PHARMACOLOGY AND TOXICOLOGY

Effect of Dimephosphone (Monophosphonate) on the Course of Pregnancy and Fetal Development in Rats

R. Kh. Khafiz'yanova, I. M. Burykin, G. N. Aleeva, and A. P. Kiyasov

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Dimephosphone injected on days 1-19 of gestation did not cause fetal death and specific abnormalities in rats and did not modulate sex differentiation of fetuses. Morphological study of fetal liver revealed no pathological changes.

Key Words: *rat; dimephosphone; pregnancy; teratogenic effect*

It was shown in the beginning of the 20th century that some natural factors promote the development of congenital abnormalities in human and animal progeny. X-rays, radioactive isotopes, some metals and inorganic elements can modify fetal development in animals and humans. On the other hand, more and more facts indicating drug effects on the genetic system of the cells and embryonic development process are accumulated [4]. Therefore, comprehensive evaluation of drug effects on the reproductive parameters of experimental animals will help to reduce the probability of manifestation of teratogenic effects of drugs.

By the present time many organophosphorus compounds (OPC) acting as inhibitors and activators of cholinesterase were synthesized, studied, and introduced into practice. One of OPC groups exhibiting no anticholinesterase effects is the group of bi- and monophosphonates with P-C-P and P-C bonds. By their chemical composition these substances are similar to pyrophosphates involved in calcium and phosphorus metabolism in the body. The therapeutic value of these drugs is determined by their capacity to inhibit osteoclast-mediated bone resorption.

Biphosphonate drugs are widely used for the treatment of Paget disease, hypercalcemia of different origin, primary and secondary hyperparathyrosis, prevention of bone resorption in patients with osteoblastomas, *etc.* However, numerous side effects of biphosphonates limit their use. Some biphosphonates produce unfavorable effect on prenatal development of the progeny [10,11].

Monophosphonates exhibit a variety of pharmacological effects: nootropic, antidepressive, stress-protective, immunomodulating, *etc*. Dimephosphone (1,1-dimethyl-3-ketobutylphosphonic acid dimethyl ether) belongs to this group of substances. This compound is widely used in medical practice as a vasoactive drug normalizing CNS function in patients with cerebrovascular disorders [5,6]. However, reproductive safety of dimephosphone remains little studied.

We studied the effects of a course of dimephosphone therapy on pregnancy and embryonic development in rats.

MATERIALS AND METHODS

The study was carried out on 22 female random-bred albino rats (200-250 g) kept under standard vivarium conditions. The day when spermatozoa were first detected in vaginal smears was considered as day 1 of gestation. Pregnant rats were divided into 2 groups. Group 1 rats received distilled water (controls) and group 2 rats received dimephosphone (Arbuzov Institute of Physical Chemistry, Kazan Research Center,

Department of Pharmacology, Department of Normal Anatomy, Kazan State Medical University. ***Address for correspondence:*** rofija_kh@mail.ru. Khafiz'yanova R. Kh.

Russian Academy of Sciences). The drug (200 mg/kg) was injected intraperitoneally on days 1-19 of gestation. General status, behavior, water and food consumption, and dynamics of body weight gain of pregnant females were evaluated daily throughout the experiment.

The rats were sacrificed under light ether narcosis on day 20 of gestation and the viscera (ovaries, uterus, liver, kidneys, heart, spleen, and adrenals) were collected and their weight coefficients were estimated. After removal of fetuses the uterus was examined. We determined the number of implantation sites, resorption bodies, live and still fetuses in the uterus and the number of corpora lutea in the ovaries. Parameters of embryonic death (indexes of pre- and postimplantation mortality) were estimated by standard formulas [9]. All embryos and their placentas were weighed and measured in order to detect external developmental abnormalities. A part of rat litter was placed into Bouin's fixative for study by the Wilson—Dyban method and the other part of the litter was fixed in 96% ethanol for studies of bone skeleton by the Dawson—Dyban method [7]. The anogenital index was estimated as the ratio of fetal anogenital distance (AGD) to the cube root of its body weight [9].

Some embryos were fixed in 4% formalin and embedded in paraffin by routine methods. Sections of fetal liver were stained with hematoxylin and eosin and with antibodies to α-smooth-muscle actin (α-SMA, Dako). Histological study was carried out under a light microscope.

The results were processed by parametrical Student's test (for normal distribution) and by nonparametrical Mann—Whitney test (when distribution was not normal) [8].

RESULTS

Evaluation of the general status of rats revealed no symptoms of general toxic effect of dimephosphone on pregnant females at all terms of the experiment. Coefficients of rat viscera on day 20 of gestation were not changed. A significant increase in ovarian weight coefficient and higher body weight gain were observed in experimental animals at the end of the experiment (Table 1).

Injection of dimephosphone in the mean daily therapeutic dose did not change the general status and behavior of pregnant rats, which confirms good tolerance of drug therapy. Previous studies with repeated injections of the drug to nonpregnant females for 1 and 6 months also demonstrated its good tolerance [6].

Dimephosphone did not modulate embryo survival. No dead fetuses or females with complete resorption of embryos were observed in both control and experimental groups. The drug had no effect on litter size, number of corpora lutea, and total number of resorption sites. The indexes of pre- and postimplantation mortality in experimental group did not differ from those in the control (Table 2).

