PROTECTIVE ACTION OF ANTIOXIDANTS ON DEVELOPMENT OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN GUINEA PIGS

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As reported previously, the development of experimental allergic encephalomyelitis (EAE) is accompanied by marked activation of lipid peroxidation (LPO), dependent on the formation of an immunologic response to injection of encephalitogenic material [9]. This suggests that intensification of LPO plays an important role in the development of pathology of membrane structures in EAE. Data have been published showing that LPO is intensified in certain diseases of the nervous system (multiple sclerosis, schizophrenia) [8, 7], in the genesis of which a definite role is played by the neuroallergic component.

The aim of this investigation was to study the action of a combination of substances on the development of EAE in guinea pigs, including: α-tocopherol, which has an antioxidant action, lithium compounds, which prevent the effects of antibrain antibodies [3], and pyridoxal phosphate, which participates in the regulation of various metabolic processes. There is evidence of pyridoxine deficiency in patients with multiple sclerosis [1].

EXPERIMENTAL METHOD

Experiments were carried out on 110 guinea pigs weighing 250-300 g. The animals were kept under standard animal house conditions. EAE was produced by means of an encephalitogenic emulsion containing homologous brain and Freund's adjuvant (5 mg BCG in 1 ml). EAE was diagnosed on the basis of the clinical picture: pareses and paralyses of the limbs and sphincters, loss of body weight. In some cases the brain and spinal cord were studied morphologically. Sections were stained with hematoxylin and eosin. Clinical manifestations of EAE developed in the control animals on the 10th-13th day after injection of the encephalitogenic emulsion and followed an acute course. As a rule all animals of the control group died during 1 week after appearance of clinical manifestations of EAE.

To prevent EAE a combination of substances was used, namely lithium hydroxybutyrate (150 mg/kg), pyridoxal phosphate (15 mg/kg), and vitamin E (100 mg/kg). These substances were injected intramuscularly into the animals over a period of 20 days: in group 1 (19 animals) starting from the 1st day, in group 2 (10 animals) starting from the 3rd day, and in group 3 (10 animals) starting from the 7th day of immunization with encephalitogenic emulsion (Table 1). During immunization with encephalitogenic emulsion (on the 7th and 14th days) the following parameters were determined in the blood of 10 control animals and of 10 animals of group 1: total peroxidase activity [5, 6], the intensity of formation of TBA-active products in erythrocyte membranes [2], and neurotropic activity of serum [4] to monitor the effect of treatment on LPO processes and the appearance of antibrain antibodies. Blood for investigation was taken from the heart.

EXPERIMENTAL RESULTS

Of 19 guinea pigs in group 1 only four animals developed EAE: a mild form in three followed by restoration of motor functions, and a severe form in one animal, followed by death. The incubation period, incidentally, was increased in these animals to 17-20 days (Table 1).

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TABLE 1. Effect of Combined Treatment on Development of EAE in Guinea Pigs

Group of animals	Total number of animals	Number of animals with paralyses	Number of animals which died	Number of animals not developing disease	Incubation period, days
1	19	4	1	14	17—20
2	10	2	—	8	17—19
3	10	10	10	—	13—16
4	5	5	3	—	10—13
5	5	5	5	—	10—13
6	5	5	5	—	10—13

Legend. Animals of groups 4, 5, and 6 received lithium hydroxybutyrate, pyridoxal phosphate, and α-tocopherol, respectively.

TABLE 2. Effect of Combined Treatment on LPO and Serum Neurotropic Activity in Guinea Pigs during Immunization with Encephalitogenic Emulsion (M ± m)

Times of investigation	TBA-active products		Total peroxidase activity, relative units		Neurotropic activity, relative units	
	control	experiment	control	experiment	control	experiment
Background	28,2±0,5	28,2±0,5	21,3±0,5	21,3±0,5	0,42±0,01	0,42±0,01
7th day	43,3±0,3*	24,9±0,6	15,6±0,2*	27,7±0,7***	0,18±0,03	0,45±0,02
14th day	57,7±1,6*	28,1±0,8	24,6±0,6**	21,8±0,5	0,24±0,02*	0,30±0,03

Legend. "Experiment" column gives data for animals of group 1. Level of TBA-active products given in nanomoles/ml of erythrocyte suspension. *$P < 0.001$, **$P < 0.002$, ***$P < 0.01$ compared with background.

On the 7th day of sensitization, the formation of TBA-active products in erythrocyte membranes under the influence of H_2O_2 was increased in animals of the control group by 55.5%, whereas in animals receiving combined treatment this parameter was identical with that for normal guinea pigs (Table 2). On the 14th day of immunization the level of TBA-active products in erythrocyte membranes of the treated animals likewise did not differ from the background values, whereas in animals (controls) with EAE it was 103.5% higher than the background values.

