The Antioxidant System in Combined Therapy of Epilepsy with Traditional Anticonvulsants and an Antioxidant α-Tocopherol

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Disturbed regulation of lipid peroxidation (LPO) is an important element in the pathogenesis of epilepsy [4,8]. Some data [3,5,8-10] suggest that initial disturbances in the system of LPO regulation arising during epileptogenesis are greatly aggravated by developing insufficiency of the antioxidant enzyme system (AOES). The strengthening of the AOES in experimental animals which can be effected via administration of endogenous superoxide dismutase (SOD) was found to inhibit the development of epileptiform activity and to alleviate it [5]. In this context studies of the AOES in the course of anticonvulsant therapy and the search for AOES-improving drugs are important.

In the present study we investigated the activity of the AOES in blood from epileptic patients in the course of combined treatment with currently used anticonvulsant drugs and an antioxidant, α-tocopherol (TP).

MATERIALS AND METHODS

Thirteen male epileptic patients (generalized seizures) aged 23 to 39 with a 7-18-year history of epilepsy were examined. Standard anticonvulsant therapy had proved to be inefficient in all patients. In different, individually adjusted combinations the patients received barbiturates, difenin, benzodiazepines, etc. Nine of them, in addition to standard anticonvulsant, were administered TP (600 mg, orally). The patients were examined 1-2 days before and 2 and 4 weeks after TP administration. The examination protocol consisted of clinical-electrophysiological tests including electroencephalography (EEG) (both the background and postphotostimulation and posthyperventilation EEG were recorded) and biochemical tests including determination of the plasma TP level and the activity of SOD, glutathione peroxidase (GSH-Px), and glutathione reductase (GS-R) in erythrocytes.

Blood was drawn from the cubital vein in the morning before breakfast. Activity of SOD, GSH-Px, and GS-R in erythrocytes and the TP level were determined as described elsewhere [9]. Blood samples from 10 male volunteers aged 23-37 without mental or somatic disorders served as controls.

RESULTS

Before TP administration all patients showed more or less expressed diffuse pathological deviations in the background EEG. Photostimulation and hyperventilation led to more frequent paroxysmal bursts. Generalized seizures became very frequent, from one per day to 1-16 paroxysms per month. The initial blood level of TP in the patients did not

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TABLE 1. Parameters of Antioxidant System in Epileptic Patients and Healthy Volunteers ($M \pm m$)

Parameter	Control (n=10)	Epilepsy (n=13)
TP, mg/ml plasma	10.6±0.8	10.1±0.7
SOD, convent. units/mg hemoglobin	3.2±0.1	2.52±0.06*
GSH−Px, nmol NADPH/min/mg hemoglobin	10.7±0.42	8.14±0.60*
GS−R, nmol NADPH/min/mg hemoglobin	8.8±0.4	8.4±0.4

Note. *: $p<0.05$ in comparison with the control

differ from the norm (Table 1). Activity of SOD and GP was decreased by 20-25% in comparison with the control, while GS-R activity remained within the normal range (Table 1).

After 2 weeks of TP administration, the blood TP level had risen 70% above the initial value (Table 2). At this time SOD activity in the blood was increased by 11%, whereas GSH-Px and GS-R activity remained unchanged (Table 2). Sleep disturbances, headache, and anxiety were reduced, volitional-emotional activity and work capacity were improved. At this stage the EEG revealed no marked deviations from the initial state.

After 4 weeks of TP administration the blood TP level in the patients surpassed the initial level by 50%, SOD activity was elevated by 15%, while still no changes in GSH-Px and GS-R were observed (Table 2). At this time the frequency of seizures decreased and the patients' general state continued to improve. Over the entire observation period (1 month of TP therapy) among the patients with an initial frequency of 1-2 seizures per month three had no new episodes, and two others had one episode each. One patient with an initial frequency of 1-2 seizures per week had 2 episodes, and a patient with an initial frequency of 2-3 seizures per week had 5 episodes. EEG data after 4 weeks of TP therapy showed that diffuse pathological activity in the background EEG was less pronounced and the frequency of paroxysmal bursts during photostimulation and hyperventilation was reduced.

