

Effects of aluminum exposure on the allergic responses and humoral immune function in rats

Yanzhu Zhu · Jinfeng Xu · Hao Sun ·
Chongwei Hu · Hansong Zhao · Bing Shao ·
Alphajoh A. Bah · Yanfei Li

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Abstract This study was conducted to assess effects of aluminum (Al) exposure on allergic responsive reactions and humoral immune function in rats. Forty male Wistar rats (5 weeks old) weighed 110–120 g were randomly allocated into four groups and were orally exposed to 0, 64.18, 128.36, and 256.72 mg/kg body weight aluminum trichloride in drinking water for 120 days. The levels of immunoglobulin (Ig) G, IgA, IgM, IgE, Complement factor (C)3, and C4 in serum were determined by ELISA and nephelometric assays at the end of experiment. The results showed that the levels of IgM, C3, and C4 were lowered, and the levels of IgG, IgA, and IgE were increased in an Al-dose dependent manner. The increased in IgE level and the decreased in C3 and C4 levels indicate that Al induces allergic responses in rats; while the increased levels in IgG and IgA and the decreased level in IgM suggest that Al disorders the humoral immune function in rats.

Keywords Aluminum exposure · Allergic responses · Humoral immune function · Rats

Introduction

The residual of available aluminum (Al) in biologically ecosystem has been substantially increasing during recent years, so Al contamination becomes an increasing problem in human society (Hewitt et al. 1990; Rengel 2004; Fewtrell et al. 2009). It was reported that Al exposure caused delayed hypersensitivity. Vaccination involving Al-containing adjuvant could trigger the cascade of immunological events which were associated with autoimmune conditions including chronic fatigue syndrome and macrophagic myofasciitis (Exley et al. 2009). Unexpectedly high incidence of persistent itching nodules and delayed hypersensitivity to Al in children were observed after the use of adsorbed vaccines (Bergfors et al. 2003). While contacting allergy to Al in patients hyposensitized with Al-containing hyposensitizing extracts (Hindsén 2005), antacids led to immunological changes in the intestine of BALB/c mice similar to human food allergy (Pali-Schöll et al. 2008).

Type 1 allergic response is mediated by immunoglobulin E (IgE) (Saini and Mac-Glashan 2002), and an increased IgE level induces type 1 allergic response. The IgE level was increased by injected vaccine containing Al to dogs (Tater et al. 2005). When the patients were exposed to Al-precipitated antigen

Y. Zhu · H. Sun · C. Hu · H. Zhao · B. Shao ·
A. A. Bah · Y. Li (✉)
College of Veterinary Medicine, Northeast Agricultural
University, Harbin 150030, China
e-mail: yanfeili200@yahoo.com.cn

J. Xu
College of Animal Sciences and Technology,
Heilongjiang Bayi Agricultural University, Daqing
163319, China

solutions, delayed hypersensitivity was observed (Lopez et al. 1994). However, little data has investigated the effect of Al on the IgE level in patients.

Complement factor 3 (C3) is important in opsonization-phagocytosis of foreign particle, solubilization and elimination of immune complex, generation of inflammatory peptides, and antibody production of B-cell. Complement factor 4 (C4) plays an important role in activation stage of classical pathway (Wouters et al. 2005). Complement activation was observed in IgE mediated response (Gerard and Gerard 2002), so the levels of complement can be used as an index to represent the allergic responses. However, the effect of Al on the complement is controversy (Ramanathan et al. 1979; Arvidsson et al. 2007).

Immunoglobulin G (IgG), IgM, and IgA are the dominant immunoglobulins in the serum, and are important indexes in humoral immune function. They are part of manifestation and material basis of antibody, and are serum globulins which have similar structures, antibody activities, and dissolve pathogen. Some studies have focused on relation between Al and humoral immune function, but it is controversy that Al induces adverse effect on the humoral immune function (Gräske et al. 2000; Synzynys et al. 2004).

Therefore, this experiment examines effects of Al exposure (as aluminum trichloride, AlCl_3) on the allergic responses (measured as IgE, C3 and C4) and humoral immune function (measured as IgG, IgM and IgA) in rats.

