Biochemical Indicators of Atherogenic and Protective Activity of Xydiphone in Experimental Animals

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Atherosclerotic plaque formation and vascular calcinosis were modeled in a subchronic experiment. Reduced HDL and elevated LDL concentrations, increased atherogenic index and albumin toxicity index, and high blood levels of triglycerides and uric acid were early markers of pathology. Xydiphone in combination with vitamin D effectively reduced these changes and the degree of vascular calcinosis.

Key Words: *markers of atherosclerosis; vascular calcinosis; xydiphone*

Atherosclerosis underlies most cases of cardiovascular disease [1,3]. Progressive disease with lipid deposition in the walls of large arteries leads to pronounced atherosclerotic changes with plaque formation in the endothelium and calcinosis of the vascular walls and atherosclerotic plaques [7,9-14]. Multifactorial pathogenesis of atherosclerosis and vascular calcinosis necessitates the search for new ways of prevention and treatment of atherosclerosis.

The purpose of the study was to determine the most informative indicators of altered metabolismunder conditions of experimental model of atherosclerotic plaque formation and vascular calcification and to evaluate the protective activity of xydiphone.

MATERIALS AND METHODS

A model of atherosclerotic plaque formation and vascular calcinosis (a piece of fishing line was surgically introduced into the thoracic aorta for 14 days) was reproduced on 50 male rats weighing 300-350 g and housed in the stationary vivarium. The animals were divided into 5 groups (10 animals per group). Groups 2-5 daily received 750 mg/kg cholesterol (CH) with food [4]; groups 3 and 5 additionally received 75,000 U vitamin D_2 per day during the first 3 days of the experiment and groups 4 and 5 received 50 mg/kg xydiphone (synthetic analogue of natural regulator of calcium metabolism) for 30 days through a tube. Serum samples were obtained after animal killing on day 30. HDL, LDL, CH, and triglycerides were assayed and atherogenic index was calculated. The state of blood albumins was evaluated using fluorescent probes [2]. Lipid peroxidation was measured by serum activity of pro-oxidant enzyme xanthine oxidase, total serum antioxidant activity, and serum MDA content [8]. In addition, serum activity of lactate dehydrogenase (LDH), creatine phosphokinase (CPK) and alkaline phosphatase, and the content of creatinine and uric acid involved in non-enzymatic antioxidant defense system were determined using standard Human kits on a Konelab biochemical analyzer.

RESULTS

Biochemical examination revealed the following changes.

In group 1 rats, the highest serum levels of LDL (0.37 mmol/liter) and high atherogenic index (2.2) characteristic of patients with atherosclerosis as well as elevated index of toxicity (0.23 rel. units) were found. Moreover, high serum levels of MDA (2.29

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U/liter), xanthine oxidase activity (280.5 mmol/liter× min) and alkaline phosphatase (377.0 U/liter) and the highest content of triglycerides (1.36 mmol/liter) and uric acid (0.64 mmol/liter) were recorded in this group. The content of HDL, CH, activity of CPK and LDH were not increased in comparison with animals of other groups. Thus, surgery aimed at simulation of atherosclerotic plaque had a negative impact on some parameters of lipid metabolism, peroxidation processes, and purine metabolism in group 1 (Table 1).

In group 2 (fishing line, CH), the highest atherogenic index (2.5), the lowest level of HDL (0.7 mmol/liter), and high levels of LDL (0.28 mmol/liter) were revealed. HDL concentration decreased by 6% in comparison with group 1, while LDL concentration increased by 30%. In 4 of 10 animals, serum CH increased by 17-20% in comparison with mean serum CH level in group 1. Serum content of triglycerides and uric acid remained high, but a slight decrease in these parameters was noted (by (14 and 6%, respectively; Table 1).

