Neuroprotector Effect of Comenic Acid against Cytotoxic Action of Glutamate *in Vitro* in Cultured Neurons of Lead-Poisoned Rat Pups

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We demonstrated an increase in glutamate neurocytotoxicity in cultured cerebellar cells from rat pups subjected to lead poisoning during the prenatal development or early lactation period. The toxic effect of glutamate was weaker, if lead was applied in combination with antioxidant comenic acid. These data are discussed in view of practical use of comenic acid for the therapy of the brain tissues subjected to lead poisoning.

Key Words: *lead poisoning; glutamate; cell culture; oxidative stress; comenic acid*

The deleterious effects of lead entering the body with food, water, air, or soil (indirectly) on vital physiological systems are widely known. The most vulnerable targets of lead are CNS tissues, especially in the developing brain. Numerous studies showed that prenatal and early postnatal exposure to lead leads to dramatic morphological abnormalities in the brain of the progeny [14] and negatively affects the higher functions of CNS [4].

One of the most important pathogenic properties of lead underlying its neurotoxic effect is its prooxidant action, which leads to ROS accumulation in cells, activates LPO processes, and inhibits antioxidant processes in cerebral tissues [7]. Deterioration of the antioxidant protective mechanisms in the nervous tissue caused by lead poisoning can decrease cytotoxicity threshold of glutamate, which is the major excitatory neurotransmitter. Glutamate cytotoxicity can provoke the oxidative stress, and it is generally viewed as a key pathogenic factor of neuron damage during acute forms of cerebral pathology (resulting from stroke or trauma) or chronic neurodegenerative diseases [1,3].

Modern pharmacological preparations against lead poisoning are little efficient due to their side effects appreciably limiting the use of these drugs [11]. Logically, the search for novel neuroprotectors against lead toxicity is of particular importantance. One of the ways to prevent the toxic action of lead is the use of antioxidants [9]. This class of biologically active compounds includes comenic acid (CA), an efficient antioxidant substance and a component of Balysum-2 anti-inflammatory drug [5].

Our aim was to examine the resistance of neurons to glutamate cytotoxicity in cultured cerebellar cells of rat pups exposed to lead during prenatal and early postnatal periods, and the neuroprotective effect of CA applied simultaneously with lead.

MATERIALS AND METHODS

The experiments were carried out on cultured cerebellar cells of 7-9-day rat pups Wistar born by rat dams

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daily administered during pregnancy with lead acetate (LA) added to the feed (6 mg/kg) alone or in combination with CA (1 mg/kg). The culturing technique was described elsewhere [6]. The experiments were performed on 7-8-day cultures *in vitro*.

Four groups of cells were examined: cells derived from intact rats (group 1) and from rats receiving to LA alone (group 2), LA with CA (group 3) and CA alone (group 4). Some cultures in each group were incubated for 10 min in balanced salt solution (pH 7.4) and then in the initial growth medium for 5 h in CO_2-incubator at 35.5°C (control). The rest cultures were incubated for 10 min in balanced salt solution supplemented with 50 or 100 μM glutamate and then 5 h in the initial growth medium (in CO_2-incubator at 35.5°C). After incubation, the cultures were fixed and examined by phase contrast microscopy under an ID 03 Invertoscope (Carl Zeiss). The neuron survival index (SI) was counted in percentage relatively to the number of live neurons in the respective control subgroup.

The data were processed statistically using Student's *t* test and presented as $M \pm m$.

RESULTS

In all control cultures, the percent of live neurons did not significantly differ and was taken for 100%. The percent of live neurons in all experimental cultures was lower due to neurotoxic effect of glutamate (Fig. 1) and was presented in percentage to this value in the corresponding control subgroup. In intact cultures (group 1), 50 and 100 μM glutamate significantly decreased SI to 65.3±9.0 and 26.2±2.1%, respectively (Fig. 1, *b*, c; Fig. 2, *a*). The toxic effect of glutamate markedly increased in group 2 cultures derived from pups exposed to LA prenatally or during lactation (Fig. 2, *b*). In this group, 50 and 100 μM glutamate dramatically decreased SI to 7.2±2.2 and 6.0±1.8%, respectively. However, in group 3 cultures, the toxic effect of glutamate was significantly lower, SI being 42.7±6.3 and 25.5±3.7% for 50 and 100 μM, respectively (Fig. 2, *c*). Administration of CA alone during prenatal development or lactation (group 4 culture) produced no significant neuroprotective effect against glutamate (Fig. 2, *d*). In this group, 50 and 100 μM glutamate decreased SI to 70.9±8.5 and 33.6±9.0%, respectively, which did not significantly differ from the corresponding values in intact group 1.

