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Prooxidant action of aluminum Ion – Stimulation of iron-mediated lipid peroxidation by aluminum

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Abstract

Prooxidant nature of aluminum ion was analyzed in relation to iron coordination. Aluminum ion effectively enhanced the formation of thiobarbituric acid-reactive substances as a marker of lipid peroxidation of microsomes from rat liver under the acidic conditions, and this metal further attenuated the antioxidant action of flavonoids such as quercetin and baicalein under neutral conditions. Autooxidation of ferrous ion was markedly inhibited by aluminum ion. Aluminum can act as a prooxidant by stabilizing reduced iron the initiating species for lipid peroxidation, and by inhibiting the antioxidant action of flavonoid.

Introduction

Aluminum is toxic in experimental animals, and is responsible for several neurological disorders (Crapper *et al.* 1973; Perl & Brody 1980; Perl *et al.* 1982; Selkoe *et al.* 1979) accompanied with an increased rate of lipid peroxidation in brain (Parkinson *et al.* 1981). Production of thiobarbituric acid-reactive substances as a marker of lipid peroxidation is increased in brain of chronically aluminum-intoxicated mice (Fraga *et al.* 1990). These findings suggest that increased lipid peroxidation could be one of the mechanisms underlying aluminum-mediated damage to the central nervous system. Stimulatory effect of aluminum on iron-mediated lipid peroxidation has been explained by binding to membrane and promotion of changes in the arrangement of membrane lipids (Gutteridge *et al.* 1985; Ohyashiki *et al.* 1993, 1996; Oteiza 1994; Oteiza *et al.* 1993; Quinlan *et al.* 1988). Here we analyzed prooxidant action of aluminum closely related to the inhibition of the autooxidation of ferrous ion as the prooxidant. Aluminum-induced oxidative injury may be ascribed to the direct stimulation of lipid peroxidation and potent inhibition of antioxidant action of flavonoids.

Materials and methods

Materials

Baicalein, quercetin, bathophenanthroline disulfonate were products of Sigma-Aldrich Japan (Tokyo, Japan). Other chemicals were obtained from commercial sources.

Preparation of liver microsomes

Microsomes were prepared from the livers of adult male rats by standard differential centrifugation techniques (Quinlan *et al.* 1988). Microsomes were suspended in 0.25 M KCl at a protein concentration of about 15 mg/ml, and were stored at −80 °C.

Lipid peroxidation of liver microsomes

Lipid peroxidation was determined by iron/ascorbic acid system with rat liver microsomes (Buege & Aust 1978). Reaction mixture of 1 ml contained 10 μM $FeCl_3$, 0.5 mM ascorbic acid, microsomal fraction of 0.2 mg, metals as chloride salts in the absence and presence of flavonoids in 60 mM Tris-HCl (pH 7.4) or Mops-KOH buffer (pH 6.8). The mixture was incubated at 37 °C for 20 min, and the lipid peroxides pro-

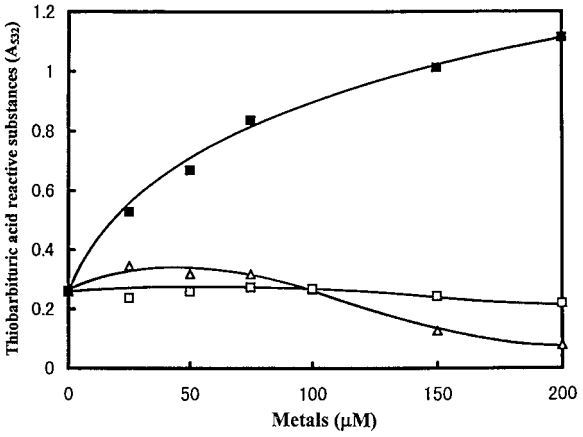

Figure 1. Effect of aluminum ion on the iron-induced lipid peroxidation of rat liver microsomes. Lipid peroxidation was induced by 10 μM $FeCl_3$, 0.5 mM ascorbic acid, 0.2 mg microsomal fraction and 60 mM Mops-KOH buffer (pH 6.8) in the presence of aluminum, zinc or cadmium ions in the reaction mixture of 1 ml. The mixture was incubated at 37 °C for 20 min, and the reaction was stopped by addition of 100% trichloroacetic acid. Lipid peroxides produced were determined as the thiobarbituric acid-reactive substances (Draper & Hadley 1990). ■, aluminum; △, zinc; □, cadmium.

