Selective induction of IL-6 by aluminum-induced oxidative stress can be prevented by selenium

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In this study the acute toxic effects of aluminum (Al) on mice have been investigated, including the interactions of Al and selenium (Se). Focus was put on the systemic effects of (co)exposure to Al and Se as a reflection of the redox status in the liver, kidney and brain.

Short-term exposure (16 h) to Al resulted in an increase in the systemic inflammation parameters IL-6 and PAI-1, whereas serum levels of TNF-α remained unaffected. The different response pattern of IL-6 and TNF-α probably indicates an increased intracellular oxidative stress and altered redox status in the liver, because the selective increase in IL-6 serves as a protective intracellular process driven by oxidative stress. The intracellular glutathione concentration GSH
decreased significantly upon Al exposure. Both the increase in IL-6 and decrease in glutathione status could be prevented by co-exposure to Se, but not the increase in PAI-1. The redox status of the kidney and brain was not markedly affected.

Therefore it was concluded that short-term exposure to Al causes adverse effects on the intracellular oxidative stress processes in the liver, as reflected by the selective increase in the IL-6 concentration. This process can be restored by co-administration of the trace element Se as a part of the glutathione redox system.

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Introduction

Aluminum (Al) as an abundant metal in the environment is one of the factors that have been implicated as a possible cause contributing to neurotoxicity [1,2], hepatotoxicity [3,4] and nephrotoxicity [5]. Chronic Al administration might be responsible for the oxidative cell damage due to its interference with mitochondrial functions and disturbing the redox status [6]. These processes can be characterized by the change in activity of intracellular anti-oxidant enzymes, such as superoxide dismutase, catalase and glutathione peroxidase (GPX), and the depletion of sulfhydryls measured by the glutathione (GSH) status. In a systematic review [7] it was shown that most studies on Al toxicity demonstrated a decrease in both the activity of GPX and the concentration of GSH. The GSH redox system depends on the trace metal selenium (Se). Se can increase the antioxidant capacity of intracellular systems [8], because of its involvement as a cofactor in glutathione peroxidase catalyzed reactions [9,10] such as a reduction of organic hydroperoxides [11] and hydrogen peroxide by GSH [12]. Since the Se status in the European population is generally rather low [13,14], a deficiency of Se might enhance early toxicity of the environmental factors that evoke oxidative stress.

Recent studies demonstrated that Al ions interfere with the metabolism of selenium and therefore systemic AI toxicity may directly (or indirectly) affect Se homeostasis in animals [15,16]. In addition, systemic toxicity induced by Al could be reflected by an increase in systemic inflammation parameters, such as interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and others.

To gain a deeper insight into these complex interactions between Al and Se, the present study investigated a possible protective effect of Se on the intracellular redox status of mouse liver, kidney, and brain after short-term exposure to Al.

Materials and methods

Experimental animals and exposure protocol

Bab/c mice weighing 20–25 g were exposed (by i.p. injection) for 16 h to AlCl3 (25 mg Al3+/kg body mass), Na2SeO3 (1.25 mg...
Se⁴⁺/kg body mass), and a combination of Se and Al of the same concentrations. The dosage of Al and Se and the duration of exposure that were the most effective in previous experiments was applied [17]. Control animals were injected with the same volume of physiological solution. Each group consisted of six animals.

After 16 h the mice were anaesthetized and terminated. All procedures were performed according to the Republic of Lithuania Law on the Care, Keeping and Use of Animals (License of State Veterinary Service for working with laboratory animals No. 0200). The organs (liver, kidney and brain) were removed, rapidly cooled on ice and homogenized in 3 volumes (weight/volume) of isolation buffer (50 mM Tris–HCl, pH 7.6; 5 mM MgCl₂; 60 mM KCl; 25 mM sucrose). The homogenates were centrifuged at 1000 g for 15 min at 4 °C. The supernatants were saved and the pellets discarded. The supernatants were centrifuged again at 12,000 × g for 15 min at 4 °C, filtered through 4 layers of gauze, poured into Eppendorf tubes and frozen at −80 °C. All the samples were shipped from Kaunas to Bilihoven on dry ice. The samples were received frozen and stored at −80 °C until analysis.

Biochemical analysis

Total glutathione (GSHtot) in the liver homogenates was measured after deproteinization followed by incubation with glutathione reductase to transform GSSG to GSH. Then the total GSH was determined by derivatization with 5,5’-dithiobis-(2-nitrobenzoic acid) (DTNB). The reaction product was measured using an autoanalyzer (LX-20 Pro, Beckman Coulter, Mijdrecht, Netherlands). IL-6, tumor necrosis factor alpha TNF-α and plasminogen activator inhibitor-1 (PAI-1) were determined with a Lincoplex Mouse Adipokine kit (Millipore) using the Luminex technique. Intra assay coefficients of variation were 3.5% for IL-6, 4.2% for TNF-α and 6.2% for PAI-1.

Statistical analysis

A one-way ANOVA with Tukey–Kramer test was performed using GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com.

Results

To study the possible mechanisms of Al toxicity under short-term exposure the following serum parameters were measured: IL-6, TNF-α, and PAI-1. A 16 h exposure of mice to Al resulted in a substantial increase (30-fold) of inflammatory cytokine IL-6 concentration in serum from 8.8 to 191 pg/mL and also in an almost twofold increase in PAI-1 from 8.7 to 14.4 μg/mL (Fig. 1). Both increments were statistically significant. The concentration of TNF-α, however, did not change after injection of AlCl₃ solution (Fig. 1).

Exposure to Se alone caused a relatively small increase in the concentration of IL-6 from 8.8 to 35 pg/mL probably due to the relatively high dose of Se. However, Se exposure had no effect on TNF-α and PAI-1.

