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EXPERIMENTAL STUDY OF THE USE OF D-PENICILLIAMINE IN CIRRHOSIS OF THE LIVER

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D-penicillamine (D-PA) is known to be an effective remedy for systemic diseases of connective tissue. In recent years the beneficial effect of D-PA in chronic hepatitis and cirrhosis of the liver has been reported [3, 9, 15]. However, there are no reports in the literature on experimental research into the use of D-PA in this pathology, although one or two communications have dealt with the biochemical study of the effect of D-PA on collagen synthesis [10, 14].

The object of the present investigation was a combined study of the action of a therapeutic dose of D-PA on different manifestations of the pathological process during the development of experimental cirrhosis and also a comparative study of the action of D-PA and prednisolone, a drug widely used in clinical practice for the treatment of chronic liver diseases.

EXPERIMENTAL METHOD

Experiments were carried out on a model of metatoxic cirrhosis of the liver of the writers' own design, using a cyclic combination of hepatotoxic agent and hepatogenous antigen [8]. In this particular model experimental cirrhosis of the liver is accompanied by more marked autoimmune manifestations and it bears a closer resemblance to active human cirrhosis of the liver than models described previously [2, 5, 6].

Experiments lasting 7.5 months were set up on 97 male chinchilla rabbits, and comprised two series of chronic experiments. In series I (37 animals) the effect of D-PA on the

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development of cirrhosis of the liver was studied. In series II (60 animals) a comparative study was made of the action of D-PA and prednisolone. Each series included groups of treated, untreated (subjected only to the action of the harmful agent), and control (intact) animals. In series I the treated animals received D-PA in a dose of 30 mg/kg. In series II the group of treated animals was divided into three subgroups: a) receiving D-PA (30 mg/kg); b) receiving prednisolone in a dose of 0.5 mg/kg; c) receiving prednisolne in a dose of 0.25 mg/kg. Treatment with these drugs began after the appearance of the early signs of formation of cirrhosis of the liver and continued for 3 months simultaneously with administration of the harmful agent. The effectiveness of treatment was assessed by intravital morphological investigation of liver tissue and by a combination of functional, biochemical, and immunologic indices. Material from repeated liver biopsies was studied histologically, histochemically, and electron microscopically. Morphological manifestations of the pathological process in the liver were assessed quantitatively in conventional units depending on the degree of severity of the trophic changes in the hepatocytes, fibrous changes, and autoimmune manifestations [4].

The oxidizing and phosphorylating functions of the hepatocyte mitochondria (MCh) were studied in the same biopsy material by a polarographic method and the concentration of cytochromes in MCh was determined by differential spectrophotometry. The total gas exchange was studied when biopsy specimens were taken; the excretory function of the liver was studied by the bromsulphthalein (BSP) test [7], indices of protein (total protein and its fractions) and lipids (β-lipoproteins) metabolism were investigated, and activity of the following serum enzymes also were determined: aldolase, alanine (GPT) and asparagine (GOT) aminotransferase, glutamate dehydrogenase (GlDH), total lactate dehydrogenase and its urea-stable fraction, alkaline phosphatase, and γ-glutamyl transpeptidase (GlTP). The enzymes were determined by a kinetic method on the Rotochem II centrifuge autoanalyzer.

The immunologic investigations included determination of immunoglobulin (Ig) fractions in the blood serum by immunoelectrophoresis and circulating liver antibodies by a modified direct Coombs' test [1], and by the lymphocyte blast transformation test (LBTT) in a 3-day culture with phytohemagglutinin [12].

The animals were tested before exposure (background) and during exposure to the harmful factors, before the beginning and at the end of treatment, given simultaneously with poisoning.

EXPERIMENTAL RESULTS

Before the beginning of treatment, after the first cycle of poisoning marked morphological changes were observed in the liver, with the development of degeneration of the hepatocytes and fibrosis of the portal tracts. After two cycles of poisoning most animals showed signs of monolobular cirrhosis of the liver. Disturbances of liver function accompanied these changes: a decrease in the albumin concentration and an increase in the β-lipoprotein and γ-globulin concentrations in the blood serum, an increase in the retention coefficient (C_r) of BSP, high blood enzyme levels, and changes in the indices of oxidative metabolism of MCh. Evidence of the development of autoimmunization was given by the appearance of circulating antiliver antibodies and of infiltration of the liver tissue by histiocytes, lymphocytes, and plasma cells and a reduction in the transforming activity of the lymphocytes.

