Correction of Endothelial Dysfunction with Impaza Preparation in Complex with Enalapril and Losartan during Modeling of NO Deficiency

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Modeling of NO deficiency by administration of L-NAME to rats led to the development of arterial hypertension and endothelial dysfunction. Pronounced endothelium and cardioprotective effects of impaza under these experimental conditions manifested more markedly during combined administration of the preparation with standard hypotensive preparations enalapril and losartan.

Key Words: *impaza; endothelium; nitric oxide; L-NAME*

Here we studied the endothelium-protective and cardioprotective effects of impaza, enalapril, losartan, and their combinations on the model of L-NAME-induced NO deficiency.

MATERIALS AND METHODS

In albino male Wistar rats weighing 250-300 g NO deficiency was modeled by intraperitoneal injection of N-nitro-L-arginine-methyl ester (L-NAME) in a dose of 25 ng/kg over 7 days [6,7]. Simultaneously, pharmacological correction of NO deficiency was performed in 10 animals by administration of impaza, enalapril, losartan, impaza+enalapril, and impaza+losartan. Impaza (ultralow doses of antibodies to endothelial NO-synthase, a mixture of homeopathic dilutions C12+C30+C200; Materia Medica Holding) was dissolved in drinking bottles (1 tablet per 100 ml water). The rats received impaza with drinking water (the animals had free access to drinking bottles), daily water consumption was 20±3 ml per animal. Enalapril maleate (Farmstandart-Leksredstva) and potassium losartan (Farmstandart-Leksredstva) were administered in dose of 0.5 and 6 mg/kg, respectively, through a gastric tube once a day for 7 days. The same doses and administration routes were used for combined treatment with these preparations. The group with experimental NO deficiency served as the control. Intact animals were also used in the study.

On day 8 of the experiment, a catheter was introduced into the left carotid artery under narcosis (sodium ethaminal, 50 mg/kg) and systolic and diastolic blood pressure (BP) and heart rate (HR) were recorded. Then, a needle was inserted into the left ventricle through the heart apex for recording of the maximum contraction and relaxation rates. Measurements were performed and processed using P213ID transducer (Gould), L-154 analog-to-digital converter, and Bioshell software.

For the study of vascular bed reactivity in experimental NO deficiency we recorded BP during endothelium-dependent and endothelium-independent vasodilation. Endothelium-independent and endothelium dependent vasodilation was induced by bolus injection of sodium nitroprusside (30 μg/kg) and acetylcholine (40 μg/kg) into the right femoral artery [1]. Coefficient of endothelial dysfunction (CED) was calculated by the formula: $CED=SBP_{NP}/SBP_{Ach}$, where SBP_{NP} is the area of a triangle above the BP recovery curve, where

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the lower leg connects the point of maximum BP drop and the point where BP curve attained the plateau during the test with nitroprusside; SBP_{Ach} is the area of a triangle above the BP recovery curve during acetylcholine test, where the lower leg is the difference between the end of bradycardic cardiac component and the point of BP recovery. CED reflects changes in vascular bed reactivity during NO deficiency modeling and makes it possible to evaluate the degree of correction of endothelial dysfunction [4,5].

Functional capacities of the myocardium were evaluated using load tests: test for adrenoreactivity (intravenous injection of epinephrine hydrochloride (1×10^{-5} mol/liter, 0.1 ml/100 g) [2] and resistance load test (30-min clamping of the ascending aorta).

Reliability of differences between the absolute parameters was evaluated using Student *t* test.

RESULTS

Blockade of NO synthase after injection of L-NAME led to the development of pronounced arterial hypertension. No significant decrease in initial BP values was noted in animals receiving impaza and its combinations with enalapril and losartan. However, a minor BP decrease was observed in the group treated with impaza+losartan.

In the control group, the ratio between endothelium-independent and endothelium-dependent vasodilation increased against the background of L-NAME treatment, CED increased to 5.4±0.6 *vs*. 1.1±0.1 in intact animals. In the group treated with impaza, CED approached the values observed in intact animals (2.1±0.2, Table 1).

Enalapril monotherapy produced less pronounced endothelium-protective effect compared to impaza monotherapy (CED=3.2±0.3). The combination of enalapril and impaza potentiated the endothelium-protective effect of each preparation (CED=1.6±0.2).

