EFFECT OF CHRONIC ALCOHOL CONSUMPTION ON THE MYOCARDIAL MICROCIRCULATORY BED

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KEY WORDS: alcohol; myocardium; capillary ultrastructure; antioxidants.

The harmful action of alcohol on the microcirculatory bed and the systemic nature of these changes have been studied [1, 2]. However, the role of disturbances of the microcirculation in the pathogenesis of the specific myocardial disease caused by alcohol, namely alcohol cardiomyopathy (ACMP), has not yet been explained.

The aim of the present investigation was to compare the ultrastructure of the myocardial capillary bed during chronic alcohol consumption in rats and in experimental ACMP and to attempt to correct it by antioxidants. These latter were given because of the hypothesis that lipid peroxidation plays a leading role in the pathogenesis of ACMP [5].

EXPERIMENTAL METHODS

Experiments were carried out on male Wistar rats weighing 150-180 g. All animals of the experimental groups were given ethanol for 12 weeks in a dose of 36% of the total calorific value of the diet, mixed with semisolid food [6]. Animals of the first group also were given an intraperitoneal injection of the specific catalase inhibitor, 3-amino-1,2,4-triazole, in a dose of 1 g/kg on alternate days to create a model of experimental ACMP [3, 6]. The animals of group 2 also received ethanol but on alternate days they were given an intraperitoneal injection of physiological saline (control to group 1). The rats of group 3, unlike those of group 1, received vitamin E in excess of its amount in the diet daily in a dose of 1 mg/kg to correct changes arising in ACMP. Animals of groups 4 and 5 were given another antioxidant, dibunol (4-methyl-2,6-di-tert-butylphenol) in doses of 100 and 10 mg/kg respectively for the same purpose. The control animals of group 6 received a diet of the same calorific value as the groups receiving ethanol, as a result of the addition of sucrose to the diet. In each group five animals were investigated. After decapitation of the rats the heart was removed and kept in cold isotonic potassium chloride solution. Material from the left ventricle was prepared for electron microscopy as described previously [3], including electron-histochemical detection of nickel as a marker of myocardial anoxia [4], and the method with colloidal lanthanum to determine membrane permeability [7].

EXPERIMENTAL RESULTS

The picture found in the myocardium of the animals of group 1 was that typical of ACMP, described by the writer previously [3]. Changes virtually identical with those in the animals of group 2 were found in the capillaries. In the rats of group 2, no characteristic signs of ACMP were observed.

Changes in the capillaries of the rats of groups 1 and 2 were as follows: stasis of blood (Fig. 1a), a great decrease in thickness of the endothelium and disturbances of its continuity, with the appearance of large "ports," so that capillary permeability was increased not only for plasma (Fig. 1b), but also for blood cells (the latter were often found in the interstices close to the capillaries; Fig. 1c). The lumen of individual capillaries was completely closed by leukocytes, and loci of adhesion of leukocyte and endotheliocyte membranes were observed (Fig. 1d). The impression was created of marked slowing of the blood flow in the capillary bed. Capillaries with smaller caliber were sometimes collapsed, and some of them had an undulating wall with numerous projections of endotheliocytes into their lumen. Marked edema of the endothelium was found in some capillaries (Fig. 2a).