Materials and methods

Ethical approval of the experimental protocol

The experiment was carried out in accordance with the *Guiding Principles in the Use of Animals in Toxicology*, which has been adopted by the Chinese Society of Toxicology. The experimental protocol was approved by the Ethics Committee on the Use and Care of Animals, Northeast Agricultural University, China.

Animals and housing conditions

Forty healthy male Wistar rats (5 weeks old) weighed 110–120 g were randomly allocated into control group (GC), low-dose group (GL), medium-dose group (GM), and high-dose group (GH), and then were

orally exposed to 0, 64.18, 128.36, and 256.72 mg/kg body weight AlCl_3 in drinking water for 120 days, respectively. Each group had 10 rats. All the rats were acclimatized for 1 week before the Al exposure.

Rats were housed in the Biomedical Research Center, Northeast Agriculture University. The housing conditions were maintained at temperature of $24 \pm 1^\circ\text{C}$, relative humidity at $55 \pm 5\%$, ventilation frequency at 18 times/h and a 12-h light/dark cycle. The rats were kept in plastic cages (5 rats per cage) with soft chip bedding. The size of all the cages is $470 \times 300 \times 150$ mm, large enough for the growth of five rats. Throughout the experiment, chips were replaced every 3 days and rats were given tap water addition ad libitum. The health status of rats was daily observed, and body weight of the rats was recorded every 10 days.

Determination of experimental parameters

After 120 days, the rats were anesthetized with ether. Then 1.0 ml blood was collected from the caudal vein of each rats, and centrifuged at 2,000 rpm for 10 min to obtain serum. The IgE level in serum was detected via solid phase sandwich Goat anti-Rat ELISA Kit (Adlitteram Diagnostic Laboratories, Inc., USA). Levels of IgM, IgG, IgA, C3, and C4 in serum were determined by nephelometric assays according to Zager et al. (2007).

Statistical analysis

Statistical analyses were done using SPSS 13.0 package programmer (SPSS Inc., Chicago, IL, USA). One-way analysis of variance was used for the determination of measurement differences between Al-treated rats and control rats. The least square means and standard deviation (SD) were presented in this paper. A *P* value of less than 0.05 was considered significant, and a *P* value of less than 0.01 was considered markedly significant.

Results

Changes of body weight

Changes in the body weight of rats are shown in Table 1. The body weight of all rats increased

Table 1 Changes in body weight (g) of rats (Mean $\pm$ SD, $n = 10$ per group)

Time (days)	0	30	60	90	120
GC	117.3 $\pm$ 1.5	265.8 $\pm$ 2.2	331.8 $\pm$ 4.8	367.3 $\pm$ 4.6	408.5 $\pm$ 3.0
GL	118.0 $\pm$ 1.4	259.0 $\pm$ 2.9*	324.0 $\pm$ 2.9*	360.3 $\pm$ 2.8*	402.3 $\pm$ 2.5*
GM	115.8 $\pm$ 2.2	207.0 $\pm$ 5.4**	272.0 $\pm$ 4.7**	323.3 $\pm$ 4.7**	375.2 $\pm$ 2.9**
GH	114.8 $\pm$ 3.5	190.3 $\pm$ 2.5**	245.5 $\pm$ 4.7**	294.3 $\pm$ 3.5**	351.8 $\pm$ 2.4**

GC control group; GL low-dose group; GM medium-dose group; GH high-dose group

* $P < 0.05$, ** $P < 0.01$ versus control group

continuously and consistently throughout the experimental period, while the body weight of Al-treated rats were significantly lower than those of in GC. The body weight of rats in GL was lower ($P < 0.05$), and that in GM and GH was significantly lower than those in GC ($P < 0.01$).

Concentrations of IgG, IgM, IgA, and IgE

As shown in Table 2, Al exposure reduced the concentration of IgM, whereas increased concentrations of IgA, IgE and IgG, in a dose dependent manner. The reductions in IgM and the increases in IgA reached significant levels ($P < 0.05$) in all the Al treatment. The effect on IgG was significant only at GH ($P < 0.01$). Both GM and GH, but not GL, had significant effects on IgE ($P < 0.01$).