The above data suggest the enhanced efficiency of anticonvulsant therapy supplemented with TP. This is in good agreement with our previous data [3] and the results obtained by other investigators [7,11]. The positive antiepileptic effect of TP is evidently related to its antioxidant and membrane-stabilizing activities. The inhibitory effect of TP on LPO processes is due to a number of properties. First, as an active acceptor of singlet oxygen, TP is capable of dismutating O_2 and thereby inhibiting the reactions of LPO initiation [2]. Second, by interacting with peroxide radicals, TP reduces their concentration, thus terminating the elongation of LPO chains [2]. Third, the antioxidant effect of TP may be mediated through protection of the protein Se against oxidation, thus preventing its substitution with less active sulfur [2]. The membrane-stabilizing effect may be activated through interaction of the hydrocarbon side chain of TP with acyl chains of membrane phospholipids [2], as well as through its ability to form complexes with free fatty acids, thus preventing the detergentlike action of the latter on the membrane [1].

By normalizing the LPO processes and stabilizing the membranes of nerve cells, TP partially reduces the contribution of at least one essential element to the general disturbances of the nervous system in epileptogenesis, namely the imbalance in the LPO regulation system. This, as already noted, mainly determines the antiepileptic activity of TP.

The increased activity of SOD found in the course of TP therapy may be attributed to several factors. If the decreased SOD activity in the blood of epileptic patients results from partial inactivation of the enzyme pool due to interaction with certain substances, peroxides among them, that are generated or accumulated during seizures, as was assumed earlier [10], the increase in SOD activity is related to a reduced content of SOD inhibitors due to TP-induced normalization of LPO. It is also possible that the effect of TP on SOD activity is not mediated through its antioxidative properties, but rather through other mechanisms. Some data in favor of this hypothesis were obtained earlier [6]. The mechanisms of SOD induction by α-tocopherol

TABLE 2. Parameters of Antioxidant System in Epileptic Patients in the Course of TP Administration ($M \pm m$, n=9)

Parameter	Initial level, %	After 2 weeks of TTP	After 4 weeks of TP
TP	100±6.7	169±16**	136±6**
SOD	100±2.5	111±3*	115±3*
GSH−Px	100±7.4	97±6.5	84±11
GS−R	100±4.8	98±6.0	106±6.5

Note. *: $p<0.05$, **: $p<0.01$ in comparison with the initial level.

have not yet been clarified. Nevertheless, activation of one of the main enzymes of the AOES is undoubtedly important for compensating for the disturbances in LPO regulation, which occur in epileptic patients [9]. This effect of TP is most likely largely responsible for its anticonvulsant properties.

Thus, the treatment of epileptic patients with α-tocopherol in addition to traditional anticonvulsants leads to increased blood activity of superoxide dismutase, which explains to a certain extent its anticonvulsant properties.

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Correction of Neuroimmune Reactions by Regulation of Lipid Peroxidation

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The activation of free-radical oxidation (FRO) is of great pathogenic importance [8] in the development of stress-induced alterations and immune disorders in which stress plays a significant role. Experimental allergic encephalomyelitis (EAE) is a model of neuroimmune damage to the brain and is accompanied by an enhancement of FRO [2]. It is known, that guinea pigs possess a high sensitivity to EAE (which may be overcome by a vitamin E-deficient diet), while albino rats show tolerance to the reproduction of this process.

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A comparative study of the stress sensitivity of these species of animals to the model of emotional-pain stress is of interest. The dynamics of autoneurosensitization was used as the criteria of resistance, and the state of FRO processes was examined in parallel.

MATERIALS AND METHODS

Experiments were carried out on male albino rats weighing 150-200g and on guinea pigs of both sexes weighing 250-300 g kept under vivarium conditions in the fall and winter period. Emotional-pain stress (EPS) was reproduced according to a

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