THERAPEUTIC EFFECT OF THE ANTIOXIDANT DIBUNOL IN EXPERIMENTAL ALCOHOL CARDIOMYOPATHY

V. D. Antonenkov, V. G. Tsyplenkova, A. M. Vikhert, and L. F. Panchenko

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An experimental model of alcohol cardiomyopathy (ACMP) is created by prolonged and combined administration of ethanol and the highly specific catalase inhibitor 3-amino-1,2,4-triazole (aminotriazole) to animals [11, 15]. As a result, rat cardiomyocytes (CMC) develop ultrastructural changes, similar on the whole to the morphological picture of ACMP in man [10, 16]. The study of the mechanism of development of the disease has shown that prolonged alcoholic intoxication is accompanied by increased activity of microperoxisomal enzymes in the rat myocardium: acetyl-CoA-oxidase and catalase. There is a parallel rise of the level of peroxidation in the heart tissue. Aminotriazole (AT), which inhibits catalase, potentiates the pro-oxidant and cardionecrotic effects of ethanol [2, 3, 13]. The information given above indicates a probable role of myocardial microperoxisomes and of lipid peroxidation (LPO) in the pathogenesis of ACMP, so that therapeutic agents with antioxidative action can be regarded as potential drugs for the prevention and treatment of this disease.

In this investigation we studied the effect of the artificial lipid antioxidant dibunol on the intensity of the morphological features of ACMP and also on the level of peroxidation in rat CMC.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing initially 160-180 g. The animals were kept on a semisolid diet balanced for the basic components (proteins, fats, carbohydrates, vitamins, mineral additives) [1]. Parallel experiments were carried out on rats of four groups: 1) control, 2) ethanol + AT, 3) dibunol, 4) ethanol + AT + dibunol. Animals of groups 2 and 4 received ethanol as a constituent of their diet (34-36% of the total calorific value of the diet, 10-12 g/kg body weight daily). AT, in the form of a 10% aqueous solution, was injected intraperitoneally in a dose of 1 g/kg 3 times a day. The animals of groups 3 and 4 were each divided into three subgroups in accordance with the dose of dibunol (2,6-di-*tert*-butyl-*p*-cresol, from "Sigma," USA): a) 100 mg/kg, b) 40 mg/kg, c) 10 mg/kg. The total duration of the experiments was 10-12 weeks. The rats were deprived of food for 18-20 h before sacrifice and the last injection of AT was given. Perfusion of the heart with isotonic KCl solution and homogenization were carried out as described previously [2, 3]. The homogenate was centrifuged at 3000 rpm for 10 min (nuclear-free homogenate). Catalase activity was determined at 25°C [8] and the concentration of reduced glutathione was measured with the aid of Ellman's reagent [14]. To investigate LPO the level of chemiluminescence and the rate of accumulation of products reacting with 2-thiobarbituric acid — TBAP [3, 13], were determined. To initiate peroxidation, 0.1 mM $FeCl_3$, 1.5 mM ADP, and 0.1 mM ascorbic acid were used in both cases [9]. The level of chemiluminescence was recorded on a scintillation counter ("Roche-Bioélectronique," France) and expressed in counts per minute on a standard sample. The rate of TBAP accumulation was expressed in nanomoles of malonic dialdehyde (535-156 mM^{-1} cm^{-1}) per minute per gram tissue. Protein was determined by Lowry's method [12]. Material for electron-microscopic study was taken from the left ventricle and processed as described previously [15], including electron-histochemical detection of nickel as a marker of ischemia of CMC [7] and the method with colloidal lanthanum to determine membrane permeability [5].

