PHARMACOLOGY AND TOXICOLOGY

Correction of Disturbances in Neuroregulatory Systems at Early Stages of Atherogenesis with Verapamil

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 129, No. 1, pp. 61-63, January, 2000
Original article submitted July 28, 1999

The effect of verapamil on morphofunctional state of the hypothalamo-pituitary neurosecretory system, adrenergic innervation of microvessels, and microcirculation in the early stages of atherogenesis was studied. Correction of functional aberrations of the neuroregulatory systems and microcirculatory disturbances with verapamil was accompanied by restoration of lipid homeostasis and less pronounced atherosclerotic alterations in major arteries.

Key Words: *verapamil; hypothalamo-pituitary neurosecretory system; adrenergic innervation of microvessels; atherosclerosis*

The hypothalamo-pituitary neurosecretory system (HPNS) and peripheral subdivision of the sympathoadrenal system (SAS), which produce bioactive substances with vasoactive and lipid mobilizing effect (vasopressin, norepinephrine), play an important role in the mechanisms of atherogenesis. There is a correlation between functional state of these systems, microcirculatory disturbances, and the degree of atherosclerotic process in major arteries during hyperlipemia [2,5,8].

In view of the priming role of microcirculatory disturbances in the development of polyorgan pathology during hyperlipidemia [4], the search for means of correction of functional disturbances in HPNS and SAS is actual. It was established that some calcium channel blockers affect lipid metabolism and modulate activity of the antioxidant system and SAS [7,10,11]. Calcium blocker verapamil is an active neuroprotector, which protects, in particularly, the neurosecretory pituitary cells [6].

Our aim was to study the effect of verapamil on morphofunctional state of the regulatory systems at the early stages of atherogenesis.

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MATERIALS AND METHODS

Experiments were performed on 30 male Chinchilla rabbits weighing 2.5-3.0 kg. Group 1 rabbits (intact controls) were maintained on a standard diet. Group 2 rabbits were given 0.3 g/kg cholesterol during 2 months, which modeled atherogenic diet (ATD) according to N. N. Anichkov. Group 3 rabbits were intramuscularly injected with verapamil (phynoptin, Orion) in a dose of 0.5 mg/kg for 10 days during the 2nd month of the diet. We previously observed marked accumulation of LDL, pronounced changes in HPNS, peripheral subdivision of SAS (adrenergic innervation of microvessels), and microcirculatory vascular bed, and initial lipidosis of the aortal intima after one month of ATD [3,5]. Plasma level of total HDL and LDL fractions were determined. The degree of atherosclerotic process was assessed by the index of atherosclerotic damage (IAD) to the aorta [13]. The hypothalamic supraoptic (SO) nuclei and neurohypophysis were studied on serial brain slices by the methods of Gomori—Maiorova, Nissl, and Milenkov. The functional state of HPNS was assessed as described elsewhere [9] by the content of Gomori-positive substance in neurosecretory cells, hypothalamo-pituitary tract, and posterior pituitary. The percentage of "bright" and

0007-4888/00/0001-51$25.00

"dark" cells in the SO nuclei and the number of pyknomorphous cells were counted. The area of neuronal nucleus and soma were determined using a Leitz-ASM semiautomatic image analyzer. The adrenergic innervation of terminal part of vascular bed was studied by the method of Falk—Ovmen. The intensity of catecholamine fluorescence was determined photometrically under a LYUMAM microscope. Microvessels were impregnated with silver nitrate (proposed by V. V. Kupriyanov) and morphometried.

RESULTS

After 2 months of atherogenic diet (group 2), plasma content of LDL increased more than 20-fold and HDL content increased 5-fold compared with those in group 1 rabbits. The index of atherosclerosis in the aorta was 20% (Fig. 1).

In the SO nuclei the number of "bright" (actively synthesizing the neurohormone) cells decreased by 25% (Table 1). In most neurons, the cytoplasm and processes contained many neurosecretory granules. Accumulation of neurosecretory substance was also observed in other parts of HPNS (hypothalamo-pituitary tract and neurohypophysis). "Dark" cells contained pyknotic and shrunk nuclei (Table 1). Numerous neurosecretory neurons were in the stage of "red pyknosis". These signs attest to decreased functional activity of HPNS after 2 months of ATD with a tendency toward exhaustion.

Morphofunctional analysis of the adrenergic component of microvessel innervation revealed a pronounced increase in the intensity of specific catecholamine luminescence (Fig. 2). Periarterial adrenergic plexuses contained primarily tortuous axons with large varicosities. Pronounced vascular, intra- and extravascular alterations were observed in all elements of the microcirculatory bed: irregularities in the diameter, tortuosity of venules, microaneurysms, intravascular cell aggregates, empty capillaries, and perivascular edema. The morphometric indices characterizing the state of microcirculatory bed were significantly changed (Fig. 2).

