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Up-regulation in the expression of renin gene by the influence of aluminium

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ABSTRACT

The excretion of aluminium in urine was significantly increased after intake of analgesics containing aluminium, confirming increased absorption and hence exposure to aluminium with such medication. The effect of aluminium on the kidney was further investigated by study of gene expression in mice. After a single dose of aluminium, an up-regulation of renin gene was found by DNA sequencing of the products of differential display analysis. The up-regulation of renin was confirmed by reverse transcription-polymerase chain reaction (RT-PCR) and Western blotting experiments in the dose dependent treatments and the time course observation after aluminium citrate injection. The up-regulation of the renin expression by aluminium is a strong indication of the influence of aluminium on the renin–angiotensin–aldosterone-system, resulting in possible induction of essential hypertension.

1. Introduction

Recently it has been implicated that the risk of hypertension increases in the aluminium exposure in manufacturing employees [1]. Aluminium is the third abundant element and the most abundant metal in the earth's crust, therefore exposure to aluminium is inevitable in daily life. Aluminium in humans comes primarily from the diet and it is known that the uptake, distribution and toxicity of aluminium are greatly dependent on its physicochemical form [2]. Aluminium appears in food not only as a natural component but also as an additive. Aluminium compounds are used in water purification, brewing and refining of sugar [3]. The major sources of aluminium intake include drinking water, food [4] and medications such as aluminium buffered aspirin products, antiperspirants, antacids, phosphate binders [5,6] and vaccines [7,8]. In these sources of aluminium exposure, buffered aspirin containing aluminium glycinate was commonly used as analgesics. Moreover, it was reported from the measurement of human volunteers that aluminium glycinate is absorbed at much a higher rate than aluminium hydroxide [5]. Therefore, it is important to know the quantity and duration of aluminium exposure after the intake of aluminium glycinate. Aluminium has no demonstrated essential function in mammals [9]. Aluminium is toxic to all organisms [10]. Aluminium accumulates in all tissues of mammals such as the kidneys, liver, heart, blood, bones and brain [11–13]. Accumulation of aluminium in biological systems has been linked to disease conditions such as dialysis encephalopathy, renal osteodystrophy, hypochromic microcytic anemia [14–16]. A relationship between aluminium and Alzheimer's disease has been suggested [4,17–21] but the mechanism remains rather unclear due to the lack of adequate animal models [22,23]. Aluminium intoxication has been reported to cause oxidative stress and a decrease in the intracellular levels of reduced glutathione [24]. The main route of aluminium excretion is urine [25–29]. Different mechanisms of renal excretion of aluminium such as glomerular filtration [30], tubular reabsorption of filtered aluminium and secretion in distal nephron [26,31] and excretion in the distal tubules [32] have been suggested. The kidneys play a major role in preventing the harmful accumulation of aluminium, excreting aluminium from the body. Diverse studies have been conducted on metabolism, excretion and the nephrotoxic effect of aluminium [25,33,34], but there has been no report on the effect of aluminium on renal gene expression. The pathological influences of aluminium may appear chronically, but the gene expression may start to be altered in an early time of the exposure to aluminium. The aims of this investigation are to study the excretion of aluminium after taking Al-containing analgesics and to study the effect of single dose of aluminium on gene expression in the kidneys of mice subjects.

2. Method

2.1. Aluminium excretion in urine

2.1.1. Human volunteers

(1) Urine was collected from six volunteers who took same meals and drinks and stayed at the same time for 3 days at

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the University Hotel which was located in Mt. Daisen in Tottori Prefecture in Japan. On the first day the control urine was collected and on the second day, volunteers took antacid of aluminium hydroxide containing 300 mg of aluminium after breakfast and the urine collection was continued to the third day.

2.2.1. Human volunteers

(1) Nine female volunteers within the same age bracket of 18–22 years who customarily took analgesics containing aluminium glycinate for relief from menstrual pain were recruited for participation in this study. Urine was collected for 2 days before and after taking analgesics containing 15.8 mg of aluminium in a day. The control volunteers ($n = 7$) took the analgesics without aluminium. A special urine collection equipment (Sumitomo Bakelite Co., Tokyo) was used to collect 1/50 of total urine at each time.

2.2.2. Human volunteers

(1) Five female volunteers within the same age bracket of 18–22 years, who customarily take analgesics containing aluminium glycinate for relief of heavy menstrual pain, were recruited for participation. Urine was collected for continuous 4 days after taking analgesics containing 31.6 mg of aluminium.

All participants received oral and written information about the aim and protocol of the study and gave their written informed consent before participation. The study protocol was approved by the Ethics Committee of Tottori University.

2.2. Analysis of aluminium in urine

Human urine was collected and a 2.0 ml aliquot was taken in a polypropylene sample tube and was heated at 80 °C for 20 min on heating block equipment after the addition of 0.5 ml ultra-pure nitric acid (Kanto Chem. Co., Tokyo) [5]. Each sample solution had been stored in the refrigerator until the measurement of aluminium. The standard solution (aluminium: 1000 μg/ml) was supplied by Wako Pure Chem. Ind., Osaka. Aluminium analysis was carried out using HITACHI 180–80 type polarized light Zeeman atomic absorption spectrometer (AAS) with a graphite furnace. The AAS conditions for sample volume of 10 μl were drying at 80–120 °C for 30 s, ashing at 800 °C or 30 s and atomizing at 2800 °C for 3 s. Disposable polypropylene tubes (PP-16, Maruemu Corp., Osaka) were used for aluminium analyses without further washing and rinsing procedure to avoid contamination from the environment. Aluminium was not detected from blank tubes. The daily excretion of aluminium was calculated from the concentrations of aluminium and the total urine volumes. The daily collection of urine was carried out in every experiment, therefore, creatinine correction was not applied.

