Original Article

Effect of taurine on toxicity of aluminum in rats

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Summary

Background & aims: A study was undertaken to investigate the effect of taurine on the toxicity of aluminum in male Wistar rats.

Methods: The rats were divided into six groups and fed different diets with or without 5% taurine and 150–300 ppm aluminum added for 2 months.

Results: It was found that the body weight of rats, the ratios of liver and kidney weight to body weight, and the level of glutathione in the liver were decreased with increasing the dose of aluminum. The levels of aluminum in the liver and kidney, the levels of TBARS in the plasma and liver, the activities of AST and ALT in the plasma and the levels of BUN and creatinine in the plasma of rats were increased with the increasing dose of aluminum. Hence, symptoms of aluminum toxicity in rats included loss of body weight, hepatotoxicity and nephrotoxicity.

Conclusions: However, these toxic effects of aluminum were significantly reduced when the rats fed diet with taurine added. Furthermore, the level of aluminum in the feces of rats treated with taurine and aluminum was higher than that of rats treated with aluminum alone. It indicated that taurine might play a role in reducing the toxic effect of aluminum in rats.

1. Introduction

Aluminum toxicity was first recognized in patients to whom aluminum was administered parenterally, i.e. through renal dialysate\textsuperscript{1} and intravenous nutrient solution.\textsuperscript{2} Aluminum is proposed to be a factor contributing to several neurological disorders such as Alzheimer’s disease.\textsuperscript{3} There is evidence that exposure to aluminum from drinking water results in cognitive impairment and an increased incidence of Alzheimer’s disease.\textsuperscript{4} To understanding of a possible enhanced bioavailability of aluminum in this type of exposure or after other exposure such as antacid intake or industrial exposure need to be considered or explored. Although these points might be debatable, there is little doubt that accumulation of aluminum leads to tissue damage.\textsuperscript{5}

That aluminum can be toxic to plants, animals, and humans is well documented, but the mechanism of action is poorly understood\textsuperscript{6–8}. A clear example is the etiology of the microcytic anemia, osteomalasia, and encephalopathy that occur in renal failure patients as a result of the progressive accumulation of aluminum in this condition.\textsuperscript{8} Despite a large body of literature on the pathological effects of aluminum in biology, the underlying causes of tissue damage in renal failure, and in most other aluminum-related disorders, are largely matters of conjecture. This is partly a result of the wide variety of experimental conditions under which aluminum has been studied, many of which are in vivo. In our laboratory, we have been investigating the effects of dietary aluminum and taurine intake on mineral metabolism in vitro using animal models.

Taurine is a sulfur containing amino acid that conjugates with bile acids in the liver\textsuperscript{9} and chemically similar to acetylcysteine, an agent for treating the heavy metal intoxication.\textsuperscript{10,11} It has been reported that taurine might possess a protective action against drug-induced injuries.\textsuperscript{10,11} Taurine may play an important role in reducing the toxic effect of copper, lead, oxidized fish oil, oxidized cholesterol and vitamin A in rats.\textsuperscript{12–17} As above description, aluminum is a modern toxic metal. Hence, it prompted us to investigate the effect of taurine on the toxicity of aluminum. In this study, the model used an evaluation of aluminum on dietary protection of taurine in the intestine was studied.
2. Materials and methods

