Preclinical Studies of General Toxic Properties of Preparations Containing Ultralow Doses of Antibodies to Endogenous Regulators

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Preclinical study of the safety of 6 preparations containing ultralow doses of antibodies to endogenous regulators showed that they are relatively safe, are well tolerated by animals in doses more than 1000-fold surpassing the therapeutic dose for humans, and produce no general toxic effect on the organism of laboratory animals.

Key Words: *ultralow doses of antibodies; acute toxicity; chronic toxicity; local irritating effect; preclinical study*

Some preparations belonging to a new class of drugs, ultralow doses of antibodies (ULD AB), are now approved for clinical use [8,9]. Here we evaluated general toxic effects (acute and chronic toxicity and local irritating effect) of 6 ULD AB preparations.

MATERIALS AND METHODS

The study was performed according to methodical recommendations for the study of general toxic effects of pharmacological substances [6].

The test preparations: anaferon (ULD AB to IFN-γ in dilutions C12+C30+C200), artrofoon (ULD AB to TNF-α in dilutions C12+C30+C200); afala (ULD AB to prostate-specific antigen in dilutions C12+C30+C200), impaza (ULD AB to NO synthase in dilutions C12+C30+C200), poetam (ULD AB to erythropoietin in dilutions C12+C30+C200), and proproten-100 (ULD AB to S-100 protein in dilution C1000) are colorless fluids, homeopathic dilutions of affinity-purified antibodies to the corresponding endogenous regulators in a solution (equivalent concentration is 10^{-24} mass fractions).

The experiments were carried out on mature albino outbred rats and mice (Laboratory of Biomodeling, Institute of Pharmacology, Tomsk Research Center) and on Chinchilla rabbits (Rassvet nursery, Tomsk). The animals were maintained according to European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasburg, 1986).

Acute toxicity of the test preparations was determined after single (artrofoon and proproten-100) or double with 3-h interval (anaferon, afala, impaza, and poetam) administration in MTD depending on the administration route [6]: intragastrically in a dose of 25 ml/kg and intraperitoneally in a dose of 50 ml/kg for mice and intragastrically in a dose of 15 ml/kg and intraperitoneally in a dose of 25 ml/kg for rats.

For evaluation of chronic toxicity, the test ULD AB preparations were administered in doses of 7.5 и 15 ml/kg (the first dose is close to the effective dose and the second dose corresponded to maximal tolerated dose for intragastric administration) 7 times a week. Controls received distilled water in a volume

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of 15 ml/kg. The rabbits received the test drugs in the dose corresponding to daily water consumption (50 ml/kg, water was not given) for 6 months. Each group included 10 animals (5 males and 5 females).

Toxicity of the test compounds was evaluated by the general state, body weight gain, body temperature, parameters of peripheral blood, bone marrow, function of the liver, kidney, CNS, heart (ECG), and by the results of morphological study of internal organs and their weights (brain, heart, lungs, liver, thymus, spleen, kidneys, gastrointestinal tract, adrenal glands, gonads, pancreas, thyroid gland, and pituitary) [2-5,10]. The study was performed after 3- and 6-month treatment with the test preparations and 2 weeks after withdrawal. The rats were euthanized by decapitation under ether narcosis. In rabbits, the blood from the marginal ear vein was drawn after 3- and 6-month treatment for evaluation of complete blood count and biochemical parameters. The animals were euthanized after 6 months (intravenous injection of saturated KCl solution, 1 ml/kg). After cardiac arrest, the animals were autopsied, the urine was sampled for biochemical analysis and internal organs were isolated for macro- and microscopic examination.

Numerical data were processed by methods of variation statistics using Student's *t* test and Mann—Whitney test.

RESULTS

Single administration of the test preparations in maximally possible doses did not induce animal mortality; therefore, the parameters of acute toxicity were not determined. In light of this, LD_{50} can be considered to be equal to the greater administered maximal tolerated dose of the preparations depending on the administration route, which suggests that these preparations can be classified as class 4 substances (low-hazard substances) according to GOST 12.1.007-76.

Evaluation of chronic toxicity showed that daily intragastric administration of the test preparations in doses of 7.5 and 15.0 ml/kg and daily intake of the test preparations in a dose of 50 ml/kg by rabbits for 6 months caused no animal death; we revealed no changes in animal behavior, appetite, excretions, and state of visible mucosa, fur, skin, *etc*. We revealed no pathological changes in the studied parameters characterizing the functions of the major internal organs and systems (body weight and temperature, peripheral blood, bone marrow, liver, kidney, CNS, ECG). Pathomorphological (macro- and microscopic) study after 6-month treatment (rats and rabbits) and 2 weeks after withdrawal (rats) revealed no pathological changes in the internal organs. Histological examination also showed that the preparations