Administration of D-PA and prednisolone in adequate doses caused stabilization of the pathological process, whereas the cirrhotic manifestations progressed in the untreated animals. Treatment with D-PA led to a marked decrease in the severity of the fibrosis (Fig. 1), which was 7.4 ± 0.4 in the untreated animals and 5.2 ± 1.0 conventional units ($P < 0.05$) in the treated animals. The intensity of the dystrophic changes also was less than in the untreated animals (6.5 ± 1.1 and 11.7 ± 1.8 respectively; $P < 0.05$). The dynamics of the morphological features of autoimmunization was similar.

The effect of D-PA on liver morphology also was revealed electron microscopically. In the first place differences were seen in connective tissue development: In the treated animals the number of mature collagen bundles was small (Fig. 2) and the collagen fibrils were loosely arranged and surrounded by floccular material, identified histochemically as glycosaminoglycans. Meanwhile in the untreated animals extensive areas of collagen fibers and bundles, characteristic of coarse connective tissue of scars, were observed (Fig. 2).

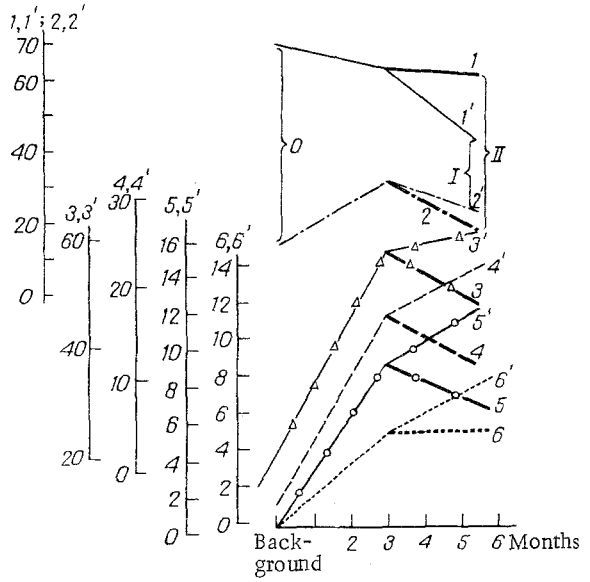

Fig. 1

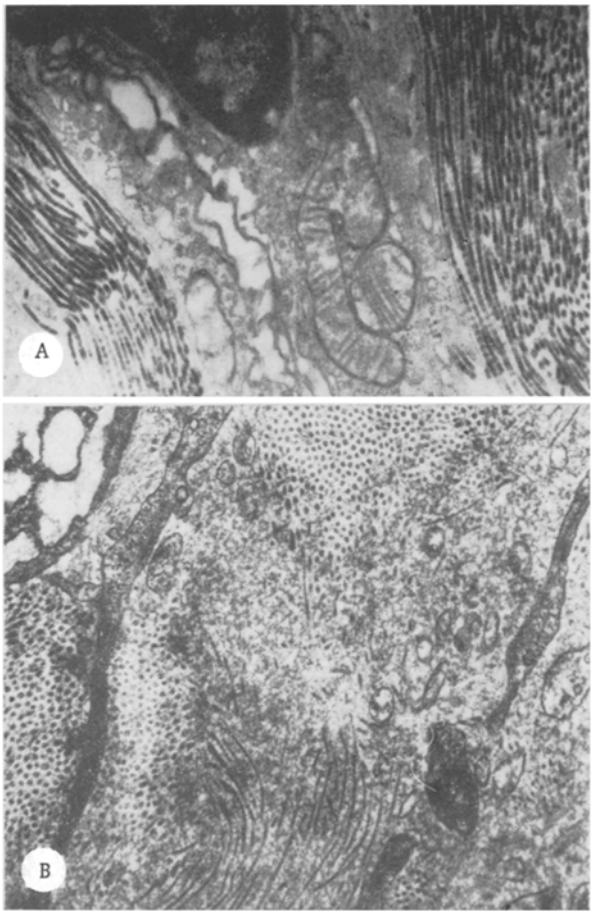

Fig. 2

Fig. 1. Functional and morphological changes in the liver during treatment of cirrhosis by D-PA. Abscissa, time of investigation (in months); ordinate: 1, 1') respiration rate of MCh in state of activity (in n-atoms O_2/min/mg protein), in the presence of succinate, glutamate, and 2,4-dinitrophenol (V_{DNP}); 2, 2') respiration rate of MCh after phosphorylation of added ADP (V_4), 0, I, II) ranges of respiratory activity of MCh ($V_{DNP} - V_4$); 3,3') β-lipoproteins (in optical density units × 100); 4, 4') BSP remaining in the blood after 15 min as a percent of its concentration determined 1 min after injection into the animals); 5, 5') dystrophy (in relative units); 6, 6') fibrosis (in relative units). Without prime — receiving treatment, with prime — without treatment. treatment.

Fig. 2. Electron micrographs of interlobular connective tissue of the liver without treatment (A) and after treatment with D-PA (B) (12,500 ×).

