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Toxicology

Effect of silicon-rich water intake on the systemic and peritoneal inflammation of rats with chronic low levels of aluminum ingestion

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ABSTRACT

Background and Objectives: Study evaluated effect of silicon-rich water intake on systemic inflammation and functional characteristics of peritoneal macrophages (PMs) of rats that were chronically exposed to dietary aluminum.

Methods: One month-old female Wistar Albino rats were administered aluminum chloride dissolved in distilled water (1.6 mg/kg body weight in 0.5 mL) by gavage for 90 days. The rats were then given standard (6 mg/L) or silicon-rich water (19 mg/L silicon) (n = 7/group). Control rats underwent sham gavage and received standard or silicon-rich water (n = 7/group). Blood was assessed for cytokine levels. Unstimulated and lipopolysaccharide (LPS)-stimulated PMs were assessed in terms of phagocytic activity and cytokine secretion in vitro.

Results: Chronic exposition to dietary aluminum and silicon-rich drinking water did not change serum TNF-α levels. Aluminum increased serum IL-2 and this was reversed by silicon-rich water. The aluminum-exposed rats had higher serum sICAM-1 than sham-gavaged, unrelated to type of water. LPS-stimulated PMs from aluminum-intoxicated animals exhibited low phagocytic activity and release of TNF-α, this was significantly improved by silicon-rich water intake. In the presence of silicon-rich water, LPS-stimulated and unstimulated PMs from aluminum-exposed rats produced significantly more IL-10.

Conclusions: Chronic ingestion of aluminum, increases systemic and peritoneal inflammation and PM dysfunction. The presence of high levels of the natural aluminum antagonist silicon in the drinking water restored IL-10 and TNF-α PM secretion, preventing prolonged inflammation. Thus, silicon intake can decrease the immunotoxicity of aluminum.

1. Introduction

Modern way of life leads to significant increasing of the aluminum levels in humans [1]. One of the potential sources of aluminum is processed food because aluminum and its compounds are used as food additives as well as in the processing, packaging, and storage of food products [2,3]. However, aluminum can also readily introduced into the body *via* water, air, or the skin because it is used in water purification [4], in medicines such as antacids [5] and buffered aspirin, and in antiperspirants [6] and various cosmetics products [7].

In 2011, the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA) increased the provisional tolerable weekly intake (PTWI) of aluminum to 2 mg/kg body weight [8]. Studies show that in many countries, daily aluminum intake is increasing, and exceed the PTWI, especially in the young [3,9].

Aluminum was considered to be a neutral element in terms of its health impact. Attention was first drawn to the potential role of aluminum as a toxic metal over 50 years ago [10]. There is increasing evidence showing that aluminum can accumulate in the body over the course of life and that it is stored predominantly in the lungs, bones, liver, kidneys, and brain. These accumulations are toxic to the local tissues; consequently, inducing a number of neurological, skeletal, hematopoietic, and immunological disorders and disease states [11].

It has been proposed that aluminum accumulation is cytotoxic and

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immunotoxic because it increases oxidative stress, lipid peroxidation, intracellular glutathione reduction, oxidative DNA damage and alter gene and inflammatory signaling [12–14]. Chronic exposure to low levels of aluminum in the drinking water can trigger various inflammatory processes [15]. Moreover, the ingestion of aluminum salts can lead to intracellular accumulation of the metal in the brain, prolonged neuroinflammation and the progression of neurodegenerative diseases [16]. Finally, if the accumulation of ingested aluminum in the skeleton is maintained for a long period of time, it may provoke osteoporosis due to inappropriate osteal macrophage activity [17,18].

Silicon is the second most abundant elements of the Earth's crust after oxygen, and exist in various forms of silicon dioxide. It is commonly found in nature and in the cell walls of diatoms. Silicic acid, $Si(OH)_4$, is water soluble form of silicon and its only biologically available form [19]. Silicic acid is a natural antagonist of aluminum and it could prevent the well-established neurotoxic and immunotoxic effects of aluminum by decreasing its bioavailability [20,21]. Indeed, a French cohort study showed that when the silicic acid concentrations in drinking water are low, aluminum ingestion increases the risk of cognitive impairment [22]. Surprisingly, the bioavailability of silicon has an exponential inverse relationship with the silicon content in all foods except various silicon-containing drinks [23]. Thus, silicon-rich water is an important source of natural and bioavailable silicic acids and could be used to reduce the adverse effects of the high aluminum intake by forming hydroxyaluminosilicates [19,24].

Therefore, our study aimed to evaluate the effect of silicon-rich water intake on the systemic inflammation of rats that were chronically exposed to dietary aluminum. The effect of silicon-rich water intake on the phagocytosis and cytokine production of peritoneal macrophages (PMs) from these rats was also assessed.

2. Methodology

2.1. Animal handling

One-month-old female Wistar Albino rats ($n = 28$) were bred at the Vivarium of the Institute of Biomedical Research, Medical faculty, Nis, under conventional laboratory conditions. The rats were handled in accordance with EU guidelines for the accommodation and care of animals used for experimental and other scientific purposes (2010/63/EU). Before initiating the experiment, the rats were housed collectively in standard laboratory cages [seven animals in each 90 × 120 × 30 cm (W × L × H) cage] for 2 weeks. The housing room was maintained at 24 °C with 42 ± 5% relative humidity and had a 12–12 h light-dark cycle (light on between 06:00 and 18:00 h). Food (standard laboratory chow with 470 μg/kg of aluminum) and tap water with aluminum concentration (37.5 μg/L) were available ad libitum. Aluminum was determined by ICP-OES (iCAP 6500 Duo, Thermo Scientific, United Kingdom). All animal experiments were approved by the Animal Ethics Board of the Medical Faculty in Nis (No: 323-07-06862/2016-05/8) and were performed according to board guidelines. The general health of the rats was monitored daily.