Concentrations of C3 and C4

As shown in Table 3, the levels of C3 and C4 decreased in a dose dependent manner. The level of C4 was significant lower than that of control group ($P < 0.05$). And the levels of C3 and C4 were markedly significant in GM and GH compared with GC ($P < 0.01$).

Table 3 Concentrations (mg/ml) of C3 and C4 in serum of rats (Mean $\pm$ SD, $n = 10$ per group)

	C3	C4
GC	0.59 $\pm$ 0.057	0.05 $\pm$ 0.028
GL	0.55 $\pm$ 1.51	0.043 $\pm$ 0.071*
GM	0.51 $\pm$ 1.24**	0.036 $\pm$ 0.014**
GH	0.50 $\pm$ 0.35**	0.033 $\pm$ 0.30**

GC control group; GL low-dose group; GM medium-dose group; GH high-dose group

* $P < 0.05$, ** $P < 0.01$ versus control group

Discussion

Al exposure via drinking water is an important route of exposure. According to Yokel et al. (2008), consumption of antacids can provide up to 5,000,000 μ g Al/day. This experiment was used to offer a service to those people at a risk of oral exposure to Al.

No died rats were discovered in the Al-treated rats during the experimental period. However, Al-treated rats were less active (dull, spirit depression) than those in GC. The reduction of the body weight gain indicates that Al exposure can suppress the growth of rats. And a similar result was found in our previous research (Li et al. 2010).

Table 2 Concentrations (mg/ml) of IgG, IgM, IgA, and IgE in serum of rats (Mean $\pm$ SD, $n = 10$ per group)

	IgG	IgA	IgM	IgE
GC	0.21 $\pm$ 1.19	0.022 $\pm$ 0.25	0.034 $\pm$ 0.25	0.0025 $\pm$ 0.057
GL	0.22 $\pm$ 1.83	0.037 $\pm$ 0.36*	0.023 $\pm$ 0.36*	0.0029 $\pm$ 0.053
GM	0.24 $\pm$ 1.46	0.03 $\pm$ 0.08**	0.022 $\pm$ 0.08**	0.0044 $\pm$ 0.022**
GH	0.31 $\pm$ 0.47**	0.061 $\pm$ 0.30**	0.018 $\pm$ 0.30**	0.0056 $\pm$ 0.031**

GC control group; GL low-dose group; GM medium-dose group; GH high-dose group

* $P < 0.05$, ** $P < 0.01$ versus control group

Although IgE represents only a small fraction of all serum antibodies, it is extremely important in allergic disorders. In this experiment, the increase of IgE was observed, indicating an allergic disorder was triggered by Al exposure. But the allergic responses were not observed in all the Al treated-rats. The level of IgE can be affected by many factors. And IL-4, which is secreted by auxiliary T lymphocyte, is one of affecting factors (Becky-Kelly et al. 2003). IL-4 cooperates with IL-13 to maintain and promote mastocyte hyperplasia, which makes mastocyte produce IgE instead of IgM. An increased level of IL-4 was induced by Al (Ulanova et al. 2001), and IL-4 promotes to produce IgE (Kips et al. 2001; Albers et al. 2003). This may contribute to the decreased IgM level and increased IgE level in this experiment. Type 1 allergic response was mediated by IgE (Saini and Mac-Glashan 2002), so Al can induce type 1 allergic response.

Al can activate complement system (Ramanathan et al. 1979), and circulating immune complex was increased after Al exposure (Zhu et al. 2011). While circulating immune complex activates the complement, immunoglobulins bind to the complement and induce complement cascade reaction. And complement activation is associated with allergic disease (Zhang and Köhl 2010).

Complement receptor type 1 (CR1) is an important complement protein receptor that binds C_{3b} and C_{4b} . CR1 may bind to circulating immune complex with attached complement proteins, and these immune complex are removed from the circulating erythrocytes when the erythrocytes circulate to sites of immune complex degradation. However, the number of erythrocytes decreased in Al-treated rats in our previous research (Zhang et al. 2010). Because CR1 present in the erythrocytes membrane, the number of CR1 decreased in Al-treated rats, and circulating immune complex with attached complement proteins had few chances to bind to CR1. So most complement binds to circulating immune complex, and they are not removed from the circulation. Though, complement was activated constantly, it was occupied by circulating immune complex.

