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Transport and toxic mechanism for aluminum citrate in human neuroblastoma SH-SY5Y cells

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Abstract

Aluminum (Al) is thought to be a risk factor for neurodegenerative disorders, but the molecular mechanism has been not clarified yet. In this study, we examined how a transport system handled transport of Al citrate, the major Al species in brain, and effect of Al citrate treatment on expression of the transporter and on susceptibility to oxidative stress in human neuroblastoma SH-SY5Y cells. Uptake of Al citrate by the cells was temperature- and concentration-dependent, and inwardly-directed Na^+-gradient-independent. Simultaneous application and preloading of L-cystine or L-glutamate inhibited and stimulated, respectively, the Al citrate uptake by SH-SY5Y cells, demonstrating kinetically that Na^+-independent L-cystine/L-glutamate exchanger, system Xc^-, is involved in its uptake. When the cells were treated with Al citrate, but not citrate, for 2 weeks, but not a day, the expression of the transporter was decreased. Although the cell viability and glutathione content of the cells were not altered by the treatment with Al citrate alone, the number of dead cells among the Al citrate-treated cells increased on exposure to oxidative stress caused by a glucose deprivation/reperfusion treatment. These findings demonstrate that Al citrate is a substrate for system Xc^-, and that chronic treatment with Al citrate causes downregulation of the transporter and increases the vulnerability of the cells to oxidative stress without a direct effect on the viability or GSH content.

Keywords: Aluminum; Neuronal cell; System Xc^-; Oxidative stress

Introduction

Chronic aluminum (Al) neurotoxicity is well known from the fact that dialysis dementia occurs in adults and children with renal insufficiency who are treated with an Al-contaminated dialysate solution or oral phosphate-binding agents that contain Al (Alfrey et al., 1976; American Academy of Pediatrics Committee on Nutrition, 1986). However, definite mechanism underlying Al toxicity has not been obtained yet, nevertheless a lot of studies have been performed in vitro and in vivo.

It has been reported that up to 90% of Al in the plasma is in a complex with transferrin, and the remainder (up to 11%) is predominantly associated with citrate (Öhman and Martin, 1994), and that its penetration into the brain appears to be mediated by receptor-mediated endocytosis and a specific transport system, respectively (Roskams and Connor, 1990; Yokel et al., 1999). The Al citrate complex is formed through coordination bonding of Al with the hydroxyl group and the two terminal carboxylates of citrate, this leaving a free carboxylate and leading to dissociation at physiological pH and is extremely stable (Martin, 1986; Yokel et al., 1999). Al citrate is the major species in the brain extracellular fluid (approximately 60%) and transported out of the brain (Öhman and Martin, 1994). For these reasons, the selection of Al species is thought to be critical for evaluating the effect on neuronal cells, especially an in vitro system. To our knowledge, however, there has no report on the use of Al citrate.

Recently, we reported that Al citrate is transported by an Na^+-independent glutamate transporter, system Xc^-, at the

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blood–brain barrier (BBB) (Nagasawa et al., 2005). Since system Xc^- exchanges extracellular L-cystine and intracellular L-glutamate (Bannai, 1986), and L-cystine is a source of the major intracellular antioxidant, glutathione (GSH), in the brain, its influx via system Xc^- is thought to be one of the rate-limiting steps in the biosynthesis of GSH (Dringen et al., 2000; Schulz et al., 2000). Therefore, chronic exposure of neuronal cells to Al citrate might increase their vulnerability to oxidative stress and might promote the development of neurodegenerative disorders.

In this study, using human neuroblastoma SH-SY5Y cells, we firstly determined whether or not Al citrate is a substrate for system Xc^-, and then evaluated the effect of chronic exposure of the cells to Al citrate on expression of system Xc^-, cell viability, cellular GSH content and/or susceptibility to oxidative stress.

Materials and methods

Chemicals

Al citrate monohydrate (purity: >99%) was purchased from Soekawa Chemicals (Tokyo, Japan). L-Glutamic acid, L-cystine, sodium azide (AZ), all-*trans* retinoic acid, diethyl maleate (DEM), citric acid, dimethyl sulfoxide (DMSO), multielement, lithium, bismuth, indium and gallium standard solutions were obtained from Wako Pure Chemical Ind. Ltd. (Osaka, Japan). 2-Deoxy-D-glucose (DOG) was purchased from Sigma (St. Louis, MO, USA). All other chemicals were of commercial or analytical grade requiring no further purification. [^{3}H]H_2O (1 mCi/37 MBq) was obtained from American Radio Chemicals, Inc., St. Louis, MO, USA. In this study, we used 25 mM HEPES-contained Hanks' balanced salt solution (HBSS, pH 7.4) and phosphate-buffered saline (PBS, pH 7.4) as the transport and wash buffers, respectively, unless otherwise stated.