2.1. Animals

Male weanling Wistar rats were purchased from the National Laboratory Animal Center. They were kept in an air-conditioned room (23 ± 1°C, 50–60% humidity) lit for 12 h per day (07:00–19:00 h). After acclimating for 2 weeks with a commercial non-purified diet (Rodent Laboratory Chow 5001, Pruida Co., USA), 36 rats were divided into six groups. Six rats in each group were assigned to receive an 8-week course of one of six formulated diets (Table 1). The diets were formulated as described previously by American Institute of Nutrition (AIN) because this formula is still commonly used in spite of new one recommended by AIN in 1993. The form of aluminum nitrate nonahydrate (analytical grade) was supplied by E. Merck (Germany). Water and food were always available. After feeding, all rats were weighed. The blood of the rats was taken at a 2 weeks interval from the tail vein. Then, the plasma samples were collected by centrifugation (2000 g, 15 min) from blood and examined for the level of thiobarbituric acid reactive substances (TBARS), the activities of aspartate transaminase (AST) and alanine transaminase (ALT) in the plasma were also assayed by a Vitalab Selectra with using an enzymatic kit. At the last 2 days of 8-week course of diets, the feces were taken at a 2 weeks interval from the tail vein. Then, the plasma was analyzed for TBARS, AST, ALT, blood urea nitrogen (BUN) and creatinine. The levels of BUN and creatinine in plasma were also assayed by a Vitalab Selectra with using an enzymatic kit.

2.2. TBARS production

Lipid peroxidation activities in blood and liver were assayed by measurements of malondialdehyde (MDA), an end product of peroxidized fatty acids and thiobarbituric acid (TBA) reaction product. Twenty µl of plasma and 20% liver homogenate were separately mixed with 1.0 ml of 0.4% TBA in 0.2% HCl and 0.15 ml of 0.2% dithiobis (2-nitrobenzoic acid) (DTNB) to yield glutathione disulfide (GSSG) and 2-nitro-5-thiobenzoic acid (TNB). GSSG is then reduced enzymatically by NADPH and glutathione reductase (GR) to regenerate GSH.

Concentrations of DNTB, NADPH and GR are chosen such that the rate of the overall reaction is linearly proportional to the concentration of total glutathione. The rate of formation of TNB is followed spectrophotometrically, and assay is calibrated using standards. If the sample is reacted with 2-vinylpyridine, GSH is derivatized, and only GSSG is detected during subsequent assay.

2.3. Levels of glutathione (GSH) measurement

GSH reacts non-enzymatically with 5, 5′dithiobis (2-nitrobenzoic acid) (DTNB) to yield glutathione disulfide (GSSG) and 2-nitro-5-thiobenzoic acid (TNB). GSSG is then reduced enzymatically by NADPH and glutathione reductase (GR) to regenerate GSH. Concentrations of DNTB, NADPH and GR are chosen such that the rate of the overall reaction is linearly proportional to the concentration of total glutathione. The rate of formation of TNB is followed spectrophotometrically, and assay is calibrated using standards. If the sample is reacted with 2-vinylpyridine, GSH is derivatized, and only GSSG is detected during subsequent assay.

2.4. Aluminum measurement

Aluminum contents of liver, kidney and feces were determined by atomic absorbance by a Hitachi Z-8100 Polarized Zeeman Atomic Absorbance Spectrophotometer (Japan).

2.5. Statistical analysis

Statistical analysis for differences among rats in the experimental groups was performed by the 2-way analysis of variance procedure and Duncan’s new multiple range tests. A P-value < 0.05 was considered statistically significant.

3. Results

The effects of taurine and aluminum on the growth of rats are shown in Fig. 1. After 2-week feeding, the weight of the rat was significantly decreased (P < 0.05) when the concentration of aluminum in diet was more than 150 ppm, but significantly increased when the diet was with taurine added (P < 0.05). It also indicated that taurine could improve the growth of rats which fed

<table>
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<th>Tau + Al 150</th>
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* Taurine: 5% taurine in diet; Al 150: 150 ppm Al in diet; Tau + Al 150: 5% taurine and 150 ppm Al in diet; Al 300: 300 ppm Al in diet; Tau + Al 300: 5% taurine and 300 ppm Al in diet.

