ORIGINAL ARTICLE

Role of sodium silicate in induction of scleroderma-related autoantibodies in brown Norway rats through oral and subcutaneous administration

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Abstract Silica hazard is a growing occupational problem and has been reported to be associated with scleroderma via case reports and occupational studies. The aim of this study is to demonstrate whether oral or subcutaneous silicate exposure can induce an autoimmunity and scleroderma susceptibility in immunosensitive rats. Sodium silicate in a dose of 3 mg in 0.2 ml NS was administered through oral and subcutaneous routes to 20 brown Norway rats. Autoantibodies including ANA, anti-RNP, anti-SCL70 and anti-centromere were measured and compared with pre- and post-challenge serum samples. Serum ANA and anti-RNP were high in significant number of rats ($P < 0.05$) of only the subcutaneous silicate group. There is an increase in the number of positive readings of autoantibodies at 14th week in comparison with the number of positive readings of autoantibodies at 7th week but P values were not significant. It may be concluded that silicate might induce autoimmunity and scleroderma and it seems to be that the longer the duration of exposure the greater the risk. This is probably the first experimental animal study demonstrating the induction of scleroderma-related autoantibodies after challenge with silicate.

Keywords Silica · Autoimmunity · Scleroderma · Anti-scl70

Introduction

Scleroderma is a multisystem disease that has been associated with a variety of chemical and environmental agents for nearly a century. The list of chemicals and environmental agents known to be associated with scleroderma has grown considerably. Occupations and industries having the potential for crystalline silica exposure include mining, quarrying tunneling foundry work, glass manufacture, abrasive blasting, ceramic, pottery production, cement and concrete production. These occupations have probably a higher risk of developing scleroderma [1, 2].

There are implants made of silicon for medical purposes such as cosmetic breast implant, cardiac valve replacement and joint implants [3–5]. One of the environmental agents is the crystalline silica such as quartz, which is a ubiquitous mineral dust found worldwide. The National Institute for Occupational Safety and Health (NIOSH) estimates that approximately three million US workers are exposed to this mineral [2].

There are some diseases that may be associated with occupational crystalline silica exposure including autoimmune diseases such as systemic sclerosis, systemic lupus erythematosus and rheumatoid arthritis [6–9]. The exact mechanism by which silica promotes or accelerates the development of autoimmune disease is unknown. In vitro studies have shown that silica can act as an adjuvant stimulating T cell response or as inducer of apoptosis. Silica is also known to cause a relative decrease in the number of regulatory T cells and tend to have cytotoxic effect on macrophage attempting to break down internalized silica particles, which causes a cascade of events, including oxidative stress, induction of proinflammatory cytokines, chronic inflammation and latent immune stimulation [10].

Compared with the number of human studies, there is less experimental research directly examining the mechanisms by which silica may affect autoimmune diseases [11].

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The objective of this study is to investigate whether challenging immunosensitive rats with oral or subcutaneous silicate will induce scleroderma-related autoantibodies or not.

Materials and methods

This study was conducted at the Research Center, College of Medicine, King Khalid University Hospital, King Saud University, Riyadh, Saudi Arabia. Brown Norway (BN) rats were purchased from Charles Rivers Laboratories, Wilmington, USA. They were kept in polycarbonate metrolon plastic cages covered with stainless steel in the animal house in the College of Medicine. They were maintained under 12-h dark–12-h light cycles and were kept under observation for 3 weeks. No evidence of sickness was observed. All rats were 8–11 weeks old at the onset of the experiment.

Treatment

Twenty rats with an average weight of 158 g were randomized into two groups each consisting of ten rats. One group received sodium silicate (NaSiO$_4$) 3 mg (equivalent to 2 mg of silica, only double dosage that was shown to exacerbate development of systemic autoimmunity in mice to ensure safety of our rats) [12]. Each dose was dissolved in sterile 0.9% NaCl solution and given as 0.2 ml subcutaneously (sc) in the dorsal side or orally (PO) once weekly for the first 5 weeks of the experiment. The remaining 10 rats received sodium silicate PO with the same dose, volume, timing and frequency as in the subcutaneous group. Prechallenge serum autoantibody results were compared with post-challenge results.