TABLE 1. Body Weight (g, Numerator) and Body Weight Gain (%, Denominator) during Afala Treatment (*M*±*m*)

Term of experiment	Control		Dose, ml/kg			
			7.5		15.0	
	males	females	males	females	males	females
Before treatment	281±5	228±6	273±7	227±5	268±6	230±3
3 months	448±14 / 61±4	359±11 / 57±5	460±12 / 73±5	334±9 / 48±3	454±22 / 72±6	325±5 / 42±2*
4 months	482±20 / 73±6	367±14 / 61±5	502±14 / 89±6	354±9 / 57±3	484±19 / 85±6	334±9 / 46±3*+
5 months	498±18 / 79±5	376±13 / 65±6	519±14 / 95±7	357±10 / 59±4	500±21 / 91±7	345±7 / 51±2*
6 months	517±14 / 83±5	386±16 / 69±6	533±14 / 101±6*	363±10 / 61±3	508±19 / 94±6	346±7 / 52±2*+
2 months after withdrawal	521±20 / 87±3	414±22 / 86±8	533±24 / 105±10	370±16 / 61±5*	537±17 / 112±7*	338±5 / 53±2*

Note. Here and in Tables 2 and 3: $p<0.05$ compared to: *control, +females of experimental groups.

produced no local irritating effects on the gastric mucosa.

At the same time, changes were produced by long-term administration of some test preparations in high doses. For instance, moderate and transient decrease in the content of immature and mature neutrophilic granulocytes in the bone marrow was noted in rats after 6-month treatment with anaferon. This was not specific toxic effect of the preparation, because the number of leukocytes in the peripheral blood was not changed.

After 6-month afala treatment, a dose-dependent decrease in body weight gain was observed in female rats by the end of the experiment (Table 1).

Long-term administration of impaza in the maximum dose produced an anabolic effect in male and female rats (Table 2).

It should be noted, that the number of eosinophils (Table 3) decreased (but remained within the normal) in rats after long-term treatment with poetam.

Thus, preclinical study of the toxic effects of ULD AB to endogenous regulators of functions showed that all test preparations are relatively safe, are well tolerated by experimental animals in doses considerably (>1000-fold) surpassing the therapeutic doses for humans, and produce no general toxic effects. The observed single disturbances after long-term administration of some preparations in the

TABLE 2. Body Weight (g, Numerator) and Body Weight Gain (%, Denominator) during Impaza Treatment (*M*±*m*)

Term of experiment	Control		Dose, ml/kg			
			7.5		15.0	
	males	females	males	females	males	females
Before	190.9±6.1	197.3±9.1	200.0±8.0	202.7±4.9	198.0±6.9	200.7±5.3
3 months	439.1±11.5 / 131.7±8.1	310.9±9.8 / 59.4±5.6	444.7±13.1 / 124.7±6.3	325.8±5.3 / 61.1±3.5	445.0±6.4 / 129.7±8.3	322.7±7.8 / 61.1±2.2
4 months	453.8±8.7 / 135.4±8.0	312.5±15.1 / 55.2±5.4	475.5±18.6 / 137.3±7.9	320.0±5.4 / 58.4±4.7	473.3±5.8 / 154.2±11.4	348.2±9.3* / 71.0±3.2*
5 months	456.3±19.5 / 135.5±8.9	326.3±17.0 / 61.6±4.7	507.3±18.1 / 153.1±7.5	328.8±5.2 / 62.7±4.7	498.9±7.9 / 168.2±13.0*	364.6±11.7 / 78.9±4.3*
6 months	506.7±6.7 / 148.6±14.9	335.0±20.2 / 77.8±9.4	538.0±17.4 / 164.8±15.8	347.5±8.5 / 64.1±5.4	525.0±9.6 / 192.9±11.1*	382.0±17.2 / 92.7±7.6
2 months after withdrawal	490.0±5.8 / 140.0±14.6	335.0±20.2 / 77.8±9.4	528.0±13.9 / 160.2±15.8	337.5±2.5 / 59.6±6.1	517.5±6.3* / 188.7±10.1*	374.0±22.1 / 87.9±5.9

TABLE 3. Peripheral Blood Eosinophil Count (g/liter) after 6-Month Poetam Treatment and 2 Weeks after Its Withdrawal (*M*±*m*)

Term of experiment	Control		Dose, ml/kg			
			7.5		15.0	
	males	females	males	females	males	females
6 months	0.87±0.24	0.43±0.09	0.24±0.09*	0.27±0.06	0.36±0.12*	0.18±0.05*
2 months after withdrawal	0.59±0.14	0.44±0.11	0.24±0.09*	0.19±0.03*	0.39±0.13	0.10±0.09*

maximum dose cannot be contraindications for their clinical use.

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