Experimental Study of Hypotensive Effect of Kardos in Rats with Inherited Hypertension

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Kardos, a preparation containing ultralow doses of antibodies to C-terminal fragment of type 1 receptor of angiotensin II, intragastrically administered to SHR rats with hereditary hypertension for 28 days reduced blood pressure by 14.8%. Kardos was not inferior to losartan and, in contrast to the latter reduced HR by 9.4%.

Key Words: *kardos; losartan; hypertension; SHR rats; ultralow doses*

The rennin-angiotensin system (RAS) participates in the regulation of physiological functions of the organism [5,7]. An important component of RAS, angiotensin II, is synthesized from angiotensin I under the effect of angiotensin-converting enzyme and chymases [3]. Angiotensin II is also involved into the regulation of blood pressure (BP), electrolyte balance in the body, and hormonal background and modulates growth processes in the organism. Most physiological effects of angiotensin II (vasoconstriction, stimulation of sympathetic innervation of the heart and vessels, secretion of aldosterone, stimulation of cell proliferation) are mediated by type 1 receptors (AT1 receptors). Activation of type 2 receptors induce opposite effects [3,8].

Increased activity of RAS can lead to the development of hypertension, heart hypertrophy, and heart failure [1,2].

Several classes of drugs can regulate activity of RAS. Inhibitors of angiotensin-converting enzyme disturbing angiotensin II synthesis and reducing its release into the circulation are widely used in clinical practice. However, these preparations produce a number of side effects (cough, skin itch, headaches, and reduced libido). Blockers of angiotensin II receptors selectively affecting AT1 receptors also induce BP drop [4].

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Here we studied the antihypertensive effect of ultralow doses of antibodies to C-terminal fragment of AT1 angiotensin II receptor (kardos) in rats with hereditary hypertension (SHR rats).

MATERIALS AND METHODS

Hypotensive effect of kardos was studied on 38 SHR male rats weighing 350±50 g (age 4.5-5 months) divided into 3 groups: group 1 rats received distilled water (2.5 ml/kg intragastrically) and rats of groups 2 and 3 intragastrically received 10 mg/kg losartan and 2.5 ml/kg kardos, respectively. The preparations were administered for 28 days. The animals were maintained under standard vivarium conditions (5-6 rats per cage, according to FELASA/ICLAS regulations) at 12-h light-dark regimen, constant ventilation, and 21-25°C, the bedding was changed weekly. The animals had water and food *ad libitum.*

On day 29, arterial and venous catheters were implanted to rats under sodium ethaminal narcosis (40 mg/kg). On the next day, mean (BP_{mean}), systolic, and diastolic BP and HR were recorded in alert rats. For the study of reflex activity of the cardiovascular system, 1.5 μg/kg sodium nitroprusside (Sigma) and after 15 min 2 μg/kg phenylephrine (Sigma) were injected intravenously. Baroreflex coefficient (BRC)

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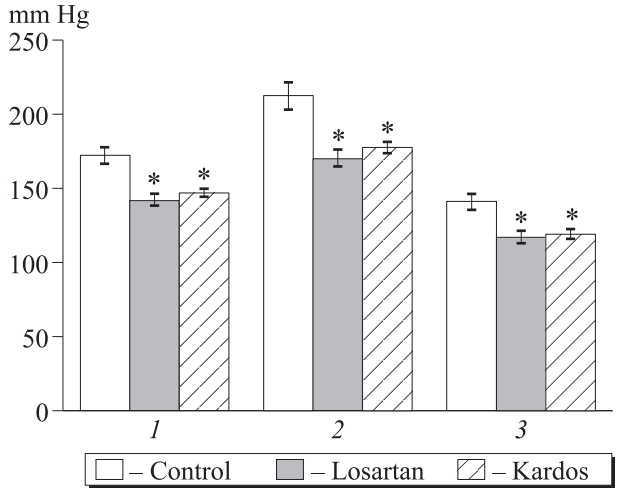

Fig. 1. Effect of kardos and losartan on BP_{mean} (*1*) and systolic (*2*) and diastolic (*3*) BP in SHR rats. Here and on Figs. 2-4: *$p<0.05$ compared to the control.