The complement level was also decreased in iron deficiency anemia (Jagadeesan and Reddy 1983). Because Al can decrease level of iron and induce anemia (Zhang et al. 2010), complement level could be decreased through this way.

The complement is helpful to remove antigen–antibody complex. C_{3b} can bind antibody of immune complex, decrease affinity between antibody and antigen, and make them separated. At the same time, interaction of Fc in circulating immune complex was changed in space, reducing formation of new immune complex. However, decreased level of the complements, and increased levels of circulating immune complex could be induced by Al, which led to lots of immune complex deposited in many organs and tissues. Lots of immune complex can cause allergic disease (Geha 1977). So Al can induce allergic responses in Al-treated rats.

After infected with pathogen, IgG plays the dominant role in humoral immune function, and resists variety of bacteria to protect the body from infection. IgM can bind to the complement to dissolve pathogen. IgA exerts antiseptis and antiviral, and also plays barricade in local mucosal immunity of the respiratory passages and the alimentary canal. The Al exposure decreased the level of IgM, and increased the levels of IgA and IgG in this experiment, suggesting that Al disordered humoral immune function.

In conclusion, the increased IgE level and decreased C3 and C4 levels indicate that Al induce allergic responses in rats. However, more work is needed to investigate the mechanism of the allergic responses induced by Al. While the increased levels of IgG and IgA, and the decreased level of IgM suggest that Al disorder the humoral immune function in Al-treated rats.

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Erratum to: Effects of aluminum exposure on the allergic responses and humoral immune function in rats

Yanzhu Zhu · Jinfeng Xu · Hao Sun ·
Chongwei Hu · Hansong Zhao · Bing Shao ·
Alphajoh A. Bah · Yanfei Li

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Regrettably, errors were introduced in Tables 2 and 3 of the above mentioned publication. The values of the

standard deviation (SD) were not properly represented. The correct representation of Tables 2 and 3 is published below and should be treated as definitive by the reader.

Table 2 Concentrations (mg/ml) of IgG, IgM, IgA, and IgE in serum of rats (mean $\pm$ SD, $n = 10$ per group)

	IgG	IgA	IgM	IgE
GC	0.21 $\pm$ 0.0119	0.022 $\pm$ 0.0025	0.034 $\pm$ 0.0025	0.0025 $\pm$ 0.00057
GL	0.22 $\pm$ 0.0183	0.037 $\pm$ 0.0036*	0.023 $\pm$ 0.0036*	0.0029 $\pm$ 0.00053
GM	0.24 $\pm$ 0.0146	0.03 $\pm$ 0.0008**	0.022 $\pm$ 0.0008**	0.0044 $\pm$ 0.00022**
GH	0.31 $\pm$ 0.0047**	0.061 $\pm$ 0.0030**	0.018 $\pm$ 0.0030**	0.0056 $\pm$ 0.00031**

GC control group; GL low-dose group; GM medium-dose group; GH high-dose group

* $P < 0.0005$, ** $P < 0.0001$ versus control group

The online version of the original article can be found under doi:[10.1007/s10534-011-9443-4](https://doi.org/10.1007/s10534-011-9443-4).

Y. Zhu · H. Sun · C. Hu · H. Zhao · B. Shao ·
A. A. Bah · Y. Li (✉)
College of Veterinary Medicine, Northeast Agricultural
University, Harbin 150030, China
e-mail: yanfeili200@yahoo.com.cn

J. Xu
College of Animal Sciences and Technology,
Heilongjiang Bayi Agricultural University, Daqing
163319, China

Table 3 Concentrations (mg/ml) of C3 and C4 in serum of rats (mean $\pm$ SD, $n = 10$ per group)

	C3	C4
GC	0.59 $\pm$ 0.00057	0.05 $\pm$ 0.00028
GL	0.55 $\pm$ 0.0151	0.043 $\pm$ 0.00071*
GM	0.51 $\pm$ 0.0124**	0.036 $\pm$ 0.00014**
GH	0.50 $\pm$ 0.0035**	0.033 $\pm$ 0.0030**

GC control group; GL low-dose group; GM medium-dose group; GH high-dose group

* $P < 0.0005$, ** $P < 0.0001$ versus control group