Antinucleolar Autoantibody Induced by Mercuric Chloride in Mice: Does Sodium Selenite Inhibit Autoantibody Production?

Abstract: Repeated exposure of subtoxic doses of mercuric chloride (HgCl_2) can induce antinucleolar autoantibodies in susceptible mice. To study the immunopathological mechanism(s), sodium selenite (Na_2SeO_3), which is known to reduce the toxicity of mercury, is injected simultaneously with HgCl_2 into mice. Equimolar Na_2SeO_3 nor the same amount of Se in Na_2SeO_3 as Hg in HgCl_2 could not reduce antinucleolar autoantibody induction by HgCl_2 . These results indicate that the mechanism of autoimmunity induction by HgCl_2 might be independent of its toxicity.

Key words: HgCl_2 — Na_2SeO_3 — Antinucleolar autoantibody

Chronic exposure to metals and their compounds is associated with severe toxic effects. While often overlooked, several metals are frequently responsible for immunological reactions, resulting in allergic contact dermatitis and/or autoimmunity in individuals who come into contact with metal products occupationally¹⁾.

Experimental models of metal-induced hypersensitivity including autoimmunity have been developed. Repeated exposure to subtoxic doses of mercuric chloride (HgCl_2) can induce anti-glomerular basement membrane antibodies or antinuclear antibodies in rats and rabbits, resulting in severe autoimmune or immune complex glomerulonephritis²⁻⁴⁾. HgCl_2 also induces antinucleolar autoantibodies (AnuA) in susceptible mice⁵⁻⁸⁾. These immunological abnormalities occur in a T cell dependent manner⁸⁻¹¹⁾. The susceptibility to HgCl_2 induced autoimmunity is mainly determined by the class II major histocompatibility complexes (MHC), though extra MHC genes may also be involved^{2, 12-15)}. In addition, HgCl_2 increases the expression of class II MHC on B cells in rats and mice¹⁶⁻¹⁸⁾.

Sodium selenite (Na_2SeO_3) protects against the toxicity of HgCl_2 . Necrotizing changes of renal tubules caused by HgCl_2 toxicity completely disappeared after the administration of Na_2SeO_3 ¹⁹⁾. Further, animals given a lethal dose of HgCl_2 together with Na_2SeO_3 survived with few pathological changes^{19, 20)}. However, its effect on autoimmunity induction by HgCl_2 had not yet been studied. Thus, we performed the present experiments.

IQI/Jcl mice were obtained from the Central Institute for Experimental Animals (Kawasaki, Japan), and were maintained in our own animal facilities under conventional conditions. Both male and female mice were supplied for the experiments at 8 to 10 weeks old.

Stock solutions of 10 mg/ml of HgCl_2 (Wako Pure Chemical, Tokyo, Japan) and Na_2SeO_3 (Wako Pure Chemical, Tokyo, Japan) in sterile 0.9% saline were prepared

at the beginning of each experiment. Before administration, aliquot of the stock solutions were diluted with sterile 0.9% saline to the desired concentrations. To avoid direct contact, mice simultaneously received subcutaneous injection of 0.1 ml HgCl_2 solution and intraperitoneal injection of 0.5 ml Na_2SeO_3 solution 3 times a week for 4 weeks. Control mice were given subcutaneous 0.1 ml and/or intraperitoneal 0.5 ml of 0.9% saline.

The mice were anesthetized with chloroform and then bled by heart puncture 2 days after the final injection. Sera were separated and stored at -20°C until use. AnuA levels were determined by indirect immuno-fluorescence using rat myoid cells (R615B2) as the substrate and fluorescein-conjugated goat-anti-mouse IgG (Cappel, West Chester, PA, USA). The sera were diluted with phosphate buffered saline by a 2-fold dilution starting at 1:10. The AnuA titer was expressed as the number of the tube with the highest dilution showing the nucleolar staining. Details of the method were described previously⁸⁾.

Since Na_2SeO_3 can reduce the toxic effects of HgCl_2 and its optimum suppressive effects occur when equimolar amounts are administered²⁰⁾, equimolar amounts of HgCl_2 and Na_2SeO_3 were injected at the first experiment. Repeated administration of 4 $\mu\text{mole/kg}$ (1.09 mg/kg) HgCl_2 induced high titer AnuA in all mice with and without injection of 4 $\mu\text{mole/kg}$ (0.69 mg/kg) Na_2SeO_3 (Table 1). Injection of Na_2SeO_3 alone did not induce any AnuA. In the second experiment, the same amounts of metals were administered. Male mice were injected with 1 mg/kg HgCl_2 (0.74 mg/kg as Hg) and 1.84 mg/kg Na_2SeO_3 (0.74 mg/kg as Se), or 0.25 mg/kg HgCl_2 (0.19 mg/kg as Hg) and 0.46 mg/kg Na_2SeO_3 (0.19 mg/kg as Se), respectively. Once again, Na_2SeO_3 did not inhibit AnuA induction by HgCl_2 (Table 2).

In these experiments, neither simultaneous injection with equimolar Na_2SeO_3 nor amounts of Se equivalent to Hg reduced AnuA induction by HgCl_2 . These results

Table 1. Effects of Na_2SeO_3 on AnuA induction by HgCl_2 (I).

Sex	Treatment	Positive/Examined	Titer
Male	Saline + Saline	0/8	
	Saline + Na_2SeO_3	0/8	
	HgCl_2 + Saline	8/8	7.5 ± 0.9
	HgCl_2 + Na_2SeO_3	10/10	7.5 ± 0.5
Female	Saline + Saline	0/7	
	Saline + Na_2SeO_3	0/7	
	HgCl_2 + Saline	7/7	6.7 ± 1.5
	HgCl_2 + Na_2SeO_3	7/7	7.6 ± 0.5

4 $\mu\text{mol/kg}$ HgCl_2 (0.1 ml/mouse, *s.c.*) and/or 4 $\mu\text{mole/kg}$ Na_2SeO_3 (0.5 ml/mouse, *i.p.*) were injected 3 times a week for 4 weeks.

Table 2. Effects of Na₂SeO₃ on AnuA induction by HgCl₂ (II).

HgCl ₂ (mg/kg)	Na ₂ SeO ₃ (mg/kg)	Positive/Examined	Titer
0	0	0/8	
	0.46	0/8	
	1.84	0/8	
0.25	0	8/8	6.4 ± 1.1
	0.46	8/8	5.9 ± 0.9
1.0	0	8/8	6.0 ± 1.0
	1.84	7/7	5.7 ± 1.0

HgCl₂ (0.1 ml/mouse, *s.c.*) and Na₂SeO₃ (0.5 ml/mouse, *i.p.*) were injected in male mice 3 times a week for 4 weeks.

suggested that the pathological mechanism of autoimmunity induction by HgCl₂ might be independent of its toxicity. Since doses of HgCl₂ necessary to induce AnuA were far less than those to induce toxic effects, it might also be possible that doses of Na₂SeO₃ used in these experiments were too small to inhibit autoimmunity induction by HgCl₂.

The possible mechanism(s) of autoimmunity induced by heavy metals and chemicals may include (1) release of autoantigen(s) in the circulation due to their toxic effects; (2) alteration of autoantigen(s) due to their combination with self antigen(s); or (3) direct effects on lymphocytes following activation of helper T cells and subsequent polyclonal activation of B cells^{2, 21, 22}. The precise mechanism of AnuA induction by HgCl₂ requires further examination.

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