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Effect of Aluminum Ingestion on Lipid Peroxidation in Rats

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The effect of aluminum ingestion on the lipid peroxidation in rat brain, lung, liver, spleen and kidney was examined. Aluminum hydroxide, 100 mg/kg body weight, was administered orally once a day for 7 d, and the amount of lipid peroxide (TBA value) and the activity of superoxide dismutase (SOD) were measured 24 h after the last administration.

Lipid peroxide was increased in the brain, to 142% of the control. TBA values in the lung, liver, spleen and kidney were similar to those in the control group. Pretreatment of rat brain, lung, liver, spleen, and kidney homogenates with aluminum chloride in an ice-bath increased the TBA value in the brain significantly compared with that of the control.

Examination of variation in SOD activity showed that the activity in the brain was decreased, while that in the kidney was increased, compared with those of the control. Activities of SOD in the lung, liver, and spleen were similar to those of the control.

These results suggest that an increase in lipid peroxidation and a decrease in activity of SOD in the brain after oral administration of aluminum hydroxide constitute one of the factors for the mechanism of brain injury by aluminum.

Keywords——aluminum ingestion; aluminum hydroxide; rat brain; brain injury; lipid peroxidation; thiobarbituric acid; superoxide dismutase

Aluminum has been known to induce neurofibrillary changes in several mammalian species.[1] Recent studies have revealed the accumulation of aluminum in the brain of patients with Alzheimer's disease and senile dementia of the Alzheimer type (SDAT).[2]

It has been reported that aluminum accumulation in the brain may be related to various neurological and behavioral disorders in animals and man.[3] The biochemical mechanisms of aluminum in the brain are poorly understood.[4] On the other hand, the tissue injuries induced by several heavy metals are thought to result in the formation of lipid peroxides in membrane.[5]

In the present work, therefore, the effect of aluminum on lipid peroxidation in the rat brain, lung, liver, spleen and kidney was examined both *in vitro*, and *in vivo* by the oral administration of aluminum hydroxide. Further, it has been reported that lipid peroxidation in tissue is inhibited by SOD, which catalyzes the dismutation of the superoxide free radical.[6] Therefore, variations in the activity of SOD after oral administration of aluminum hydroxide were examined in the same organs.

Experimental

Materials——2-Thiobarbituric acid was purchased from Wako Pure Chemicals Ind., Ltd. Malonaldehyde bisdimethyl acetal was the product of Tokyo Kasei Kogyo Campany, Ltd. Xanthine oxidase was Grade I (from buttermilk) from Sigma Chemical Co., and was used as a suspension in 2.3 M $(NH_4)_2SO_4$. $Al(OH)_3$ solution was a homogeneous suspension in distilled water at a concentration of 10 mg/ml (as Al mg/ml). Other reagents were commercial extra-pure grade products.

Preparation of Tissue Homogenates——Male Wistar strain rats, 6—7 weeks of age and weighing 190—200 g, were used. The animals were fed on a solid food CE-2 (CLEA Japan, Inc.,) and water *ad libitum*. Rats were divided into two groups of 4 animals each. Rats of group 1 were given $Al(OH)_3$ solution orally through a stomach tube at a dose of 100 mg/kg body weight (as Al mg/kg) once a day for 7 d. Rats in group 2 served as controls and were given an appropriate volume of distilled water orally in place of $Al(OH)_3$ solution. The animals were sacrificed by decapitation 24 h after the final oral administration, and the brain, lung, liver,

spleen and kidney were rapidly removed. The liver and lung were perfused immediately with ice-cold 1.15% KCl. Brain, spleen and kidney were removed without being perfused. The organs were washed with ice-cold 1.15% KCl, and blood and moisture were wiped off. Each organ was minced finely, and homogenized with ice-cold 1.15% KCl to make a 25%(w/v) homogenate by a glass homogenizer.

Measurement of Lipid Peroxidation——Lipid peroxide in various tissue homogenates was measured by the TBA method of Uchiyama *et al.*[7]

To 0.1 ml of 25% whole homogenate, 0.2 ml of 8.1% SDS, 1.5 ml of 1% phosphoric acid and 0.2 ml of distilled water were added, followed by 1.0 ml of 0.6% TBA, and the mixture was heated for 45 min in a boiling-water bath. After the reaction, the mixture was cooled in an ice-bath, and then the colored TBA reactants were extracted with 4.0 ml of *n*-butanol. After centrifugation, the optical density of the *n*-butanol layer was measured at 535 nm with a Hitachi spectrophotometer (Model 200-10); the results were expressed as nmol of malonaldehyde formed.

Effect of Addition of Aluminum Chloride on TBA Value——A 0.1 ml aliquot of 25% whole homogenate was pretreated with 0.1 ml of aluminum chloride dissolved in distilled water (5—100 μmol/ml) and 0.1 ml of 0.05 M Tris–HCl buffer (pH 7.4) in an ice-bath for 10 min. After the pretreatment, the mixture was added to 0.2 ml of 8.1% SDS, and lipid peroxides were measured as described above. As a control, the same mixture but using 0.05 M Tris–HCl buffer (pH 4.0) in place of $AlCl_3$ solution was processed in the same way.