On the 7th day of sensitization total serum peroxidase activity in animals of the control group was 26.8% lower than the background values, whereas in animals receiving combined treatment it was 30.4% higher than in normal guinea pigs (Table 2). On the 14th day of sensitization total peroxidase activity in animals with EAE was 15.5% higher than the background values, whereas in guinea pigs of group 1 it was the same as in normal animals (Table 2).

Serum neurotropic activity of animals of the control group could be detected as early as on the 7th day of sensitization. During further development of EAE neurotropic activity decreased a little. In treated animals neurotropic activity could not be detected on the 7th day and did not appear until the 10th-14th day (Table 2).

In group 2 EAE developed on the 17th-18th day of sensitization in only two of the 10 animals and it followed a mild course. Motor functions of these animals were restored by the 22nd-25th days of immunization.

Combined treatment started on the 7th day of immunization gave no protective effect. Clinical manifestations of EAE developed on the 13th-16th day. All the animals died during the first 4 days after appearance of signs of EAE.

In subsequent versions of the experiment the substances, in the doses indicated above, were given separately. In these experiments there was no protective effect. The incubation period was 10-13 days. Only in two animals receiving lithium hydroxybutyrate was recovery of motor functions observed on the 22nd-24th day after the beginning of immunization. All the remaining 13 animals died during the first week.

Morphological investigations were undertaken on control animals and also on guinea pigs of groups 1 and 2 (five animals from each group). Perivascular infiltrates, fibrinoid necrosis of the walls of some vessels, and marked signs of edema were found in the brain of all five animals in the control group. In two animals of group 1 and one animal of group 2 solitary small perivascular infiltrates were found.

The positive effect of combined treatment confirms the view expressed above that activation of LPO is initial in character and that antibrain antibodies play an important role in the genesis of membrane lesions in neuroallergy. The absence of a prophylactic effect when the substance was given starting on the 7th day of immunization with encephalitogenic emulsion was evidently due to the fact that LPO processes were already activated in the animals' brain at that stage of immunization [9] and that irreversible changes manifested as significant morphological and functional changes in the cell and, in particular, as activation of the lysosomal apparatus and injury to myelin, had taken place. Meanwhile, administration of each substance separately, starting with the 1st day of immunization, gave no appreciable effect and did not prevent the development of EAE.

The results suggest that the use of this combination of substances in diseases of the nervous system in whose genesis an important role is played by the neuroallergic component may have a beneficial effect for the prevention of natural exacerbations.

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QUANTITATIVE ENZYME-HISTOCHEMICAL CHANGES IN RED AND WHITE SKELETAL MUSCLE FIBERS IN THE LIMBS DURING TEMPORARY ISCHEMIA AND POSTISCHEMIC RECIRCULATION

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The dynamics of changes in metabolism of ischemized skeletal muscles of the limbs has so far been examined morphologically mainly at the descriptive level [5, 7, 9]. Quantitative enzyme histochemical studies of skeletal muscle tissue during ischemia have been few in number and have not allowed for muscle heterogeneity [4] and, in particular, changes in red and white muscle fibers (RMF and WMF, respectively) have been studied only in chronic ischemia of the limbs [2].

The dynamics of metabolic processes in skeletal muscle was studied in the present investigation, taking into account heterogeneity of the muscle, during temporary acute occlusion of the main limb arteries and early postischemic recirculation.

EXPERIMENTAL METHOD

Acute occlusion of the hind-limb arteries was created by the method in [1] in experiments on 69 mongrel dogs of both sexes, weighing 13-18 kg. The duration of ischemia of the limbs was 3, 6, 9, and 12 h, and revascularization for 2 h was carried out after each period of ischemia. Enzyme-histochemical investigation of the soleus muscle was undertaken on frozen sections 10 μ thick. Activity of the following enzymes was determined: succinate dehydrogenase (SDH) — the Krebs' cycle, lactate dehydrogenase (LDH) — anaerobic glycolysis, glutamate dehydrogenase (GDH) and β-hydroxybutyrate dehydrogenase (HBDH) — protein and lipid catabolism, and NAD- and NADP-diaphorases. Activity of oxidoreductases was demonstrated with nitro-BT by the usual methods [11]. ATPase activity was determined by the calcium method [10]. To differentiate between muscle fibers when determining enzyme activiy, the classification given in [10, 15] was used. Changes in enzyme activity were assessed quantitatively with an MIF-7 integrating photometric microscope, based on the logarithmic screen method [3]. The object was measured under oil immersion in monochromatic light at a wavelength of 546 nm (magnification 600). From one transverse section 11 RMF and 11 WMF were investigated: Each fiber was subjected to photometry separately in three regions, followed by calculation of arithmetic mean values and standard error.

EXPERIMENTAL RESULTS

It is generally accepted that the main source of energy of WMF (fast contracting) is anaerobic glycolysis, whereas that of RMF (slowly contracting) is metabolic processes of the Krebs' cycle and processes associated with protein and lipid metabolism [8, 12, 14, 15]. The results of the present cytophotometric investigations confirmed this enzyme-histochemical het-

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