Differences between the groups were discovered also in relation to the oxidative function of hepatocyte MCh. In the untreated animals the velocities of oxidation of the different substrates (V_{DNP}), uncoupled by 2,4-dinitrophenol, were significantly reduced compared with the control, stimulation of respiration by ADP was reduced, and the quantity of oxygen initially consumed on addition of MCh to incubation medium was increased (Fig. 1). Meanwhile, in the treated animals, no significant differences were found from the controls in the levels of oxidative and phosphorylating functions of MCh. The action of D-PA was most clearly reflected by the range of respiratory activity (Fig. 1), which was twice as wide in the treated animals (II) as in the untreated (I), and some close to that observed in intact rabbits (0). In the treated animals, moreover, a smaller decrease was observed in the concentration of cytochromes (Fig. 3), reflecting a decrease in the number of respiratory chains.

The intensity of the gas exchange of the body as a whole was significantly higher (8.3 ± 0.7 mg/min/kg) in the treated animals than in the untreated (6.9 ± 0.5 ml/min/kg) and it corresponded to the level in the control group (8.6 ± 0.9 ml/min/kg).

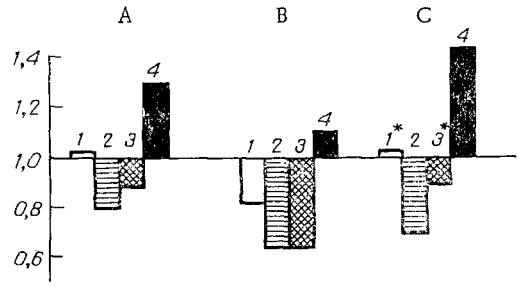

Fig. 3. Content of cytochromes in liver mitochondria of untreated and treated rabbits. 1, 2, 3, 4) Cytochromes a, b, c_1, and c, respectively (in relative units). A) Before treatment; B) without treatment; C) after treatment. *P < 0.05.

Analysis of the state of the liver function (Fig. 1) showed that in the untreated animals there was a greater decrease in the albumins and an increase in the concentration of β-lipoproteins and γ-globulins and an increase in C_r of BSP. The differences between the two groups for the two last indices were significant. The increase in activity of various serum enzymes (GPT, GOT, GlDH, GlTP) was less marked in the treated animals, although differences between the groups were not significant.

A characteristic feature of the action of D-PA was a change in the immunologic indices of the blood serum. In treated animals the titer of liver antibodies was significantly lower (0.17 ± 0.036) than in the untreated animals (0.36 ± 0.054; P = 0.02), in which, however, a smaller increase in the concentrations of IgM and IgA was noted. The results of LBTT of the peripheral blood demonstrated a significant decrease in the level of this reaction in the untreated animals compared with the controls (21.37 ± 2.12 and 37.8 ± 0.69 respectively; P < 0.01), whereas in the treated animals this value was substantially unchanged.

