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Effect of aluminum ions on the activities of $tRNA^{Leu}$ and leucyl-tRNA synthetase in mouse liver *in vivo* and *in vitro*

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The object of the study was to evaluate the effect of aluminum ions (Al^{3+}) on activity of $tRNA^{Leu}$ and leucyl-tRNA synthetase from mouse liver *in vivo* and *in vitro*. Experiments were done on white laboratory mice using intraperitoneal injections of 0.5 LD_{50} $AlCl_3$ solution (25 mg Al^{3+}/kg body mass). The acceptor activity of $tRNA^{Leu}$ was evaluated by the formation of [^{14}C]-leucyl-$tRNA^{Leu}$. The leucyl-tRNA synthetase activity was measured by the initial rate of $tRNA^{Leu}$ aminoacylation. We determined the effect of Al^{3+} on the activities of translation machinery components in mouse liver *in vivo* and *in vitro*. 16 h after treatment with Al^{3+} the acceptor activity of $tRNA^{Leu}$ decreased by 23%. However, Al^{3+} caused an increase of leucyl-tRNA synthetase activity in the total preparation of aminoacyl-tRNA synthetases by 20%. We supposed that the increase in leucyl-tRNA synthetase activity is part of compensatory response to the diminished activity of $tRNA^{Leu}$ *in vivo*. Aluminum ions inhibit the acceptor activity of $tRNA^{Leu}$ *in vitro* by 15–20% in the presence of different concentrations of Al^{3+} (5–40 μM). On the other hand, even low concentrations of Al^{3+} (5–10 μM) inhibit the activity of leucyl-tRNA synthetase by 19–25%. In the presence of 40 μM of Al^{3+} activity of this enzyme was only 40% as compared to control. These data show that Al^{3+} has a significant influence on the components of translation machinery in mouse liver *in vivo* and *in vitro*.

Key words: aluminum, mouse liver, tRNA, aminoacyl-tRNA synthetase

INTRODUCTION

Although aluminum (Al(III)) is the most abundant metal in the Earth's crust, no living organism has been known to utilize it. In fact, this ubiquitous element is often considered toxic. This metal has been extensively investigated for a potential role in the etiology of various diseases. The exact mechanism of aluminum toxicity is not known, but accumulating evidences suggest that this metal can potentiate oxidative and inflammatory events, eventually leading to tissue damage [1, 2]. Aluminum causes an oxidative stress within brain tissue, leading to the formation of Alzheimer-like neurofibrillary tangles [3, 4]. Aluminum can impair cellular energy transfer processes by interfering with phosphate and ATP metabolism [5, 6]. Toxic effects of aluminum result from its competition with other metal ions in enzymes and proteins. As the aluminum ion substitutes a metal at its binding site, the function of the protein is changed and the metabolism of the cell is altered [7]. Magnesium (II) has been shown to be one of the cations most affected by aluminum interference [8]. Some studies suggest that translation machinery is a target for Al action. Aluminum can bind to phosphorylated bases on DNA, induce considerable changes in chromatin structure and disrupt protein synthesis and catabolism [9, 10]. There is no uniform effect of aluminum ions on protein synthesis – they can enhance synthesis of certain proteins [11] but inhibit synthesis of others [12]. Expression of heat-shock or stress proteins, including ubiquitin, can be induced by aluminum in cell culture experiments [13]. At the early stages of intoxication Al ions activate protein synthesis in liver and kidneys, while in skeletal muscle and heart they do not [14]. The present study deals with the effects of Al on the total protein synthesis and on the activity of $tRNA^{Leu}$ and leucyl-tRNA synthetase in mouse liver *in vivo* and *in vitro*.

MATERIALS AND METHODS

Experiments were done on white laboratory mice of 20–25 g body mass. To study the effect of Al^{3+}, mice were

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injected i.p. with 0.5 LD_{50} $AlCl_3$ solution in deionised water (25 mg Al^{3+}/kg body mass). Control animals received an injection of the same volume of physiological solution. After 16 h the mice were anaesthetized and terminated according to the rules defined by the European convention for the protection of vertebrate animals used for experimental and other scientific purposes (License No 0028).