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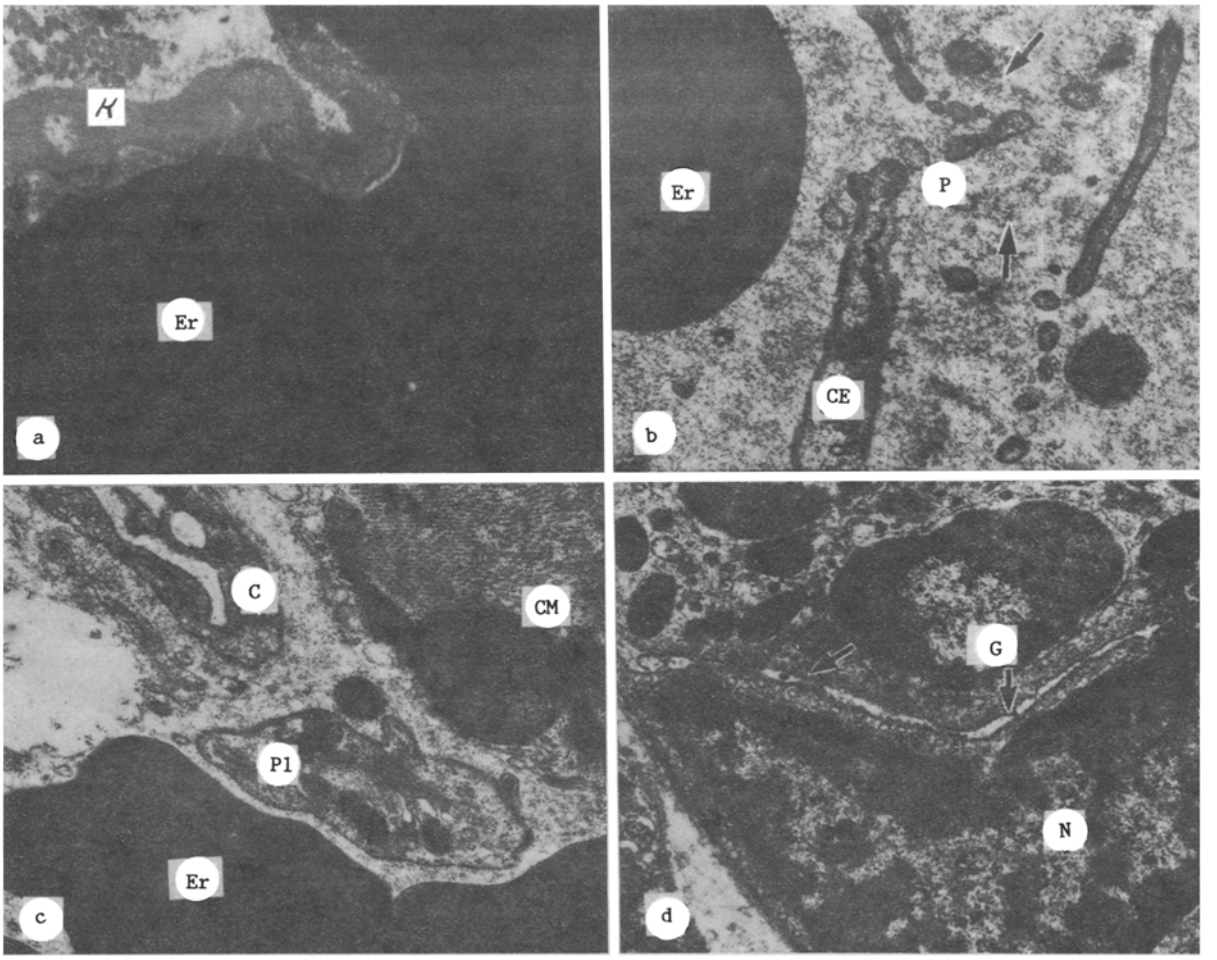

Fig. 1. Ultrastructure of myocardial capillaries of rats with chronic alcoholic intoxication and with experimental ACMP. a) Capillaries (C) congested with erythrocytes (Er) (20,000 ×); b) appearance of large "ports" (P) in capillary endothelium (CE), outflow of plasma proteins from capillaries (arrows). Er) Erythrocyte in capillary lumen (26,000 ×); c) appearance of blood cells outside capillaries (C). Er) Erythrocyte, Pl) platelet, CM) cardiomyocyte (18,000 ×); d) granulocyte (G) covering capillary lumen. N) Nucleus of endotheliocyte. Arrows indicate sites of adhesion of leukocyte and endotheliocyte membranes (20,000 ×).

Besides dilated capillaries, occluded by blood cells, the reaction for nickel also revealed cardiomyocytes (CMC) in a state of anoxia. In the animals of groups 1 and 2 colloidal lanthanum passed through the sarcolemma of some CMC but it was not found in the endotheliocytes. All changes in the microcirculatory bed described above were identical in groups 1 and 2, but were sometimes more marked in group 2 (without any signs of ACMP). In the rats of group 3 (receiving extra vitamin E) relative integrity of the CMC ultrastructure was observed by comparison with that in the animals of group 1; however, changes in the capillaries were analogous to those in the rats of groups 1 and 2. The walls of some capillaries were so overstretched that sometimes they were in almost direct contact with the sarcolemma of CMC, in which considerable accumulation of nickel was observed, evidence of their anoxia (Fig. 2b). Colloidal lanthanum was found in individual CMC with destructive changes, but it was not found in the capillary endothelium.

In the animals of group 4, treated with dibunol in a dose of 100 mg/kg, in most capillaries changes similar to those in the rats of groups 1, 2, and 3 were found. The difference was that marked interstitial perivascular sclerosis developed around some capillaries (Fig. 2b). In this group, unlike in groups 1, 2, and 3, colloidal lanthanum in capillaries free from a "sleeve" of this kind, penetrated into the endotheliocytes and even into the capillary lumen, although fixation was not done through the blood stream (Fig. 2d). Virtually no colloidal lanthanum was found in CMC. CMC with considerable accumulation of nickel were seen.

