Some Aspects of Toxicological Activity of Kardosten after Chronic 6-Month Administration to Rats

V. I. Petrov, L. I. Bugaeva, S. A. Lebedeva, E. V. Lavrova, Yu. L. Dugina*, I. A. Kheifets*, Yu. A. Zabolotneva*, S. A. Sergeeva*, and O. I. Epstein*

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Chronic treatment of rats with kardosten for 6 months had a positive effect on some ECG values and behavior without disordering the hepatorenal functions. All effects of the drug observed during a course of treatment were leveled and the parameters reached the basal values within 2 weeks after drug discontinuation, this confirming its safety.

Key Words: *chronic toxicity; ultralow dose antibodies to type 1 angiotensin II receptor C-terminal fragment; chronic cardiac failure*

Kardosten is an ultralow dose preparation of affinely purified antibodies to type 1 angiotensin II receptor C-terminal fragment (Materia Medica Holding), characterized by antihypertensive and cardioprotective effects and used for the treatment of chronic cardiac failure (CCF) [2,3,5,7]. Previous toxicological studies showed that the drug is not cumulated and by toxicity can be referred to low toxic substances, because it caused no animal death after intragastric and intraperitoneal administration to mice and rats in doses maximally allowed by methodological recommendations of the Ministry of Health and Social Development of the Russian Federation (2005) [4]. The study revealed no changes in the behavioral, neuromuscular, and autonomic reactions. Just short-term (3-4 h after kardosten injection) trends to higher motor and behavioral activity, respiration rate, improvement of blood supply to peripheral tissues were recorded. Rapid onset and leveling of these effects and the absence of cumulative properties indicate good bioavailability of the drug, its rapid inactivation and elimination from the body.

Department of Pharmacology, S. M. Kirov Military Medical Academy, St. Petersburg, Russia; *Materia Medica Holding Research-and-Production Company, Moscow, Russia. ***Address for correspondence:*** i.v.zarubina@inbox.ru. I. V. Zarubina

We studied the effects of kardosten on behavior, cardiovascular system, and functional activities of the liver and kidneys of rats during 6-month course treatment.

MATERIALS AND METHODS

Experiments were carried out on 90 outbred adult albino rats of both genders initially weighing 160-180 g. Toxicological studies were carried out in accordance with "Manual for Experimental (Preclinical) Studies of New Pharmacological Substances" (2005). Before the study, the rats were divided into 3 groups, 30 per group (15 females and 15 males). The animals received a course of kardosten intragastrically for 6 months in a dose of 5 ml/kg (group 2) and 15 ml/kg (group 3). Controls (group 1) received distilled water in the same volume intragastrically (through a metal tube) throughout the entire experiment. The effects of the drug on behavior, cardiovascular system, detoxifying and excretory functions of the liver and kidneys were evaluated on days 3, 6, and 2 weeks after drug discontinuation. General status and body weight gain were evaluated throughout the study.

Behavioral activity of rats was studied in the open field test by the common method. Cardiovascular sta-

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TABLE 1. Effects of Kardosten on Rat Behavior in the Open Field Test (*M*±*m*)

Animal gender; parameters		Course of								
		3 months			6 months			1 month after drug discontinuation		
		control	kardosten, 5 ml/kg	kardosten, 15 ml/kg	control	kardosten, 5 ml/kg	kardosten, 15 ml/kg	control	kardosten, 5 ml/kg	kardosten, 15 ml/kg
Males	horizontal activity	21.70±1.82	16.40±2.54	21.60±2.35	24.80±1.82	14.30±1.78*	9.20±1.44*	22.29±3.18	21.63±1.85	20.83±2.09
	vertical activity	4.60±0.95	5.40±0.91	7.70±1.31	5.60±0.54	4.70±0.46	4.20±1.14	6.43±1.00	6.38±1.45	5.83±1.45
	hole activity	2.90±0.72	3.3±0.6	2.90±0.74	3.60±0.54	4.70±0.87	1.00±0.33	2.14±0.83	3.13±0.67	1.67±0.33
	grooming	4.70±1.03	5.20±0.85	5.30±1.26	4.60±0.31	2.40±0.34*	4.30±0.65	5.14±0.83	4.00±0.65	3.50±0.62
	defecation	1.20±0.57	0.80±0.36	1.90±0.81	2.00±0.37	0.60±0.24	1.00±0.58	0.14±0.14	0.24±0.12	0.50±0.24
Females	horizontal activity	30.90±3.46	23.00±1.41	30.30±2.42	15.40±1.59	12.80±0.88	15.30±1.08	16.00±1.58	15.60±1.78	15.20±1.59
	vertical activity	10.90±1.45	7.5±1.9	8.10±1.05	4.80±0.76	4.00±0.68	3.9±0.7	4.8±0.8	4.40±0.51	4.00±0.71
	hole activity	3.10±0.48	2.80±0.42	4.70±0.75	3.90±0.62	2.30±0.56	1.80±0.46*	3.20±0.58	2.6±0.6	3.00±0.71
	grooming	5.50±0.93	4.10±1.06	3.6±0.6	4.0±1.0	3.30±0.49	3.00±0.53	5.60±0.81	4.20±0.73	4.40±0.51
	defecation	1.0±0.7	0.80±0.47	0.10±0.02	1.70±0.76	1.60±0.73	1.10±0.59	1.20±0.37	1.40±0.51	1.60±0.24