2.2. Experimental design

A chronic aluminum ingestion model with four treatment arms was employed. Thus, to generate aluminum-intoxicated rats, the rats were administered aluminum chloride ($AlCl_3$) in the form of solution in distilled water on a daily basis for 90 days. Every day, each rat received volume of 0.5 mL of fresly prepared aluminum solution by oral gavage with a ball-tip needle, which correspond to dose of 1.6 mg/kg bw of rats. To prepare the daily solution, the average rat body weight was determined by weighing three rats from each group every week. Appropriate solution of aluminum chloride-6-hydrate ($AlCl_3 \times 6H_2O$, Mr 241.43 pro analysis, Centrohem) was then prepared. The amount of $AlCl_3$ to be administered was established on the basis of the reported aluminum oral uptake in EU countries: these values range from 0.06 mg/day to as high as 3500–5200 mg/day. The very high levels are the result of consuming aluminum-containing antacids [25].

The 28 study rats were divided into four groups of seven animals. The control rats only received standard tap water (SW group; 6 mg/L of silicon determined by ICP-OES (iCAP 6500 Duo, Thermo Scientific, United Kingdom) or experimental water (EW group; 19 mg/L of silicon) *ad libitum*. The experimental water was made by adding solution of sodium-silicate (sodium silicate, Si 1.00 g/L, Merck, Germany) in standard tap water. The Al + SW and Al + EW rats were chronically intoxicated with soluble aluminum chloride by gavage, while drinking *ad libitum* either the standard or the experimental water respectively. The control rats (SW and EW) all underwent sham gavage by 0.5 mL of corresponding water every day. After 90 days of treatment, the animals were anesthetized by intraperitoneally injecting Ketamin HCl (50 mg/kg) and were then sacrificed. During the course of the study, none of the animals were subjected to procedures that caused pain or discomfort.

2.3. Isolation of peritoneal cells

Peritoneal cells were obtained as previously described [26]. In brief, cells were harvested from the rats by peritoneal lavage with 20 mL of ice cold phosphate-buffered saline/tetrasodium-ethylenediaminetetraacetate (PBS/NaEDTA). The harvested cells were then purified by using 40% OptiPrep gradient. The purity of the peritoneal macrophage population obtained by this method was 85%, as determined by staining with FITC-conjugated anti-CD68 antibodies (AbD Serotec, Oxford, UK). The purified cells were then seeded in 96-well plates (10^5 cells per well) and cultured in standard conditions (5% CO_2, 37 °C). In parallel, the purified cells were stimulated with lipopolysaccharide (LPS) (10 μg/mL). The cytokine release of the PMs were measured after 24 h and phagocytic activity after 72 h cultivation *in vitro*.

2.4. Detection of cytokines in the serum and cell culture medium

Blood samples were drawn by cardiac puncture from the animals in terminal anesthesia and were placed in vials with EDTA. The serum was separated and subjected to ELISA. The following serum cytokines were measured: tumor necrosis factor alpha (TNF-α), interleukins (IL)-2 and −10, soluble intercellular adhesion molecule-1(sICAM-1), and soluble vascular cell adhesion molecule-1 (sVCAM-1). All ELISAs were performed according to the instructions of the manufacturer (R&D Systems, Minneapolis, USA). The sensitivity of the TNF-α (Cat. No. RTA00), IL-2 (Cat. No. R2000), IL-10 (Cat. No. R1000), sICAM-1/CD54 (Cat. No. RIC100), and sVCAM-1/CD106 (Cat. No. DVC00) ELISAs was up to 5 pg/mL, up to 15 pg/mL, up to 10 pg/mL, 1.2–4.1 pg/mL (assay range = 31.2–2.000 pg/mL), and 0.17–1.26 ng/mL (assay range = 6.3–200 ng/mL), respectively. The amount of TNF-α, IL-10, and sICAM-1 that was released by the unstimulated or LPS-stimulated PMs was determined by subjecting the cell supernatants after 24 h cultivation in vitro, to the same ELISAs described above.

2.5. Phagocytosis assay

The phagocytic ability of the cultured PMs was assessed by measuring Natural Red uptake according to the technique described by Chen et al. [27]. Thus, the PMs were placed in a 96-well plate and cultured at 37 °C, 5% CO_2 for 72 h, after which 50 μL of Neutral Red was added to each well (dilution = 1:300). After incubating the plates for 4 h, the supernatants were discarded and the cells were washed three times with PBS (pH 7.2–7.4). The washed macrophages were then resuspended in 100 μL/well of cell lysis solution (ethanol and 1% acetic acid at a ratio of 1:1) and cultured for 2 h. The phagocytic activity of the cells was determined by measuring the absorbance at 540/650 nm by using an ELISA reader (Thermo Labsystems, Multiscan Ascent, Canada). All determinations were conducted in quadruplicate.