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ORIGINAL ARTICLE

Dental amalgam as one of the risk factors in autoimmune diseases

Jirina Bártová [1], Jarmila Procházková [1], Zuzana Krátká [1], Klára Benetková [1], Zora Venclíková [1] & Ivan Šterzl [2]

1. Institute of Dental Research, lst Medical Faculty, Charles University and General Faculty Hospital Prague, CZECH REPUBLIC.
2. Institute of Endocrinology, Prague, CZECH REPUBLIC.

Correspondence to: Jirina Bártová
Institute of Dental Research, lst Medical Faculty
Charles University and General Faculty Hospital Prague
Vinohradská 48, 120 60 Prague 2, Czech Republic
PHONE: +420 2 24256718
FAX: +420 2 24247034
E-MAIL: jirina.bartova@post.cz

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Abstract

BACKGROUND: Experimental and clinical data published recently show that dental amalgam can give rise to undesirable immunological responses in susceptible individuals. In genetically susceptible strains of experimental animals, mercury and silver can induce autoimmune responses. Sera of patients sensitive to mercury were found to have a higher incidence of autoantibodies relative to controls.

OBJECTIVE: The aim of this study was to determine possible presence of antinuclear SSB/La autoantibodies after the *in vitro* stimulation of peripheral blood lymphocytes with $HgCl_2$.

METHODS: Lymphocytes were obtained from patients with autoimmune thyroiditis and increased response to mercury *in vitro*. Mononuclear cells were cultivated for 6 days with 100 µl $HgCl_2$ solution or with pure medium and the levels of antinuclear autoantibodies SSB/La were assayed by a commercial SSB/La ELISA kit.

RESULTS: Increased production of SSB/La autoantibodies in the media following stimulation of peripheral blood lymphocytes with $HgCl_2$ was found in all cases. Using the Student's paired test, the results were significant on the $p=0.05$ significance level.

CONCLUSION: Results imply that, in some patients with thyroiditis, mercury from dental amalgam can stimulate the production of antinuclear antibodies. Dental amalgam may be a risk factor in some patients with autoimmune disease.

Introduction

Recent experimental and clinical data show that, although amalgam alloys have been used as filling material in dentistry for more than 150 years and have never been associated with damage to healthy subjects, in susceptible individuals they can give rise to undesirable immunological responses. Both local and systemic changes in tolerance have been described not only for mercury but also for other metals that form parts of the amalgam, i.e. for silver, tin, copper and zinc [1].

Experiments show that in genetically susceptible strains of animals mercury and silver can induce autoimmune responses. Administration of $HgCl_2$ to genetically susceptible mice and rats brings about deposition of immunocomplexes in the kidney, production of antinuclear autoantibodies and increased serum IgG and IgE levels [2,3,4]. In the Brown-Norway mercury sensitive rat strain, introduction of four amalgam fillings into the lower molars brought about the development of an autoimmune syndrome within four weeks. This syndrome is characterised by deposition of immunocomplexes in the kidney and in the vessel walls and a multiple increase in the levels of mercury with selenium in the kidney, spleen, cerebellum, liver and thymus [5]. Sera of patients sensitive to mercury were found to have a higher incidence of autoantibodies relative to controls [6]. Šterzl et al. [7] described an enhanced response to mercury and nickel in patients with chronic fatigue syndrome and with autoimmune thyroiditis, increased levels of antinuclear autoantibodies in the sera and an improvement in their medical condition following the replacement of amalgam fillings with composites.

In this report we have studied the production of antinuclear SSB/La autoantibodies in lymphocyte cultures from patients with autoimmune thyroiditis and proven sensitivity to mercury.

Materials and methods

The group of patients consisted of 12 individuals with autoimmune thyroiditis and a proven increased response to mercury in a lymphocyte proliferation test modified for metals by Stejskal (MELISA test) [8]. The informed consent according to the Helsinki declaration was obtained from all patients.

To document autoantibody production, volumes of 5 ml blood were collected from all patients into Vacutainer test tubes with beads. The blood was defibrinated by shaking and diluted 1:1 with RPMI 1640 tissue culture medium containing Hepes (Gibco BRL), supplemented with 400 μg gentamycin (Krka, Yugoslavia) and 50mg glutamine (Sevapharma, Prague) in 100 ml medium. Mononuclear cells were separated by centrifugation on a Ficoll-Paque (Pharmacia Uppsala) gradient at 600g for 30 min; the buff coat was removed by aspiration, washed and suspended in 5 ml RPMI 1640 medium containing 20% of autologous inactivated serum (inactivation 56°C/45 minutes). The samples were then cultivated for 30 minutes in reclining 50 ml culture flasks (Falcon) at 37°C in 5% CO_2 atmosphere to remove adherent cells. After cultivation and counting, the cells were diluted to a concentration of 10^6/ml in RPMI 1640 medium with 5% of inactivated autologous serum. A 1 ml volume of the diluted cells was cultivated for 6 days with 100 μl $HgCl_2$ solution [in a concentration given by Stejskal [8]] or with pure medium. After 6 days the media from the tissue cultures were collected for autoantibody assay and stored until examination at –20°C.

