REDUCTION OF VOLUNTARY ETHANOL CONSUMPTION BY A LONG-ACTING ZINC PREPARATION

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Evidence of the development of zinc deficiency [8] and of excessive accumulation of copper [6, 10] and iron [16] in the liver, as well as of a raised copper and iron concentration in the blood serum under the influence of chronic alcohol intoxication (CAI) has recently been obtained. The zinc content in brain structures of patients with alcoholism are deficient in zinc to the extent of 15-30% [13]. The zinc content is also depressed in the liver and blood [14] of these patients. We [6] found an increase in the copper content in the brain of rats with CAI for 1.5 and 3 months, and also in the mature offspring of these rats [5].

Changes in the content of trace elements in the body as a whole and in the brain in particular may affect the formation of alcohol dependence. This hypothesis is based on the fact that voluntary consumption of ethanol is increased in rats transferred from an ordinary to a zinc-deficient diet [11], and also on data obtained by Gurtovenko and co-workers [3] and our own observations [5].

We have shown for the first time that the level of preference for ethanol by rats and mice is lowered by the action of zinc in physiological doses (50 μg/g). Direct correlation has been demonstrated between the antialcoholic effect of zinc and its content in the animal brain.

The aim of this investigation was to study the effect of zinc, introduced into the animal body as a highly dispersed powder (HDP) of the metal, which has the property of being stored at the site of injection and of being gradually dispersed into the internal medium of the recipient over along period of time.

METHODS

Experiments were carried out on 42 noninbred male albino rats weighing initially 120-140 g. The animals were divided into two equal groups. Rats of the experimental group consumed 10% ethanol solution as the sole source of fluid, whereas rats of the control group were given water for 8 months [4]. Next, 10 animals from each group were decapitated and concentrations of trace elements were studied by atomic-absorption spectrophotometry after wet incineration [9] on the S-5000 apparatus (Perkin—Elmer, USA). The concentration of malonic dialdehyde (MDA) in the brain [12] and catalase activity in the blood and brain [1] were determined. In the remaining rats, consumption of ethanol solution was measured under free choice conditions with water. On the 7th day of testing, eight rats of the experimental group were given a subcutaneous injection of zinc HDP in the form of 0.15-0.3 ml of suspension in a dose of 5 mg/kg, and another eight rats of this group received the corresponding volume of distilled water.

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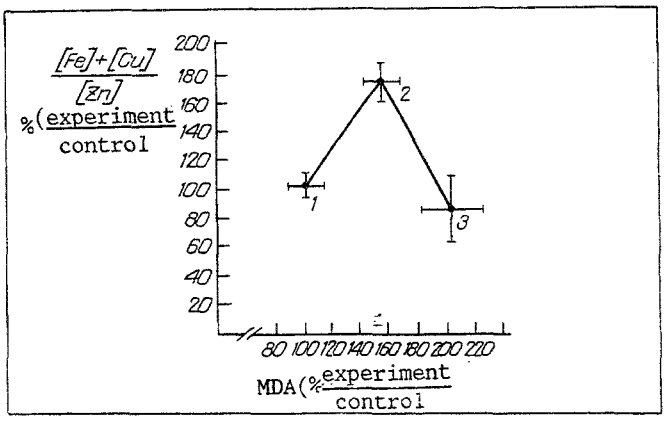

Fig. 1. Relationship between [Fe] + [Cu]/[Zn] ratio and MDA level in rat brain. 1) Control; 2) alcohol intoxication (8 months); 3) after injection of highly dispersed zinc powder.

TABLE 1. Content of Iron, Copper, and Zinc (in mg/kg wet weight of tissue) in Brain of Rats with Chronic Alcohol Intoxication (8 months) and Subcutaneous Injection of Zinc HDP in a Dose of 5 mg/kg (M ± m, n = 5-6)

Group	Iron	Copper	Zinc
Control	2,69±0,37	3,13±0,43	19,75±4,75
Experiment (CAI)	1,97±0,34	2,41±0,13	8,62±1,22*
CAI + Zinc HDP	1,38±0,75	2,32±0,38	14,62±5,99

Note. Here and in Table 2, values for which $p < 0.05$ are indicated by an asterisk.