Blood sampling

Blood samples were obtained from the retroorbital vein plexus and collected in heparinized tubes prior to intervention and at the 7th and 14th weeks post intervention, respectively.

Serological studies

Serum ANA was detected by indirect immunofluorescence (IF) using the Immco Diagnostic, Inc. (60 Pineview Drive, Buffalo, NY 14228, USA) test kit. ANA/HEP-2 test system is a pre-standardized kit designed for the quantitative and semi-qualitative detection of anti-nuclear antibodies in rats. In this test, rat serum samples were initially diluted 1:10 with phosphate buffer saline (PBS) by adding 20 µl of sera to 180 µl PBS as screening dilution. Slides were removed

from storage and allowed to warm to room temperature 20–25°C. Slides containing tissue culture substrates were used as an antigen source. Each well was identified with the appropriate sera and controls (positive and negative). With a suitable dispenser, one drop of approximately 20 µl of diluted test sera and controls was added on the cells in the respective wells and spread over the entire area of the well. Slides were incubated in moist chamber at room temperature 20–25°C for 30 min, and were them removed at one time and gently rinsed with a stream of PBS, avoiding direct stream of PBS into the test wells. The slides were then placed in the staining dish and washed in PBS for 5 min intervals with change of buffer PBS. A magnetic stirrer was used for mixing the setup. Slides were removed from PBS one at a time and blotted (with blotters provided) by wiping the reverse slide with an absorbent wipe. One drop (approximately 20 µl) of conjugate (Rat IgG F(ab)$_2$ (H + L) fragment affinity purified Fluorescein conjugate (AP 136 G Chemicon International Temecula, CA 92590 lot No. 12036741) was added and the slides incubated and washed as previously mentioned. 3–5 drops of mounting media were applied to each slide and cover-slipped. Slides were examined immediately with an appropriate fluorescent microscope. Detection of apple green fluorescent is positive. Results were evaluated against positive and negative controls. Interpretation of the result depends on the pattern observed and any titer above 1:10 was considered significant (1:10 the baseline titer).

Other serum autoantibodies including anti-RNP, anti-SCL70 and anti-centromere were detected by enzyme-linked immunosorbent assay (ELISA) using the Immco Diagnostic, Inc. test kit. ImmuLisa is an in vitro diagnostic ELISA, designed for the detection and quantitation of IgG antibodies to (anti-RNP, anti-SCL70, anti-centromere) in rat serum. Purified specific antigens were bound to the wells of a polystyrene microwell plate under conditions that will preserve the antigen in its nature form. The unreacted sites of the polystyrene were blocked to reduce non-specific binding. Serum samples and test reagents were allowed to come to room temperature (20–25°C) before starting with the test procedure. 100 µl of pre-diluted positive and negative controls and 1:100 serum dilutions (5 µl of rat serum + 500 µl of serum diluents) were added to separate wells. The plates were incubated for 30 min at room temperature. (To allow antibodies to bind to immobilized antigen.) Unbound samples were washed away from microwells using automatic washer, as per manufacturer's instruction. Anti-Rat IgG Alk. Phos. Conjugate (AP 136 A) from Chemicon International Temecula, CA 92590 lot No. 0606033637) was diluted 1:5,000 (as an optimal working dilution), 100 µl was added and incubated for 30 min at room temperature. Microwells were washed as previously mentioned. 100 µl of substrates was added

into each well in the same order and timing as for the conjugates and incubated for 30 min. The reactions were stopped by the addition of stop solution using the same order and timing. Absorbance values were read within 1 h from adding stop solution.

Interpretation of results

In this study, the significant cut-off value for the immunofluorescent (IF) test of serum ANA was taken as 1:10 positive fluorescence [13]. However, significant reading for the ELISA test was taken as an absorbance reading of 0.205 dilution for anti-RNP, 0.296 for anti-SCL70, and 0.120 for anti-centromere [14].