2.3. Animal treatment

Adult male ICR mice maintained in a temperature controlled room (22–24 °C) with 12 h light:12 h dark cycle in the Division of Laboratory Animal Science in the Research Center for Bioscience and Technology of Tottori University. Animals were fed *ad libitum* dried rice and de-ionized and distilled water to limit aluminium exposure from food and water. Urine was collected using plastic metabolic cage, separating urine and feces.

Animals were divided into treatment groups comprising two control groups, the first control group received 1 ml of sodium saline (Sigma–Aldrich, MO, USA) and the second control group received sodium citrate (Wako, Japan) at the dose of 5 mg/100 g body weight. The aluminium treatment groups received single dose of aluminium citrate at 5 mg Al/100 g body weight (Soekawa chemicals, Japan) intraperitoneal (IP) injection. Urine was also collected from aluminium-treated mouse and the control mouse in metabolic cages for the measurement of aluminium excretion. To avoid the influence of the stress to animals in the metabolic cage the gene expression in kidneys was examined for the animals in normal cages.

There were no observable differences between the control group and the treated groups in their daily consumption rates. Careful observation of the mice after treatment showed that there were no abnormal changes in behavior between aluminium treated and control mice.

The protocols were approved by the committee on animal research of Tottori University. The investigation conforms to the guiding principles in the use of animals in Toxicology, adopted by the Society of Toxicology in 1989. Mice were anaesthetized and killed by decapitation at time intervals from 12 to 72 h after treatment.

2.4. Differential display analysis

Differential display analysis was carried out on kidneys of ICR mice 72 h after IP treatment with aluminium–citrate (at 5.0 mg aluminium/100 g body weight) [35]. RNA was isolated using TRIzol extraction kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Total RNA (1 μg) was treated with DNase I and then annealed to 50 ng of random primers at 65 °C. Reverse transcription was carried out with Superscript II reverse transcriptase (RNase H minus), 10 mM DTT (dithiothreitol) (Wako, Japan), Recombinant Ribonuclease Inhibitor (RNaseOUT, Invitrogen) and 1 mM dNTPs at 42 °C for 1 h. Reaction mixtures were diluted in DEPC-TE (0.1% diethyl pyrocarbonate, 10mM Tris-HCl, pH8.0, 1mM EDTA) to 95 μl followed by heat inactivation at 65 °C for 15 min. Quantitative PCR (qPCR) reactions (15 μl) contained 1 μl cDNA, 7.5 μl of iQ SYBR Green Supermix (Bio-Rad), 0.15 μl uracil DNA glycosylase (Invitrogen) and 200 nM each of forward and reverse primers. Primer sequences are listed as follows:

Downstream primers: 5′ Rhodamine labeled-TnAA, 5′ Rhodamine labeled- TnAC, 5′Rhodamine labeled-TnCA, 5′Rhodamine labeled-TnCC, 5′Rhodamine labeled-TnGA, 5′Rhodamine labeled-TnGC ($n = 13–15$).

Upstream primers: 5′-GATCATAGCC-3′, 5′-CTGCTTCATG-3′, 5′-GATCCAGTAC-3′, 5′-GATCGCATTG-3′, 5′-CTTGATTGCC-3′, 5′-AGGTGACCGT-3′, 5′-GATCATGGTC-3′, 5′-TTTTGGCTCC-3′, 5′-GTTTTCGCAG-3′, 5′-GTTGCGATCC-3′, 5′-GATCTGACAC-3′, 5′-CTGATCCATG-3′, 5′-TGGATTGGTC-3′, 5′-GGAACCAATC-3′, 5′-GATGAATCGA-3′, 5′-TCGGTCATAG-3′, 5′-GATCTGACTG-3′, 5′-TCGATACAGG-3′, 5′-TACAAGCAGG-3′, 5′-GATCAAGTCC-3′, 5′-GATCTCAGAC-3′, 5′-AGCCAGCGAA-3′, 5′-CAAACGTCGG-3′, 5′-CTTTCTACCC-3′.

2.5. RT-PCR

Kidneys were rapidly excised, weighed, cut in half and frozen in RNA*later* (Ambion, Austin, TX, USA) to maintain RNA. Total RNA was purified from kidney tissues using RNeasy spin columns (Qiagen, Hilden Germany). RT-PCR was performed using standard protocols [36].

Reverse transcription was carried out using; 2 μl total RNA extract from kidneys, 4 μl 5× first strand buffer, 2 μl 0.1 M DTT, 10 μl 2 mM DNTPs, 1 μl random primers, 1 μl RNase inhibitor, 1 μl M-MLV reverse transcriptase (Invitrogen) at 37 °C for 1 h, 95 °C for 5 min the reaction mixtures were kept at 4 °C until polymerase chain reaction (PCR). PCR was carried out using 25 μl Premix Taq