

Preliminary Communication

Mercury exposure in protein A immunoadsorption

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Abstract

Background. Immunoadsorption is increasingly used to treat antibody-mediated autoimmune diseases. To prevent microbial growth during storage, reusable protein A–Sepharose gel columns are primed with ethyl mercury thiosalicylate (thiomersal, 0.1% solution) and rinsed with phosphate buffer before use. In this study, we tested the hypothesis of systemic mercury exposure in protein A immunoadsorption.

Methods. Whole blood mercury levels were measured by atomic absorption spectroscopy before and after protein A immunoadsorption (11 patients, 26 treatments), anti-IgG immunoadsorption (eight patients, 13 treatments) and LDL apheresis (DALI and Therasorb systems; nine patients, 14 treatments).

Results. Patients treated with protein A immunoadsorption had significantly elevated baseline mercury levels compared with the other groups, which were not different from healthy controls. Following protein A immunoadsorption, mercury levels increased from $5.9 \pm 1.4 \mu\text{g/l}$ (mean \pm SEM, normal, $< 5 \mu\text{g/l}$) to $32.3 \pm 5.7 \mu\text{g/l}$, $P < 0.001$). In one intensively treated patient, acute neurological toxicity developed at a mercury level of $107 \mu\text{g/l}$. Symptoms abated slowly and did not recur after switching to a thiomersal-free system and chelation therapy. No mercury release to patients occurred in anti-IgG immunoadsorption or LDL apheresis treatments.

Conclusion. This preliminary report suggests that protein A immunoadsorption columns primed with thiomersal during storage may cause a sustained increase of systemic mercury concentrations, which exceed current safety recommendations in a proportion of patients. Considering the potential for mercury-induced toxicity, every effort should be undertaken to reduce systemic mercury exposure, either by adding chelators to the rinsing solution or ideally by replacement of thiomersal.

Keywords: immunoadsorption; mercury; thiomersal; toxicity; tremor

Introduction

Immunoadsorption is a novel adsorption technique for semi-selective extracorporeal removal of circulating autoantibodies in disorders such as recurrent kidney graft rejection due to HLA hypersensitization [1], renal autoimmune disorders [2], haemophilia with inhibitors to factor VIII or IX [3], congestive heart failure [4] and neurological autoimmune diseases including myasthenia gravis and Guillain–Barre syndrome [5]. Current apheresis systems employ reusable columns designed for long-term storage (20–50 treatment sessions). To prevent microbial growth, staphylococcal protein A–Sepharose (PA) columns are primed with ethyl mercury thiosalicylic acid (buffered thiomersal 0.1% solution) during storage and rinsed with phosphate buffer before use [6]. In contrast, anti-IgG immunoadsorbents are stored after priming with phosphate-buffered saline (PBS)–sodium azide [7].

The long half-life of ethylmercury could theoretically result in accumulation and toxicity during chronic application, as discussed in the context of thiomersal-containing vaccines [8]. Following recommendations from the US Public Health Service and the American Academy of Pediatrics, thiomersal has been largely replaced in infant vaccines, although no clear evidence of potential health and development problems has been demonstrated so far [8]. Organic mercury toxicity affects mainly the central nervous system (CNS), with symptoms such as lethargy, loss of appetite, weight loss, tremor, memory loss, sleep disturbance, emotional lability and confusion [9]. Furthermore, mercury may cause significant damage to the haematopoietic and renal system [9]. Since PA immunoadsorption has been used successfully for several years at our department, the lack of long-term safety data led us to investigate the hypothesis of a thiomersal-related mercury release during apheresis.

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Subjects and methods

Subjects and treatment characteristics

All studies were performed at the Apheresis Clinic, Division of Nephrology, Department of Medicine III, University of Vienna. Patients treated by immunoabsorption were included in clinical trials which were approved by the Ethics committee of the Vienna Medical School. Written informed consent was obtained before initiation of treatment. Patients on LDL apheresis were treated for homozygous ($n=1$) or heterozygous familiar hypercholesterolaemia ($n=8$) on a routine basis. Vascular access was established by puncturing peripheral veins or haemodialysis shunts using 15–17 gauge needles (Bionic Medizintechnik GmbH, Friedrichsdorf, Germany). No mercury-containing skin disinfectants were used. Blood flow rates were set to 50–80 ml/min. Anticoagulation consisted of citrate dextrose, formula A (ACD-A, Baxter, Munich, Germany); with a citrate: blood ratio of 1:22 (4.5%). In addition, standard heparin (Heparin Immuno, Baxter-Immuno, Vienna, Austria) was administered (2000 U bolus, followed by 1500 U/h infusion). For plasma separation, the COBE Spectra (Cobe Laboratories, Zovantem, Belgium) was used. Clinical characteristics of the patients are summarized in Table 1; further details are given below.

