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 lipopoly-saccharide (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 $90 \times 120 \times 30$ 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 freshly 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.

Table 1
Average body weight of the rats at the end of the 90-day study period.

	N	Mean	SD	SE	95% CI for mean
EW	7	295.0	20.7	8.4	273.2–316.7
Al + EW	7	270.0	29.6	12.1	238.8–301.1
SW	7	300.0	6.3	2.5	293.3–306.6
Al + SW	7	278.3	29.9	12.2	246.9–309.7

Al + SW = aluminum-intoxicated with standard water supply; Al + EW = aluminum-intoxicated with silicon-rich water supply; SW = sham-gavaged rats with standard water supply; EW = sham-gavaged rats with silicon-rich water supply; SD = standard deviation; SE = standard error; CI = confidence intervals. Student's *t*-test shows the four groups did not differ in terms of body weight.

2.6. Statistical analysis

All data were presented as means $\pm$ SD or means $\pm$ SE of means. The Al + SW, Al + EW, SW and EW animal groups were compared in terms of 90-day body weights, serum cytokine levels, and PM cytokine production and phagocytosis by using the Student's *t*-test or ANOVA with post hock analysis. P values of < 0.05 were considered to indicate statistical significance. The data were analyzed by using a commercially available statistics software package (SPSS 16.0® for Windows, Chicago, USA).

3. Results

3.1. Effect of chronic aluminum exposure and silicon intake on body weight

The average weights of the Al + SW, Al + EW, SW, and EW rats at the end of the 90-day study period are shown in Table 1. Average weight of rats was from 270 to 300 g, and examined groups did not differ significantly in terms of body weight.

3.2. Effect of aluminum exposure and silicon intake on serum cytokines

Fig. 1 shows the serum TNF-α levels of the rats at the end of the 90-day study period. The difference in serum TNF-α levels (mean $\pm$ SE) was not observed between aluminum-intoxicated and sham gavaged rats, with high silicon (5.1 $\pm$ 1.6 vs. 9.3 $\pm$ 2.8 pg/mL) and low silicon (6.4 $\pm$ 1.1 vs. 6.7 $\pm$ 1.7 pg/mL) content in the drinking water. Thus, aluminum and silicon-rich water intake did not significantly change the systemic TNF-α levels.

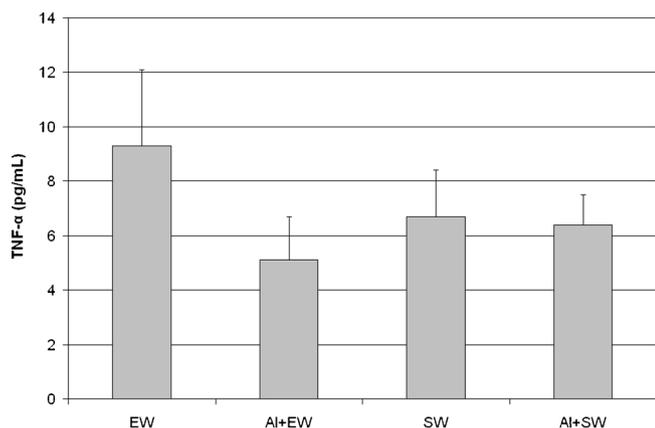Fig. 2 shows the serum levels of IL-2 in the rats. The Al + SW rats

Fig. 2. Concentration of IL-2 in serum of the rats at the end of the study period. Al + SW = aluminum-intoxicated with standard water supply; Al + EW = aluminum-intoxicated with silicon-rich water supply; SW = sham-gavaged rats with standard water supply; EW = sham-gavaged rats with silicon-rich water supply. The data are expressed as mean $\pm$ standard error (n = 7). The Al + SW rats had significantly higher TNF-α levels than the SW controls. Al + SW rats also had higher IL-2 levels than the Al + EW rats. There was no difference between the Al + EW and EW and the EW and SW rats (*P < 0.05 vs. SW and Al + EW) as determined by Student's *t*-test.

had significantly higher systemic IL-2 levels than the SW controls (50.3 $\pm$ 12.3 vs. 21.3 $\pm$ 1.8 pg/mL, p < 0.05) and AL + EW (50.3 $\pm$ 21.3 vs. 16.8 $\pm$ 2.8 pg/mL, p < 0.05). This effect of aluminum was not observed in the Al + EW rats when compared to the EW controls (16.8 $\pm$ 2.8 vs. 16.7 $\pm$ 6.1). Thus, the silicon-rich water supply suppressed the ability of chronic aluminum ingestion to increase serum IL-2 levels.