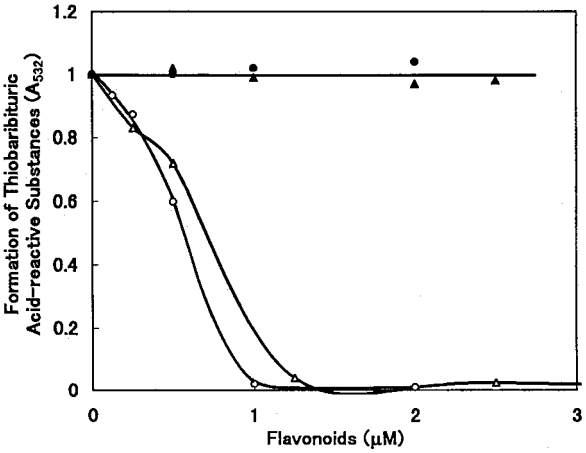

Figure 2. Effect of flavonoids on the iron-induced lipid peroxidation of rat liver microsomes in the absence and presence of aluminum ion. Conditions of lipid peroxidation were similar to those of the legend to Fig. 1 except that pH of the incubation buffer was replaced with Tris-HCl (pH 7.4). ◯, ●, baicalein added; △, ▲, quercetin added. Open and closed symbols indicate the conditions without or with 0.2 mM $AlCl_3$, respectively.

duced were determined as thiobarbituric acid-reactive substances (Draper & Hadley 1990).

Autooxidation of Fe^{2+} ion

Fe^{2+} autooxidation was evaluated by determining the Fe^{2+} concentration on the basis of the bathophenanthroline disulfonate method described previously (Yoshino & Murakami 1998).

Results

We examined the effect of aluminum ion on the formation of thiobarbituric acid-reactive substances as an index of microsomal lipid peroxidation. Figure 1 shows that aluminum ion enhanced the production of thiobarbituric acid-reactive substances under the acidic conditions (pH 6.85), but Cd^{2+} and Zn^{2+} ions showed little or no enhancement of lipid peroxidation.

We further examined the effect of aluminum on the antioxidant action of flavonoids, a group of polyphenolic compounds ubiquitously found in plants. Baicalein and quercetin markedly inhibited the lipid peroxidation, but addition of aluminum completely cancelled the flavonoid-induced inhibition of the formation of thiobarbituric aicd-reactive substances under neutral conditions (Figure 2). Effect of increasing concentrations of aluminum on the lipid peroxidation was analyzed. Baicalein of 8 μM or quercetin of 10 μM inhibited lipid peroxidation completely, and the addition of increasing concentrations of aluminum stimulated the formation of thiobarbituric acid-reactive substances (Figure 3). The concentration of aluminum required for half maximal stimulation of lipid peroxidation was about 15 μM. However, the increase in Zn^{2+} and Cd^{2+} concentrations did not enhance the lipid peroxidation under the same conditions.

The effect of aluminum on the rate of Fe^{2+} autooxidation was examined. Aluminum inhibited the rate of Fe^{2+} oxidation completely, but Zn^{2+} and Cd^{2+} showed little or no effect on the Fe^{2+} autooxidation (Figure 4).

Discussion

Aluminum is abundant in the earth's crust, but is present in very small amounts in living organisms because of its insolubility of aluminum hydroxide complexes at neutral pH (Ganrot 1986; Macdonald & Martin 1988; Martin 1986). Aluminum is not an essential element for mammals and microorganisms, but causes impairment of energy metabolism (Womack & Colowick 1979; Yoshino *et al.* 1990, 1998) relating to some neurological and skeletal disorders (Crapper *et al.* 1973; Parkinson *et al.* 1981; Perl &

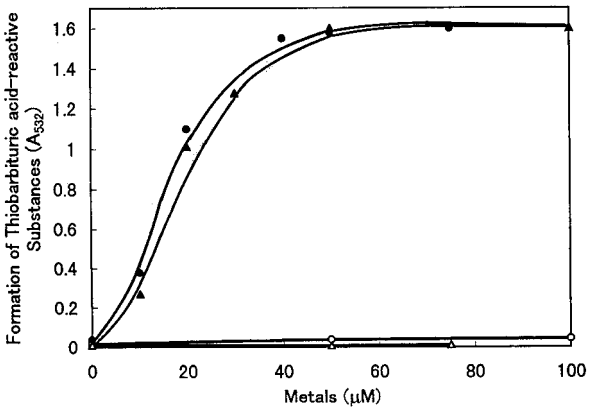

Figure 3. Effect of increasing concentrations of metal cations on the lipid peroxidation of rat liver microsomes in the presence of flavonoids. Conditions of lipid peroxidation were similar to those of Figure 2 except that metal concentrations were varied in the presence of 8 μM baicalein or 10 μM of quercetin. •, ◯, 8 μM baicalein added; ▲, △, 10 μM quercetin added. Open and closed symbols indicate the presence of $ZnCl_2$ and $AlCl_3$, respectively.