Of the 11 patients treated by PA column-based immunoabsorption (Immunosorba[®], Fresenius Medical Care, Germany), nine were on regular treatment and two were investigated when treatment was newly initiated or re-established. Clinical diagnoses were recurrent rejection of renal allografts due to polyreactive HLA antibodies ($n=4$), systemic lupus erythematosus ($n=2$), myasthenia refractory to standard treatment ($n=2$), and Goodpasture's syndrome, Wegener's disease and endocrine orbitopathy due to autoimmune hyperthyroidism ($n=1$, respectively). Mean plasma volume processed per treatment was 6890 ml (range, 6000–7500 ml). Column performance index (CPI) was calculated by the microprocessor-controlled Citem 10[®] apheresis monitor from buffer kinetics measured by a pH-sensitive electrode [6]. Following preservation with phosphate-buffered, 0.1% thiomersal (pH 7.0), columns were stored between +2 and +8°C between applications. Provided there is proper aseptic technique, preservation and storage, columns are deemed suitable for reuse for the same patient for up to 12 months from the date of manufacture [6].

Nine patients were treated by antibody-based IgG immunoabsorption (Ig-Therasorb, PlasmaSelect, Teterow, Germany). Clinical diagnoses were recurrent rejection of renal allografts due to polyreactive HLA antibodies ($n=3$), systemic lupus erythematosus ($n=2$), myasthenia refractory to standard treatment ($n=2$) and endocrine orbitopathy ($n=1$). Mean plasma volume processed per treatment was 6780 ml (range, 6800–8000 ml).

Six patients were treated by LDL apheresis at weekly intervals using the Ig-Therasorb system (PlasmaSelect, Teterow, Germany). Plasma volume processed was 6000 ml per treatment.

Three patients were treated by LDL apheresis at weekly intervals using direct adsorption of lipoproteins from whole blood (DALI system, Fresenius Medical Care, St Wendel, Germany). Blood volume processed was 9990 ml per treatment.

Analytical methods, normal limits

Venous blood samples were collected immediately before and after treatment using mercury-free materials. We also collected pre- and post-column plasma samples in a proportion of PA treatments. Mercury levels in whole blood, plasma and serum were measured in duplicate using atomic absorption spectrometry, hydride method (Perkin Elmer MHS-20 spectrophotometer, Perkin Elmer, Norwalk, USA). Control material for the metal analysis (Recipe, Munich, Germany) was multiply determined in each analytical series. The upper normal limit for mercury in whole blood ($<5 \mu\text{g/l}$) was established in a large sample of healthy Austrian adults where occupational mercury exposure had been excluded. The upper long-term safety level for mercury was established at $<15 \mu\text{g/l}$. Standard chemical parameters were measured by a Hitachi Autoanalyzer (Roche Diagnostics, Mannheim, Germany), and blood counts were measured by a Sysmex Hematology Analyzer (TAO Medical Electronics Company, Kobe, Japan).

Statistical methods

Data are presented as means (SD) unless indicated otherwise. Normal distribution of samples was tested with the Kolmogorov–Smirnov test. We used ANOVA (analysis of variance) or ANOVA on ranks to investigate potential

Table 1. Clinical characteristics of the study population

Parameter	Protein A immunoabsorption	Anti-IgG immunoabsorption	LDL apheresis	<i>P</i> -value
<i>n</i>	11	8	9	–
Age, years	46 ± 12	41 ± 11	53 ± 11	0.11
Total number of treatments	52 ± 60	72 ± 71	191 ± 160	0.03
Body weight, kg	60 ± 13	68 ± 17	80 ± 16	0.03
Height, cm	164 ± 8	174 ± 5	171 ± 13	0.07
Creatinine, mg/dl	3.1 ± 3.0	4.3 ± 3.5	1.1 ± 0.2	0.06
Blood urea nitrogen, mg/dl	45 ± 32	30 ± 13	16 ± 2	0.02
Bilirubin, mg/dl	0.5 ± 0.3	0.3 ± 0.1	0.5 ± 0.2	0.13
Haemoglobin, g/dl	9.9 ± 2.1	9.1 ± 1.1	12.4 ± 1.4	<0.001
Blood mercury levels before treatment, $\mu\text{g/l}$	5.9 ± 7.2	1.4 ± 0.9	2.3 ± 2.6	<0.001

Data are represented as means ± SD.

differences between treatment groups. All pairwise multiple comparison procedures (Dunn's test) were used to explore significant group differences further. Analysis of treatment effects was performed with Wilcoxon signed rank test or paired *t*-test, as appropriate. In patients on PA immunoadsorption, the relationship between clinical data, treatment-related parameters and the increase of mercury levels during treatment was investigated using Spearman correlation. *P*-values of <0.05 (two-tailed test) were considered statistically significant.

