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Effect of exposure to silica in inducing autoimmune hepatitis: an experimental animal-controlled study

S.M. AL-MOGAIREN[1], A. AL SHEIKH[2], S. HUSAIN[2], A.S. AL ARFAJ[1], K. AL SWAT[3], M. HAMDANI[1], M. HEFNAWY[4], B. AL MOHIMED[5], A.W. AL HAMMAD[6],

[1]Rheumatology Division, Department of Medicine, [2]Department of Pathology, [3]Hepatology Division, Department of Medicine, [4]Department of Pharmacology, [5]Department of Biochemistry, [6]Department of Immunology, [7]Department of Family and Community Medicine, [8]Department of Physiology, College of Medicine, King Saud University, Riyadh, Saudi Arabia

Abstract. – OBJECTIVE: The aim of this experimental study is to investigate the effect of subcutaneous and oral sodium silicate in inducing the autoimmune hepatitis.

MATERIALS AND METHODS: Twelve Brown Norway rats were studied, six rats were challenged with Sodium Silicate and the rest were challenged with normal saline as a control group. At 14th week post-sodium silicate or normal saline exposure, the rats were sacrificed. Histopathological studies were conducted in six positive autoantibodies responding silicate group rats and then compared with an equal number of negative autoantibodies responding control rats.

RESULTS: The liver findings from sodium silicate group of animals showed a histopathological reaction in 3/6 (50%) compared with 0/6 of the corresponding control saline group (p = 0.09). However, the absolute differences in the percentage between the two groups was 50%, the subcutaneous sodium silicate sub-group showed hepatic tissue response close to being statistically significant level (p = 0.05).

CONCLUSIONS: After correlating the results with autoantibodies including serum antinuclear antibodies and anti ribo-nucleoprotein response of the same rats, it is concluded that sodium silicate play a role in inducing the autoimmune hepatitis in a genetically susceptible rat model.

Key Words:
Silicate, Silica, Autoimmunity, Auto-antibodies, ANA, Anti-RNP, Liver, Autoimmune hepatitis.

Introduction

Epidemiologic and experimental research suggest a potential role of occupational exposure in the development of systemic autoimmune diseases such as systemic lupus erythematosus. A plausible association has been identified in studies of occupational silica exposure and SLE, complemented by experimental studies in lupus-prone mice exploring potential mechanisms related to apoptosis and immune dysregulation[1-4]. Occupations involving risk of silica exposures include: mining, quarrying, tunneling, glass manufacture, ceramics, pottery production, cement and concrete production[5-6].

There are implants made of silicon for medical purposes such as cosmetic breast implants, cardiac valve replacement, and joint implants[7-9]. The liver may be target of the hazards of toxin and drugs. Our previous studies[10-12] showed that serum antinuclear antibodies (ANA) titer at 14th week post-silicate challenge was high in (8/10) rats (p = 0.007) in subcutaneous silicate group, while the oral silicate group showed high titers in (3/10) rats (p = 0.10). Serum antiribo-nucleoprotein (anti-RNP) levels were detectable in (7/10) rats (p < 0.007) in subcutaneous silicate group, while the oral silicate group detectable in (2/10) rats (p = 0.23). On the other hand, all antibody titers including ANA and anti-RNP were almost all undetectable in the corresponding control groups.

The aim of the present study is to determine effect of sodium silicate in inducing autoimmune hepatitis in immune-sensitive rats, through the correlation of the liver histopathological features with the previous serum autoantibodies responses of the same rats.

Materials and Methods

Twelve Brown Norway rats (BN) (average weight of 157 gm) were purchased from Charles

Corresponding Author: Sultan M. Al Mogairen, MD; e-mail: almogairin@gmail.com

Rivers Laboratories, Raleigh, NC, USA. They were kept in polycarbonate metrolon plastic cages covered with a stainless steel cover in the animal house at the College of Medicine, King Saud University, Riyadh, Saudi Arabia. The rats were exposed to 12 hours of darkness and 12 hours of light daily, and were kept under observation for three weeks. No evidence of sickness was observed. All rats were 8-11 weeks old at the onset of the experiment. There were a total number of 12 rats divided into two main groups including the silicate and the control normal saline groups. There are four sub-groups, the first and the second sub-groups (six rats) are called the subcutaneous silicate sub-group (three rats) and the oral silicate sub-group (three rats). The third and the fourth sub-group (six rats) are called the subcutaneous normal saline control sub-group (three rats) and the oral normal saline control sub-group (three rats).

