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THE USE OF MINI PIGS — A NEW KIND OF EXPERIMENTAL ANIMAL — TO STUDY THE EMBRYOTROPIC ACTION OF PHENAZEPAM

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Experiments on a Siberian breed of mini pigs showed that phenazepam, in a dose of 1 mg/kg internally throughout the period of organogenesis, has neither embryotoxic nor teratogenic action. Parallel determinations of the blood level of the compound were made in pregnant animals. It can be concluded from the results of this investigation that mini pigs are a promising species with which to test the embryotropic activity of drugs.

KEY WORDS: embryotropic action; mini pigs; phenazepam.

In many countries, mini pigs are being used with increasing frequency as experimental animals in recent years. The reason is that the structure and functions of the principal physiological systems and the character of feeding and metabolism, including drug metabolism of these animals are closer to those of man than in any other experimental animal except, perhaps, monkeys [6]. It is difficult to enumerate all the fields of application of mini pigs in medico-biological research. Appropriate surveys on this subject can be consulted [2, 4, 6].

The study of the embryotropic action of drugs is made much more difficult because of the absence of a perfect experimental model. Experiments nowadays are carried out chiefly on small laboratory animals (rodents), which are cheap and convenient from the technical point of view. However, the results of such experiments have limited prognostic value for extrapolation to man. The use of mini pigs in this branch of research is a promising development, for these animals different from ordinary pigs in their more precocious development and higher fertility, and the physiology of reproduction and embryogenesis of these animals has been adequately studied. Mini pigs readily tolerate such surgical procedures as removal of fetuses at different stages of pregnancy.

Various factors, including genetic, hypoxia, irradiation, and virus infections, may cause teratogenesis both in man and in pigs. Malformations induced in pigs are similar clinically and pathologically to those observed in man. The difference in structure of the placenta (epitheliochorial in the pig, hemochorial in man) is not a serious obstacle, for the distance between the maternal and fetal blood is very small because of the development of sub- and intraepithelial capillaries in the maternal and fetal parts of the placenta.

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TABLE 1. Effect of Phenazepam on Embryogenesis in Mini Pigs

Experimental conditions	Pig. No.	Weight at sacrifice, kg	Stage of pregnancy at sacrifice, days	Number of piglets		Weight of piglets, g	Length of piglets, cm
				alive	dead		
Phenazepam, 1 mg/kg	1	47	110	7	0	506,0±29,1	21,5±0,3
	2	40	110	6	2	548,3±33,2	22,1±0,5
	3	65	108	7	0	557,8±19,1	22,1±0,3
	4	50	106	11	0	456,8±17,9	20,8±0,3
	5	34	109	6	0	510,0±13,4	21,2±0,3
Mean						508,9±18,5	21,4±0,24
Control	1	30	109	5	1	326,0±20,4	17,1±0,2
	2	60	107	8	1	205,0±8,1	15,5±0,3
	3	50	109	9	0	425,6±13,7	20,4±0,2
	4	40	106	8	0	556,0±22,2	21,4±0,5
Mean						391,3±74,5	19,2±1,4

The first experiments in which mini pigs were used to study teratogenesis showed a definite similarity between the developmental anomalies and those observed in man [8]. Attempts have been made to determine the periods of embryogenesis most sensitive to drugs [5].

This paper gives the results of a study of the embryotoxic and teratogenic action of the new Soviet tranquilizer phenazepam.

METHODS

Mini pigs of the Siberian breed [1] were used in the experiments. The animals were given phenazepam in a dose of 1 mg/kg once a day on a piece of bread during the period of organogenesis (from the 11th to the 35th days of pregnancy inclusive). The blood level of phenazepam was determined in parallel tests on pregnant animals. Blood was taken from a vein of the ear 30, 60, 120, and 240 min after the first and last doses of the drug. Phenazepam was determined quantitatively by a gas-chromatographic method on the Tsvet-110 chromatograph with a detector with constant recombination velocity. To judge the embryotropic action of the drug the animals were killed on the 3rd-7th day before giving birth to their young, i.e., on the 106th-110th day of pregnancy. The state of the reproductive organs was investigated at autopsy. The piglets were weighed, the cranial caudal length measured, and macroscopic inspection was carried out to reveal any developmental anomalies. Dawson's method [3], modified by the writers for a large test object, was used to define the skeletal defects. The number of centers of ossification was counted in the sternum and skull, the number of vertebrae and ribs, and the length of the limb bones of the shoulder and pelvic girdles was measured. To detect anomalies of the internal organs the piglets were autopsied, inspected macroscopically, and their organs studied histopathologically.