Note. Here and in Table 2: *p<0.05 compared to control.

TABLE 2. Effects of Kardosten on ECG Parameters

Animal gender; parameters		Course of								
		3 months			6 months			1 month after drug discontinuation		
		control	kardosten, 5 ml/kg	kardosten, 15 ml/kg	control	kardosten, 5 ml/kg	kardosten, 15 ml/kg	control	kardosten, 5 ml/kg	kardosten, 15 ml/kg
Males	*R*, mV	0.23±0.07	0.31±0.02	0.33±0.04	0.29±0.03	0.30±0.01*	0.43±0.05	0.22±0.03	0.22±0.01	0.21±0.02
	R-R, sec	0.15±0.01	0.18±0.01	0.190±0.015	0.150±0.003	0.180±0.003*	0.18±0.01	0.140±0.003	0.140±0.003	0.15±0.02
	QRS, sec	0.016±0.001	0.014±0.001	0.015±0.001	0.016±0.001	0.015±0.001	0.015±0.002	0.014±0.001	0.015±0.001	0.013±0.001
	QRST, sec	0.045±0.001	0.041±0.006	0.040±0.006	0.045±0.001	0.043±0.004	0.042±0.008	0.034±0.003	0.039±0.001	0.035±0.003
	heart rate, bpm	392.90±17.86	334.00±10.73	313.80±23.18	391.70±8.33	327.50±5.85*	341.40±17.56	445.10±10.99	429.70±10.99	417.50±50.38
Females	*R*, mV	0.27±0.02	0.31±0.02	0.29±0.04	0.27±0.02	0.29±0.02	0.30±0.01	0.23±0.06	0.26±0.01	0.24±0.01
	R-R, sec	0.14±0.01	0.16±0.01	0.17±0.01	0.15±0.01	0.170±0.003	0.18±0.01*	0.140±0.009	0.140±0.003	0.140±0.006
	QRS, sec	0.015±0.001	0.014±0.001	0.013±0.001	0.015±0.001	0.014±0.001	0.013±0.001	0.013±0.001	0.015±0.001	0.015±0.001
	QRST, sec	0.043±0.004	0.045±0.001	0.041±0.003	0.039±0.005	0.041±0.002	0.042±0.001	0.034±0.002	0.037±0.003	0.036±0.004
	heart rate, bpm	421.70±25.22	378.20±25.21	362.10±19.78	401.20±15.48	360.30±7.35	334.00±10.73*	442.90±29.74	439.30±9.52	422.50±17.78

tus was evaluated by ECG, the functions of the liver and kidneys were evaluated by classical bromsulfalein and phenol red load tests.