In group 3 (fishing line, CH and vitamin D_2), parameters of serum albumin (total albumin concentration TAC and effective albumin concentration EAC) slightly decreased and toxicity index (0.27 rel. units) increased in comparison with those in group 2. However, we observed a pronounced decrease in LDL concentration (0.28 *vs.* 0.23 mmol/liter) and atherogenic index (2.5 *vs*. 2.1). LDH activity remained high, while activity of CPK and xanthine oxidase increased. Increased MDA content (1.93 *vs.* 2.1 U/liter) in comparison with group 2 and reduced serum antioxidant activity (71.98 *vs.* 61.66%) were revealed. This complex of changes indicated the activation of the peroxidation processes in the serum of group 3 animals which is typical for exposure to high doses of vitamin D_2. Nevertheless, several positive effects were observed in comparison with serum indicators in group 2 (fishing line, CH). First of all, it concerned serum HDL level (0.7 *vs.* 0.77 mmol/liter in group 3) as well as reduced LDL and atherogenic index. Further decline in alkaline phosphatase activity (280 *vs.* 266 U/liter in group 3), serum triglycerides (1.164 *vs.* 0.904 mmol/liter in group 3),) and uric acid (0.596 *vs.* 0.501 mmol/liter in group 3) was recorded. Blood CH and creatinine remained almost unchanged. The findings suggest the dual action of vitamin D_2 in this experiment: on the one hand, enhancement of peroxidation processes that may increase the risk of damage to the vascular endothelium and CH deposition; on the other hand, the positive effect of subtoxic doses of vitamin D2 was evident possibly due to its effect on calcium homeostasis and purine metabolism.

TABLE 1. Biochemical and Morphometric Characteristics of Experimental Atherosclerosis

Indicator	Group				
	1	2	3	4	5
HDL, mmol/liter	0.76±0.06	0.70±0.04	0.77±0.04	0.86±0.07^{x}	0.77±0.07
LDL, mmol/liter	0.37±0.03	0.28±0.02^{+}	0.23±0.01^{+}	0.28±0.02	0.25±0.04
Atherogenic index, arb. unit	2.20±0.06	2.62±0.11	2.17±0.15	2.09±0.11^{x}	2.32±0.22
Index of toxicity, arb. unit	0.23±0.07	0.22±0.04	0.27±0.05	0.24±0.06	0.11±0.01^{o}
MDA, U/liter	2.29±0.57	1.93±0.38	2.10±0.28	2.19±0.28	2.40±0.42
Antioxidant activity, %	65.80±5.16	71.98±2.83	61.66±4.95	62.54±4.15	59.00±4.83
LDH, U/liter	333.00±32.60	414.30±12.50^{+}	415.70±42.00^{+}	442.60±42.84	484.20±42.56
CPK, U/liter	52.00±4.51	56.60±5.29	67.00±17.27^{+}	55.90±7.03	52.90±8.14
Xanthine oxidase, mmol/liter×min	280.50±22.70	261.50±23.70	249.66±24.85	256.30±12.30	277.30±15.60
Alkaline phosphatase, U/liter	377.00±26.60	278.40±14.50^{+}	285.00±19.57^{+}	319.00±25.90	301.00±21.54
CH, mmol/liter	2.43±0.15	2.46±0.13	2.39±0.18	2.64±0.18	2.48±0.26
Triglycerides, mmol/liter	1.27±0.16	1.26±0.11	0.90±0.09^{+}	0.85±0.06^{x}	0.86±0.11
Uric acid, mmol/liter	0.64±0.09	0.60±0.06	0.50±0.03	0.52±0.03	0.39±0.04^{o}
Number of granules*	3600±350	4738±560^{+}	7016±736^{+}	1980±424^{x}	1898±315^{o}
Σ granule area*	4431±400	5827±400^{+}	11646±1100^{+}	2336±380^{x}	2277±395^{o}

Note. * Number of calcium granules and Σ granule area per aortic cross-sectional area. $p<0.05$ in comparison with $^{+}$group 1, ^{x}group 2, ^{o}group 3.

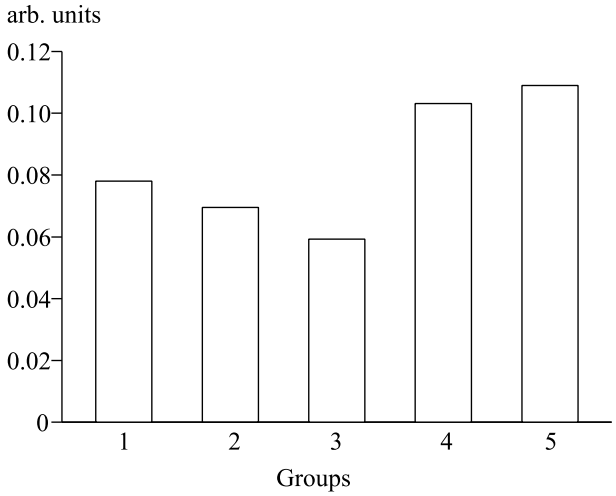

Fig. 1. Mean optical density of calcium salt granules in the aortic wall of experimental animals.

