied drug. On gestation day 20, the animals were euthanized under etheric anaesthesia by cervical vertebrae dislocation. Then, a laparotomy was performed, and the uterine horns and ovaries were isolated and transferred into a plate filled with the saline solution. The yellow bodies in the ovaries, as well as the number of live, dead and resorbed foetuses in the uterine horns were identified. The fetal crown-rump length and weight, as well as the size and weight of placenta, were measured, and the fetoplacental index was calculated.

Evaluation criteria of reproductive toxicity

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 yellow bodies during pregnancy and the number of live foetuses. Then, the resulting value was identified as the percentage share of the yellow bodies during pregnancy. Pre-implantation mortality was determined using the difference between the number of yellow bodies and the number of implantation sites and resulting value was identified as the percentage share of the yellow bodies. 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 yellow bodies.

Statistical analysis

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

Statistical processing of the results was performed using Microsoft Excel. The Kolmogorov-Smirnov test was performed to verify the normality of distribution. The parametric data are described in terms of averages (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 evaluated using one-way ANOVA test followed by Tukey HSD test and Kruskal-Wallis test (H-test) in the cases of deviation in the distribution law from normal. Relevant changes were considered as significant, where the confidence level exceeded 95% ($p < 0.05$).

RESULTS

This study showed that the clinical status of female rats in the experimental groups with daily administration of intranasal acyclovir during pregnancy did not differ from that of control

animals. All animals consumed food, and responded adequately to tactile, sound and photic stimuli. The condition of skin cover and mucous membranes in rats from the experimental group did not visually differ from that in the control group. The intranasal administration of acyclovir did not affect the term of pregnancy. The normal gestation course in the control and experimental groups of animals was confirmed by weekly weighing of pregnant rats from gestation day 1 through day 19. No statistically significant differences were found in the weight gain of female rats (Figure 1).

The fetal respiration was spontaneous after the rupture of fetal membranes and cord cutting; the skin cover was pink. The skin surface was wrinkled, with large folds, the back was straightened, and the mouth, eyes and ears were closed.

The organometry analysis of placenta development in the experimental group of animals receiving acyclovir at a concentration of 0.1 mg/m^3 (Table 1) shows that the average placenta weight and diameter did not differ significantly from those in the control group. The morphometrical fetal parameters in this experimental group did not differ significantly from those in the control group.

Placenta size in the group exposed to acyclovir at a concentration of 1.0 mg/m^3 (Table 1) was 6.7% lower vs control group. In addition, this experimental group, however, showed a slightly (up to 1%) significant increase in fetal crown-rump length with no significant differences in fetal weight, and a decrease of 7.7% in the number of litter.

Based on the above data (Tables 1 and 2), parameters of embryotoxic effect such as the number of yellow bodies, the number of implantation sites and pre-implantation mortality were not statistically different in the experimental group and the control group. Post-implantation and total mortality parameters in both experimental groups did not differ from those in the parallel control group.

The dams in the experimental group receiving acyclovir at a concentration of 1.0 mg/m^3 showed a tendency towards decreased intrauterine survival rate by 7.8% vs control group.

DISCUSSION

This study did not find any clinical sign of maternal toxicity, as well as body weight loss and changes in food intake. Consequently, it can be

