Comparative Study of Potential Endothelioprotectors and Impaza in Modeled Nitric Oxide Deficiency

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Experimental NO deficiency induced by L-NAME injection led to the development of arterial hypertension, endothelial dysfunction, and cardiomyocyte hypertrophy and reduced blood content of nitrates and nitrites. Impaza, NO donors, activators of NO-synthase, antioxidants, and antihypertensive preparations produced endothelium-protective effect of different degree.

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

Here we compared endothelium-dependent effects of impaza and other preparation correcting endothelial dysfunction in experimental NO deficiency.

MATERIALS AND METHODS

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 to albino male Wistar rats weighing 250-300 g [9.12]. In parallel, the studied endothelium protectors were administered to animals of different groups daily for 7 days 1 h before injection of L-NAME. Controls received L-NAME only. Intact animals were also used in the study. Each group consisted of 10 animals. The degree of correction of endothelial dysfunction with the test preparations was evaluated on day 8 of the experiment.

For pharmacological correction of NO deficiency, impaza (ultralow doses of antibodies to NO synthase, a mixture of homeopathic dilutions C12+C30+C200; Materia Medica Holding) was dissolved in drinking water (1 tablet per 100 ml water); the animals had free access to drinking bottles; the volume of consumed fluid was 20±3 ml/rat per day. Endotheliotropic effects of impaza were compared to those of endogenous and synthetic NO donors: L-arginine (Ajinomoto Co. Inc.) in a dose of 200 mg/kg intraperitoneally, isosorbide mononitrate (Istituto Lusofarma) in a dose of 4 mg/kg intragastrically, GYaL-279 (nitroindole derivative) in a dose of 50 mg/kg intraperitoneally and RE-420 (dithiocarbamate derivative) in a dose of 50 mg/kg intraperitoneally (both preparations were synthesized in Russian Research Center for Antibiotics by a research group headed by V. G. Granik), and nebivolol (Berlin-Chemie/Menarini Group) in a dose of 0.5 mg/kg intragastrically; activators of NO-synthase: resveratrol (Greensyn™ (Guangzhou) Co., Ltd) in a dose of 2 mg/kg intraperitoneally, furostanol glycosides of cultured plant cells *Dioscorea deltoidea* (DM-05, synthesized at Institute of Plant Physiology, Russian Academy of Sciences under supervision of A. M. Nosov) in a dose of 1 mg/kg intraperitoneally, mildronate (Grindex) in a dose of 80 mg/kg intragastrically; antioxidants: mexicor (EkoFarmInvest) in a dose of 30 mg/kg in-

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tramuscularly, mexidol (Ellara) in a dose of 30 mg/kg intramuscularly, ascorbic acid (Hebei Welcome Pharmaceutical Co. Ltd) in a dose of 30 mg/kg intragastrically, ascorbic acid-titanium metallocomplex compound (πQ510, synthesized by E. A. Parfenov, Institute of Experimental Diagnostics and Therapy of Tumors, N. N. Blokhin Russian Cancer Research Center) in a daily dose of 30 mg/kg intraperitoneally; and cardiotropic preparations with documented pleiotropic endothelium-protective effect (EPE) enalapril (Berlin-Chemie AG) in a dose of 0.5 mg/kg intragastrically, losartan (Farmstandart-Lekstedstva) in a dose of 6 mg/kg intragastrically, amlodipine (Farmstandart-Lekstedstva) in a dose of 0.5 mg/kg intragastrically, indapamide (Farmstandart-Lekstedstva) in a dose of 2 mg/kg intragastrically.

The animals were narcotized with sodium ethaminal (50 mg/kg). Systolic and diastolic blood pressure (BP) and HR were recorded via a catheter inserted into the carotid artery and coupled with P213ID transducer (Gould) using Bioshell software. Reactivity of the vascular wall was evaluated in functional tests with acetylcholine (40 μg/kg) and sodium nitroprusside (30 μg/kg) using coefficient of endothelial dysfunction (CED) [5,8] calculated by the formula: $CED=SBP_{NP}/SBP_{Ach}$, where SBP_{NP} and SBP_{Ach} are the areas of triangles above the BP recovery curves in tests with nitroprusside and acetylcholine, respectively. We calculated CED in each animal in the intact and control (after NO-synthase blockade) groups; the values differed by 5 times (1.1 in intact animals and 5.4 in controls). The EPE of the test preparations can be classified by the decrease in CED within the above range of CED values.