For the measurement of protein synthesis [^{14}C]-leucine was injected i.p. 1 h before termination. Total tRNA and post-mitochondrial supernatant (source of leucyl-tRNA synthetase) were obtained from mouse liver as described earlier [15]. Protein concentration was determined by the Warburg method. To evaluate the effect of Al^{3+} ions on the activity of the components of the translation system, we have used a reaction mixture in which one of the translation system components (tRNA or aminoacyl-tRNA synthetase) was isolated from mouse liver treated with Al^{3+} and the other component was isolated from Al-nontreated mice. The acceptor activity of $tRNA^{Leu}$ was evaluated by the formation of [^{14}C]-leucyl-$tRNA^{Leu}$ in the 0.1 ml of reaction mixture (100 mM Tris-HCl, pH 7.5; 10 mM $MgCl_2$; 10 mM KCl; 4 mM ATP; 0.2 mM [^{14}C]-leucine; 0.25 mg protein of post-mitochondrial supernatant; 0.05 mg total tRNA). The duration of aminoacylation reaction for determination of tRNA acceptor activity was 20 min. The activity of leucyl-tRNA synthetase was measured in post-mitochondrial supernatant by the initial rate of $tRNA^{Leu}$ aminoacylation with [^{14}C]-leucine (duration of aminoacylation reaction was 3 min) as described earlier [15]. The radioactivity was measured with a liquid scintillation counter. To evaluate the effects of aluminum ions on the activity of $tRNA^{Leu}$ and leucyl-tRNA synthetase *in vitro*, different concentrations of aluminum chloride were directly added into the reaction mixtures.

For statistical comparison between control and experimental groups Student's t test was used. Changes were statistically significant when $p < 0.05$.

RESULTS AND DISCUSSION

Despite numerous investigations, the molecular mechanisms underlying the Al^{3+} toxicity have not yet been clarified. Thus, this study is one of the first attempts to evaluate the effects of Al^{3+} on protein synthesis system components in mouse liver *in vivo* and *in vitro*. It is known that activity and fidelity of the translation process largely depend on the components of the translation machinery. Aminoacyl-tRNA synthetases catalyze the covalent attachment of an amino acid to its cognate tRNA [16]. In our previous report [14] we have shown that the most evident alterations in the total protein synthesis were obtained 16 h after administration of $AlCl_3$; the protein synthesis intensity under 16 h intoxication increased almost twice. Therefore we chose this duration of intoxication for the determination of the effect of Al^{3+} on the activity of $tRNA^{Leu}$ and leucyl-tRNA synthetases from mouse liver. The results (Fig. 1) showed that after treatment with Al^{3+} the acceptor activity of $tRNA^{Leu}$ decreased by 23%. However, Al^{3+} caused an increase of leucyl-tRNA synthetase activity in the total preparation of aminoacyl-tRNA synthetases *in vivo* by 20% (Fig 1). It was unexpected to find the opposite effect of aluminum on the acceptor activity of tRNA (an inhibition) (Fig. 1) versus the intensity of total protein synthesis (an activation) after the same duration of intoxication. Some studies have shown Al^{3+} to disturb cellular energy transfer processes [10]. Aminoacylation of tRNA is an energy-requiring reaction [16]; therefore we supposed that the decreased activity of $tRNA^{Leu}$ might be associated with impaired cellular energy metabolism.

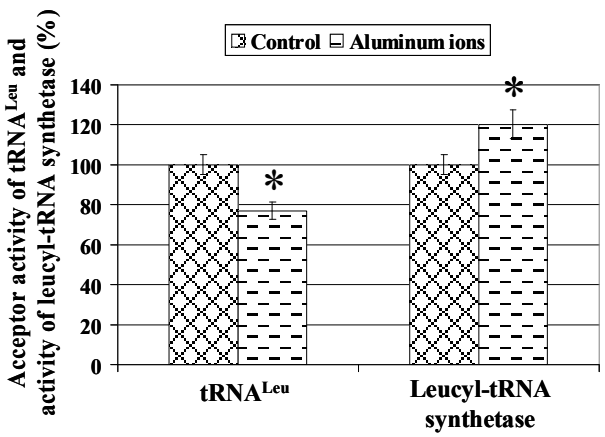

Fig. 1. Activity of mouse liver $tRNA^{Leu}$ and leucyl-tRNA synthetase in norm (control) and 16 h after $AlCl_3$ solution (25 mg Al^{3+}/kg body mass) injection. Data represent results of 8–10 separate experiments

* Differences between control and Al-treated group are statistically significant.

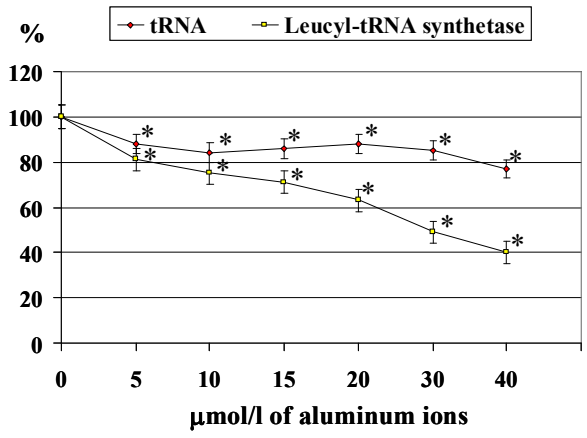

Fig. 2. The effect of Al^{3+} on the acceptor activity of $tRNA^{Leu}$ and on the activity of leucyl-tRNA synthetase from mouse liver *in vitro*. Data represent results of 8–10 separate experiments. Acceptor activity of $tRNA^{Leu}$ and activity of leucyl-tRNA synthetase of the control group is equated to 100%

* Difference between control and Al-treated group is statistically significant.