Fig. 1. Effect of aluminum and taurine on the body weight of rats. a–f: values in the same week with different superscript are significantly different (P < 0.05). The lettering (a, b, c, d, e, f) in the figure means the statistical different groups in the same week.
diets with 150–300 ppm aluminum added. The effects of taurine and aluminum on the ratios of liver and kidney weight to body weight in rats are shown in Fig. 2. After 8-week feeding, the ratios of liver and kidney weight to body weight of rats fed with aluminum diet (either 150 or 300 ppm) were more significantly decreased than those of rats fed with control diet and taurine diet. However, the ratios of liver and kidney weight to body weight in rats fed diet taurine and aluminum added were not significantly different from those of rats fed diet aluminum added. This means that taurine might not significantly reduce the toxicity of aluminum in the rats based on the ratios of liver and kidney weight to body weight. The effects of taurine and aluminum on the activities of AST and ALT in the plasma are shown in Fig. 3 and Fig. 4. It was found that the activities of AST and ALT in the plasma of rats fed with aluminum added were gradually increased with the feeding time course. The activities of AST and ALT in the plasma of rats are increased with the increasing level of aluminum. The activities of AST and ALT in those rats fed diet with taurine added were significantly to reduce the toxicity of aluminum (\( P < 0.05 \)), indicating taurine might play protective effect on aluminum toxicity in rats. The effects of taurine and aluminum on the TBARS production in the plasma in rats are shown in Fig. 5. After 8-week feeding, the level of TBARS in the plasma of rats fed with added of aluminum was higher than that of control group (\( P < 0.05 \)). After 8-week feeding, the level of TBARS in the liver of rats fed with added of aluminum was also higher than that of control group (Fig. 6) (\( P < 0.05 \)). The level of GSH in the liver of rats fed diet with aluminum added only was less than that of rats fed diet with aluminum added only (\( P < 0.05 \)), but the plasma of rats was not (\( P > 0.05 \)). The level of GSH in the liver of rats was decreased with the increasing the concentration of aluminum in diet. The level of GSH in the liver of rats fed diet with the taurine and aluminum added was higher than that of rats fed diet with only aluminum added (\( P < 0.05 \)) (Fig. 6). The effects of taurine and aluminum in the plasma of rats are also shown in Fig. 6. After 8-week feeding, the level of GSH in the plasma of rats fed with aluminum added than in control group. The level of BUN and creatinine in the plasma of rats was increased with the
increasing dose of aluminum in the diet. When the diet was with taurine added, the level of BUN and creatinine was significantly reduced \((P < 0.05)\). The effects of taurine and aluminum on the level of aluminum in the liver, kidney and feces of rats are shown in Fig. 7. After 8-week feeding, the level of aluminum in the liver, kidney and feces was obviously higher in the groups fed diet with aluminum added than in control group. The level of aluminum in the liver, kidney and feces was increased with the increasing dose of aluminum in the diet. When the diet was with taurine added, the level of aluminum in the liver and kidney was significantly reduced, and the level of aluminum in the feces was slightly increased \((P < 0.05)\).

4. Discussion

In this study, the toxic effect of aluminum in rats was not severe than that of copper and lead. The symptoms of aluminum toxicity in rats included reduced body weight, ratios of liver and kidney weight to body weight, and level of GSH in the liver, and increasing activities of AST and ALT in the plasma, levels of TBARS, BUN and creatinine in the plasma and/or liver, and concentrations of aluminum in the liver and kidney of rats. In the clinical plasma examination, the activities of AST and ALT in plasma represent biomarkers for liver functions. The activities of AST and ALT in the plasma of rats were significantly elevated by aluminum, indicating aluminum-related injury to the liver. This result is also reported by other papers. Since taurine significantly reduced the AST and ALT activities in the plasma of rat, the hepatic injury by aluminum could be ameliorated by taurine. This result was similar to that of body weight in rats. However, the levels of TBARS and GSH in the...
liver are additional indicators of liver injury. TBARS is an end product of lipid peroxidation. The level of TBARS of the plasma and liver was increased with the increasing dose of aluminum and the level of TBARS of the plasma was also increased with exposure time. The data do not prove that the mechanism of aluminum injury is by lipid peroxidation but it is strongly suggestive that it plays an important role. Other paper also indicated that aluminum might increase the level of TBARS in the tissues of experimental animal.4,25 The level of TBARS in the plasma and liver of rat was significantly reduced when the rats were fed diet with the taurine added. The level of GSH in the liver of rats was reduced by aluminum, which was similar to that of other report.26,27 While it was raised significantly when the rats were fed diet with the taurine added. It means that taurine may play an important role in the metabolism of GSH and in preventing lipid peroxidation but these related mechanism should be studied further. On the other hand, the levels of BUN and creatinine in the plasma of rats are tested as indicators for kidney functions.25,28 Judging from both indicators and the ratio of kidney weight to body weight, aluminum significantly induced the dysfunction of kidney. Although with taurine added in diet did not ameliorate the ratio of kidney weight to body weight, the accumulated amount of aluminum was higher in the kidney than in the liver, which was the same as previous report.29