TABLE 1. Effect of Dimephosphone on Body Weight Gain and Visceral Weight Coefficients in Rats (*M*±*m*)

Parameter	Control	Dimephosphone
Body weight gain	67.69±6.87	95.00±14.57*
Weight coefficient for:		
ovaries	0.56±0.08	0.88±0.06*
uterus	14.78±2.35	19.09±1.62
liver	61.43±5.81	77.31±5.48
kidneys	7.52±0.47	7.42±0.71
heart	4.92±0.40	4.32±0.40
spleen	5.56±0.78	6.52±0.80
adrenals	0.39±0.01	0.44±0.04

Note. Here and in Tables 2 and 3: *$p<0.05$ compared to the control.

These findings attest to the absence of embryotoxic effects of dimephosphone injected to pregnant females.

It is assumed that spontaneous abortions can be a manifestation of embryotoxic effects of drugs in humans [12]. The incidence of spontaneous abortions increased in women employed in the production of OPC and toxic chemicals and contacting with these substances during pregnancy. Decreased embryo survival was observed in experiments on laboratory rats and mice treated with anticholinesterase OPC [1,3]. However, the study of the teratogenic effects of biphosphonates in doses causing no toxic effects revealed no embryotoxic effect in these animals [10]. Presumably, the

TABLE 2. Embryo Survival after a Course of Dimephosphone Injections to Pregnant Rats (*M*±*m*)

Number of	Control	Dimephosphone
Pregnant rats	13	10
Rats with live fetuses	13	10
Rats with dead fetuses	0	0
Rats with complete resorption	0	0
Corpora lutea	11.76±0.41	12.80±0.58
Sites of resorption		
early	0.50±0.33	0.60±0.60
late	0.50±0.23	0*
total	1.00±0.46	0.60±0.60
Mortality index		
preimplantation	0.22±0.06	0.20±0.04
postimplantation	0.08±0.04	0.05±0.05

embryotoxic effect of OPC depends on their pharmacodynamic and pharmacokinetic characteristics.

General delay of fetal growth is a manifestation of the teratogenic effect of biphosphonates [10]. In contrast to biphosphonates, dimephosphone had no effect on the weight and length of the placentas and fetuses, both male and female (Table 3). These data suggest that dimephosphone administered for 19 days did not modulate fetal growth and development.

Some authors reported the effects of chemicals on sex differentiation processes. A relationship between abnormal sex differentiation of some animals leading to sterility of males and females and concentration of polychlorinated biphenyls with estrogen activity was noted. The effects of the test drug on sex differentiation processes can be evaluated by AGD and male/female ratio [9]. Dimephosphone did not modify these parameters. The course of monophosphonate treatment did not lead to changes in the anogenital index of fetuses (Table 3).

Our findings suggest that the drug has no effect on fetal sex differentiation processes, which is in line with the data on biphosphonates [10,11,14].

Dimephosphone injected to pregnant rats did not cause specific congenital developmental abnormalities of fetal viscera (brain, heart, lungs, liver, spleen). No external abnormalities of the facial skull and limbs were seen at macroscopic examination. The incidence of variations in the development of the viscera, such as dilatation of renal pelvises and ureters, was virtually the same in the control and experimental fetuses and corresponded to published data [7,9]. The study of the bone system in both groups showed normal ossification and the absence of abnormalities in the development of vertebrae, ribs, sternum, pelvic bones, and other parts of the skeleton. The effect of dimephosphone monophosphonate on the bone system differs from the effects of biphosphonates. No effects of dimephosphone on the bone development were detected. According to published data, biphosphonates impair the formation and growth of the bone system [10, 11,14].

The embryo is highly sensitive to the damaging effects of teratogens because of immaturity of mechanisms responsible for detoxification and elimination of foreign substances from the body (*e.g.* immaturity of the liver detoxification systems). It was shown that morphological changes appear in embryonic tissues under the effects of drugs used in doses lower than the doses causing a teratogenic effect. This can be explained by anatomical structure of embryonic vessels: blood flowing from the placenta passes through the liver.

Injection of dimephosphone in a total dose of 3800 mg/kg did not cause pronounced morphological disorders in liver structure. Histological examination of the liver from experimental fetuses showed minor decrease in hepatocyte size and increase in the number of hemopoietic cells. Damage to mature liver cells stimulates proliferation of Ito cells, whose marker is desmin [2]. However, the study of expression of the proliferating cell nuclear antigen in the embryonic liver is little informative because of increased number of dividing cells. Moreover, transitory expression of desmin is observed in embryonic hepatoblasts [13]. Presumably, the key point in liver injury is associated with transdifferentiation of sinusoidal Ito cells into myofibroblasts, connective tissue precursors expressing α-SMA [2]. Immunohistochemical study of fetal liver tissue from experimental animals showed no expression of α-SMA. This fact indicates the absence of damaging effect of dimephosphone on rat embryonic liver.

Hence, our data indicate that long-term treatment with monophosphonate dimephosphone had no embryolethal, embryotoxic, and teratogenic effects and this drug can be used in pregnant women.

TABLE 3. Morphometric Parameters and Parameters of Sex Differentiation in Rat Fetuses after Dimephosphone Treatment during Gestation ($M \pm m$)

Parameter	Control	Dimephosphone
Fetal weight		
male	2.81±0.20	2.61±0.12
female	2.71±0.16	2.48±0.09
Fetal length		
male	38.61±0.77	38.51±0.67
female	37.69±0.71	37.63±0.51
Placental weight		
male	0.62±0.02	0.62±0.03
female	0.59±0.01	0.61±0.02
Placental diameter		
male	14.38±0.27	13.64±0.10*
female	14.08±0.28	13.68±0.29
Male/female index	1.14	1.23
AGD, mm		
male	2.07±0.06	2.11±0.06
female	1.12±0.02	1.06±0.02
Anogenital index		
male	1.47±0.02	1.53±0.02
female	0.81±0.008	0.78±0.02

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