Statistical analysis

Statistical differences between nickel-treated and corresponding control groups were calculated using the Fisher's exact tests. P values of <0.05 were considered significant.

Results

The prechallenge (baseline) results of serum autoantibodies including ANA, anti-RNP, anti-SCL70 and anti-centromere serum autoantibodies are shown in Figs. 1, 2. All titers in both groups were negative except one rat in the subcutaneous group 1/10 (10%) with positive serum ANA titer. These prechallenge results were considered as a control to the post-challenge results.

Figure 1 for the oral silicate group showed only one rat 1/10 (10%) ($P = 0.5$) at 7th week post-silicate challenge and 3/10 rats (30%) ($P = 0.10$) at 14th week with positive titer of serum ANA. Anti-RNP was detectable in one rat 1/10 (10%) ($P = 0.5$) at 7th week and 2/10 rats (20%) ($P = 0.23$) at 14th week. Anti-SCL70 level was significant
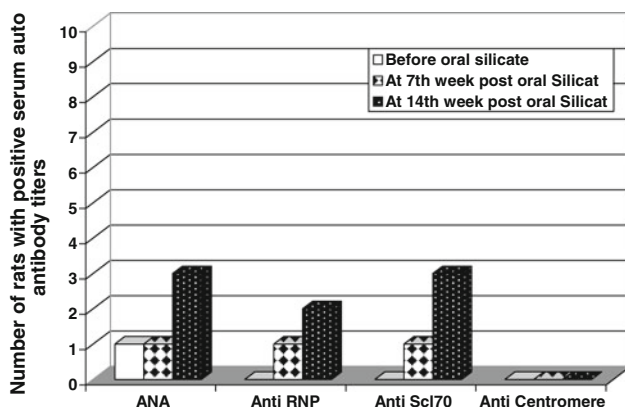

Fig. 1 Number of rats with positive serum auto antibody titers prior and post oral silicat challenges
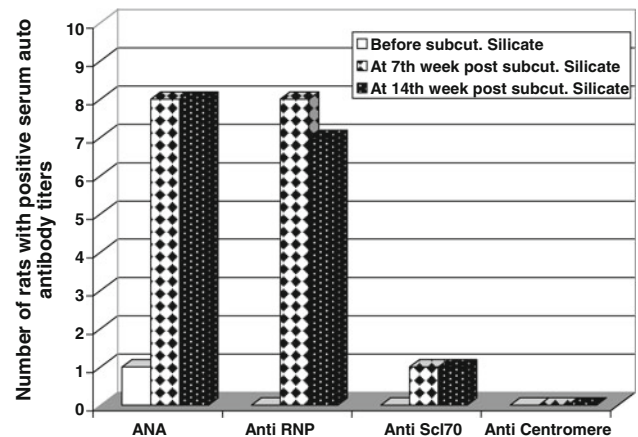

Fig. 2 Number of rats with positive serum auto antibody titers prior and post subcut silicat challenges

in only one rat at 7th week and in 3/10 rats (30%) ($P = 0.10$) at 14th week. Anti-centromere levels were undetectable in all rats at 7th and 14th week.

In Fig. 2 for the subcutaneous silicate group; serum ANA titers were high in 8/10 rats (80%) ($P < 0.005$) at 7th and 14th week post-subcutaneous silicate challenge. Anti-RNP titers were significant in 8/10 rats (80%) ($P < 0.0004$) at 7th week and in 7/10 rats (70%) ($P < 0.0075$) at 14th week. Anti-SCL70 levels were detectable in one rat 1/10 (10%) ($P = 0.5$) at 7th and 14th week. Anti-centromere levels were undetectable in all rats in both post-challenge samples.

P value for comparison of the number of positive results of autoantibodies at 14th week with the number of positive results of autoantibodies at 7th week were shown in Tables 1, 2.

Discussion

Environmental crystalline silica exposure has been associated with formation of autoantibodies and development of systemic autoimmune diseases [15–17]. Silica exposure significantly elevated ANA level autoantibodies to histone, but had little effect on dsDNA autoantibody level [12–18].