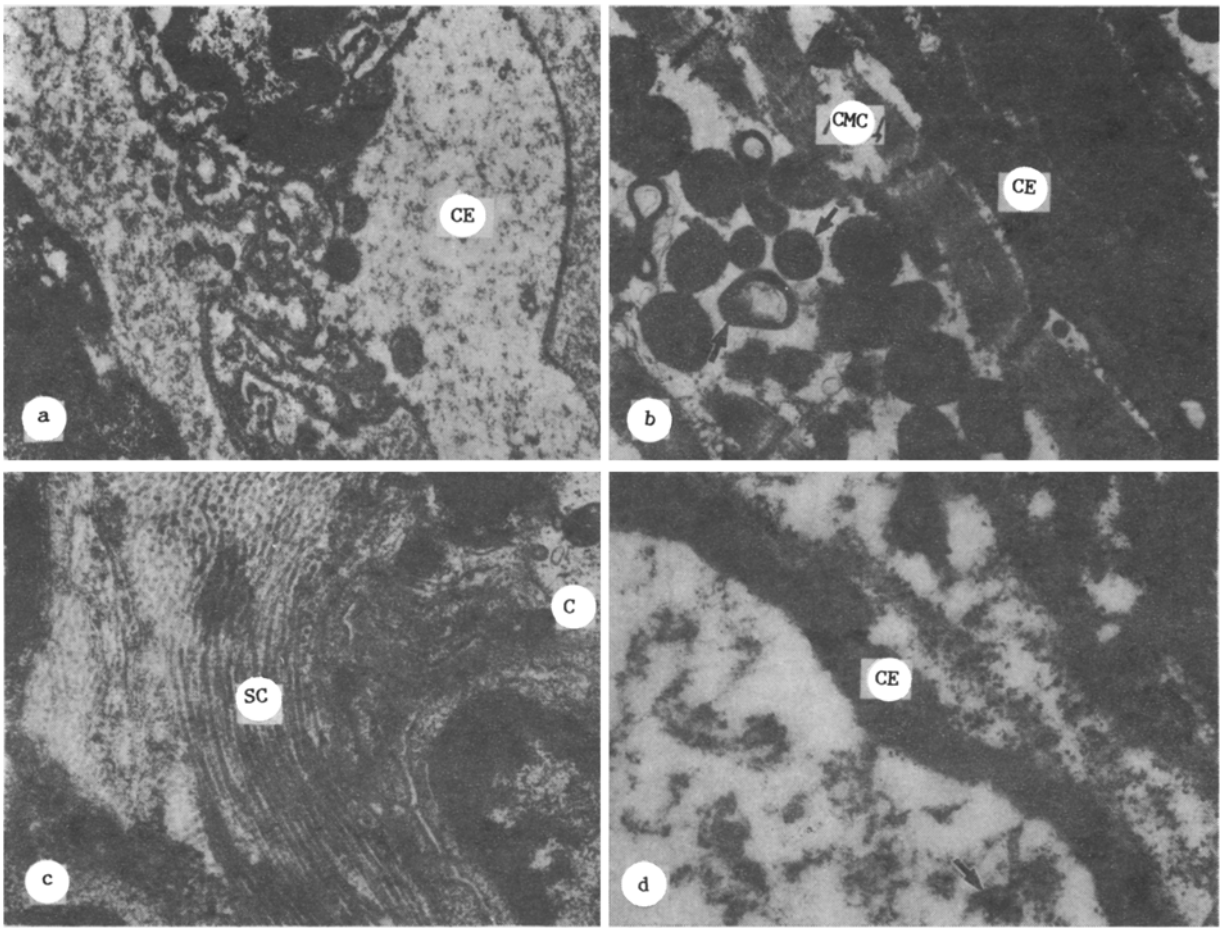

Fig. 2. Ultrastructure of myocardial capillaries of rats with experimental ACMP and during its treatment by antioxidants. a) Marked edema of capillary endothelium (CE) in experimental ACMP (18,000 ×); b) thinning of capillary endothelium (EC) in group treated with vitamin E. CMC) Cardiomyocyte with evidence of anoxia. Arrows indicate complexes of nickel dimethylglyoxime (13,000 ×); c) perivascular myocardial sclerosis (SC) after injection of 100 mg/kg dibunol into animals with experimental ACMP. C) Capillary (20,000 ×); d) appearance of colloidal lanthanum particles (arrows) in pinocytotic vesicles of capillary endothelium (CE) and capillary lumen after injection of dibunol into animals with ACMP (38,000 ×).