The serum concentration of IL-10 was mostly undetectable in all experimental groups. Only sham gavaged rats with the standard water supply had measurable average level of IL-10 (10.9 $\pm$ 3.55 pg/mL) (data not shown).

Figs. 3 and 4 show the serum levels of sICAM-1 and sVCAM-1 in the rats, respectively. Chronic aluminum ingestion significantly increased the sICAM-1 levels in rats with silicon-rich water (748.9 $\pm$ 75.9 vs. 512.7 $\pm$ 15.7 pg/mL, p < 0.05) as well as in rats with low-silicon water (584.5 $\pm$ 74.8 vs. 444.6 $\pm$ 108.4 pg/mL, p < 0.05).

However, the aluminum intoxicated Al + EW and Al + SW rats had similar sVCAM-1 levels as their control groups (109.9 $\pm$ 7.9 vs. 114.9 $\pm$ 5.9 ng/mL, NS) and (114.4 $\pm$ 8.2 vs. 112.4 $\pm$ 9.7 ng/mL,

Fig. 1. Concentration of TNF-α in the serum of the rats at the end of the study period. Al + SW = aluminum-intoxicated with standard water; Al + EW = aluminum-intoxicated with silicon-rich water; SW = sham-gavaged rats with standard water; EW = sham-gavaged rats with silicon-rich water. The data are expressed as mean $\pm$ standard error (n = 7). Significant differences in TNF-α levels between the Al + EW, Al + SW, EW and SW rats were not observed, as determined by Student's *t*-test.
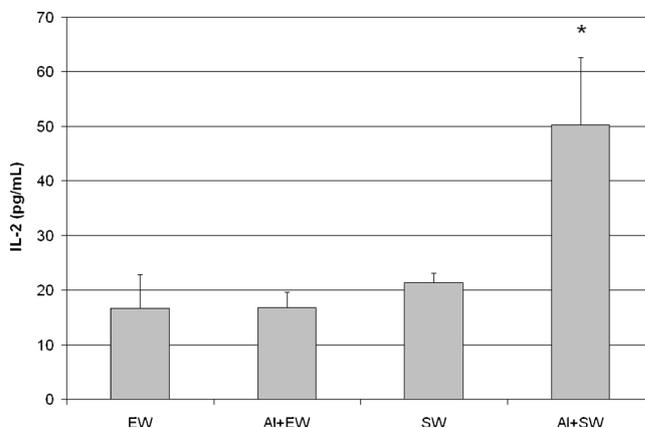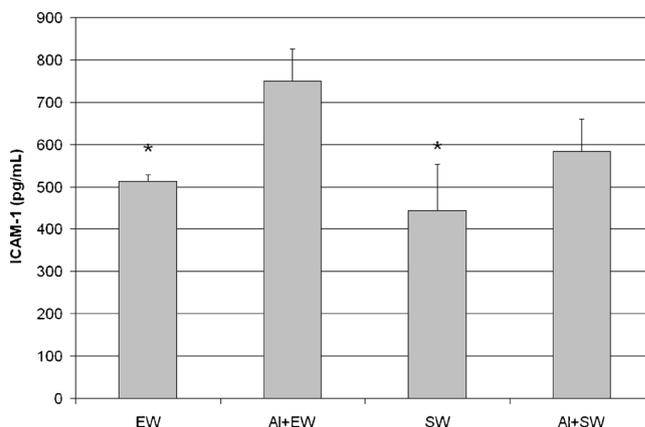
Fig. 3. Concentration of sICAM-1 in the serum of the rats at the end of the study period. Al + SW = aluminum-intoxicated with standard water supply; Al + EW = aluminum-intoxicated with silicon-rich water supply; SW = sham-gavaged rats with standard water supply; EW = sham-gavaged rats with silicon-rich water supply. The data are expressed as mean $\pm$ standard error (n = 7). The Al + EW and Al + SW rats had significantly higher sICAM-1 levels than their control groups (*P < 0.05 vs. EW and SW), as determined by Student's *t*-test.
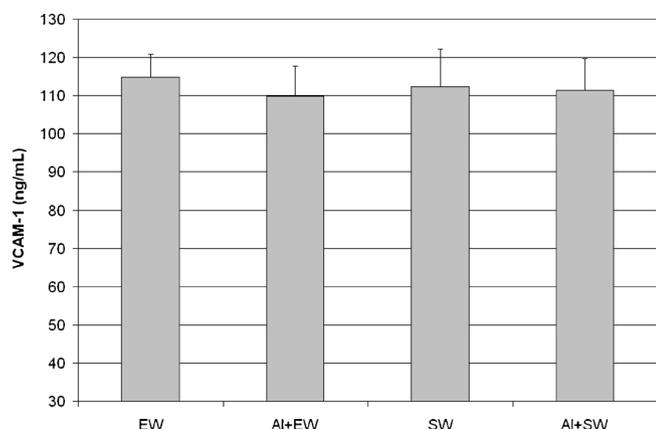
Fig. 4. Concentration of sVCAM-1 in serum of the rats at the end of the study period. Al + SW = aluminum-intoxicated with standard water supply; Al + EW = aluminum-intoxicated with silicon-rich water supply; SW = sham-gavaged rats with standard water supply; EW = sham-gavaged rats with silicon-rich water supply. The data are expressed as mean ± standard error (n = 7). The four groups did not differ in terms of sVCAM-1 levels, as determined by Student's *t*-test.