Results

Clinical and demographic characteristics of the study population are presented in Table 1. Since there was no difference between patients treated with LDL Therasorb or DALI systems with respect to clinical parameters or mercury concentrations, these groups were merged into an 'LDL apheresis' group to facilitate analysis. Patients treated with immunoadsorption were slightly younger and showed lower values for body weight and haematocrit and higher values for blood urea nitrogen (Table 1). There was a trend towards higher plasma creatinine compared with patients treated with LDL apheresis. The total number of treatment episodes was higher in the group on LDL apheresis. Groups were otherwise comparable with respect to most other baseline characteristics. However, there were significant differences in mercury levels between groups.

Patients treated by PA immunoadsorption showed elevated mercury levels already at baseline (Table 2), exceeding the upper normal range of 5 µg/l in eight out of 26 treatment sessions compared with none in the IgG-Therasorb, one in the LDL-Therasorb and none in the DALI treatment groups, respectively (*P* < 0.05 for comparison between PA and other groups). In contrast, baseline mercury level was 1.1 µg/l in a haemodialysis patient in whom treatment was re-established after a 3 month break (No. 1, Table 3) and 1.4 µg/l in a patient in whom PA treatment was newly initiated (No. 6, Table 3). Blood mercury levels increased during all PA treatment sessions (Table 2, Figure 1). Post-treatment values exceeded our upper safety level of 15 µg/l in 13 out of 26 PA treatments (mean value, 50.6 µg/l), but in no other modality (Figure 1). The increase in blood mercury concentrations during treatment was significantly correlated with total number of treatments (*R* = 0.65; *P* < 0.001), baseline mercury concentrations (*R* = 0.47, *P* = 0.01) and haematocrit (*R* = 0.52; *P* = 0.02), but not with other treatment-related parameters such as column performance index, plasma volume treated or clinical variables including age, sex, body weight, and markers of hepatic or renal function (*P* = NS, respectively; data not shown). Renal function did not appreciably affect mercury concentrations: in patients with serum creatinine values > 2 mg/dl, mercury concentrations increased by 249%, compared with +520% in patients with serum creatinine values of ≤ 2 mg/dl (medians, *P* = NS).

Table 2. Blood mercury levels before and after treatment

Treatment	Mercury levels before treatment, µg/l	Mercury levels after treatment, µg/l	<i>P</i> -value
Protein A immunoadsorption	5.9 ± 7.2 (0.2–37.6)	32.3 ± 27.9 (7.2–107)	< 0.001
Anti-IgG immunoadsorption	1.4 ± 0.9 (0.1–3.1)	1.3 ± 1.2 (0.1–4.5)	0.38
LDL apheresis	2.3 ± 2.6 (0.2–8.2)	2.4 ± 2.7 (0.2–8.2)	0.31

Data are represented as means ± SD (range).

Table 3. Serial evaluation of mercury levels in patients in whom protein A immunoadsorption was repeated within 10 days

Patient	Day	Blood mercury level before immunoadsorption (µg/l)	Blood mercury level after immunoadsorption (µg/l)
1	0	1.1	43.8
	2	10.4	39.2
2	0	4.6	53
	10	10.4	90.5
3	0	2.2	14.5
	2	4.1	14.3
4	0	11.5	68
	1	37.6	107
5	0	3.3	7.4
	1	2.7	9.8
6	0	1.4	7.2
	4	6.8	13.1
	5	11.4	12.5
	21	2.2	14.5
	23	4.1	14.3
	26	3.6	24

A detailed analysis of seven PA treatment sessions in four patients confirmed that mercury concentrations were consistently increased downstream of the PA columns, clearly suggesting mercury release from thiomersal-primed columns (Table 4). As expected, mercury release was maximal at the start of treatment and declined thereafter.

To exclude potential bias by different haematocrits, we also measured serum concentrations of mercury, which yielded essentially similar findings (means, 4.3 µg/l before vs 37.6 µg/l following PA treatment, *P* < 0.001; 1.3 µg/l vs 1.1 µg/dl in anti-IgG immunoadsorption and 1.0 µg/l vs 0.6 µg/l for LDL apheresis, *P* = NS, respectively).