Assay of SSB/La autoantibodies

SSB/La antinuclear autoantibodies were assayed by a commercial SSB/La ELISA kit from Binding Site Birmingham (Imunotech, Prague). Volumes of 100 μl of media after cultivation of cells with $HgCl_2$ or with pure medium were applied into the wells of plates with bound antibody. The assay then proceeded according to the supplier's instructions.

Evaluation

In view of the possible occurrence of autoantibodies in the autologous serum present in the medium, we compared the levels of autoantibodies in media after stimulation of mononuclear cells with $HgCl_2$ with the levels of autoantibodies after stimulation of mononuclear cells with medium alone. Statistical evaluation was done using Student's paired *t-test* at a $p=0.05$ significance level.

Results

The results are given in Figure 1. In all cases, we found increased production of SSB/La autoantibodies in media after stimulation of peripheral blood lymphocytes with $HgCl_2$ in comparison with those stimulated with medium alone. The results are significant at a $p=0.05$ significance level.

Discussion and conclusion

Autoimmune diseases are multifactorial afflictions associated with a variety of predisposing factors – genetic, hormonal, immunological and environmental [9,10]. Environmental factors include bacterial and viral infections [11], smoking [12] and a large group of factors include vaccines and chemical substances [13]. The possible association between silicon breast implants and autoimmune disorders was described in some patients [14].

Autoimmune diseases are characterized by the presence of autoantibodies to various cellular and tissue components. The SSB/La antigen is predominantly a nuclear protein and is present in almost all human and animal tissues [15]. The occurrence of antinuclear autoantibodies is characteristic for some severe autoimmune diseases such as SLE, Sjögren's syndrome and some other diseases of the connective tissue. Our results imply that, as in sensitive experimental animals, mercury can also stimulate the production of antinuclear SSB/La autoantibodies in patients

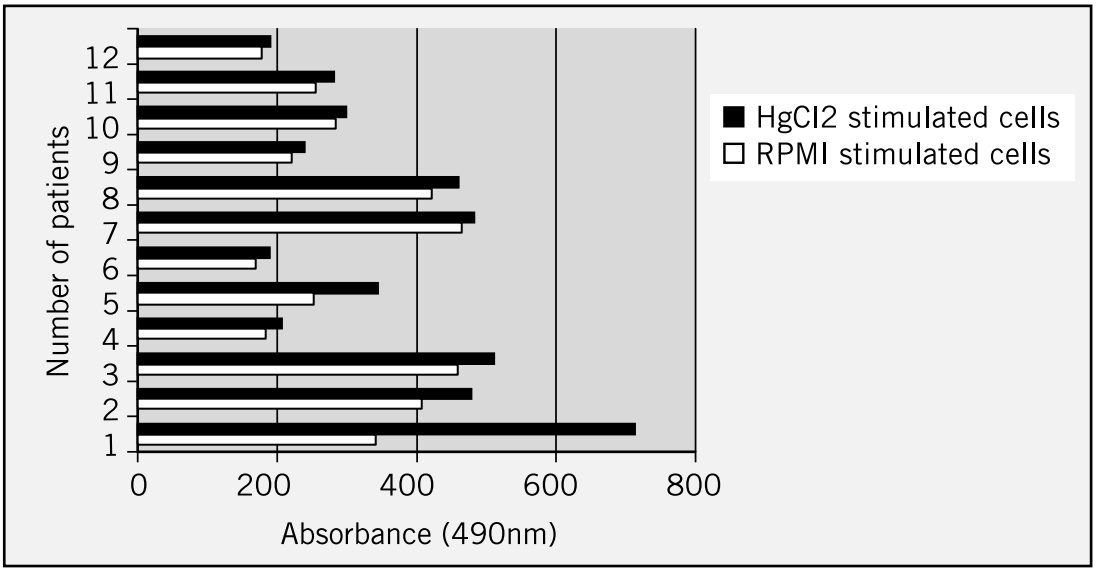

Fig. 1. Effect of $HgCl_2$ stimulation on SSB/La production by lymphocytes isolated from peripheral blood in patients with thyroiditis. (The results are significant at a p=0.05 significance level.)

with proven mercury intolerance. The production of antinuclear autoantibodies takes place via a polyclonal activation of B lymphocytes. The undesirable response to mercury thus involves not only the previously described delayed hypersensitivity type response but also a polyclonal activation of B lymphocytes with activation of multiple clones of diverse specificity. For this reason we could prove an increased level of SSB/La autoantibodies in media from mercury-sensitive patients with autoimmune thyroiditis after stimulation of peripheral blood lymphocytes with $HgCl_2$. The proven increased production of SSB/La autoantibodies is typical for Sjögren's syndrome, which is manifested by increased dental caries experience. In sensitive patients with autoimmune disease, mercury released from amalgam fillings can thus secondarily affect the immune system and thereby also the course of the disease. The presence of antinuclear autoantibodies in the sera of our patients lends support to the role of polyclonal activation due to dental amalgam. Our results implicate dental amalgam as a risk factor in autoimmune disease. For this reason, these patients should receive special dental care including careful choice of the dental material.

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