Co-exposure to Se + Al, compared with exposure to Al only, showed a fourfold statistically significant decrease of the IL-6 concentration from 191 to 57 pg/mL (p < 0.001). This difference was not statistically significant compared with the exposure to Se alone. The PAI-1 concentration did not decrease upon exposure to Se + Al compared with exposure to Al only. The increase in the level of PAI-1 was still statistically significant compared to the control group. The concentration of TNF-α was not affected upon co-exposure to Se + Al.

To investigate the intracellular toxicity of Al ions in the target tissues, the glutathione status in liver, kidney and brain was determined by measuring the concentration of the total glutathione, which was corrected for protein content. As shown in Fig. 2, a statistically significant decrease of about 50% upon treatment with Al was observed only in the liver.

The exposure to Se alone resulted in a small but significant increase in the hepatic GSH status. Co-exposure to Se + Al showed a 20% decrease in the GSH status compared to the status after exposure to Se only. This level was still significantly higher than that of the Al group. No statistically significant effects were observed in the GSH status in the kidney and brain although in the kidney a 50% increase in the GSH concentration was observed for both the Se and the Se + Al group.

Discussion

Short-term exposure to Al results in an increase in several systemic parameters. Both the concentrations of IL-6 and PAI-1 are increased whereas the concentration of TNF-α does not change. This pattern points to a local toxicity or inflammation, rather than a systemic effect. Since the liver, as well as the kidney and brain, is one of the main target tissues for Al, the observed toxic pattern could account for the acute toxicity in this tissue with the induction of acute-phase reactions. The increase in PAI-1 concentration could be explained by the liver’s response to Al exposure. It has been shown that up-regulation of PAI-1 expression can occur in a variety of liver injury models [18]. This result is in agreement with the release of hepatic ALAT to the circulation upon exposure to Al [19,20].

The increased concentration of serum IL-6 usually correlates with an increase in TNF-α concentration, both having a similar origin in the inflammatory processes. However, in this study only the concentration of IL-6 increased substantially upon short-term exposure to Al, whereas the concentration of TNF-α was not
affected. IL-6 is a multifunctional protein produced by various kinds of cells, including hepatic Kupffer cells. It plays a major role in the mediation of the inflammatory and immune responses initiated by infection or injury. Another function of this protein is an anti-inflammatory action on hepatocytes stimulating liver regeneration and repair through the STAT 3 pathway [21]. It has been suggested that by this pathway IL-6 is involved in the repair process responding to oxidative stress and depletion of reduced glutathione [22,23]. The protective effect of IL-6 against oxidative stress and mitochondrial dysfunction has been proven by the increased toxicity of reactive oxygen species in IL-6 deficient mice [24,25]. These studies demonstrated a specific induction of IL-6 as a response to the disturbed redox status. This observation is in agreement with the intracellular decrease in GSH in the liver observed in this study. The hepatic production and release of IL-6 could explain the fast response of the liver upon administration of Al. TNF-α also plays a central role in inflammation and apoptosis and is produced by many cells including hepatic natural T cells. Short-term exposure to Al and other metals, which enter the body in negligible quantities, apparently does not affect these processes.

The acute effects by which Al ions can disturb cellular metabolism is the induction of oxidative stress and disturbance of intracellular redox system as was described previously [26–28]. Therefore the GSH status as a marker of mouse liver redox system activity was studied. Exposure to Al decreased the concentration of GSHtotal significantly to about 50%, whereas co-exposure to Se + Al prevented the decrease of this parameter. Administration of Al did not result, however, in changes of the redox status in the similar samples from the brain and had only a non-significant effect in the samples from the kidney. Apparently the GSH status in the kidney is not optimal and can still be increased by exposure to excessive Se.

The mechanism by which Al causes tissue damage by an oxidation-mediated process is probably caused by an increase in the iron content due to the competitive binding of Al to transferrin, an iron binding and transport protein. It was shown previously [29–31] that upon exposure to Al the iron content in several tissues, especially in the liver, increased substantially. Since iron is the most probable initiator of oxidative stress reactions in tissues, the Al induced increase in oxidative stress could be mediated by iron. The rapid increase of the iron content in tissues cannot be matched by a similar increase in the synthesis of ferritin. As a result iron ions will exist in the non-protein bound form [32] and therefore show reductivity. Other authors also show an iron-mediated dose-dependent increase in the IL-6 concentration [33].

The increase of these oxidative stress processes can be counterbalanced by restoration of the disturbed antioxidant system. Sanchez-Iglesias et al. [34] showed also an increase in oxidative stress and a decrease in activity of anti-oxidant enzymes and enzymes of the redox status (glutathione peroxidase) under exposure to Al. Apparently, as shown is this study, restoration of the redox status can be accomplished by an increase of the concentration of Se, which increases the activity of glutathione peroxidase.

This oxidative stress-mediated mechanism is much more likely than assuming a direct competition between Se and Al for binding site in iron-binding proteins. Since the applied dose of Al was 20 times higher than that of Se, the competitive effects could not be expected. Unfortunately, no material was left for assessing the iron status of the mice.

From the results of this study it can be concluded that short-term exposure to Al is hepatotoxic and causes local hepatic inflammation due to an increase in oxidative stress and a decrease in redox (glutathione) status in mouse liver. The involvement of oxidative stress processes was confirmed by the substantial and selective increase in the concentration of IL-6 (not for TNF-α). In addition, Se can prevent the intracellular oxidative stress caused by Al. In this study a short-term treatment with Al had no effect on the kidney and brain, but the possibility of Al involvement in the onset of cognitive diseases by chronic iron-mediated processes cannot be excluded.

Conflict of interest

There is no conflict of interest.

References