One patient with myasthenic crisis who had been intensively treated by PA immunoadsorption (39 sessions within 4 months) developed clinical signs of mercury toxicity, with intention tremor and emotional lability emerging during treatment. A neurological work-up did not disclose other potential causes of tremor. Symptoms continued at gradually declining intensity for ~1 week after the last treatment. Since the patient's myasthenia had been otherwise improving

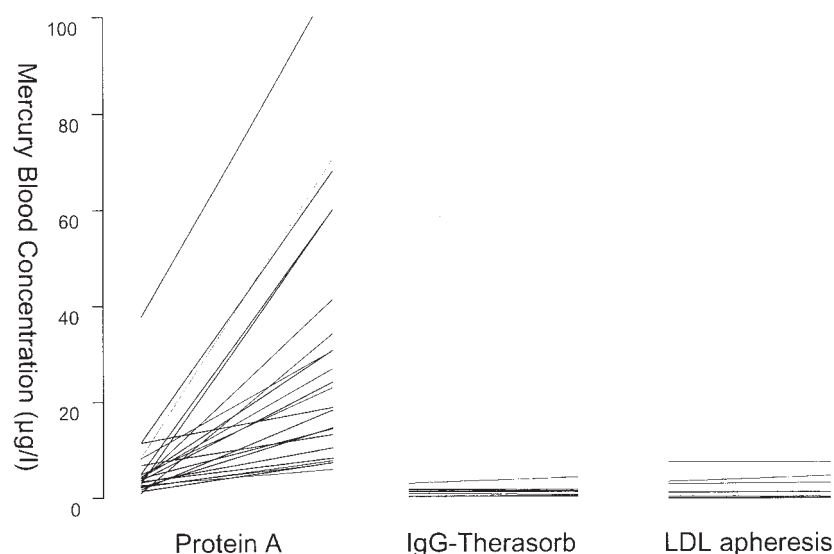


Fig. 1. Individual blood mercury concentrations before (left) and after immunoadsorption or LDL apheresis treatments. Patients treated with protein A immunoadsorption show elevated mercury concentrations at baseline, with further increments during treatment. In IgG-Therasorb or LDL apheresis treatment, no change is observed.

Table 4. Serial evaluation of plasma mercury levels during protein A immunoadsorption (seven treatment sessions)

	Start of treatment, cycles 1/2	During treatment, cycles 9/10	End of treatment, last two cycles
Mercury pre-column, µg/l	1.6 ± 2.3	4.9 ± 4.9	4.0 ± 4.1
Mercury post-column, µg/l	62.4 ± 73.3	13.3 ± 9.3	8.3 ± 5.5
Column performance index	41.6 ± 4.6	–	39.6 ± 7.3

Data are represented as means ± SD.

during immunoadsorption, we decided to continue treatment using a thiomersal-free system (IgG-Therasorb). Seventeen days after the last PA session, pre-treatment mercury level was still 14.4 µg/l, clearly indicating long-term toxicity. Fortunately, symptoms did not recur on chelating therapy with dimercaprol. Immunoadsorption treatment was discontinued after 7 months as the patient was transferred to another hospital.

Discussion

This preliminary report provides first evidence that immunoadsorption using thiomersal-primed PA sepharose columns can cause a sustained increase in systemic mercury concentrations, which exceeded current safety recommendations in a significant proportion of patients. The magnitude of mercury increase during treatment could not be predicted by clinical parameters including weight, renal function or clinical

diagnosis, but showed significant correlations with total number of treatments and pre-treatment concentrations of mercury and haematocrit, indicating that blood mercury levels were related to the dose of PA treatment and the amount of red blood cells, as the major binding site in the circulation [9]. However, analysis of mercury concentration in serum yielded similar results. Mercury levels found in some patients after and occasionally before PA immunoadsorption were comparable with or even higher than those reported for occupational exposure [9]. Fortunately, only one patient developed clinical signs of toxicity, and most had only moderate pre-treatment concentrations. Given that mercury exposure was restricted to patients on PA devices, sources other than thiomersal are very unlikely. We have informed the health authorities and other centres in Austria using PA immunoadsorption, but only one additional event of toxicity has been reported so far. The practical handling of PA immunoadsorption at our centre was thoroughly reviewed and checked by the manufacturers, without any errors being identified. Inappropriate contamination during set-up was also excluded as the nurses handling PA devices every working day for ~6 years had a median blood mercury concentration of only 0.9 µg/l ($n=6$, range, 0.6–2.1).