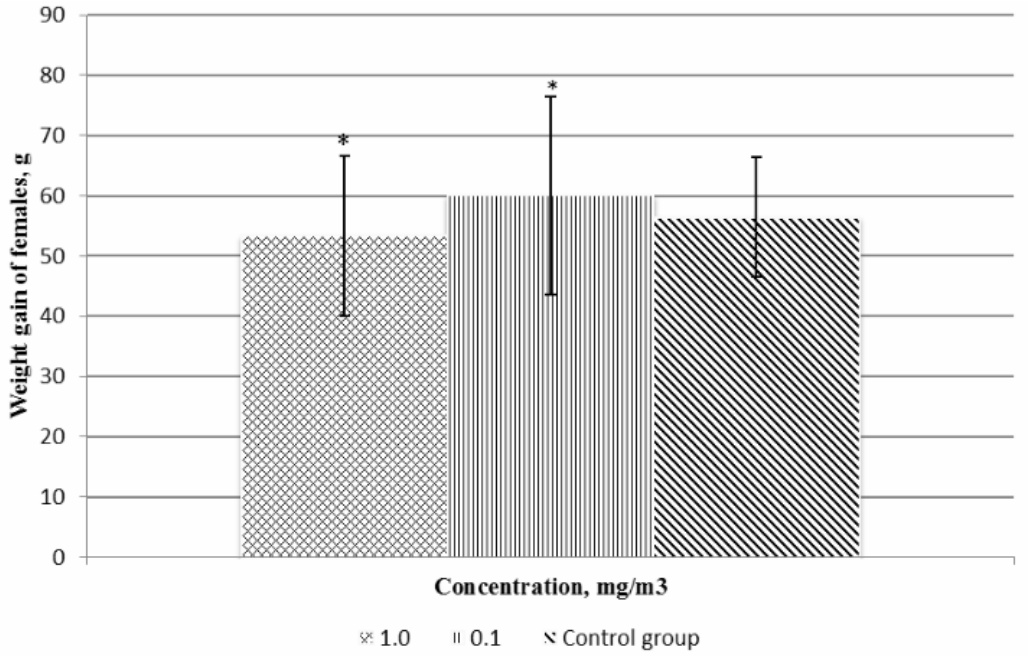

Figure 1. Effects of acyclovir on maternal weight gain (1-19 days). Data are reported as mean ± S.D. *$p < 0.05$, Tukey-Kramer Test, 10 animals per group.

Table 1. Fetal effect of acyclovir after inhaled administration once daily in pregnant female rats at the concentrations of 1.0 mg/m^3 and 0.1 mg/m^3 from gestation day 1 through day 19.

Parameter	Animal group		
	Control	**Exposed to acyclovir at a concentration of 1.0 mg/m^3**	**Exposed to acyclovir at a concentration of 0.1 mg/m^3**
Fetal weight, g (M ± SD)	3.6 ± 0.40	3.6 ± 0.40	3.4 ± 0.41
Crown-Rump Length, mm (M ± SD)	35.5 ± 2.21	35.7 ± 1.95*	34.8 ± 2.12
Placenta weight, g (M ± SD)	0.53 ± 0.09	0.48 ± 0.09	0.47 ± 0.08
Placenta size, mm (Me [Q1;Q3])	Me 15 [25% -13; 75% - 15]	Me 14 [25% -13; 75% - 15]*	Me 15 [25% -14; 75% - 15]
Yellow bodies count per 1 dam, units (M ± SD)	12.6 ± 0.98	12.33 ± 2.35	12.38 ± 0.74
Number of implantation sites per 1 dam, units (M ± SD)	10.7 ± 2.43	10.3 ± 1.93	10.6 ± 2.45
Number of live foetuses per 1 dam, units (M ± SD)	10.4 ± 2.57	9.6 ± 2.40*	10.2 ± 2.30
Number of resorption sites per 1 dam, units (Me [Q1;Q3])	Me 1 [25% -0; 75% - 1]	Me 0 [25% -0; 75% - 1]	Me 0 [25% -0; 75% - 1]
Fetal-placental index (M ± SD)	0.15 ± 0.04	0.13 ± 0.03	0.14 ± 0.03

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

Table 2. Estimated embryotoxicity parameters after inhaled administration of acyclovir in female rats during pregnancy.

Parameter	Control	Experimental groups	
		Exposed to acyclovir at a concentration of 1.0 mg/m^3	**Exposed to acyclovir at a concentration of 0.1 mg/m^3**
Total embryonal mortality, % (Me [Q1;Q3])	Me 8.3 [25% -3.8; 75% - 30.4]	Me 15.4 [25% -14.3; 75% - 28.6]	Me 12.5 [25% -6.3; 75% - 23.1]
Pre-implantation mortality, % (Me [Q1;Q3])	Me 8.3 [25% -3.8; 75% - 22.6]	Me 9.1 [25% -7.1; 75% - 25.0]	Me 8.0 [25% -0; 75% - 15.7]
Post-implantation mortality, % (Me [Q1;Q3])	Me 0 [25% -0; 75% - 5.0]	Me 0 [25% -0; 75% - 11.0]	Me 0 [25% -0; 75% - 8.5]
Intrauterine survival rate, % (Me [Q1;Q3])	Me 91.7 [25% -69.6; 75% - 96.2]	Me 84.6 [25% -71.4; 75% - 85.7]*	Me 87.5 [25% -76.9; 75% - 93.8]

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

concluded that acyclovir given at the study concentrations had no toxic effect on female rats.

The group of animals receiving acyclovir at a concentration of 0.1 mg/m^3 showed formation of the maturated fetal-placental complex, which contributed to fetal development, with somatometry parameters not statistically significantly different from the control values and normal average values for these parameters in the laboratory animals [14].