NS), assuming neither chronic exposure to aluminum nor the water supply influenced serum sVCAM-1 concentrations.

3.3. Effect of aluminum exposure and silicon intake on PM function

The phagocytic activity of the PMs from the four rat groups is shown in Fig. 5. Chronic exposure to aluminum significantly decreased PM phagocytic activity in non-stimulated and LPS-stimulated phagocytes. The non-stimulated Al + EW (0.07 ± 0.006 vs. 0.23 ± 0.01, p < 0.05) and LPS-stimulated Al + EW (0.12 ± 0.009 vs. 0.25 ± 0.01, p < 0.05) exhibited much less phagocytosis of Natural Red particles than their controls. Thus, aluminum exposure markedly downregulated macrophage phagocytosis. Moreover, the non-stimulated and LPS-stimulated PM from Al + SW rats had significantly less phagocytic activity than the Al + EW rats (0.04 ± 0.003 vs. 0.07 ± 0.006, for non-stimulated, p < 0.05) and (0.05 ± 0.008 vs. 0.12 ± 0.009, for LPS-stimulated, p < 0.05), which suggests that a silicon-rich water supply somewhat protected the phagocytic capability
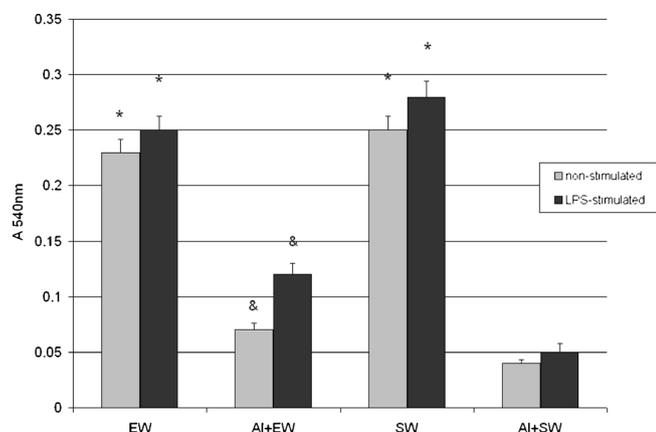

Fig. 5. Phagocytic activity of PMs from the four rat groups, as measured by Neutral Red uptake.
Al + SW = aluminum-intoxicated with standard water supply; Al + EW = aluminum-intoxicated with silicon-rich water supply; SW = sham-gavaged rats with standard water supply; EW = sham-gavaged rats with silicon-rich water supply. The data are expressed as mean ± standard error (n = 7). The PMs from Al + EW and Al + SW rats were less phagocytic than the PMs from their controls (*P < 0.05 vs. Al + EW and Al + SW, as determined by Student's *t*-test. In addition, the PMs from the Al + SW rats were less phagocytic than the PMs from the Al + EW rats (&P < 0.05 vs. Al + SW), as determined by Student's *t*-test.
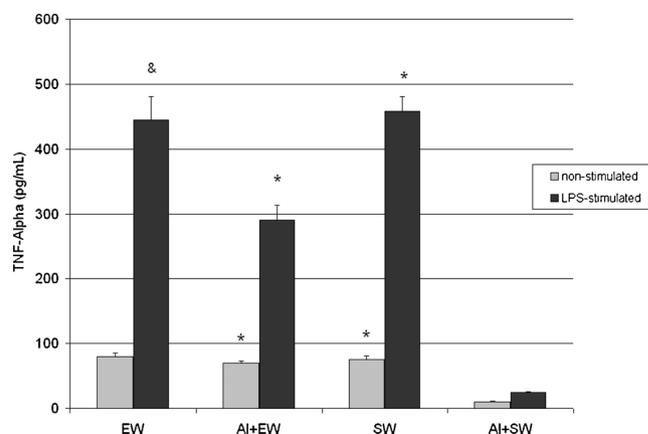