We previously showed that poisoning with LA during pregnancy or lactation up-regulates ROS production and results in accumulation of MDA (LPO end-product) in the brain of progeny accompanied by inhibition of the glutathione antioxidant system (GAS) [2]. There are data showing that the pathogenetic effect of lead on CNS, the developing brain included, is mainly caused by activation of oxidative processes and disturbances in GAS mechanisms [15]. One of the key mechanisms underlying neuronal death during glutamate toxicity is the development of the oxidative stress inflicted by up-regulation of ROS production and activation of LPO processes [10]. Inhibition of GAS is known to enhance vulnerability of neurons to ROS destructive action [8]. Thus, greater neuronal vulnerability in group 2 (unprotected lead poisoning) to glutamate can be explained by degradation of GAS caused by previous chronic lead poisoning of these neurons *in vivo*. The use of antioxidants moderates the pro-oxidant action of lead on the brain [9] and protects the neurons *in vitro* against glutamate toxicity [12]. We showed that CA known due to its high antioxidant activity impedes glutamate-induced neuron destruction *in vitro* [13]. Moreover, the presence of CA simultane-

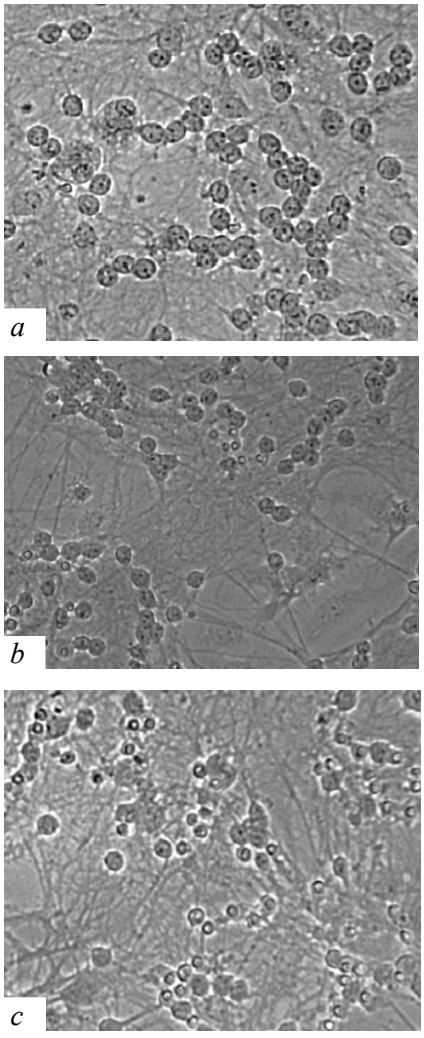

Fig. 1. Cytotoxic effect of glutamate on cerebellar cultured cells from intact (group 1) rat pups. Scale 50 μ; unstained culture, phase contrast. *a*) control: numerous round-shape intact granular cerebellar cells with projections. No destruction signs; *b*) 50 μM glutamate; *c*) 100 μM glutamate. Arrows in *b* and *c* mark pyknotic nuclei of dead neurons.