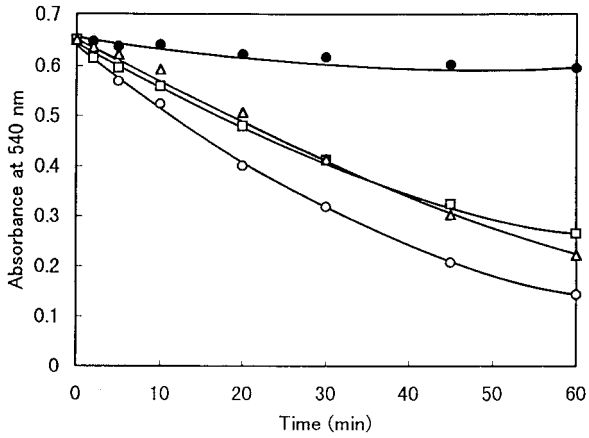

Figure 4. Effect of metal cations on the autooxidation of ferrous ion. Iron autooxidation was followed by determining the ferrous ion concentration with the bathophenanthroline disulfonate (Yoshino & Murakami 1998). The samples of 1 ml contained 10 mM Tris-HCl buffer (pH 7.1), 0.05 mM $FeSO_4$, and 0.1 mM metals as chloride salts. All incubations were carried out at 37 °C. The reaction was started by addition of $FeSO_4$. Aliquots of 0.2 ml were mixed with 0.1 ml of 1 mM bathophenanthroline disulfonate at appropriate intervals, and the absorbance at 540 nm was measured. ◯, No addition; • $AlCl_3$ added; □, $ZnCl_2$ added; △ $CdCl_2$ added.

Brody 1980; Perl *et al.* 1982; Selkoe *et al.* 1979). Lipid peroxidation also may be responsible for the toxic effect of aluminum (Parkinson *et al.* 1981). Aluminum was demonstrated to stimulate iron-dependent lipid peroxidation (Fraga *et al.* 1990; Gutteridge *et al.* 1985; Quinlan *et al.* 1988); however, aluminum is not a transition metal with the ability to undergo valence changes, and thus direct generation of reactive oxygen species by aluminum should be excluded. Enhancement of iron-dependent lipid peroxidation by aluminum has been considered to depend on phospholipid composition (Ohyashiki *et al.* 1993, 1996; Oteiza 1994; Oteiza *et al.* 1993; Xie & Yokel 1996). The inner negatively charged face of the plasma membrane is the target of iron-mediated oxidative injury (Xie & Yokel 1996), and the interaction between aluminum and phosphatidyl-serine with higher negative charge seems to play a key role in the aluminum effect on lipid peroxidation (Ohyashiki *et al.* 1993, 1996).

Recent studies have indicated that aluminum can enhance the ability of transition metals to promote the generation of reactive oxygen species in cerebral synaptosomes (Bondy *et al.* 1998). Enhancement by aluminum of reactive oxygen species formation causing lipid peroxidation was shown to be related to the stabilization of iron as ferrous ion the prooxidant (Bondy *et al.* 1998). In this paper we presented a direct relationship between the inhibition of the ferrous ion oxidation by aluminum and the enhanced lipid peroxidation. The aluminum-mediated inhibition of the oxidation of ferrous ion causing oxidative damages to membrane lipid, DNA and protein seems to be related to the colloidal form of aluminum ion. Aluminum as a colloidal form of the octahedral hexahydrate, $Al(H_2O)_6^{3+}$ formed under acidic conditions may prohibit the electron transfer from reduced iron to oxygen molecule. Furthermore, under neutral conditions aluminum acts as a prooxidant by attenuating the action of flavonoids, which play a physiological antioxidant in cells (Das 1994). Neutral solutions give an $Al(OH)_3$ precipitate that redissolves in basic solutions, owing to formation of tetrahedral $Al(OH)^{4-}$ (Evans *et al.* 1992, Martin 1986). Interaction of this aluminum species with flavonoids may inhibit the antioxidant action of the polyphenolics by iron-chelation.

Aluminum ion shows a potent inhibitory effect of NADP-isocitrate dehydrogenase from yeast and porcine heart (Yoshino & Murakami 1992; Yoshino *et al.* 1992). NADP-isocitrate dehydrogenase acts as an enzyme of the citric acid cycle and further as an antioxidant enzyme that produces reduced NADP for the regeneration of reduced glutathione. Inhibition by aluminum of the NADP-isocitrate dehydrogenase with potent NADPH-generating activity may cause an oxidative injury to cells. The prooxidant properties of aluminum was concluded to be due to the stabilization of reduced iron the initiating species for lipid peroxidation and further to attenuation of the antioxidant

action of flavonoids in addition to the inhibition of NADPH-generating reactions.

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