Fig. 6. Production of TNF-α by PMs from the four rat groups.
Al + SW = aluminum-intoxicated with standard water supply; Al + EW = aluminum-intoxicated with silicon-rich water supply; SW = sham-gavaged rats with standard water supply; EW = sham-gavaged rats with silicon-rich water supply. The data are expressed as mean ± standard error (n = 7). The LPS-stimulated and non-stimulated Al + SW PMs produced less TNF-α than the SW control and Al + EW PMs (*P < 0.05 vs. AL + SW). The LPS-stimulated Al + EW PMs produced less TNF-α than the EW PMs (&p < 0.05 vs. Al + EW). The unstimulated Al + EW PMs produced similar TNF-α as the EW control. P values were determined by Student's *t*-test.

of the macrophages in aluminum-exposed rats. These differences were all observed regardless of whether or not the macrophages were stimulated with LPS.

The unstimulated and LPS-stimulated PMs from the Al + SW rats released significantly less TNF-α than the SW PMs (10.0 ± 0.8 vs. 75.1 ± 6.0 pg/mL, for non-stimulated, p < 0.05) and (25.0 ± 1.25 vs. 458.0 ± 22.9 pg/mL, for LPS-stimulated, p < 0.05). The unstimulated PMs from Al + EW rats produced similar TNF-α as the EW control (70.0 ± 3.5 vs. 80.0 ± 6.0, pg/mL, NS), but LPS-stimulated Al + EW PMs produced less TNF-α than the EW PMs (290.0 ± 23.2 vs. 445.0 ± 35.6 pg/mL, p < 0.05). Moreover, the unstimulated and LPS-stimulated PMs from the aluminum intoxicated rats with low-silicon water (Al + SW, 10.0 ± 0.8 and 25.0 ± 1.25 pg/mL) produced significantly less TNF-α than intoxicated rats with high-silicon water (Al + EW) (70.0 ± 3.5 and 290.0 ± 23.2 pg/mL) (p < 0.05). Hence, suppressive effect of aluminum exposure on PM TNF-α production was partially (in the LPS-stimulated PMs) or wholly (in the unstimulated PMs) ameliorated by silicon-rich water intake (Fig. 6).

In the presence of silicon-rich water, both unstimulated and LPS-stimulated PMs from aluminum-exposed rats produced significantly more IL-10 than the sham-gavaged control rats (5.1 ± 0.28 vs. 1.21 ± 0.06 pg/mL, for non-stimulated, p < 0.05) and (28.4 ± 1.38 vs. 10.0 ± 0.4 pg/mL, for LPS-stimulated, p < 0.05). This was not observed in the aluminum-exposed rats that received standard water (1.15 ± 0.08 vs. 1.34 ± 0.07 pg/mL, for non-stimulated, NS) and (22.1 ± 1.2 vs. 30.2 ± 1.7 pg/mL, for LPS-stimulated, NS). Silicon-rich water intake also significantly decreased LPS-stimulated PMs production of IL-10 in the sham-gavage control (EW) compared to aluminum intoxicated (Al + EW) (10.0 ± 0.4 vs. 28.4 ± 1.38 pg/mL, p < 0.05) and low silicon water control (SW) (10.0 ± 0.4 vs. 30.2 ± 1.7 pg/mL, p < 0.05) (Fig. 7).

4. Discussion

The silica content in tap waters commonly ranges from 4.2 to 22.4 mg/L [22]. Accordingly, in the present study, we used waters containing high and low silicon levels.