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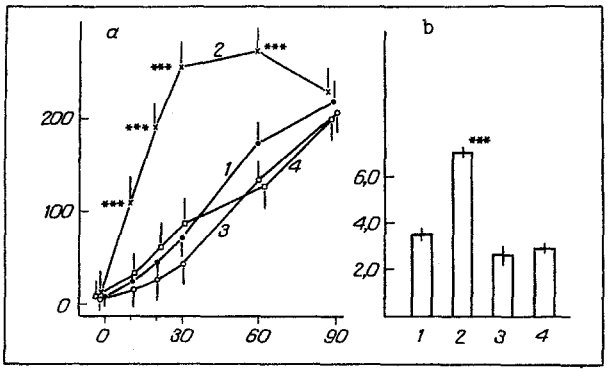

Fig. 1. Characteristics of peroxidation in rat myocardium during long-term administration of ethanol, AT, and dibunol. a) Trace of intensity of chemiluminescence; abscissa, time after addition of initiators of peroxidation (in min); ordinate, level of chemiluminescence (in cpm · 10^{-6} per sample). Nuclear-free homogenate of heart tissue was used (final protein concentration in sample 0.9-1.1 mg/ml). b) Determination of TBAP in nuclear-free heart homogenate (0.6-0.8 mg/ml). Ordinate, rate of accumulation of TBAP (in mmoles malonic dialdehyde per minute per gram tissue). Group of animals: 1) control, 2) ethanol + AT, 3) dibunol, 4) ethanol + AT + dibunol in a dose of 40 mg/kg (number of animals in each group 4-6). Significance of differences from control: $^{**}p < 0.01$, $^{***}p < 0.001$.

EXPERIMENTAL RESULTS

As the writers showed previously [3, 6] and as the present study confirmed, during the combined action of ethanol and the catalase inhibitor AT, considerable activation of LPO took place in the rat myocardium. In group 2 there was a parallel rise of the level of chemiluminescence and the rate of TBAP accumulation (Fig. 1). Catalase activity in the nuclear-free heart tissue homogenate fell by about half (group 1, 78 ± 0.10 unit/g tissue; group 2, 0.36 ± 0.05 unit/g; $p < 0.001$, $n = 6$), whereas the concentration of reduced glutathione was unchanged (group 1, 0.72 ± 0.09 μmole/g; group 2, 0.78 ± 0.12 μmole/g). A decrease of 8-10% ($p < 0.05$) in the relative mass of the heart (mass of organ/body weight) was observed, evidence of the development of dystrophic changes in the myocardium. This last observation was confirmed by electron-microscopic study of the myocardium, described by the writers previously [15]. Lysis of virtually all myofibrils, destruction of mitochondria, marked vacuolation of the sarcoplasm, and accumulation of lysosomes and lipofuscin was observed in some CMC, marked lipid infiltration of CMC was observed, and fat cells appeared in the interstices of the myocardium. Colloidal lanthanum passed through the damaged sarcolemma of 25-30% of CMC but was not found in capillary endotheliocytes (Fig. 2a). Characteristic changes also were found in the blood capillaries: thinning of the capillary wall with disturbance of its continuity, the lumen of some capillaries was completely occupied by blood cells [4]. Besides dilated blood vessels and obstructed lumen, the reaction for endogenous nickel revealed ischemic CMC (Fig. 2b). The disturbances listed above were absent in group 1 (control) and group 3 (dibunol). No deviations from the control values were found in group 3 during the study of intensity of peroxidation (Fig. 1), of catalase activity (0.76 ± 0.12 units/g) and glutathione concentration (0.69 ± 0.06 μmole/g).