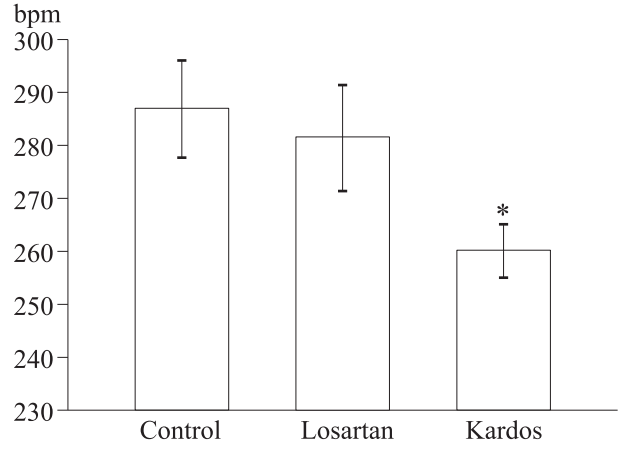

Fig. 2. Effect of kardos and losartan on HR in SHR rats.

was calculated as the ratio of ΔHR to ΔBP_{mean} in response to sodium nitroprusside and phenylephrine administration. For evaluation of the effect of preparations on RAS, angiotensin II (1 pmol/liter, Sigma) was injected intravenously 1 h after baroreflex testing. For evaluation of the contribution of NO into BP formation, a non-selective inhibitor of NO synthesis L-NAME (5 mg/kg, Sigma) was injected 1 h after angiotensin II.

The data were processed statistically using nonparametric Mann—Whitney test.

RESULTS

Course treatment with losartan (group 3) and kardos (group 2) reduced BP_{mean} by 14.8 and 17.7%, respectively, compared to the control ($p<0.05$, Fig. 1). Lower values of BP_{mean} in both groups were determined by a decrease in both systolic and diastolic BP. In group 3 rats, systolic and diastolic BP were below the control values by 16.5% ($p<0.05$) and 15.6% ($p<0.05$), respectively, in group 2 rats the corresponding decrease was 20% ($p<0.05$) and 17.1% ($p<0.05$, Fig. 1). In contrast to losartan, kardos reduced HR by 9.4% ($p<0.05$) compared to the control (Fig. 2).

Thus, kardos was not inferior to losartan by its hypotensive effect in SHR rats and in contrast to the latter exhibited a negative chronotropic effect.

For evaluation of the effect of kardos on reactions of the cardiovascular system we studied changes in BRC in response to BP increase caused by α_1-adrenoreceptor agonist phenylephrine and to BP drop caused by NO donor sodium nitroprusside.

Changes in BP_{mean} and HR in animals receiving distilled water, losartan, and kardos in response to sodium nitroprusside and phenylephrine revealed no significant changes between the experimental groups (Tables 1 and 2). BRC in response to BP rise cau-

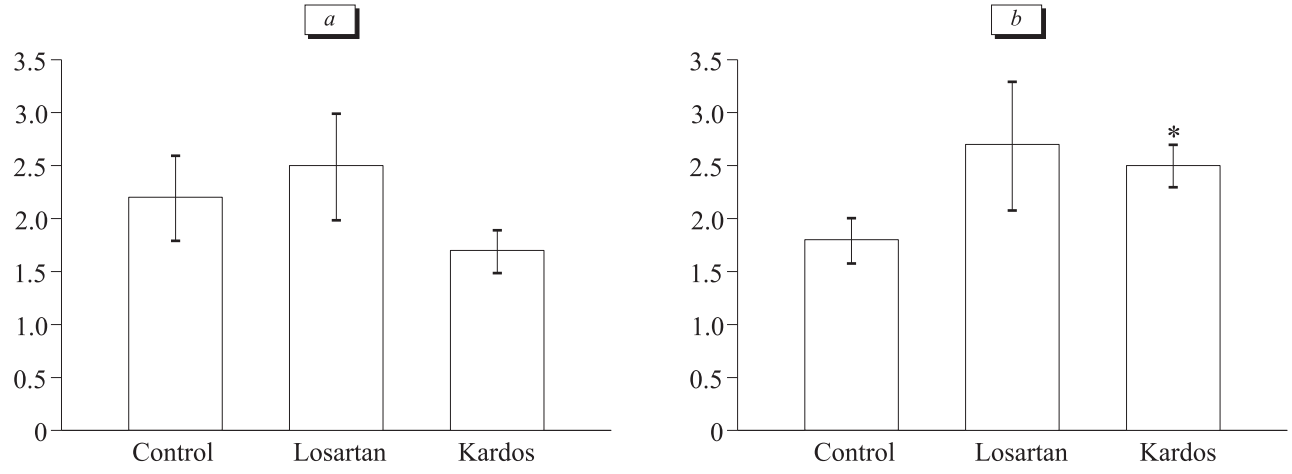

Fig. 3. Effect of kardos and losartan on BRC in SHR rats after administration of phenylephrine (*a*) and sodium nitroprusside (*b*).

sed by phenylephrine in groups 2 and 3 did not differ from the control. At the same time, in group 3 BRC increased by 38% ($p<0.05$ compared to the control) in response to injection of sodium nitroprusside (Fig. 3).

