40. ^{26}Al Tracer Experiment by Accelerator Mass Spectrometry and its Application to the Studies for Amyotrophic Lateral Sclerosis and Alzheimer's Disease. I.

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Abstract: Accelerator mass spectrometry (AMS) was applied for ^{26}Al tracer experiment to study the aluminum toxicity and metabolism in rats. To investigate the cause of amyotrophic lateral sclerosis (ALS) and Alzheimer's disease, the aluminum incorporation into the brain (cerebrum) was studied by AMS using ^{26}Al as a tracer. When ^{26}Al was intraperitoneally injected into rats, a considerable amount of ^{26}Al was incorporated into the cerebrum after 5–35 days of the injection.

Key words: Accelerator mass spectrometry; amyotrophic lateral sclerosis; Alzheimer's disease; ^{26}Al tracer; aluminum toxicity.

Introduction. By using a tandem accelerator equipped at the Research Center for Nuclear Science and Technology in the University of Tokyo, we have developed accelerator mass spectrometry (AMS) systems to detect ^{10}Be , ^{14}C , and ^{26}Al , which have been successfully applied in various scientific fields.^{7)–9), 12)} Beginning in early 1990, we started new research to investigate the pathogenesis of amyotrophic lateral sclerosis (ALS)¹⁵⁾ and Alzheimer's disease by AMS using ^{26}Al as a tracer. Meirav *et al.* have reported that intravenously-administered ^{26}Al in rat serum and urine can be detected by AMS.¹¹⁾ However, there have been no reports describing the *in vivo* distribution and turnover of ^{26}Al in tissues or organs, including the brain.

The accumulation of aluminum in the central nervous system is reported to be the cause of Alzheimer's disease and ALS.^{1), 15)} Furthermore, aluminum, one of the most abundant elements in the Earth's crust, is going to be dissolved by acidic rain from the crust, and ingested into our bodies through the drinking water and food.⁶⁾

On the contrary, many authors still claimed that aluminum accumulation in the brains of patients with Alzheimer's disease occurred after extensive death or degeneration of nerve cells by amyloid deposition or by neurofibrillary tangle

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formation, and denied the pathogenicity of aluminum in this disease.^{5),13),14)} Therefore, aluminum tracer experiment is essential to clarify the cause of Alzheimer's disease and ALS.

Aluminum has a single stable isotope (^{27}Al) and six prevalent radioactive isotopes (^{24}Al , ^{25}Al , ^{26}Al , ^{28}Al , ^{29}Al and ^{30}Al). However, the half-lives of ^{24}Al , ^{25}Al , ^{28}Al , ^{29}Al , and ^{30}Al are 2.14 sec, 7.24 sec, 2.31 min, 6.6 min, and 3.3 sec, respectively, and too short for tracer experiments in the living organisms. On the other hand, the half-life of ^{26}Al is 7.2×10^5 yr, and too long for the detection of decay using conventional gamma-ray counting techniques.

Using AMS, we can detect as few as 10^6 atoms of ^{26}Al . Moreover, this method enables us to detect the long-lived isotope (^{26}Al) as a tracer with sensitivity 10^5 times greater than that of the gamma-ray decay counting techniques.^{2),10)}

In this paper, we report an ^{26}Al tracer experiment, which was not successful until AMS has been applied. We also describe ^{26}Al incorporation into the rat brain (cerebrum) after intraperitoneal injection.

Materials and methods. Seven-to 8-month-old male Sprague-Dawley rats weighing 570–670 g were anesthetized with ether and intraperitoneally injected with 1 ml of a solution containing 10 dpm of ^{26}Al and 2.75 mg of ^{27}Al in 0.45 M sodium acetate-hydrochloric acid buffer (pH 4.3). The animals were sacrificed under ether anesthesia 5, 10, 15, 25 and 35 days after injection. The liver, blood and cerebrum were quickly removed and freeze-dried with a vacuum dryer. A sample (under 300 mg dry weight) was digested with concentrated nitric acid (3 ml) first at 80°C for 3 hours then at 140°C for 4 hours in a Teflon sealed vessel inserted in a stainless steel bomb. After cooling to the room temperature, the sample was added to 1 ml of perchloric acid (60%), heated on a hot-plate at 90°C for 4 hours, and added to about 1 mg of ^{27}Al as carrier. Aluminum fractions were purified by cation exchange, treated with pure ammonia water to convert the aluminum to aluminum oxide at 1000°C , and mixed with pure silver powder.¹²⁾ The sample aluminum oxide was pressed into a sample holder of the ion source, and examined with the tandem accelerator.