In group 4b, in which a combination of ethanol, AT, and dibunol (40 mg/kg) was given, no marked activation of LPO could be observed (Fig. 1), although catalase activity was depressed compared with the control (0.36 ± 0.05 unit/g, $p < 0.001$, $n = 5$). The glutathione level was unchanged (0.68 ± 0.05 μmole/g). Similar results were obtained with dibunol in a dose of 100 mg/kg (group 4a). In the myocardium of the rats of this subgroup, considerable destructive changes were found in 10% of CMC, where they affected mainly the mitochondria, which were swollen, their cristae were damaged, and myelin-like formations were present. Colloidal lanthanum was found in 1% of CMC, evidence of stabilization of their membranes. Features characteristic of intracellular regeneration were found in most CMC: an increase in the number of mitochondria, large nucleoli were found in large nuclei, many ribosomes and polysomes were found, with large masses of glycogen. The diameter of the CMC showed a tendency to increase (group 1, 18.4 ± 0.5 μm; group 2, 16.0 ± 0.7 μm; group 4a, 22.5 ± 3.8 μm; $p > 0.5$). Meanwhile, considerable

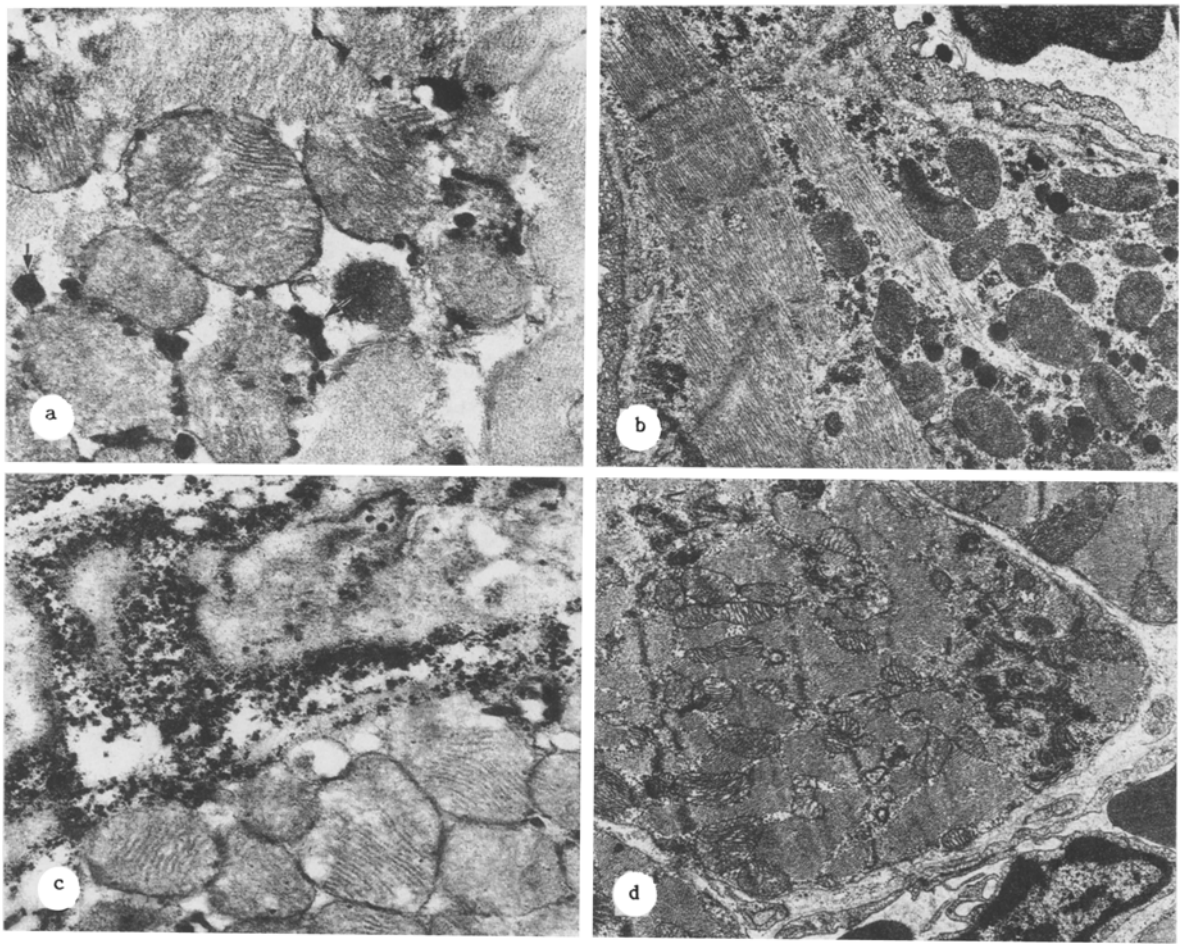

Fig. 2. Ultrastructure of myocardium in experimental ACMP and effect of dibunol. a) ACMP, penetration of colloidal lanthanum particles into sarcoplasm of CMC (arrows). 20,000×; b) Reaction to endogenous nickel in experimental ACMP (arrows). 10,000×; c) Treatment with dibunol in a dose of 40 mg/kg. Colloidal lanthanum does not penetrate into CMC but accumulates on cell boundaries (arrows). 20,000×; d) Absence of reaction for nickel in CMC after treatment with dibunol in a dose of 40 mg/kg. 8000×.

perivascular sclerosis developed in the interstices of the myocardium of the animals of group 4a, and the capillaries were sometimes immured in a muff composed of densely packed collagen fibers. Close to the vessels, nerve fibers and terminals, virtually free from vesicles, were found also to be immured in the connective-tissue sheath. With a decrease in the dose of dibunol to 40 mg/kg (group 4b) the most favorable picture of the myocardial ultrastructure was observed. The number of CMC in which changes characteristic of ACMP were discovered was minimal (5%). No perivascular sclerosis could be found around the capillaries and colloidal lanthanum did not penetrate into CMC (Fig. 2c). The reaction for nickel did not reveal any ischemic CMC (Fig. 2d). A large number of damaged CMC (20%) was observed in the subgroup of animals receiving dibunol in a dose of 10 mg/kg (group 4c). Interstitial sclerosis was absent, but considerable changes were observed in the microcirculatory bed: edema of the endothelium, disturbance of its integrity, occlusion of the capillary lumen, escape of plasma and blood cells outside the capillary wall [4]. The biochemical changes in this subgroup were not analyzed.