By its endothelium-protective effect losartan was inferior to impaza and superior to enalapril. In the group receiving losartan CED was 2.5±0.2. Impaza produced an additive endothelium-protective effect in combination with losartan, CED=1.5±0.2 in this group, *i.e.* maximally approached the values observed in intact animals.

Our findings suggest that impaza corrects endothelial dysfunction and has no effect on the development of arterial hypertension. It was found that combinations impaza+enalapril and impaza+losartan also did not reduce BP, but produced additional endothelium-protective effects.

Test for adrenoreactivity showed that systolic pressure in the left ventricle (SPLV) in the control group (247.3±4.8 mm Hg) considerably surpassed that in intact animals (199.2±8.3 mm Hg). Impaza reduced SPLV to 211.1±10.2 mm Hg. Enalapril also prevented adrenoreactivity. Losartan did not reduce SPLV during the test with epinephrine; adrenoreactivity in this group was significantly higher than in animals receiving impaza (Table 2).

Combined treatment with impaza and enalapril during the test for adrenoreactivity reduced the absolute values of SPLV to a level observed in intact animals (194.3±7.3 mm Hg). In animals receiving impaza and losartan, SPLV was 203.2±7.8 mm Hg, *i.e.* also approached the value observed in intact animals, but was slightly higher than in the group receiving enalapril+impaza.

During resistance load test, the parameter of exhaustion of myocardial reserve was calculated as the ratio of SPLV increments by the 5th and 25th seconds of aorta clamping (in %).

TABLE 1. BP and CED during Modeling and Correction of NO Deficiency with L-NAME (*M*±*m*)

Group	BP, mm Hg		HR, bpm	CED
	systolic	diastolic		
Intact	137.7±3.7	101.9±4.3	420.0±9.0	1.1±0.1
Control	190.3±6.7	145.0±3.9	428.0±11.0	5.4±0.6*
Impaza	184.3±7.0	136.7±6.5	417.1±13.1	2.1±0.2*+
Enalapril, 0.5 mg/kg	183.9±11.4	138.9±7.0	377.0±9.0	3.2±0.3+o
Impaza+enalapril, 0.5 mg/kg	190.3±6.8	149.0±5.1	421.5±9.4	1.6±0.2+
Losartan, 6 mg/kg	192.2±10.5	138.2±2.4	381.0±9.0	2.5±0.2+
Impaza+losartan, 6 mg/kg	177.9±12.7	133.9±7.6	390.8±5.1	1.5±0.2+

Note. Here and in Table 2: $p<0.05$ compared to: *intact animals, +control, oimpaza.

TABLE 2. Effect of Impaza, Enalapril, Losartan, and Their Combinations on Functional Capacities of the Myocardium during Loading Tests against the Background of Experimental L-NAME-Induced Endothelial Dysfunction (*M*±*m*, *n*=10)

Group	Adrenoreactivity, mm Hg	Exhaustion of myocardial reserve, %
Intact	199.2±8.3	83.6
Control	247.3±4.8*	66.0*
Impaza	211.1±10.2+	82.9+
Enalapril, 0.5 mg/kg	216.2±10.8+	82.7+
Impaza+enalapril, 0.5 mg/kg	194.3±7.3+	89.7+
Losartan, 6 mg/kg	242.7±4.7*o	88.3+
Impaza+losartan, 6 mg/kg	203.2±7.8+	92.0+

In intact animals this parameter was 83.6%, and in the control group it was 66.0%. In animals receiving impaza, this parameter considerably differed from that observed in the control group and was close to that in intact animals (82.9%).

Monotherapy with enalapril and impaza produced similar effects on the parameter of exhaustion of myocardial reserve (82.7 и 82.9%, respectively). The effect of monotherapy with losartan was more pronounced (88.3%) compared to impaza. In animals receiving impaza+enalapril and impaza+losartan, this parameter was 89.7 and 92%, respectively, which confirms the pronounced cardiotropic effect of the latter combination.

Thus, evaluation of the functional status of the myocardium during load tests demonstrated pronounced cardioprotective effect of impaza in combination with enalapril and losartan, which manifested in reduced adrenoreactivity and decreased SPLV during resistance load testing compared to the corresponding parameters in control animals.

Impaza exhibited endothelium- and cardioprotective effects. These effects became more potent when impaza was administered in combination with standard hypotensive preparations enalapril and losartan. The results of this study allow us to recommend these combinations for wide clinical testing.

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