Cell culture

In this study, the human neuroblastoma SH-SY5Y cells used as model neuronal cells were purchased from American Type Tissue Cultures (Manassas, VA). SH-SY5Y cells were cultured in a mixture of Eagle's minimum essential medium (EMEM; Nissui, Japan) and F-12 Nutrient medium (GIBCO BRL®, Life Technologies, Grand Island, NY, USA) (1:1; v/v) containing 10% fetal bovine serum (CELLect® Gold; ICN Biomedicals, Inc., Aurora, Ohio, USA) on collagen type I-coated well plates or dishes to about confluency. The passage number of SH-SY5Y cells used in this study was between 32 and 38 and the culturing days between their passages were more than 3 weeks to avoid its transformation. In uptake experiments, SH-SY5Y cells were cultured for 6–10 days after their passages. Before use for uptake experiments and RNA extraction, evaluation of protein expression, and assays for the viability and death of cells, SH-SY5Y cells were treated with 10 μM all-*trans* retinoic acid for 3 days to allow differentiation into neuronal cells (Ross et al., 1983). The [^{3}H]water space of SH-SY5Y cells was determined using [^{3}H]H_2O, and was estimated to be 1.03 μL/mg protein.

Uptake experiments

Uptake experiments were performed by the method reported previously (Nagasawa et al., 2005). Firstly, cells cultured in 6-well plates were washed three times with PBS warmed to 37 °C, and then preincubated for 10 min with HEPES-HBSS at the designated temperature. As for experiments on Na^+-gradient, cells were preincubated with 10 mM AZ plus 10 mM DOG in a choline-replaced, glucose-free transport buffer for 20 min, and then used for uptake experiments. The uptake reaction was initiated by the addition of the designated concentration of Al citrate, with or without an inhibitor, to the preincubated cells. After appropriate time intervals, the reaction was terminated by the addition of excess ice-cold PBS, and then the cells were washed twice with ice-cold PBS and stored at −80 °C until the assay.

Determination of Al concentrations

As reported previously (Nagasawa et al., 2005), to 0.8 mL samples, 40 μL of 69% nitric acid and 200 μL of a 2 μg/mL 1% nitric acid gallium solution, as an internal standard for assaying, were added. After vigorous mixing and centrifugation at 11,700×*g* for 5 min at 25 °C, 1 mL of the supernatant was taken, mixed with 3 mL of 0.55% nitric acid, and then used for measurement of the Al concentration in cells with an inductively coupled plasma mass spectrometer (ICP-MS; ICPM-8500, Shimadzu, Kyoto, Japan). Standard Al samples were prepared by dilution of the multielement standard solution. The recovery rate and coefficient of variation of the reproducibility were approximately 100% and 6.2%, respectively, and the standard curves for the assay showed excellent linearity (r^2>0.999). The protein concentration in each well was determined by the method reported by Bradford (1976) with bovine serum albumin as a standard, and was used to correct the amount of Al taken up in each well.

Cell treatment

Cells were treated with 50 or 500 μM of Al citrate, which were basically based upon the report of Matsuzaki et al. (2004), for a day or 2 weeks in the culture medium, or with 100 or 200 μM DEM in a 0.1% DMSO solution for a day. To assay the vulnerability of cells to oxidative stress, cells were subjected to glucose deprivation/reperfusion using a balanced salt solution (BSS(+)) (3.1 mM KCl, 134 mM NaCl, 1.2 mM $CaCl_2$, 1.2 mM $MgSO_4$, 0.25 mM KH_2PO_4, 15.7 mM $NaHCO_3$, 5 mM PIPES, pH 7.2) or glucose-free BSS containing 10 mM DOG (BSS(−), pH 7.2). Glucose deprivation was initiated by two washes in BSS(+) or BSS(−), and then the cells were incubated in the respective BSS for 4 h under a 5% CO_2 atmosphere. Thereafter, the cells were cultured in the culture medium for a day, meaning glucose reperfusion (Suh et al., 2003).