Biochemical parameters of blood serum in group 4 (fishing line, CH, xydiphone) were significantly better than in group 2 (fishing line, CH). HDL content increased by 23% (0.7 *vs.* 0.86 mmol/liter), atherogenic index was decreased (2.62 *vs.* 2.09 rel. units). Albumin binding reserve remained high (81.28%) despite moderate decrease in EAC and TAC. The index of toxicity remained moderately increased (0.22 and 0.24 rel. units respectively; Table 1). Thus, the findings attest to a positive effect of xydiphone on experimental atherosclerosis manifesting in improvement of lipid and purine metabolism despite the known ability of xydiphone to retain a certain amount of peroxides in the cell. The latter can be completely eliminated by simultaneous administration of antioxidants in age-appropriate doses (vitamin E *etc.*).

Group 5 (fishing line, CH, vitamin D_2, xydiphone) showed some unexpected and highly reliable positive effects. Albumin index of toxicity considerably (by more than 2 times) decreased in all animals in comparison with other groups (0.23, 0.22, 0.27, 0.233 and 0.11 rel. units, respectively) though reserve binding capacity for albumin (detoxifying potential) was significantly increased (90% in comparison with 81-82% in other groups). Atherogenic index was almost unchanged in comparison with group 3, but serum HDL was somewhat (4-5%) decreased (Table 1). Marked (by 24%) reduction in blood serum level of uric acid in group 5 in comparison with group 3 was reported, and it was the lowest level of uric acid in comparison with all other groups (0.64, 0.6, 0.5, 0.52, and 0.39 mmol/liter respectively). In our opinion, despite the lack of antioxidants in the medical complex, xydiphone combined with a short course of subtoxic doses of vitamin D_2 had the best effect on different aspects of metabolism, which alterations are usually accompanied by high risk of atherosclerosis.

Comparison of biochemical data with morphometric parameters of calcium deposits in the aortic wall revealed strong positive correlations between the size of granules of calcium salts, on the one hand, and the levels of LDL (r=0.90-0.91), xanthine oxidase (r=0.61-0.65), alkaline phosphatase (r=0.90-0.91), triglycerides (r=0.60-0.62), and uric acid (r=0.64-0.66), on the other. In contrast to the size, the density of calcifications inversely correlated with atherogenic index (r=-0.61), antioxidant activity (r=-0.98), and the levels of triglycerides (r=-0.8) and uric acid (r=-0.79), *i.e.* granules showed a higher density of calcium deposits with a decrease in antioxidant activity, levels of triglycerides and uric acid as well as atherogenic index, which can be considered a factor normalizing calcium deposition in the cells as have been revealed under the influence of calcium regulator, xydiphone [5,6]. This effect is probably due to the formation of storage depot for calcium in mitochondria under the impact of regulators of calcium metabolism. The results of morphometric analysis of calcium salts deposited as microconglomerates are presented in Table 1 and Figure 1.

The following conclusions can be made from these findings. Reduced HDL and increased LDL, atherogenic index, albumin toxicity index, serum levels of triglycerides and uric acid together with increased activity of alkaline phosphatase proved to be biochemical risk factors for atherosclerosis. Subtoxic doses of vitamin D_2 introduced *per os* at the beginning of the experiment resulted in increased toxicity index, serum levels of MDA and CPK after 1 month. However, a moderate decrease in activity of xanthine oxidase and triglycerides and uric acid contents was reported. Xydiphone used for medicinal purposes increased HDL levels and reduced atherogenic index, levels of triglycerides, and uric acid. Application of two regulators of calcium metabolism, namely xydiphone therapeutic doses for a long time and short initial course of subtoxic doses of vitamin D_2 decreased LDL and atherogenic index by the end of the month, high significantly reduced albumin index of toxicity as well as blood levels of uric acid and triglycerides. The investigated biochemical parameters of blood significantly correlated with the degree of calcium deposition in the aortic wall, and the size and density of calcifications depended on experimental conditions.

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