RESULTS

The results are given in Table 1. Clearly phenazepam, in a dose of 1 mg/kg, in the period of organogenesis had no embryotoxic action: Embryonic death was observed in solitary cases both in the experimental and in the control animals. The number of piglets in the litter was practically the same. The mean weight and the craniocaudal length of the piglets from the experimental animals were greater than in the control. This may have been due to the lower mobility of the pregnant animals receiving phenazepam for a fairly long period (24 days). Examination of the fetuses by Dawson's method revealed no developmental defects of the skeleton. Macroscopic inspection followed by microscopic study of the most important organs of the cardiovascular (heart, great vessels), respiratory (lungs), digestive (liver, exocrine part of the pancreas), urinary (kidneys), endocrine (adrenals, pancreatic islets), and reproductive (testes, ovaries) systems, and also of the spleen (an organ of hematopoiesis and immunogenesis) and brain revealed no differences between their structure in the experimental and control animals.

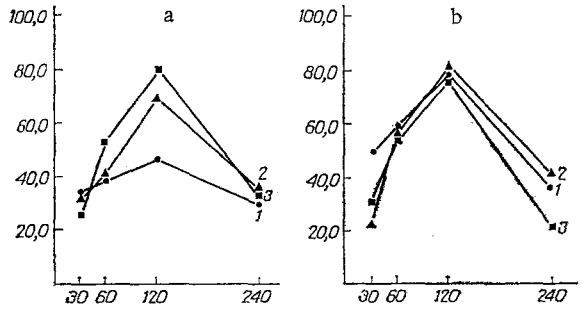

Fig. 1. Pharmacokinetics of phenazepam in blood of pregnant mini pigs during administration of single (a) and repeated (b) doses. 1, 2, 3) Nos. of experimental animals. Ordinate, blood phenazepam concentration (in mg/ml); abscissa, time after injection of phenazepam (in min).

Phenazepam in a dose of 1 mg/kg thus had neither embryotoxic nor teratogenic action.

In the dose used (1 mg/kg) phenazepam had a marked action on the behavior and state of the pregnant animals; they differed also in their sensitivity to the drug. On average 10-15 min after the first dose of the drug the animals showed general depression and disturbance of movement coordination. The animals fell asleep after 20-40 min. The sleep lasted about 6 h. The severity of the action of the drug correlated with its blood level in the animals (Fig. 1). Phenazepam was detected in the blood 30 min after injection; its concentration then rose to reach a maximum 2 h after injection, when sleep was deepest. The blood concentration of phenazepam was considerably reduced after 4 h. On administration of the second dose of phenazepam its sedative action was weaker and the sleep became less deep. The character of the pharmacokinetic curves for repeated administration was the same as for a single dose. For instance, when the blood concentration of phenazepam was determined on the 24th day of administration a maximum was reached also 2 h after injection. No cumulation of the drug was found.

It should be emphasized that the state and behavior of the animals after receiving phenazepam, like the character of the pharmacokinetic curves, showed definite coincidence with those observed in man after administration of compounds of the benzodiazepine series [7-9]. This increases the prognostic importance of the experimental data, especially when it is necessary to interpret whether the side effect being studied depends for its origin on the concentration of the drug in the blood serum.

It can be concluded from these investigations that the use of mini pigs is a promising method for testing the embryotropic activity of therapeutic substances. This conclusion is also supported by the relatively low cost of these animals and of their upkeep, and their ability to tolerate various experimental manipulations.

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