TABLE 2. Changes in Coefficient of Preference for Ethanol under the Influence of a Zinc HDP Depot (5 mg/kg) in Rats Exposed to Ethanol Intoxication for 8 Months (M ± m)

Group	Coefficient of preference for ethanol		
	During 1-6 days before injection of zinc HDP	During 1-5 days after injection of zinc HDP	During 6-14 days after injection of zinc HDP
Control	0,44±0,02	0,46±0,03	0,47±0,02
Experiment	0,50±0,03	0,32±0,03*	0,36±0,04*

The suspension of zinc HDP was prepared on a UZDN-2T ultrasonic disintegrator, working on 44 KHz, 0.5 A, for 10 min with cooling [2]. The zinc HDP consisted of particles 50-100 nm in diameter, homogeneous by composition, and virtually free from impurities (99.99% zinc). The preparation is distinguished by low toxicity compared with inorganic salts and by its prolonged action if given by subcutaneous injection; in a dose of 5 mg/kg no areas of infiltration of necrosis are formed at the site of injection.

Consumption of ethanol solution and water under free choice conditions continued to be recorded for 14 days. The coefficient of preference for ethanol was calculated by the equation

$$K = V_{ethanol}/(V_{ethanol} + V_{water}),$$

where V denotes the volume (in ml/kg body weight per day).

The results were subjected to statistical analysis by the Fisher—Student test.

RESULTS

Chronic alcohol intoxication of the rats for 8 months led to a significant fall of the zinc content and a tendency for the iron content to fall in the brain (Table 1).

In the brain of rats with chronic alcohol intoxication a tendency was noted for the MDA concentration to rise (8.70 ± 1.31 nmoles/g tissue compared with 6.12 ± 0.45 in the control), whereas catalase activity (2.33 ± 0.49 mg H_2O_2/mg tissue) did not differ from the control level (1.79 ± 0.29).

To discover correlation between the change in the level of lipid peroxidation (LPO) and the concentrations of trace elements in the rat brain, an attempt was made to find a direct relationship between the MDA level in the tissues and the ratio of metals activating LPO (iron, copper) and the LPO inhibitor (zinc) [2]. Iron and copper are involved in the Haber—Weiss cycle, in the initiation, branching, and detachment of lipid oxidation chains, whereas zinc is involved as their functional antagonist [15].

The experiments showed (Fig. 1) that the change in MDA content in the brain is proportional to the change in the ratio ([Fe] + [Cu])/[Zn]. This confirms the view that imbalance between these trace elements has a role in the activation of LPO in the brain in CAI. Increased catalase activity in the brain does not compensate accumulation of LPO products.

Accumulation of MDA in the brain, which is to some degree due to an imbalance of iron, copper, and zinc, can be regarded on the whole as an important factor in the pathogenesis of functional and structural disturbances in the brain in chronic ethanol intoxication.

A single subcutaneous injection of zinc HDP into rats exposed to ethanol for 8 months, in a dose of 5 mg/kg caused (Table 2) a decrease in the coefficient of preference for ethanol both during the first 5 days after injection (by 36%) and also later — between the 6th and 14th days (by 28%).

Reduction of the voluntary ethanol consumption by rats was combined with normalization of the zinc content and a tendency for the iron content in the brain to decrease (Table 1), and by elevation of the MDA level to 12.65 ± 1.36 nmoles/g tissue ($p < 0.001$) compared with normal and $p < 0.05$ compared with the main group. Catalase activity in the brain did not change significantly (2.05 ± 0.64 mg H_2O_2/mg tissue). As Fig. 1 shows, injection of zinc HDP led to a sharp change in the relationship between the MDA level and concentrations of iron, copper, and zinc in the brain compared with animals subjected to CAI. The possibility cannot be ruled out that accumulation of MDA in the brain may cause potentiation of the toxic action of ethanol on the brain and, correspondingly, may limit tolerance and reduce the total consumption of ethanol. The MDA accumulation which was established was evidently not connected with the activating influence of Fe^{2+} or Cu^{2+} on LPO, but more with a decrease in the intensity of enzymic oxidation of LPO products.

The results of these investigations are evidence, first, that LPO is involved in the mechanism of preference for ethanol and, second, that these mechanisms can be regulated by substances which possess an inhibitory of activating effect on LPO processes, including variable valency metals. It was shown for the first time that the creation of a depot of zinc HDP in the body, guaranteeing slow release of the element into the body in near-physiological doses, may prove to be one way of exerting a lasting therapeutic action on ethanol dependence.

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