Stable NO metabolites (total content of nitrites and nitrates measured colorimetrically in deproteinated serum) served as biochemical markers of endothelial dysfunction [4].

Histological examination of the heart was performed for morphological verification of the modeled pathological processes and for complex evaluation of drug efficiency [1].

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

RESULTS

Blockade of endothelial NO-synthase with L-NAME led to the development of pronounced arterial hypertension, which manifested in elevation of systolic/diastolic BP and CED (Table 1). The content of stable NO metabolites in the serum sharply decreased. This parameter in animals with pathology was 61.2±2.7 μM *vs*. 114.1±4.2 μM in intact rats ($p<0.05$). Macroscopic examination of the heart in rats with experimental NO deficiency revealed induration of the wall. The absolute heart weight in this group increased to 1047±95 mg (*vs*. 930±50 mg in intact rats) and the relative heart weight increased to 3.07 mg/g (*vs*. 3.79 mg/g in intact rats). The increase in the relative heart weight compared to intact group was 19%. Ventricular index (separate weighing) varied from 0.33 to 0.54 and was similar to that in intact animals, which together with the increase in heart weight attests to even hypertrophy of the ventricular walls. We also observed hypertrophy of cardiomyocytes in the left ventricle 18.4±0.9 μ (*vs*. 9.1±0.1 μ in intact animals; $p<0.05$); spasm of arterioles and thickening of their walls were also noted. Staining with Rego hematoxylin revealed focal segmentary or total contracture-type damage to cardiomyocytes.

Reduced expression of cGMP-protein kinase determining incompetence of NO/cGMP-dependent regulation of Ca^{2+} transport in hypertrophied cardiomyocyte is a trigger mechanism in the development of myocardial hypertrophy in arterial hypertension caused by administration of L-NAME [12]. It should be noted that 7 days of rat life are equal to 219 days of human life [3], *i.e.* high BP during this period under conditions of NO deficiency can be a quite real cause of myocardial hypertrophy.

Experimental studies confirmed the presence of EPE in all studied preparations (Table 1). All preparations reliably reduced CED, which attests to recovery of the balance between endothelium-dependent and endothelium-independent vasodilation, but did not significantly modulate HR (this parameter remained within the range of 390-410 bpm).

NO donors exhibited pronounced antihypertensive effect and EPE. Activators of NO synthase predominantly produced EPE, which can be explained by more pronounced pathogenetically directed correction of NO deficiency during modeling of this pathology.

Antioxidants produced less pronounced EPE and moderate antihypertensive effect, which can be related to increased bioavailability, but not the amount of produced NO.

The standard antihypertensive preparations produced no antihypertensive effect and exhibited endothelium tropic activity. This can be explained by the use of low dose of these preparations corresponding to the starting dose in the therapy of arterial hypertension in clinical practice. In this group, the maximum endotheliotropic activity was produced by losartan and amlodipine [3].

Impaza produced no antihypertensive effects under these experimental conditions, but exhibited EPE, which was intermediate between NO-synthase activators and NO donors and was superior to that of standard antihypertensive preparations. The increase

TABLE 1. BP and CED during Pharmacological Correction of L-NAME-Induced NO Deficiency with Various Endothelium-Protective Preparations ($M \pm m$, n=10)