All of the above groups were selected for liver histological studies from our previous studies, where the first and second silicate subgroups showed positive autoantibodies responses including serum ANA and anti-RNP. On the other hand, the third and fourth control, normal saline sub-groups showed overall negative autoantibody responses. After 14 weeks of exposure to sodium silicate or normal saline, the above selected sub-groups of rats were sacrificed and then the livers were dissected. The tissues were then processed in the Tissue-Tek vacuum infiltration processor and stained using hematoxylin and eosin stain. The slides were then examined blindly by a histopathologist using light microscope.

Statistical Analysis

Statistical differences between silicate and the corresponding control group were calculated using Fisher's exact test. p-value < 0.05 was considered significant.

Results

The maximum serum antibody titers of the selected rats from our previous studies are shown in Table I. ANA titers were significantly high in 4 out of 6 of the silicate group. Anti RNP titers were positive also in 4/6 of the silicate group. All titers of ANA and anti RNP in central groups are negative.

Histopathological results are shown in Table II. Liver changes become negative if all of the parameters shown in Table II are negative, otherwise, they remain positive. Positive liver changes, shown in Figure 1, were observed in 3/3 (p = 0.05) of subcutaneous sodium silicate subgroup. On the other hand, there were no tissue changes in the oral sodium silicate sub-group.

Upon comparison of both silicate groups (subcutaneous and oral subgroups), with the control normal saline group, positive hepatic changes were observed in 3/6 (50%) of the silicate group. In contrast, the positive liver changes were not observed in the normal saline group 0/6 (p = 0.09).

Discussion

Silica exposure has been reported to induce autoimmune responses and is associated with in-

Rats	Cut-off of significant titer ANA (1/10) significant	Cut-off of significant titer anti-RNP (0.205) significant
Silicate SC1	1/80	0.835
Silicate SC2	1/80	0.482
Silicate SC3	1/80	0.550
Silicate PO1	1/20	-ve
Silicate PO2	-ve	-ve
Silicate PO3	-ve	0.336
Control SC1	-ve	-ve
Control SC2	-ve	-ve
Control SC3	-ve	-ve
Control PO1	-ve	-ve
Control PO2	-ve	-ve
Control PO3	-ve	-ve

SC = subcutaneous, PO = per oral, -ve = negative.

Table II. Histopathological features of silicate-tested and control groups.

Lobular activity	Interface hepatitis	Portal inflammation	Liver of the following rats
+ve	-ve	-ve	Silicate SC1
+ve	-ve	+ve	Silicate SC2
+ve	-ve	-ve	Silicate SC3
-ve	-ve	-ve	Silicate PO1
-ve	-ve	-ve	Silicate PO2
-ve	-ve	-ve	Silicate PO3
-ve	-ve	-ve	ControlSC1
-ve	-ve	-ve	ControlSC2
-ve	-ve	-ve	ControlSC3
-ve	-ve	-ve	ControlPO1
-ve	-ve	-ve	ControlPO2
-ve	-ve	-ve	ControlPO3

-ve = 0 = negative, +ve = 1 = mild, ++ = 2 = moderate, +++ = 3 = severe; SC = subcutaneous; PO = per-oral.

creased occurrence of systemic autoimmune diseases[13-16]. The exact mechanism by which silica promotes or accelerates the development of autoimmune diseases is unknown[1-4]. Many cytokines are involved in the onset and progression of silicosis such as TGF, it is an important cytokines involved in the pathogenesis of fibrosis. It stimulate the synthesis and deposition of extracellular matrix and collagen synthesis in fibroblasts, thus, causing fibrosis[17]. In humans, the pathogenesis of autoimmune liver diseases involves several pathways, though most of the evidence supports a central role for alteration in T cell function in the pathogenesis of autoimmune hepatitis (AIH), although abnormalities in B cell function also may be important[18]. Regulatory CD4 (+) CD25 (+) T cells (Tregs) are defective numerically and functionally in AIH. Investigators have shown reduced function of CD4+25+ regulatory T cell fraction in silicosis patients[19]. *In vitro* studies have shown that silica can act as adjuvant stimulating T-cell responses or an inducer of apoptosis[20-22].

