Effect of Antioxidants pQ510 and Resveratrol on Regulatory Function of the Endothelium in Rats with Modeled Arterial Hypertension

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We studied the effects of antioxidants resveratrol and pQ510 on physiological parameters and the state of endothelial NO-synthase as a marker of the regulatory function of the endothelium in the aorta of rats with modeled arterial hypertension. The antioxidants promoted recovery of stable NO metabolites in rat serum and maintained expression of endothelial NO-synthase at a normal level. These effects were confirmed by correction of blood pressure and endothelium-dependent vascular dilation assessed by endothelial dysfunction coefficient.

Key Words: *endothelial NO-synthase; nitric oxide; resveratrol; ðQ510; endothelial dysfunction*

Nitric oxide NO synthesized by endothelial NO-synthase (eNOS) is a major regulator of the vascular tone. Arterial hypertension, an important risk factor of cardiovascular diseases, is characterized by production of extra amount of reactive oxygen species (ROS) interacting with NO and reducing its bioavailability; oxidation of NO to peroxynitrite by ROS induces oxidative stress [3]. Bioavailability of NO can be maintained by inhibition of oxidative stress, therefore agents with antioxidant properties (superoxide ion traps) inactivating free radicals increase NO bioavailability and improve regulation of the vascular tone.

Here we studied the effect of natural antioxidant resveratrol (3,4,5'-trihydroxystilbene) and synthetic antioxidant pQ510 (a coordination compound of ascorbic acid with titanium) on physiological parameters and state of eNOS as a marker of regulatory function of the endothelium in the aorta of rats with modeled arterial hypertension.

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MATERIALS AND METHODS

Experiments were carried out on male Wistar rats weighing 250-300 g (Stolbovaya nusrery, State Research Center of Biomedical Technologies, Russian Academy of Medical Sciences). The rats were divided into one control and three experimental groups (10 rats per group). Group 1 (control) consisted of intact rats. The experimental groups 2-4 comprised rats with induced arterial hypertension receiving no therapy (group 2) or treated with resveratrol (group 3) or pQ510 (group 4). Arterial hypertension was induced by daily administration of 25 mg/kg eNOS inhibitor L-NAME (N^{ω}-nitro-L-arginine methyl ester, Sigma) for 7 days through a gastric tube [7].

The rats were anesthetized on day 7 and a catheter for blood pressure (BP) measurements was introduced into the left carotid artery. Systolic and diastolic BP and HR were continuously monitored

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and mean BP was calculated using a transducer and Bioshell software.

Endothelium-dependent and endothelium-independent vasodilation reactions were recorded after bolus intravenous injection of acetylcholine (40 μg/kg) and sodium nitroprusside (30 μg/kg), respectively. The ratio of the area under curve of endothelium-independent vasodilation (characterizes BP recovery after administration of sodium nitroprusside) to the area under curve of endothelium-dependent vasodilation (characterizes BP recovery after administration of acetylcholine) was taken as a coefficient of endothelial dysfunction (K_{ed}).

Group 3 rats received daily intraperitoneal injections of resveratrol (Greensyn (Guangzhou) Co., Ltd.) in a dose of 2 μg/kg for 7 days against the background blockade of eNOS with L-NAME. The dose of resveratrol corresponded to its maximum content in wine, *i.e.* 10 μM (recommended dose for prophylaxis of complications of cardiovascular diseases is 300-400 g/day [13] or 2 mg/kg).

Metallocomplex compound pQ510 (titanium modified with ascorbate) was synthesized and kindly provided by Dr. E. A. Parfenov (Research Institute of Experimental Tumor Diagnostics and Therapy, Russian Oncology Research Center, Russian Academy of Medical Sciences). pQ510 was injected intraperitoneally in a daily dose of 30 μg/kg for 7 days. The dose is equal to 10% LD_{50}, which was determined as an effective dose producing antihypoxic and bronchodilating effects [1].

Expression of eNOS in rat aorta was evaluated using polyclonal mouse antibodies to eNOS (BD Transduction Laboratories) and enhanced chemiluminescence assay [4,5]. After sacrifice, an aortal segment (20-30 mg) was washed in 0.9% NaCl saline and stored in liquid nitrogen. Before assay, the aortal segment was weighted and minced in a porcelain mortar with addition of liquid nitrogen. The proteins were extracted with 10-fold volume denaturing buffer (6.25 mM tris pH 6.8, 2% sodium dodecyl sulfate, 5% mercaptoethanol, 10% glycerol, 0.005% bromphenol blue) followed by 3-min boiling. The samples were separated by electrophoresis in 7.5% PAAG and transferred onto nitrocellulose membrane for Western blot analysis [5]. The membrane was incubated overnight with mouse polyclonal antibodies against eNOS, washed, incubated with secondary biotinylated antimouse antibodies, visualized using an enhanced chemiluminescence detection system, and exposed on X-ray film for 24 h. The film was developed, and the bands were scanned to calculate their intensity using a TotalLab software (Amersham). Routinely prepared samples containing 1 million cultured endothelial cells of bovine aorta were used as the control (band intensity was taken as 100%).

The concentration of final stable NO metabolites (the sum of nitrate and nitrite ions NO_x) in rat blood serum was measured using modified Griess reaction [2] after serum deproteinization with 2-fold volume of ethanol and preliminary reduction of nitrate to nitrite with vanadium chloride. Optical density was recorded at λ=540 nm [9].