Group		BP, mm Hg		CED
		systolic	diastolic	
Intact		137.7±3.7	101.9±4.3	1.1±0.1
Control (L-NAME, 25 mg/kg, 7 days)		190.3±6.7*	145.0±3.9*	5.4±0.6*
NO donors	L-arginine, 200 mg/kg	177.6±9.6+	140.1±6.4	2.5±0.2+
	isosorbide mononitrate, 4 mg/kg	151.1±11.2+	119.6±6.3+	1.7±0.1+
	GYaL-279, 50 mg/kg	156.3±8.9+	116.9±11.3+	1.8±0.1+
	RE-420, 50 mg/kg	148.8±10.3+	114.5±4.2+	1.9±0.2+
	nebivolol, 0.5 mg/kg	149.8±13.3+	112.4±8.6+	2.0±0.3+
Activators of NO synthase	resveratrol, 2 mg/kg	183.8±15.0	140.0±8.3	1.2±0.1+
	DM-05, 1 mg/kg	167.5±6.3+	128.7±4.3+	1.6±0.2+
	mildronate, 80 mg/kg	161.3±8.7+	122.4±5.9+	1.7±0.2+
Antioxidants	mexicor, 30 mg/kg	158.1±9.3+	126.6±5.2+	2.9±0.1+
	mexidol, 30 mg/kg	160.1±3.4+	125.5±4.7+	2.9±0.5+
	ascorbic acid, 30 mg/kg	187.4±6.1	137.7±3.8	2.0±0.1+
	πQ 510, 30 mg/kg	164.4±10.5+	130.8±7.7	1.9±0.7+
Basic antihypertensive preparations	enalapril, 0.5 mg/kg	183.9±11.4	138.9±7.0	3.3±0.3+
	losartan, 6 mg/kg	192.2±10.5	138.2±2.4	2.5±0.3+
	amlodipine, 0.5 mg/kg	186.3±15.2	131.1±7.0	2.1±0.3+
	indapamide, 2 mg/kg	191.4±10.0	140.4±2.4	3.5±0.4+
Ultralow doses of antibodies	impaza	184.3±7.0	136.7±6.5	2.1±0.2+

Note. *p<0.05 compared to: *intact rats, +control.

in the concentration of nitrite ions to 107.3±5.5 μM confirms the effect of impaza on activation of NO synthesis, while the decrease in cardiomyocyte diameter to 13.1±0.2 μ attests to cardioprotective effects of the preparation.

Thus, under conditions of experimental NO deficiency impaza exhibited pronounced EPE comparable or superior to that of other drugs, which allows us to recommend this preparation for the use in cardiological practice. Moreover, our experimental findings confirm the concept of pathogenetically substantiated approach to the correction of NO deficiency, which takes into account the pharmacological influences on different targets on the systemic and organ levels. These results drove us to a conclusion that administration of impaza in addition to basis therapy of arterial hypertension is reasonable.

REFERENCES

1. G. G. Avtandilov, *Fundamentals of Pathologic Anatomy Practice* [in Russian], Moscow (1994).
2. A. S. Belous, M. V. Pokrovskii, V. I. Kochkarov, *et al*., *Kuban. Nauch. Med. Vestn.,* No. 1-2, 23-26 (2007).
3. I. P. Zapadnyuk, V. I. Zapadnyuk, and E. A. Zakhariya, *Laboratory Animals* [in Russian], Kiev (1974).
4. V. S. Zodionchenko, T. V. Adasheva, and A. P. Sandomirskaya, *Russ. Med. Zh*., No. 1, 11-15 (2002).
5. V. A. Metel'skaya, *Klin. Lab. Diagn*., No. 9, 86 (2004).
6. V. A. Metel'skaya, N. G. Gumanova, O. A. Litinskaya*, et al., Vopr. Biol. Med. Farm. Khimii*, No. 2, 34-39 (2004).
7. M. V. Pokrovskii, V. I. Kochkarov, T. G. Pokrovskaya*, et al., Kuban. Nauch. Med. Vestn.,* No. 10, 72-77 (2006).
8. *S 2 2301015 RU A 61 B5/02*. A Method for Evaluation of Endothelial Dysfunction. M. V. Pokrovskii, T. G. Pokrovskaya, and V. I. Kochkarev, No. 2005113243/14; Subm. 04.05.2005/17 Inventions (Applications and Patents), No. 17 (2007).

9. L. Y. Deng, G. Thibault, and E. L. Schiffrin, *Clin. Exp. Hypertens*., **15**, No. 3, 527-537 (1993).
10. N. Gokce, *J. Nutr.,* **134**, No. 10, Suppl., 2807S-2811S (2004).
11. S. Kaplan, J. A. Morgan, G. Bisleri, *et al.,* // *Ann. Thorac. Surg*., **80**, No. 5, 1773-1778 (2005).
12. J. B. Laursen, S. Rajagopalan, Z. Galis, *et al., Circulation*, **95**, No. 3, 588-593 (1997).
13. L. Mazzetti, C. Ruocco, L. Giovannelli, *et al., Br. J. Pharmacol*., **134**, No. 3, 596-602 (2001).
14. A. Palloshi, G. Fragasso, P. Piatti, *et al., Am. J. Cardiol*., **93**, No. 7, 933-935 (2004).