Activity of Superoxide Dismutase——Each organ was homogenized with 3 vol. of 0.01 M Tris–HCl buffer (pH 7.8), and centrifuged at 20000 ***g*** for 20 min. After the centrifugation, the supernatant was added to 0.25 vol. of ethanol and 0.15 vol. of chloroform in the cold. The resulting supernatant, obtained by centrifugation, was dialyzed against 0.01 M Tris–HCl buffer (pH 7.8), and used for SOD and protein analyses. The activity of SOD was measured by the xanthine–xanthine oxidase method of Imanari *et al.*[8] One unit of SOD activity was defined as the amount which produced a 50% inhibition of the rate of change in absorbance. Protein was determined by the method of Lowry *et al.*,[9] using bovine serum albumin as a standard.

Results and Discussion

Lipid Peroxidation in Various Organs

As shown in Table I, oral administration of aluminum hydroxide resulted in an increase of TBA value in the brain, to 142% of the control. On the other hand, there was hardly any effect on the lung, liver, spleen or kidney. Thus, the effect of orally administered aluminum was organ-specific.

TABLE I. Lipid Peroxidation in Various Organs after Oral Administration of $Al(OH)_3$ for 7 d

Organ	TBA value, nmol/25 mg wet weight			
	Control	$Al(OH)_3$, *p.o.*	% of control	*p*
Brain	3.43±0.39	4.86±0.49	142	<0.01
Lung	4.73±1.23	5.03±0.61	106	N.S.
Liver	13.3±2.75	11.0±2.09	83	N.S.
Spleen	7.12±0.40	6.42±0.68	90	N.S.
Kidney	5.15±0.95	5.59±0.68	109	N.S.

Rats were given aluminium hydroxide solution (100 mg of Al/kg) *p.o.* daily for 7 d. In the control group, distilled water was given in place of $Al(OH)_3$ solution. Each value is the mean ± S.D. obtained from four individual animals.

Effect of Addition of Aluminum Chloride on TBA Value

It has been reported that ferric ion increases the TBA value in various tissues by acting as a catalyzer on the reaction of TBA with lipid peroxides *in vitro*.[10] Therefore, the effect of addition of aluminum chloride on the reaction of TBA with lipid peroxides in various organs was examined. As shown in Table II, the TBA value in the brain was significantly increased by the addition of aluminum chloride. When 5 μmol of aluminum was added, the TBA value reached 260% of the control value.

Among the organs evaluated, only the brain responded to the addition of aluminum with a significant increase in TBA value. Further, as shown in Table III, aluminum chloride did

not affect the reaction of TBA with malonaldehyde. Though the mechanisms of action of aluminum administered *in vivo* are not clear, it may be surmised that the increase of TBA value in the brain by oral administration of aluminum hydroxide is probably not due only to the result of the chemical reaction of aluminum with TBA and lipid peroxides. These points require further elucidation.

TABLE II. Effect of Addition of Aluminum Choride on Values in Various Organs

$AlCl_3$ (μmol)	TBAvalue, nmol/25 mg wet weight				
	Brain	Lung	Liver	Spleen	Kidney
—	3.50(100)	4.72(100)	13.8(100)	7.44(100)	5.50(100)
0.5	3.40(97)	4.89(104)	12.4(90)	8.32(112)	5.08(92)
1.0	5.04(144)	4.37(93)	13.3(96)	8.60(116)	5.85(106)
5.0	9.10(260)	5.00(106)	13.4(97)	8.64(116)	5.89(107)
10.0	8.23(235)	4.92(104)	13.0(94)	8.66(116)	5.39(98)

Each value is the mean of three experiments. Numbers in parentheses represent percentages relative to the control.

Activity of Superoxide Dismutase in Various Organs

The effect of oral administration of aluminum hydroxide on the activity of SOD in various organs was examined, and the results are shown in Table IV. SOD activity in the brain was 71% and that in the kidney was 146% of the control values. There was hardly any effect on the other organs.

If the increase of lipid peroxides in the brain dose lead to the development of neurological disorders, then the increase of lipid peroxides and the decrease of SOD activity in the brain after oral administration of aluminum hydroxide may be involved in the mechanism of brain injury by aluminum. Further work is in progress on the effect of aluminum on SOD in rat brain and kidney.

TABLE III. Effect of Aluminum Chloride on the Reaction of TBA with Malonaldehyde

$AlCl_3$ (μmol)	TBA value[a] (nmol/reaction tube)	% of control
—	9.69	100
0.1	9.98	103
0.5	9.40	97
1.0	9.57	99
5.0	9.45	98
10.0	9.23	95
50.0	9.18	95

a) 10 nmol of malonaldehyde was reacted with TBA. Each valus is the mean of three experiments.

TABLE IV. Activity of Superoxide Dismutase in Various Organs after Oral Administration of $Al(OH)_3$ for 7 d

Organ	Activity, unit/mg protein		% of control	p
	Control	$Al(OH)_3$, *p.o.*		
Brain	68.9±7.97	49.0±6.83	71	<0.01
Lung	47.1±4.28	45.6±6.10	97	N.S.
Liver	699±67.9	727±71.6	104	N.S.
Spleen	203±31.1	182±18.1	90	N.S.
Kidney	318±26.1	464±38.4	146	<10.01

Rats were given aluminum hydroxide solution (100 mg of Al/kg) *p.o.* daily for 7d. In the control group, distilled water was given in place of $Al(OH)_3$ solution. Each value is the mean ± S.D. obtained from four individual animals

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