During the period of progression of cirrhosis of the liver D-PA thus caused, first, definite inhibition of the fibrous changes and, later, a decrease in the degree of dystrophic changes in the hepatocytes; it had a protective action on mitochondrial function, improved the functional state of the liver, and reduced the manifestations of autoimmunization.

To assess the therapeutic value of D-PA, the results of its action were compared with those of the action of prednisolone, a known immunodepressant. Two doses of the hormone were tested — 0.25 and 0.5 mg/kg. The last dose, on the first month of treatment, had no positive effect on the morphological and functional state of the liver, the indices of which differed only a little from those of the untreated animals. Further administration of the hormone led to death of all the animals after 2-3 months from intercurrent diseases. In most animals potentiation of the cirrhotic changes and damage to the kidneys were observed, and a few rabbits showed atrophy of the adrenal cortex. A dose of prednisolone of 0.25 mg/kg gave a marked therapeutic effect: The degenerative and fibrous changes in the liver tissue were reduced. Prednisolone depressed the morphological indices of autoimmunization by a greater degree than D-PA, but as regards the other immunologic test and also functional disturbances, no significant difference could be found between the animals treated with D-PA and those treated with prednisolone.

It can be concluded from these results that D-PA and prednisolone have a therapeutic action in experimental cirrhosis of the liver. The antiinflammatory, antisclerotic, and immunodepressive effects of the preparations were established. The primary stage in the mechanism of action of D-PA is inhibition of maturation of collagen, in agreement with data in the literature [13, 14], whereas its antiinflammatory and immunodepressive effects are evidently manifested later. Prednisolone acts primarily as an immunodepressant, reducing dystrophic and necrobiotic changes in the hepatocytes, but the evidence of the present experiments shows that preference must be awarded to D-PA, since prednisolone gives rise to serious side effects as a hormonal preparation, and overdosage may be extremely dangerous.

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EXPERIMENTAL ASPECTS OF THE THERAPEUTIC ACTION OF UROKINASE IN THROMBOEMBOLIC STATES

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KEY WORDS: thromboembolic complications; urokinase; fibrinolytic action; thrombolytic action.

The problem of thrombosis and embolism occupies an important position in modern medicine and has recently become one of considerable urgency because of the increased incidence of cardiovascular diseases and the development of thoracic and vascular surgery, which are attended by a high risk of development of thromboembolic complications. Much evidence has recently been gathered on the surgical and combined (together with fibrinolytic agents) methods of treatment of thrombosis and embolism [2-7]. However, besides the use of fibrinolysin, an approach that is beginning to be more intensively pursued is lytic therapy by means of urokinase, an activator of fibrinolysis which is so far the most promising of the thrombolytic agents [8-10].

The object of this investigation was to study the effect of urokinase on the fibrinolysis system and on the thrombolytic process under experimental conditions and to assess thrombolytic treatment during induction of endogenous fibrinolysis with urokinase.

A highly active preparation of urokinase with specific activity of 30,000-40,000 i.u./mg protein was isolated from healthy human urine by methods of adsorption, ion-exchange chromatography and gel filtration.

To study the fibrinolytic and thrombolytic action of urokinase experiments were carried out on rabbits weighing 2-2.5 kg. Local thrombosis was produced in 8 rabbits by injection of thrombin into a segment of the marginal vein of the ear. The presence of a thrombus in the vein was detected by phlebography, in the form of a filling defect in the vessel (Fig. 1a). Under the influence of urokinase, lysis of the clot took place (Fig. 1b). In 15 rabbits disseminated microthrombosis was induced by heterologous blood transfusion. In this group of animals, after production of thrombosis and after injection of urokinase, the following indices of the fibrinolytic system of the blood was studied: the time of lysis of a clot of euglobulin fractions, fibrinogen content, plasminogen level, degree of activity of antiplasmins, and thrombin time, indicating the combined action of fibrinogen degradation products.

Experiments also were carried out on 72 albino rats in which massive microthrombosis was induced by injection of heterologous blood (2.5 ml human blood/100 g body weight). These

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