On the whole the results indicate that the artificial lipid antioxidant dibunol possesses prophylactic and therapeutic actions on a model of ACMP. A particularly marked positive effect was obtained with relatively high doses of the drug, namely 40 and 100 mg/kg body weight. Dibunol did not affect catalase activity or the reduced glutathione concentration, but when administered to animals receiving ethanol and AT, a decrease in the intensity of peroxidation processes was observed, down to the characteristic level of control values. The antioxidant prevented the development of changes in the ultrastructure of CMC characteristic of ACMP. This fact is evidence that peroxidation plays the primary role in the pathogenesis of this disease.

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EXPERIMENTAL STUDY OF ORIENTED ANTIBIOTIC TRANSPORT IN SUPPURRTIVE-INFLAMMATORY DISEASES OF THE LIVER AND BILIARY TRACT

Zh. Sh. Zhumadilov, N. D. Skuba, and R. V. Makarenkova

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The relatively high frequency of suppurative-inflammatory complications in surgery of the biliary tract, in which they are the main cause of death, despite the widespread use of modern antibiotics, makes the search not only for new preparations, but also for more effective methods of administering them, an urgent problem [2, 3]. The attention of research workers in recent years has been drawn to the development of methods of oriented transport of therapeutic and diagnostic preparations directly into a pathological focus [1, 7]. Oriented transport of antibiotics directly into the zone where they exert their chemotherapeutic action would allow the systemic toxicity of these substances to be reduced, while enhancing their therapeutic effectiveness. These problems have not been studied experimentally. The urgency of the problem also is due to the fact that none of the existing methods of antibiotic administration enables a therapeutic concentration of the preparation to be maintained for a long time in the liver and portal system [5]. As regards the treatment of inflammatory diseases of the liver and biliary tract, the use of erythrocyte ghost carriers is particularly interesting, for it has been shown [7] that they are ingested by erythrophagocytic cells of

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