RESULTS

No differences in the general status and body weight gain in animals treated with kardosten for 6 months and controls were detected. Testing of animals in the open field showed behavioral shifts, which depended on the drug dose and duration of treatment. After 3 months of treatment (Table 1), horizontal activity and defecation rate in group 2 rats decreased by 25 and 20-30%, respectively. The orientation and exploratory activity (rearing episodes and hole reflex) virtually did not change. In group 3, changes in behavioral activity depended on animal gender. In females, the number of explored holes increased by 30%, emotional behavior (grooming and defecation acts) decreased by 35%, and number of horizontal ventures did not change in comparison with the control. In males, horizontal activity did not change either, while the number of rearing episodes increased by 10-15%. Later studies (after a 6-month course) showed lower horizontal and exploratory activities of different degree in males and females. In males, horizontal activity decreased significantly depending on the dose (by 42.3 and 62.9% in groups 2 and 3, respectively). In females, hole activity decreased in a dose-dependent manner (by 41 and 50% in groups 2 and 3, respectively). These changes were completely leveled, reaching the level in the control, within 2 weeks after the drug discontinuation.

Hence, it seems that kardosten modulates central mechanisms of behavior regulation during a 6 months course of treatment. The formation of such elements of behavioral reactions as horizontal activity, hole reflex (exploratory activity), grooming and defecation acts depends on activities of the central monoaminergic and cholinergic structures [1,6]. Heterospecificity of drug interactions with these structural receptors depends on drug dose and duration of treatment. The effects of kardosten on horizontal activity, hole reflex, defecation acts were shown, which attests to possible drug interactions with monoaminergic structures of the brain.

Analysis of ECG (Table 2) showed significant changes in cardiac conductivity and contractile activity irrespective of animal gender. The *R* wave amplitude characterizing the force of heart contractions increased by 8-11%, while *QRS* interval shortened by 6-13% and the *R-R* interval increased by 20%. Heart rate decreased by 13-15%. These aspects of positive inotropic and negative chronotropic effects of kardosten not depending on its dose and duration of treatment seem to indicate a positive effect of the drug on the myocardium. The increase of cardiac contractions force under the effect of kardosten paralleled by the diastole prolongation was leveled 2 weeks after the drug discontinuation, this, in turn, indicating that the drug had no negative effects on the myocardium.

Drug treatment was associated with stimulation of renal excretory function and hepatic absorption, excretion, and detoxifying functions. Hexenal test showed shorter narcotic-induced sleep in animals of both groups treated with kardosten, by 35% in males and by 20% in females. Bromsulfaleine test showed reduction of the stain concentration in the blood in comparison with the control by 27% (drug dose 5 ml/kg) and 20% (drug dose 15 ml/kg) in females and by 10 and 30%, respectively, in males. Phenol red test showed a trend to activation of the renal excretory function. Urine (and phenol red) excretion increased by 12 and 30% after a 6-month treatment in daily doses of 5 and 15 ml/kg, respectively. The parameters normalized after kardosten discontinuation.

Hence, a 6-month course of kardosten in doses of 5 and 15 ml/kg had a reversible mild depriming effect on rat behavior, presumably mediated through the central monoaminergic structures. The drug had a positive effect on heart work and exhibited positive inotropic and negative chronotropic effects, which implies the effects on energy resources and oxygen consumption by the myocardium. No pathological effects on the renal and hepatic functions were recorded over 6 months of kardosten treatment. The drug had a positive effect on liver biotransformation processes and renal excretory function. All effects of kardosten recorded in rats over a 6-month course of treatment were leveled and reached the initial values within 2 weeks after the drug was discontinued, which proves its high safety.

REFERENCES

1. G. V. Kovalyov, *Nootropic Drugs* [in Russian], Volgograd (1990).
2. A. L. Markel', D. R. Kudryashova, A. V. Martyushev, *et al.*, *Byull. Eksp. Biol. Med.*, Suppl. No. 4, 64-66 (2002).
3. V. I. Petrov, V. V. Ivanenko, N. A. Davydova, *et al.*, *Serdechn. Nedost.*, **8**, No. 4, 175-177 (2007).
4. *Manual of Experimental (Preclinical) Studies of New Drugs: Training Aid for Medical Upgrading*, Ed. R. U. Khabriev [in Russian], version 2, Moscow (2005).
5. I. N. Tyurenkov, A. N. Nazvanova, N. G. Chepurina, *et al.*, *Byull. Eksp. Biol. Med.*, **143**, No. 4, 411-413 (2007).
6. V. N. Stock, *Drug Therapy in Neurology. Practical Guide* [in Russian], version 2, Moscow (2000).
7. O. I. Epstein, *Ultralow Doses (A History of a Study)* [in Russian], Moscow (2008).