Why did toxicity occur in one particular patient? Although the exact reasons were not identified, we speculated on the following potential mechanisms. Most importantly, treatment by PA immunoadsorption had been performed intensively for short intervals during the previous 4 months, probably causing a gradual mercury increase in the CNS. This is a typical feature of lipophilic aryl mercury compounds, which accumulate in the CNS even at moderate plasma levels and lead to symptoms only after a variable latency period [9]. We also speculated that medical treatment of myasthenia and our practice of vitamin infusions

during treatment could possibly have concealed or delayed clinical toxicity considering that neostigmin markedly reduced symptoms of methylmercury intoxication and pyridoxine-thiols apparently remove methylmercury from the brain [9].

It is equally unclear why plasma levels exceeded 50 µg/l in only six of 26 treatments. According to our working hypothesis, PA columns are gradually loaded with thiol-containing plasma proteins, as temporary binding of serum albumin is a well-known feature of these columns. Since thiols are avid mercury scavengers, phosphate buffer is apparently insufficient to rinse all mercury from protein-loaded columns. Apart from treatment dose, we were unable to identify further individual factors responsible for the increase of mercury levels during treatment. Citrate anticoagulation appears unlikely, as traces of mercury should also have been released from the body in the patients in the other groups.

Although toxic mercury levels were not restricted exclusively to older columns with a low CPI, our findings have potential implications for approval of medical products, which is usually performed using new and unused devices. To ensure long-term safety, more thorough and lengthy examinations in a different range of patients seem advisable. According to the manufacturer's information, the PA system has been approved on the basis of post-treatment mercury levels of <20 µg/l, which held true for only 57% of treatments in our patients.

Our data do not allow us to draw firm conclusions as to potential long-term consequences. The observation that mercury levels were not normalized between treatments in a considerable proportion of patients could clearly indicate a potential risk of long-term accumulation. Due to the short duration of exposure and comparatively low baseline levels, long-term toxicity seems unlikely in the vast majority of our patients, however. Accordingly, clinical experience with the PA system over >10 years suggests a very low risk of toxicity. In addition, ethyl mercury is less neurotoxic than methyl mercury and its half-life in blood appeared closer to 10 days (as recently suggested [10]) than 50 days, as traditionally assumed for methyl mercury [9]. Estimated half-life was not appreciably influenced by renal function, confirming a major role for faecal elimination [11].

Given that chronic and systemic diseases are present in most patients on PA treatment, it is essentially impossible to judge on clinical grounds whether low-level mercury toxicity is present. Blood levels only weakly represent organ and particularly brain concentrations of mercury. A high level of suspicion seems to be justified, as occupational mercury toxicity may occur even at levels <15 µg/l, such as observed in 50% of our patients post-treatment. Dentists with an average blood level of 9.8 µg/l were found to have significantly worse performance than controls on a number of neurobehavioural tests measuring motor speed, visual scanning, visual memory, visuomotor coordination and speed, features consistent with central and peripheral

mercury neurotoxicity [12]. Piikivi and Tolonen reported that exposed workers with blood mercury levels of 12 µg/l had electroencephalographic (EEG) abnormalities on visual and computerized analysis, with significantly slower and more attenuated EEGs compared with controls [13]. Workers exposed to mercury vapour exhibited pre-clinical alterations in postural and intentional tremor at mean blood levels of 24 µg/l [14].

The clinical implications of our findings are obvious: first, if neurological symptoms should newly emerge or deteriorate in patients treated by PA immunoadsorption, investigation for potential mercury toxicity is recommended. Secondly, to reduce long-term organic mercury exposure safely given increasing environmental and food contamination [15], thiomersal should ideally be replaced by less toxic disinfectants, a goal successfully accomplished for infant vaccines [8]. As a first step, rinsing solutions should be modified to remove mercury more effectively. Such modifications could include thiol-containing agents such as glutathione, *N*-acetylcysteine or albumin, or chelators including EDTA, penicillamine, BAL or dimercaprol [9]. Assuming mercury release during treatment (Table 4), even higher blood levels than observed at the end of treatment are to be expected during treatment. Consequently, we have initiated serial determinations of mercury and will investigate the effects of additives to the rinsing solution or modifications of the rinsing procedure unless safe alternatives to thiomersal become clinically available, which seems to be the most desirable long-term solution.

Acknowledgements. The authors are indebted to the nursing team of the Apheresis Unit, Department of Medicine III, University of Vienna, for their invaluable help in performing this study. We also thank J. Jäger for mercury analysis and Professor W. Druml for referring patients.

Conflict of interest statement. None declared.

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Received for publication: 29.7.03

Accepted in revised form: 17.9.03