Our previous studies[10-12] show a significant number of rats ($p < 0.05$) in the subcutaneous silicate group had a high titer of ANA and anti-RNP. In contrast, the oral silicate group had an insignificant number of rats with positive titer. In the present study, the liver histopathological analyses showed a positive tissue reaction in all subcutaneous silicate sub-group approaching a significant level with $p = 0.05$. Lack of tissue changes in the oral silicate group was probably due to the processing of the sodium silica substance by gastrointestinal microfold cells (M-cells)[23]. Comparing both silicate subgroups (n = 6) with control normal saline group including subcutaneous and oral subgroups (n = 6) revealed a difference equal to 50% (50%-0%). Although this is clinically useful, we did not achieve statistical significance ($p = 0.09$), which probably indicate the need for a more prolonged period of exposure to silicate.

The significant serum auto-antibody responses particularly ANA and anti-RNP suggest that autoimmunity likely contributes in the liver histopathological changes. Neither paucity nor lack of portal plasma cells infiltration (Figure 1) precludes the diagnosis of AIH[24,25]. To our knowl-

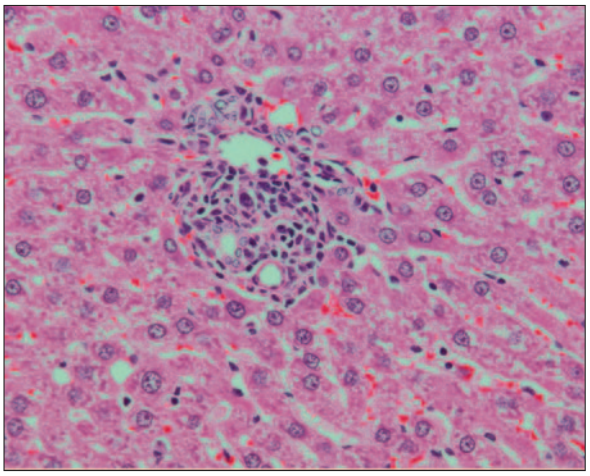

Figure 1. Histology of rat liver with portal vein inflammatory infiltration with predominantly lymphocytic cells and paucity of plasma cell. Histology of rat liver hematoxylin and eosin stain ×400 magnification.

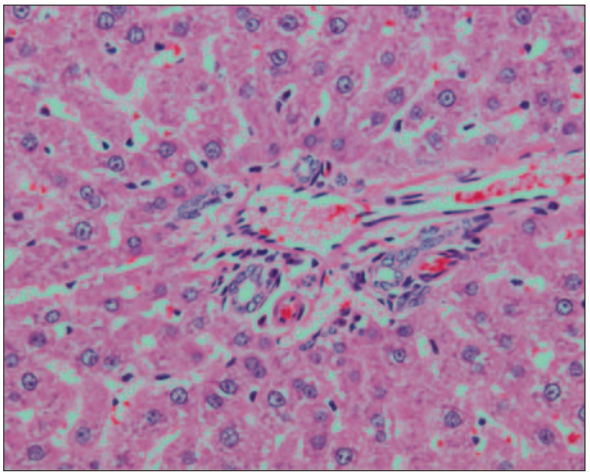

Figure 2. Histology of rat liver with no evidence of portal or lobular inflammation. Histology of rat liver hematoxylin and eosin stain ×400. Normal liver histopathological features of control group.

edge, this is the first study showing liver histological changes secondary to silicate exposure with correlation to the serum autoantibodies responses.

Conclusions

The silica exposure may promote the development of lupus autoimmune hepatitis in immunosensitive rats. There is possibility of long lag time between exposure to the trigger silicate and the onset of the disease. The exact mechanism of silica induced autoimmune hepatitis is not yet well identified. As the majority of evidence on silica and autoimmune disease stems from human studies, further experimental research is needed in order to identify mechanisms involved in autoimmune responses to silica.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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