Since the aluminum contents in the liver, blood and cerebrum⁴⁾ are negligible compared to the amount of carrier ^{27}Al added to the samples, ^{26}Al concentrations in samples can simply be determined from the $^{26}\text{Al}/^{27}\text{Al}$ ratios measured by the AMS method. As we have already reported the details of the AMS system,^{7)-9),12)} we illustrate here only the performances of the system, in which the "internal beam monitor method" enables fairly precise measurement of $^{26}\text{Al}/^{27}\text{Al}$ ratios. The reproducibility of measurement is better than $\pm 3\%$ and the abundance sensitivity, which was derived from measurement of background level ratio, is about $^{26}\text{Al}/^{27}\text{Al} = 1 \times 10^{-13}$. The minimum detectable amount is about 10^6 atoms of ^{26}Al , that is equal to a detection efficiency of about $10^{-5,9),12)}$

Results. Table I summarizes the sample names, the dry weight of samples measured, the weight of carrier added, $^{26}\text{Al}/^{27}\text{Al}$ ratios measured, ratios of ^{26}Al contents in unit weight of samples over the injected ^{26}Al amounts, and the total aluminum amounts incorporated into the whole liver or in the whole cerebrum.

The relative parts of the dosed ^{26}Al contained in the liver, blood and cerebrum are shown in Fig. 1 as a function of time after injection. Error bars show one standard errors including the ^{26}Al counting statistics. Since the samples were obtained from different rats, the data fluctuated pretty much.

The concentrations of ^{26}Al in the liver and blood decreased rapidly within

Fig. 1. Relative parts of the dosed ^{26}Al contained in the liver, blood and cerebrum (brain) as a function of time after injection.

Table I

Sample name (tissue-days)	Dry wt. measured (mg)	Carrier added (μg)	$^{26}\text{Al}/^{27}\text{Al}$ ($\times 10^{-11}$)	$^{26}\text{Al}/\text{g sample}$ $/^{26}\text{Al injected}$ ($\times 10^{-4}$)	Aluminum in whole tissue (μg)
brains					
B-5*	339.8	1107	0.357 ± 0.039	0.493 ± 0.054	0.051 ± 0.006
B-5**	287.0	1120	0.107 ± 0.014	0.177 ± 0.023	0.016 ± 0.002
B-10	370.8	1055	0.401 ± 0.044	0.484 ± 0.053	0.055 ± 0.006
B-15	318.5	1081	0.297 ± 0.029	0.428 ± 0.042	0.044 ± 0.004
B-25	312.4	1012	0.233 ± 0.033	0.320 ± 0.045	0.030 ± 0.004
B-35	294.9	1075	0.363 ± 0.052	0.561 ± 0.081	0.050 ± 0.007
blood					
B L-5	465.8	1121	0.756 ± 0.061	0.771 ± 0.062	
B L-10	561.4	1082	0.882 ± 0.196	0.721 ± 0.160	
B L-15	485.8	1021	0.162 ± 0.024	0.145 ± 0.022	
B L-25	488.1	1085	0.595 ± 0.025	0.561 ± 0.023	
B L-35	561.5	996	0.633 ± 0.043	0.476 ± 0.032	
livers					
L-5	902.7	1084	636.0 ± 40.6	311 ± 20	ca. 350
L-10	499.5	1126	115.1 ± 7.6	99.3 ± 6.5	ca. 110
L-25	663.1	1093	14.00 ± 0.58	9.79 ± 0.66	7.2 ± 0.5
L-35	681.5	995	31.61 ± 1.19	19.56 ± 0.73	29.0 ± 1.1

Summarized data of ^{26}Al -AMS together with the ratios ^{26}Al contents in unit weight of samples over the injected ^{26}Al amounts and the total aluminum amounts incorporated in the whole liver, or in the whole cerebrum.

5–10 days after injection, but were rather stationary or slightly increased at 25–35 days. On the other hand, the concentration of ^{26}Al in the cerebrum showed a slight increase at 5–35 days after injection. Since the blood remained in the blood vessels of the cerebrum samples, some part of the ^{26}Al in the cerebrum might have actually originated in the blood. However, the dry weight of the blood contained in the cerebrum is less than 15% of the overall cerebrum dry weight,³⁾ and, moreover, the ^{26}Al concentration of the cerebrum was higher than that of the blood at 15 days and 35 days after injection.

Discussion. From the data described above, it is concluded that a considerable amount of ^{26}Al was incorporated into the cerebrum within 5 days after intraperitoneal injection. The fact that ^{26}Al concentrations in the cerebrum showed a gradual increase over 5–35 days following injection indicates the accumulation of the intraperitoneally-injected aluminum in the brain, and supports the theory that Alzheimer's disease and ALS are caused by irreversible accumulation of aluminum in the central nervous system.^{1),15)}

The amount of aluminum incorporated in the cerebrum found in this experiment was estimated to be on the order of $0.05\text{ }\mu\text{g}$ per single intraperitoneal injection of 2.75 mg aluminum.

The amount of ^{26}Al in the cerebrum was on the order of $1\times 10^{-4}\text{ dpm/sample}$, which corresponds to 4×10^8 atoms of ^{26}Al . Since such a small amount of ^{26}Al could not be measured by conventional decay counting techniques, we had not been able to perform ^{26}Al tracer experiments until the AMS method was developed.

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