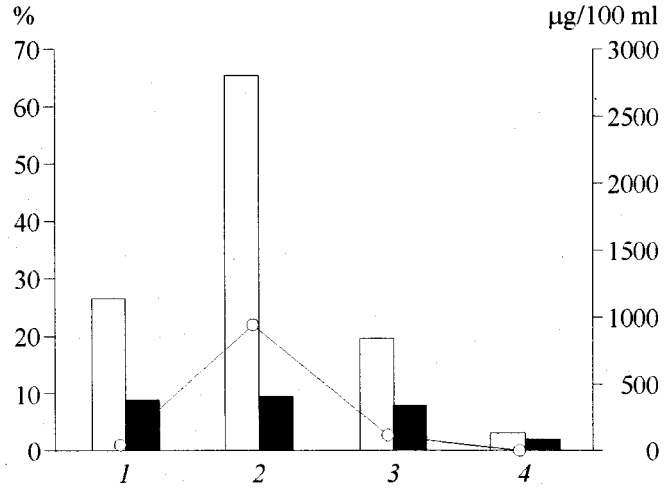

Fig. 1. Content of HDL and LDL and index of atherosclerosis in the aorta in lipoproteinemia and its correction with verapamil. Left ordinate: index (%) of atherosclerotic lesion of the aorta (curve). Right ordinate: content of LDL (light bars) and HDL (dark bars) in μg/100 ml. *1*) 1-month atherosclerotic diet (ATD). Here and in Fig. 2: 2) 2-month ATD, 3) 2-month ATD+verapamil.

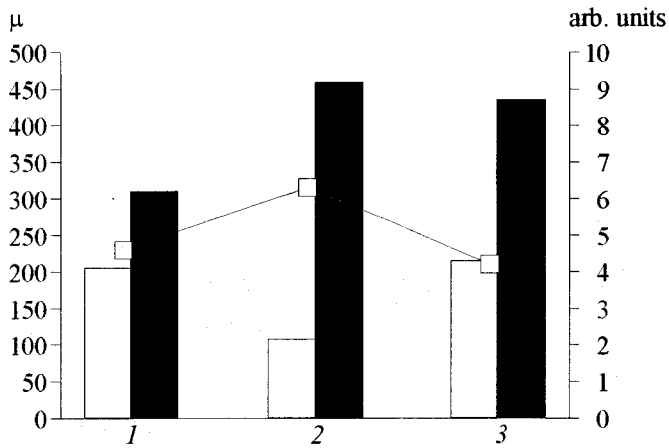

Fig. 2. Vascular cross-section area and intensity of catecholamine luminescence in lipoproteinemia and its correction with verapamil. Left ordinate: cross-section area (μ^2) of arterioles (light bars) and venules (dark bars). Right ordinate: intensity of catecholamine luminescence (arb. units) plotted by line. *1*) Control.

TABLE 1. Effect of Verapamil on Morphometric Indices of Hypothalamic SO Nuclei Characterizing HPNS State (*M*±*m*)

Group	"Bright"/ "dark" cells, %	Area, μ^2	
		nucleus	soma
Control (intact rabbits)	69/31	39.3±1.7	113.5±2.7
ADT	44/56	34.5±0.8	121.2±7.3
ADT+verapamil	60/40	37±1.9	118.1±6.4

Note. ATD: atherogenic diet. Each group comprised 10 rabbits.

After 2-month ATD, the level of LDL in verapamil-treated rabbits (group 3) was 3.2-fold lower than in group 2 rabbits, the content of HDL in group 3 rabbits 4-fold surpassed the control value, while IAD was 2.7% (Fig. 1).

In HPNS, the ratio of "bright" and "dark" cells virtually did not differ from normal. The number of pyknomorphous cells decreased. The size of nuclei and somata of neurosecretory cells was similar to those of intact rabbits (Table 1), which suggested recovery of the neurosecretory function in SO neurons.

The intensity of specific catecholamine luminescence in adrenergic fibers innervating microvessels and axonal structure returned to normal. Spastic alterations in arterioles in these rabbits disappeared against the background of venular dilation, while the number of active exchange capillaries increased.

Therefore, functional recovery of HPNS and peripheral subdivision of SAS was accompanied by normalization of lipid homeostasis and microcirculation, and pronounced decrease in the index of atherosclerosis in the aorta. It can be assumed that the antiatherogenic action of verapamil is mediated by its positive effect on the neuroregulatory systems. These data suggest that verapamil can be beneficially used for the prophylaxis and therapy of atherosclerosis.

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