Meta-analysis done in Janowsky et al.'s study showed that there was no evidence of an association between breast implants in general, or silicon-gel-filled breast implants specifically, and any of the individual connective-tissue diseases, all definite connective-tissue disease combined or other autoimmune or rheumatoid conditions. From a public health prospective, breast implants appear to have minimal effect on the number of women in whom connective-tissue diseases develop, and the elimination of the implants would not be likely to reduce the incidence of connective-tissue disease [3].

The present study showed that a significant number of rats in the subcutaneous silicate group had a high titer of

Table 1 Serum anti-nuclear antibodies (ANA) titers and absorbance value (OD) for other auto-antibodies following oral challenge of rats with sodium silicate

RATS PO	ANA		Anti-RNP		Anti-ScL70		Anti-centromere	
	7 weeks	14 weeks	7 weeks	14 weeks	7 weeks	14 weeks	7 weeks	14 weeks
1	1/20	-ve	-ve	-ve	-ve	-ve	-ve	-ve
2	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
3	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
4	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
5	-ve	1/80	-ve	-ve	-ve	-ve	-ve	-ve
6	-ve	1/80	-ve	-ve	-ve	0.296	-ve	-ve
7	-ve	1/40	-ve	-ve	-ve	-ve	-ve	-ve
8	-ve	-ve	-ve	0.336	1.200	0.333	-ve	-ve
9	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
10	-ve	-ve	0.760	0.595	-ve	0.395	-ve	-ve
P value	0.29102		0.5		0.29102		0.9999	

OD optical density, PO per oral, -ve negative

P value for comparison between the number of positive titers of autoantibodies at 14th week with the number of positive titers of autoantibodies at 7th week

Table 2 Serum anti-nuclear antibodies (ANA) titers and absorbance value (OD) for other auto-antibodies following subcutaneous challenge of rats with sodium silicate

RATS SC	ANA		Anti-RNP		Anti-ScL70		Anti-centromere	
	7 weeks	14 weeks	7 weeks	14 weeks	7 weeks	14 weeks	7 weeks	14 weeks
1	1/20	1/80	0.380	0.550	-ve	-ve	-ve	-ve
2	1/20	1/80	0.319	0.482	-ve	-ve	-ve	-ve
3	1/20	1/160	0.835	0.604	-ve	-ve	-ve	-ve
4	1/10	1/40	0.740	0.632	-ve	-ve	-ve	-ve
5	-ve	-ve	0.225	-ve	-ve	-ve	-ve	-ve
6	1/20	-ve	-ve	-ve	-ve	-ve	0.102	0.104
7	-ve	1/80	-ve	-ve	-ve	-ve	-ve	-ve
8	1/20	1/160	0.345	0.665	-ve	-ve	-ve	-ve
9	1/20	1/40	0.333	0.458	-ve	-ve	-ve	-ve
10	1/20	1/40	0.342	0.385	1.02	0.703	-ve	0.104
P value	0.70898		0.5		0.76315		0.5	

SC subcutaneous, -ve negative

P value for comparison between the numbers of positive titers of autoantibodies at 14th week with the number positive titers of autoantibodies at 7th week

ANA and anti-RNP. In contrast, the oral silicate group had an insignificant number of rats with positive titer. Although the response was slower but have increased with time and this has been observed in our previous study which may indicate that the oral silicate probably has been processed in the gastrointestinal system. This could be related to the specialized M cells found in the follicular associated epithelium of intestinal peyer patches [18, 19].

Both oral and subcutaneous silicate groups showed that the most specific autoantibodies of scleroderma including anti-SCL70 and anti-centromere probably need more time to achieve a significant level.

It is probably recommended to inquire patients with autoimmune diseases about occupations and industries having the potential risks for silica exposure and also to inquire about prosthetic implants such as prosthetic cardiac valve and joint implants.

In conclusion, silica might induce autoimmunity and it is probably one of the potential risk factor in inducing scleroderma.

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