Lipid peroxidation is a chemical mechanism capable of disrupting the structure and the function of the biological membranes that occurs as a result of free radical attack on lipids. When reactive oxygen species (ROS) begin to accumulate, hepatic cells exhibit a defensive mechanism by using various antioxidant enzymes. The main detoxifying systems for peroxides are catalase and GSH.30 Catalase is an antioxidant enzyme, which destroys H2O2 that can form a highly reactive hydroxyl radical in presence of iron as a catalyst.31 By participating in the glutathione redox cycle, GSH together with GSH-Px convert H2O2 and lipid peroxides to non-toxic products. Reduced activity of one or more antioxidant systems due to the direct toxic effect of aluminum leads to increase lipid peroxidation, oxidative stress and hepatotoxicity. In the current study, aluminum depleted GSH stores and reduced catalase and GSH-Px activities. These results are in harmony with other investigations.32 For example, aluminum induced hepatotoxicity was exacerbated by GSH depletion. In the current study, depletion of GSH stores can account for the inhibition of GSH-Px activity. In addition, high levels of peroxides may explain catalase activity inhibition.33 On the other hand, taurine can also function as a regulator of intracellular calcium homeostasis34 that can be disturbed due to aluminum toxicity. Taurine has been shown to protect against endothelial cell death by modulating intracellular calcium fluxes.35 Finally, taurine may ameliorate aluminum induced hepatic injury by enhancing the activities of endogenous antioxidants. Support for this concept comes from our results, which show that taurine produced a remarkable significant increase in hepatic GSH level and GSH-Px and catalase activities. This could be attributed to the role of taurine in maintaining a normal IGF-I level36 (Dawson et al., 1999) and its antioxidant action against lipid peroxidation, thus conserving the internal antioxidants system. The stimulatory effect of taurine on endogenous antioxidants was reported by others.37,38
Together, the results of the present study demonstrate that administration of taurine has a therapeutic role in preventing cyclosporine-induced hepatotoxicity, possibly through its unique cytoprotective properties such as antioxidant activity.

Accumulation of aluminum is the net consequence of uptake, biotransformation and elimination processes within an individual. Once aluminum is absorbed, it may be transformed into aluminum-thioene or metallothioene. Although the half-life of aluminum-thioene in the liver and kidney is not known exactly, it is many years. With continued retention, there is progressive accumulation in these tissues. The accumulated amount of aluminum in the tissue was effectively reduced by taurine. Taurine is a special amino acid, which possesses an amino group and a sulfonate group. These functional groups might bind with heavy metals, and then stimulated the excretion of such compounds. In this study, it was also found that the amount of aluminum in the feces of rats fed with taurine added was slightly increased. There is no evidence that taurine directly reduces the production of free radicals but it may well operate by binding aluminum which is then not absorbed or is more rapidly excreted. In other words it may act by reducing the overall bioavailability of aluminum or the intracellular availability of absorbed aluminum. Hence, dietary taurine may play a role to reduce the toxic effect of aluminum in the liver and kidney of rats.

Conflicts of interest

All authors have no Conflict of interest.

Acknowledgement

Y-H Yeh conducted the study design, experiment and wrote the manuscript. Y-T Lee and H-S Hsieh analyzed the data and statistical analysis. D-F Hwang performed data interpretation. All authors contributed to this study and reviewed the manuscript.

References