In our study, we exposed rats to a maximum dose of 1.6 mg dissolved aluminum per kg of body weight per day. These levels are similar to the aluminum exposure levels of approximately 5% of Americans [28] and European high aluminum food consumers [29,30].
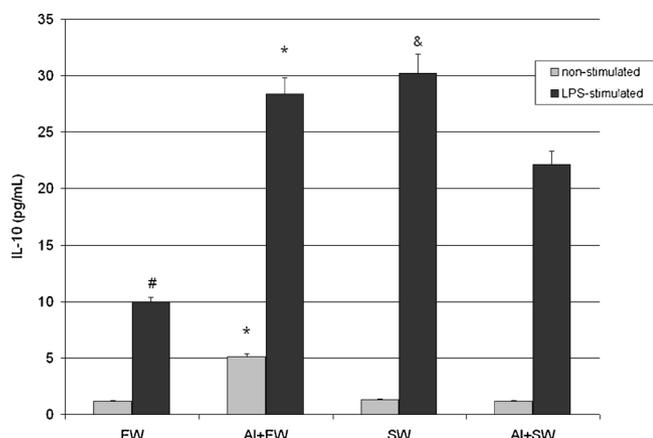
Fig. 7. Production of IL-10 by PMs from the four rat groups.
Al + SW = aluminum-intoxicated with standard water supply; Al + EW = aluminum-intoxicated with silicon-rich water supply; SW = sham-gavaged rats with standard water supply; EW = sham-gavaged rats with silicon-rich water supply. The data are expressed as mean ± standard error (n = 7). The unstimulated and LPS-stimulated Al + EW PMs produced more IL-10 than the EW control and Al + SW PMs (*P < 0.05 vs. Al + SW and EW). The LPS-stimulated EW PMs produced less IL-10 than the SW PMs (#P < 0.05 vs. SW). The LPS-stimulated SW PMs produced more IL-10 than the Al + SW PMs (&P < 0.05 vs. Al + SW).

The Al levels used in the study is in range as used in the other experimental models of aluminum exposure at human dietary levels [31]. The aluminum dose to which we exposed our rats is also much lower than the doses needed to generate adverse neurological effects in rats and mice (100–200 mg aluminum/kg/day) [32]. At even higher concentrations, aluminum disturbs bone and liver development of rodents and reduces their body weight [25]. We found that 3 months of exposure to the dietary levels of aluminum used in our study did not significantly influence the body weight of the rats. This indicates that this dose had low overt toxicity.

It has been suggested that inflammatory bowel disease (IBD) associates with high aluminum intake [33]. Orally administered aluminum at the daily dose we used (*i.e.* 1.5 mg/kg body weight daily) worsens intestinal inflammation and decreases the epithelial cell renewal; particularly in IL-10-deficient mice [34]. Indeed, several other lines of evidence suggest that IL-10 plays an important role in the pathogenesis of IBD. First, IL-10-deficient mice develop colitis and increased numbers of *Clostridium* species adhere to their colonic mucosa [35]. Moreover, *Lactobacillus* may ameliorate colitis by upregulating the expression of IL-10 and tight junction proteins [36]. Our study also showed that when aluminum-exposed rats were given silicon-rich water, their unstimulated and LPS-stimulated PMs produced significantly more IL-10 than the control PMs; this difference relative to the control PMs was not observed in the PMs from aluminum-exposed rats given standard water. Since PMs are a phagocyte population that binds to the intestine, our results suggest silicon-rich water could be useful for treating, and/or preventing, aluminum-induced intestinal inflammation in IBD. Notably, we also found that LPS-stimulated PMs from sham-gavaged rats with standard water produced significantly more IL-10 than the PMs from sham-gavaged rats with silicon-rich water. This suggests that silicon-rich water could help to boost the protective inflammatory intestinal response in presence of bacterial LPS antigen stimulation, knowing that induction of IL-10 can be exploited by certain pathogens to facilitate infection [37].

Aluminum ingestion increases intestinal permeability, which elevates the bacterial load in the peritoneal lymph nodes without changing the intestinal flora [34]. This then activates various inflammatory responses that increase the serum concentration of IL-2, which plays a central role in the development of cell-mediated immunity and increases phagocytic activity [38]. In our study, aluminum uptake increased the serum IL-2 levels. This may reflect the damage to the

intestinal barrier that is inflicted by the ingested aluminum. We also found that silicon-rich water intake completely reversed the aluminum-induced serum IL-2 levels. Silicon (as silicic acid) not only restricts the gastro-intestinal absorption and biological availability of aluminum [39], but it facilitates the removal of aluminum *via* the urine and decreases the body burden of aluminum [24]. Our results indicate that silicon-rich water probably improves systemic inflammation by directly modulating immune cells as well as by decreasing absorption and increasing aluminum removal.