In the animals of group 5, receiving dibunol in a dose of 10 mg/kg, interstitial sclerosis was not discovered. The number of damaged CMC was smaller than in group 1 but greater than in group 4. Colloidal lanthanum did not penetrate into CMC but was found in the endotheliocytes of individual capillaries. The reaction for nickel did not find its accumulation in CMC, but it was observed to accumulate in the interstices and in the endothelium of the venous section of the capillary bed and in the capillary lumen. Changes described for groups 1, 2, and 3 were preserved in most cases.

The results of this investigation suggest that the first step in the pathogenesis of ACMP is damage to the microcirculatory bed. Changes in the capillaries were similar both in experimental ACMP and in chronic alcoholic intoxication (group 2). Injection of the antioxidants restored the ultrastructure of CMC to some degree, but the capillary bed remained virtually the same as in the untreated animals.

Combination treatment, including antioxidants and preparation normalizing capillary permeability, must therefore be given to correct the myocardial pathology arising in ACMP, and the influence of the harmful factor (alcohol) on the myocardium must also be completely eliminated. A further study of the methods of treatment of experimental ACMP must be aimed at choosing doses of antioxidants.

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LITERATURE CITED

1. A. M. Vikhert and V. G. Tsyplenkova, Arkh. Patol., No. 1, 14 (1984).
2. V. N. Kochegurov, Zh. Nevropatol. Psikhiat., No. 6, 905 (1981).
3. V. G. Tsyplenkova, A. M. Vikhert, and V. V. Stepantsov, Byull. Éksp. Biol. Med., No. 9, 367 (1985).
4. I. Balogh, E. Somogyi, P. Sotonyi, et al., Z. Rechtsmed., 90, 7 (1983).
5. L. Garcia-Bunuel, Med. Hypothes., 13, 217 (1984).
6. M. Kino, J. Mol. Cell. Cardiol., 13, 5 (1981).
7. J. R. Revel and M. J. Karnovsky, J. Cell Biol., 33, 7 (1967).

MORPHOMETRIC ANALYSIS OF CARDIOMYOCYTE MITOCHONDRIA IN NORMAL RATS AND DURING POSTISCHEMIC REPERFUSION

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Swelling of the mitochondria (MC) is invariably observed in the cardiomyocytes in the course of their damage during ischemia and reperfusion [1, 2, 5]. However the general principles governing this process are unknown.

The aim of this investigation was a morphometric analysis of the size, shape, and number of MC in cardiomyocytes under normal conditions and during postischemic reperfusion.

EXPERIMENTAL METHOD

Experiments were carried out on eight male Wistar rats weighing 250-300 g. The heart was removed under thiopental anesthesia, the aorta and left atrium were cannulated, and the left side of the heart was perfused with Krebs–Henseleit buffer by the method in [4]. The four rats in the control group were perfused for 90 min. The hearts of four experimental animals, after control perfusion for 10 min, were subjected to total normothermic ischemia for 30 min and reperfusion for 40 min. At the end of perfusion, a piece of myocardium from the apex of the left ventricle was fixed in 2.5% glutaraldehyde solution, postfixed with OsO_4, dehydrated, and embedded in Epon–Araldite. Sections through the myocardium were stained with lead citrate and uranyl acetate and examined in the ÉVM-100L electron microscope under a magnification of 23,000. Using a multipurpose test grid [7] with 270 control points the bulk density of MC (V_V), the relative surface density of MC (S_V), and the number of mitochondrial profiles per square micron area of section (n) were determined on the photographic plates. The results were used to calculate the diameter (D), length (L), and volume (V_{1mc}) of one mitochondrion, the number of MC in 1 μ^3 of sarcoplasma (N), and the surface area of one MC (S_{1mc}) and of all MC in 1 μ^3 in sarcoplasm (S). In longitudinal sections through the cardiomyocytes the length of MC (l_{mc}) and of the sarcomeres (l_{sarc}) was measured, and in sections not cut longitudinally, the diameter of MC (d_{mc}) was measured. The results were subjected to statistical analysis (95% confidence limits).

EXPERIMENTAL RESULTS

During postischemic reperfusion the cardiomyocyte MC had a significantly higher bulk density and lower relative surface density than in the control (Table 1). The number of

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