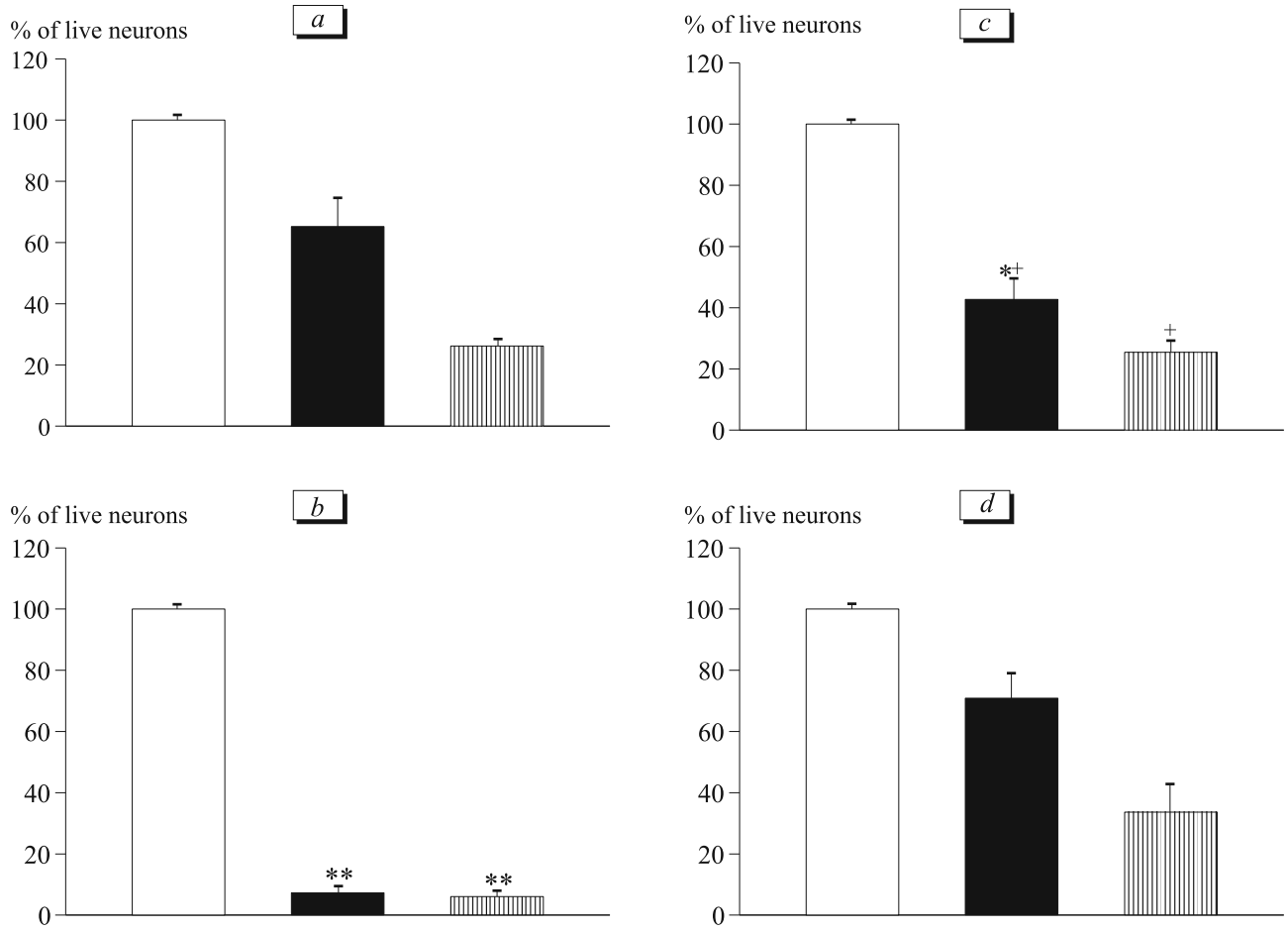

Fig. 2. Glutamate cytotoxic effect in cerebellar cultured cells exposed *in vivo* to LA and/or CA during prenatal period or lactation. *a*) group 1 (*n*=6-16); *b*) group 2 (*n*=16-20); *c*) group 3 (*n*=14-15); *d*) group 4 (*n*=9-13). Open bars – control; dark bars –50 μM glutamate; dashed bars – 100 μM glutamate. **p*≤0.05, ***p*≤0.001 compared to group 1; +*p*≤0.001 compared to group 2.

ously with lead in pregnant rats prevented disturbances in the antioxidant protective mechanisms in cerebral tissue of the progeny [2]. This fact can explain the higher neuronal antiglutamate resistance in group 3 in comparison with group 2.

In our previous experiments, administration of CA to lead-free pregnant rats produced no significant effect on activity of the brain antioxidant systems in the progeny [2] attesting to the absence of the side effects. Maintenance of the unchanged antioxidant status is also corroborated by similarity in the degree of glutamate cytotoxic effect in groups 1 and 4.

In conclusion, it should be stressed that the data obtained substantiate experimentally the feasibility of practical use of CA in the therapy of cerebral tissues during lead poisoning, which can dangerously elevate the risk of the development of acute and chronic forms of cerebral pathology related to glutamate neurotoxicity.

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