Macrophages and T lymphocytes secrete IL-2 and TNF-α to promote the production of T and B lymphocytes and to regulate immune functions. Several studies have assessed the effects of aluminum on these cytokines. First, when human splenic T lymphocytes are treated with high aluminum content *in vitro* for 48 h, their IL-2 and TNF-α production drops [40]. Second, short-term exposure of mice to low levels of aluminum does not change TNF-α level [41]. However, when rats chronically ingest much higher levels of aluminum, the aluminum significantly reduces the production of IL-2 and TNF-α by the spleen [42]. By contrast, we found that rats that were chronically exposed to a low aluminum dose had increased serum IL-2 and unchanged serum TNF-α levels. Silicon-rich water did not change the serum TNF-α level, while reversing the aluminum-induced increase in serum IL-2 levels.

We assessed the TNF-α cytokine production of the PMs from our rats to characterize the inflammatory response and activation of macrophages, in the peritoneal compartment. We found that PMs from rats that were chronically exposed to aluminum produced little TNF-α, regardless whether the PMs were unstimulated or stimulated with LPS. Zhuang et al. reported that a similar aluminum exposure protocol decreased the serum TNF-α levels and the mRNA expression of TNF-α in the PMs [43]. Similarly, our results show that chronic ingestion of a low aluminum dose did not influence serum TNF-α levels but did significantly reduce its production by PMs. This indicates that rats exposed to aluminum exhibit a high level of inflammation and inhibition of cellular immunity in the peritoneal compartment; moreover, the phagocytic activity of the PMs was strongly suppressed. In line with this, when monocyte-derived cells are recruited to the gut, they promote the development of colitis by expressing TNFα [44].

An important finding of our study is that PMs from aluminum-exposed rats treated with silicon-rich water, reversed the aluminum-induced reduction of TNF-α production. Thus, silicon-rich water can prevent aluminum-induced systemic inflammation and peritoneal immunosupression. This may reflect its ability to shape monocyte plasticity, thereby altering the balance between their proinflammatory and antiinflammatory properties.

Chronic exposure to aluminum inhibits PM function by inhibiting adhesion, chemotaxis, and phagocytic activity [45]. Similarly, we found that chronic exposure to aluminum significantly decreased PM phagocytic activity. On the other hand, when these aluminum-intoxicated animals were treated with silicon-rich water, the phagocytic activity and cytokine production of the PMs were significantly upregulated. It can be assumed that decreased PM phagocytosis could favor the intestinal inflammation in persons with chronic aluminum ingestion.

While little is known about the effect of ingested aluminum on endothelial function, it has been shown that aluminum can elicit a proinflammatory response and affect endothelial adhesive properties by *in vitro* increasing expression of VCAM-1 and ICAM-1 [46]. Indeed, we found that chronic aluminum ingestion significantly increased serum sICAM-1 levels, and that the water used did not change this. Chronic exposure to aluminum did not significantly affect serum sVCAM-1 levels. Notably, apart from its function as adhesive molecules, ICAM-1 also has immunoreactive properties and increases non-major histocompatibility complex-restricted T cytotoxicity [47].

The exposure of human endothelial cells to high silicon concentration enhances expression of adhesion molecules *in vitro* [48]. However, lower concentration of silicon in the drinking water that we used

(19 mg/L) seems do not affect endothelial function.

Thus, our data and those of others suggest that excess aluminum intake alters immune responses and this may be influenced by the accumulation of aluminum in different organs and tissues. When the aluminum ingestion is chronic, the gut and its associated lymphovascular structures are exposed to aluminum for a prolonged duration and this could initiate systemic and local peritoneal inflammatory responses. Silicon-rich water intake may minimize, reverse, or prevent these effects.

5. Conclusions

In this study, we showed that chronic ingestion of aluminum, which commonly present in water and food, increases systemic and peritoneal inflammation. The presence of high levels of the natural aluminum antagonist silicon in the drinking water significantly modulated the immune responses induced by chronic aluminum intoxication: it restored IL-10 and TNF-α PM secretion, thereby preventing prolonged inflammation. Thus, silicon intake can decrease the immunotoxicity of aluminum.

New and noteworthy

Chronic ingestion of aluminum initiates systemic and local peritoneal inflammatory responses. Silicon-rich water intake reverse the aluminum-induced increase in serum IL-2 levels and silicon intake decrease immunotoxicity of aluminum by restoring phagocytosis and cytokine production of peritoneal macrophages.

Conflict of interest

The authors have declared that no competing interest exists.

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The funding source had no involvement in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

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