During the experimental period, all rats were maintained on nitrite-free diet. The nitrite-free food (Laboratorkorm Ltd.) was balanced by amino acids, mineral substances, and vitamins. The absence of nitrate contaminants was verified by laboratory tests.

Significance of differences was assessed by Student's *t* test at $p<0.05$.

RESULTS

NO_x concentration reflecting eNOS activity under the specified diet in the experimental rats with modeled arterial hypertension was almost 2-fold lower than in the control group (Table 1). At the same time, the level of eNOS did not significantly differ in the experimental and control rats. Thus, L-NAME did not affect the amount of eNOS, but almost 2-fold reduced its activity ($p<0.05$).

In group 2 rats, all functional parameters (systolic and diastolic BP, mean BP, endothelium-dependent and endothelium-independent vasodilation, and HR) indicated arterial hypertension. In these animals, L-NAME-induced blockade of eNOS significantly decreased endothelium-dependent vasodilation (to 695.0±87.6 *vs*. 1268.0±74.8 rel. unit in intact controls, $p<0.05$). The area under curve of endothelium-independent vasodilation after injection of nitroprusside was 1375.0±93.7 rel. unit *vs.* 3323.0± 116.7 rel. unit in the control ($p<0.05$). The coefficient of endothelial dysfunction in these rats was almost 5-fold higher than in the control group (5.4±0.6 *vs*. 1.1±0.1, respectively, $p<0.05$; Table 1).

In rats with modeled arterial hypertension receiving resveratrol, NO_x concentration and eNOS expression increased and virtually returned to control values observed in intact animals, *i.e.* resveratrol promoted recovery of eNOS activity (Table 1). This conclusion was also confirmed by functional tests: K_{ed} in rats with modeled arterial hypertension receiving resveratrol returned to normal level, while BP and HR tended to decrease (Table 1).

In rats with modeled arterial hypertension receiving antioxidant pQ510, the content of NO_x also returned to the initial level. This antioxidant restored K_{ed} and reduced BP.

TABLE 1. Effect of Resveratrol and pQ510 on Endothelial Function in Rats

Group	Pharmacological test	eNOS, %	NO_x, μM	K_{ed} rel. units	Systolic BP, mm Hg	Diastolic BP, mm Hg	Mean BP, mm Hg
Intact (group 1)	Initial	72.9±11.2	114.1±10.5	1.1±0.1	137.0±3.7	101.9±4.3	119.0±4.6
	Ach				84.3±4.5	38.7±2.8	61.5±3.6
	SN				83.0±3.7	42.1±4.4	62.50±4.05
L-NAME, 25 mg/kg (group 2)							
	Initial	79.4±9.5	61.2±8.5*	5.4±0.6*	190.3±6.7	145±3.9*	167.0±5.3*
	Ach				110.6±5.2	82.8±6.6*	96.7±5.9*
	SN				88.7±4.7	50.8±4.2	69.70±4.45
L-NAME, 25 mg/kg and resveratrol, 2 μg/kg (group 3)							
	Initial	71.5±5.4	107.0±9.1+	1.2±0.1+	183.8±15.0	140.0±8.3	161.9±11.6
	Ach				55.2±7.7+	32.6±3.3+	43.9±5.5+
	SN				86.0±7.9	49.0±9.9	67.5±8.9
L-NAME, 25 mg/kg and pQ510, 30 μg/kg (group 4)							
	Initial	—	124.0±9.3+	1.4±0.1+	164.8±10.5	130.0±7.7	147.4±9.1
	Ach				86.2±5.2+	50.8±2.9+	68.50±4.05+
	SN				82.6±7.3	40.8±3.4	61.70±5.35

Note. Ach: acetylcholine; SN: sodium nitroprusside. $p<0.05$ compared to *control group 1 and to +group 2 rats injected with L-NAME.

Various antioxidants are widely examined to find the means preventing the development of oxidative stress and inhibiting its negative effects. For example, the study of antioxidant properties of resveratrol (a natural polyphenol compound present in grape, red wine, and in some medical herbs) revealed its capacity to protect endothelial cells from the negative action of peroxynitrite [6]. Resveratrol normalized systolic BP and induced vasodilation in rats [8], which agrees with our data. Long-term administration of resveratrol to rats maintained on fructose diet prevented cardiovascular fisorders and up-regulated eNOS activity [10].

Ascorbic acid, a widely used antioxidant, decreases the level of free radicals and up-regulates NO production due to activation of eNOS [14]. In senile patients (older than 78 years) suffering from arterial hypertension, treatment with ascorbic acid administered in a dose of 600 mg/day for 6 months markedly reduced systolic blood pressure and pulse pressure in ambulatory blood pressure monitoring, which was accompanied by an increase in the serum levels of ascorbic acid and decreases in the levels of C-reactive protein and modified low-density lipoproteins [11]. In patients with arterial hypertension, ascorbic acid improves endothelial function (increases endothelium-dependent vasodilation) [12].

Thus, our findings agree with published data and show that resveratrol and pQ510 exert a pronounced therapeutic effect on the regulatory function of the endothelium in arterial hypertension and promotes recovery of eNOS activity, which is seen from elevation of NO_x concentration (a product of reaction catalyzed by this enzyme). Moreover, recovery of NO-producing function of the endothelium during antioxidant therapy was accompanied by improvement of endothelium-dependent vasodilation and BP normalization. This phenomenon can be related to an increase in NO bioavailability, because antioxidants prevent its breakdown by moderating the oxidative stress.

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