Moreover, aluminum can substitute magnesium ions which participate in the stabilization of a functionally active tRNA structure [8]. At the same time of intoxication, Al^{3+} caused an increase of leucyl-tRNA synthetase activity in the total preparations of aminoacyl-tRNA synthetase *in vivo* by 20% (Fig. 2). We suppose that the increase in leucyl-tRNA synthetase activity might be part of a response mechanism to maintain the normal level of protein synthesis.

The effect of heavy metals depends on how many their ions enter the cell and what concentration of ions is directly interacting with the system examined. The mechanism of metal ions action can be indirect but mediated through other systems capable to exert the regulatory effect on the components of translation machinery. With the aim to evaluate whether aluminum ions directly affect components of the protein synthesis system, preparation of total tRNA and total preparation of aminoacyl-tRNA synthetases isolated from the control mouse liver were added into the reaction mixture of aminoacylation. Solution of different concentrations of aluminum chloride was directly added into the reaction mixture. Conditions of the aminoacylation reaction were optimal for determination of tRNA or aminoacyl-tRNA synthetase activity.

In such system, aluminum ions are in a direct interaction with the components of translation machinery. The results presented in Fig. 2 demonstrate a mild inhibition (15–20%) of the acceptor activity of $tRNA^{Leu}$ in the presence of different concentrations (5–40 μM) of Al ions (Fig. 2). Aluminum ions showed an opposite effect on the activity of leucyl-tRNA synthetase *in vivo* and *in vitro*. Even low concentrations of Al^{3+} (5–10 μM) inhibit the activity of leucyl-tRNA synthetase by 19–25%. In the presence of 40 μM of Al^{3+}, activity of this enzyme was only 40% as compared to control. Thus, the data that aluminum ions affect the components of translation machinery was supported by the results of experiments *in vitro* using the cell-free system of translation from the liver.

Thus, according to the data of other researchers and of this report, there is no doubt that Al^{3+} exerts a significant influence on the total protein synthesis [14] and on the components of translation machinery in mouse liver. The question what the molecular mechanisms are behind Al^{3+} toxicity still remains open and needs further investigations.

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ALIUMINIO JONŲ POVEIKIS PELIŲ KEPENŲ $tRNR^{LEU}$ IR LEUCIL-tRNR-SINTETAZĖS AKTYVUMUI *in vivo* IR *in vitro*

Santrauka

Šio darbo tikslas buvo įvertinti aliuminio jonų (Al^{3+}) poveikį $tRNR^{Leu}$ and leucil-tRNR-sintetazės aktyvumui pelių kepenyse *in vivo* ir *in vitro*. Eksperimentus atlikome su baltosiomis laboratorinėmis pelėmis suleidę joms į pilvo ertmę 0,5 LD_{50} $AlCl_3$ tirpalo (25 mg Al^{3+}/kg kūno masės). $tRNR^{Leu}$ akceptinis aktyvumas buvo nustatytas pagal [^{14}C]-leucil-$tRNR^{Leu}$ susidarymą. Leucil-tRNR-sintetazės aktyvumas buvo įvertintas pagal pradinį $tRNR^{Leu}$ aminoacilinimo reakcijos greitį. Buvo nustatytas Al^{3+} poveikis transliacijos aparato komponentų aktyvumui pelės kepenyse *in vivo* ir *in vitro*. Praėjus 16 val. nuo $AlCl_3$ tirpalo sušvirkštimo $tRNR^{Leu}$ akceptinis aktyvumas sumažėjo 23%, o leucil-tRNR-sintetazės aktyvumas padidėjo 20%. Manome, kad leucil-tRNR-sintetazės aktyvumas padidėja dėl kompensacinio ląstelės atsako į sumažėjusį $tRNR^{Leu}$ aktyvumą. Eksperimentuose *in vitro* buvo nustatyta, kad esant įvairioms aliuminio jonų koncentracijoms (5–40 μM), $tRNR^{Leu}$ akceptinis aktyvumas sumažėja 15–20%. Tuo tarpu esant netgi labai mažoms Al^{3+} koncentracijoms (5–10 μM), leucil-tRNR-sintetazės aktyvumas slopinamas 19–25%. Kai Al^{3+} koncentracija – 40 μM Al^{3+}, šio fermento aktyvumas sudaro tik 40% kontrolės dydžio. Eksperimentų rezultatai rodo, kad aliuminio jonai veikia pelių kepenų transliacijos aparato komponentų aktyvumą *in vivo* ir *in vitro*.