Kardos had no effect on BP_{mean} response to administration of angiotensin II. It should be noted that BP_{mean} in group 2 after injection of angiotensin II was significantly lower than in the control group and group 3 (Fig. 4 and Table 3). No significant differences by the time of attaining maximum BP_{mean} between the experimental groups were noted (Table 3).

Neither kardos, nor losartan modulated BP rise caused by intravenous bolus injection of L-NAME.

Thus, daily intragastrical administration of kardos for 28 days significantly decreased BP_{mean} and systolic and diastolic BP. The hypotesnsive effect of the preparation in a dose of 2.5 ml/kg was comparable to the hypotensive effect of losartan in a dose of 10 mg/kg. In contrast to losartan, the hypotensive effect of kardos was associated with a significant decrease in HR, which attests to normalization of cardiac activity. The development of hypertension is often accompanied by severe tachycardia, which reduces filling of heart ventricles and impairs heart output; therefore, the

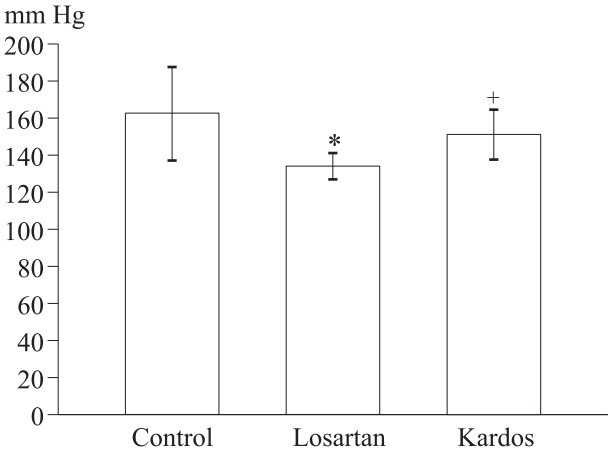

Fig. 4. Effect of kardos on changes in BP_{mean} in response to administration of angiotensin II. $^{+}p<0.05$ compared to losartan.

TABLE 1. BP_{mean} and HR before (Baseline) and after Injection of Phenylephrine (Experiment, *M±m*)

Group	BP_{mean}, mm Hg				HR, bpm		
	baseline	experiment	Δ, mm Hg	Δ, %	baseline	experiment	Δ, bpm
Control	161.3±17.1	173.0±21.9	11.7±6.5	7.1±3.5	286.8±28.2	265.7±28.4	-21.2±6.2
Losartan	138.5±10.7	147.8±10.5	9.3±1.5	6.8±1.1	275.8±29.4	252.3±18.9	-23.5±13.6
Kardos	150.2±11.8	162.5±14.0	12.4±1.4	8.2±0.9	269.7±7.2	249.3±5.5	-20.5±2.4

TABLE 2. BP_{mean} and HR before (Baseline) and after Injection of Sodium Nitroprusside (Experiment, *M±m*)

Group	BP_{mean}, mm Hg				HR, bpm		
	baseline	experiment	Δ, mm Hg	Δ, %	baseline	experiment	Δ, bpm
Control	173.5±23.1	148.0±22.4	-25.5±4.8	-14.8±2.9	282.8±25.5	329.0±21.5	46.2±17.2
Losartan	144.1±13.8	122.9±11.1	-21.3±5.3	-14.7±3.1	291.4±37.6	342.9±26.5	51.4±21.6
Kardos	147.5±11.9	124.6±11.3	-22.9±4.9	-15.5±3.0	263.1±19.4	318.1±28.4	55.0±17.0

TABLE 3. Reaction of BP_{mean} to Administration of Angiotensin II and the Time of Attaining Maximum BP_{mean} (*M±m*)

Group	BP_{mean}, mm Hg	Maximum deviation of BP_{mean}, mm Hg	Δ, mm Hg	Δ, %	Time of attaining maximum, sec
Control	162.7±26.1	227.9±21.2	65.2±4.4	41.9±4.6	29.0±1.5
Losartan	134.0±7.1*	184.8±12.3*	50.8±3.2*	37.9±2.3	26.8±1.7
Kardos	151.2±13.6^{+}	220.4±8.5^{+}	69.2±2.7^{+}	45.8±3.2	25.6±1.5

Note. $p<0.05$ compared to: *control, $^{+}$losartan.

decrease in HR is a favorable effect of the preparation in patients with arterial hypertension. The effect of kardos is most likely mediated by a peripheral mechanism, because the attempt to detect its central component by studying the baroreflex response to hypertension caused by phenylephrine failed. This can attest to partial recovery of normal physiological reaction aimed at the maintenance of working BP. The detected effects of the course treatment with kardos attest to its hypotensive activity, which was not inferior to that of losartan and to its direct chronotropic influence on the heart, which can be used in the therapy of patients with chronic heart failure and arrhythmia.

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