Reduced placenta size in the experimental group of animals after exposure to acyclovir at a concentration of 1.0 mg/m^3 (Table 1) shows a delay in placenta formation. It is generally recognized that the changes occurring in maternal body under extreme effects cause adaptive changes in the primary organs. In addition, this experimental group, with a significant decrease in the number of live foetuses per a dam, showed a tendency towards increased crown-rump length. These parameters do not exceed normal physiological limits [14], but at the same time affect the intrauterine survival rate. This may indicate the abnormal compensatory and adaptive system response: maternal body — placenta — foetus, and may be considered as an adaptation of the pregnant female body to ensure better fetal nutrition under conditions of destabilizing factors. The data obtained are in line with other studies [10, 11], which also showed a decrease in female fertility after exposure to both subcutaneous and intravenous acyclovir. The embryotoxicity induced by acyclovir at a concentration of 1.0 mg/m^3 may be associated with physiological changes in homeostasis, which resulted in such a negative fetal effect. Our study did not determine the concentration of acyclovir in the biological media. However, the data obtained from other studies on drug uptake in the amniotic fluid, fetal and placental tissues [15] of pregnant rats after intravenous administration of acyclovir confirm our assumptions.

CONCLUSION

To summarize the study results, acyclovir can be considered as a reproductive toxicant in accordance with EU Directive 93/21/EC under H361 code: “May cause potential harm to fertility or unborn child”. Further prospective epidemiological studies are required to assess the reproductive risk associated with this compound with respect to female employees of the chemical and pharmaceutical enterprises.

CONFLICT OF INTEREST STATEMENT

None to declare.

ABBREVIATIONS

IUPAC : International Union of Pure and Applied Chemistry
NIOSH : National Institute for Occupational Safety and Health

REFERENCES

1. Attaianese, E. and Duca, G. 2012, Work, 41, 1733-1738.
2. Binks, S. P. 2003, Occup. Med. (Lond.), 53, 363-370.
3. Gathuru, I. M., Buchanich, J. M., Marsh, G. M. and Dolan, D. G. 2015, Pharmaceut. Reg. Affairs, 4, 145.
4. Oddone, E., Negri, S., Morandi, F. and Imbriani, M. 2016, Compr. Anal. Chem., 73, 589-621.
5. Heron, R. J. L. and Pickering, F. C. 2003, Occup. Med. (Lond.), 53, 357-362.
6. Champmartin, C. and Clerc, F. 2014, J. Occup. Environ. Hyg., 11(2), 85-92.
7. Connor, T. H., MacKenzie, B. A., DeBord, D. G., Trout D. B. and O’Callaghan J. P. 2014, NIOSH List of antineoplastic and other hazardous drugs in healthcare settings. Atlanta, USA: National Institute of Occupational Safety and Health (NIOSH), Department of Health and Human Services, Center for Disease Control and Prevention; 2014. Report: 2014-138. www.cdc.gov/niosh/docs/2014-138/pdfs/2014-138.pdf
8. Elion, G. B. 1983, J. Antimicrob. Chemother., 12, 9-17.
9. Frenkel, L. M., Brown, Z. A., Bryson, Y. J., Corey, L., Unadkat, J. D., Hensleigh, P. A., Arvin, A. M., Prober, C. G. and Connor, J. D. 1991, Am. J. Obstet. Gynecol., 164, 569-576.
10. Komesu, M. C., Brentegani, L. G., Azoubel, R., Lopes, R. A. and Sala, M. A. 1995, Braz. Dent. J., 6(2), 91-94.

11. Snoor, J. M. and Kameel, M. 2013, JSMC, 3, 104-107.
12. Moore, H. L. Jr., Szczech, G. M., Rodwell, D. E., Kapp, R. W. Jr., de Miranda, P. and Tucker, W. E. Jr. 1983, Fundam. Appl. Toxicol., 3(6), 560-568.
13. Marcondes, F. K., Bianchi, F. J. and Tanno, A. P. 2002, Braz. J. Biol., 62(4a), 609-614.
14. Trakhtenberg, I., Sova, R., Sheftel, V. and Onikienko, F. 1978, Normal Parameters in Laboratory Animals in Toxicological Experiments (Modern concepts and methodological approaches, baseline parameters and constants) [Russian]. Moscow, Medicine, 208.
15. Brown, S. D., Bartlett, M. G. and White C. A. 2003, Antimicrob. Agents Chemother., 47(3), 991-996.