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Neuroimmunotoxic Effects of Occupational Exposure to Lead and Mercury

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Abstract. Occupational exposure to lead and mercury continues to pose a threat to workers, making early detection of their neurotoxic effects a pressing concern. The levels of sera autoantibodies to nervous system proteins can be of good value in this purpose. This study was carried out to detect subclinical neurotoxicity caused by occupational exposure to lead (n = 27) and mercury (n = 24) using blood levels autoantibodies against neural proteins. The auto-antibodies used were the IgG and IgM types of anti-NF68, anti-NF160, anti-NF200, anti-MBP and anti-GFAP. Compared to the controls (n = 30), both exposed groups showed higher percentage of positive titers for anti-NF68 (IgG), anti-NF200 (IgM), anti-GFAP (IgG, IgM) and anti-MBP (IgM). Higher median values were obtained for anti-NF200 (IgG, IgM), anti-GFAP (IgM) and anti-MBP (IgM). Anti-NF160 was not affected by exposure. Determination of sera neuroantibodies levels is recommended in the biological monitoring of lead and mercury exposure.

Keywords: Neurofilaments, autoantibodies, occupational exposure.

Introduction

Many occupational exposures have been suggested as playing a role in the etiology of nervous system diseases. Evidence has appeared in literature suggesting that lead (Pb) and mercury (Hg) may play a role in the onset of amyotrophic lateral sclerosis (ALS) and multiple sclerosis (MS) (Mano, *et al*.,

1990; Sienka, *et al*., 1990 and Arman, *et al.*, 1991). In these disorders neurotypic and glyotypic proteins have been used to detect and characterize the cellular response to toxicant-induced injury (O'Callaghan, 1992). For example, glial fibrillary acidic protein (GFAP), the astrocytic intermediate filament, has been expressed at low exposure levels of neurotoxicants (El-Fawal, *et al.*, 1992; Evans, *et al.*, 1992 and Little, *et al*., 1998).

Literature suggests that autoantibodies may appear in blood during neurotoxic insult. The mechanism proposed was as follows: In the presence of axonal degeneration, demyelination and glial degeneration such as that precipitated by heavy metals like lead or mercury or other neurotoxicants in either centralor peripheral nervous system, proteins specifically these structures are presented as antigens with the subsequent autoantibodies formation (Manzo, *et al*., 2001; Haggqvist, *et al.,* 2005 and Rowley & Monestier, 2005).

The present study was carried out to detect the value of assessing the levels of blood autoantibodies to neuro-axonal neurofilaments (NF), the astrocyte glial fibrillary acidic protein (GFAP) and the myelin sheath basic protein (MBP) as early and sensitive biomarkers for the subclinical neurotoxicity caused by occupational exposure to lead and mercury.

Material and Methods

The present study included a total number of 51 non smoking male workers. They represented two groups, 27 workers were exposed to lead in a battery manufacturing company and 24 other workers were exposed to mercury in a neon light bulb factory. A group of 30 workers was selected from a food preservation company as controls. They had no history of occupational exposure to any pollutants but showing the same demographic characteristics as those exposed.

Each subject was interviewed for his personal, social and occupational history, smoking habits and nutritional status. Special emphasis was given to the history of nervous system complaints. Positive cases were excluded.

For each subject, the body mass index (BMI) was calculated by dividing the weight in kilograms by the squared height in meters. A blood sample of 10 ml was collected by venepuncture using a heparinized vacutainer (for the determination of lead and neuroautoantibodies levels). A spot urine sample was taken from each subject in a dry clean glass bottle and stored refrigerated until analysis (for the determination of mercury and creatinine levels).

Determination of the Biological Indices of Exposure

Lead in Blood (PbB)

The atomic absorption spectrophotometric technique was used for the determination of PbB according to the method described by Niosh (1974). Blood (3 ml) was digested by concentrated nitric and perchloric acids. The residue was dissolved in diluted nitric acid and measurements were carried on using a PYE Unicam atomic absorption spectrophotometer SP-90.

Mercury in Urine (HgU)

Levels of mercury in urine were determined using the cold vapor atomic absorption spectrophotometric technique (Lindestedt, 1970). In a special conical flask, one ml urine was digested by concentrated sulfuric acid and potassium permanganate (5%). The flask was left stoppered overnight at room temperature. After 24 hours, the excess potassium permanganate was reduced by 20% hydroxylamine hydrochloride solution. The clear colorless sample was transferred to measurement in the PYE Unicam atomic absorption spectrophotometer SP-90. The concentration of mercury in urine was referred to its creatinine content.

Blood Autoantibodies to Neuroproteins

This includes the determination of autoantibodies titers (IgG and IgM) of the neurofilament triplet proteins (NF68, NF160 and NF200), glial fibrillary acidic protein (GFAP) and myelin basic protein in serum by the enzyme linked immunosorbent assay (ELISA) using the method of Tanaka, *et al.* (1989) modified by El-Fawal, *et al.* (1993).

Statistical Analysis

Non parametric analysis was used since the data were not normally distributed (Sachs, 1982). A statistical package (Mystat, Systat Inc., USA, 1995) was used in an IBM-compatible computer. The Wilcoxon-Mann-Whitney test (U-test) was used for testing the significance when controls and exposed subjects were compared. Z test was used for comparing proportions.

Results and Discussion

The personal characteristics of the studied population are shown in Table 1. They include age, duration of exposure and body mass index (BMI). Age, duration of exposure and BMI did not differ significantly between each of the exposed groups and the control subjects. This indicates that the sample population (exposed workers and control subjects) is homogenous and control subjects match well with the exposed groups.

Table 1. Personal characteristics of the different exposure groups and the control subjects (Median + S.E. of Median).

Personal characteristics		Exposed groups		Controls n = 30
		Lead n = 27	Mercury n = 24	
Age (years)	Median	39.00	35.00	32.00
	SE	1.44	0.58	2.02
	U	0.50	0.28	
Duration of exposure (years)	Median	14.00	11.50	–
	SE	0.87	0.29	–
	U	–	–	–
Body mass index	Median	27.40	6.30	26.0
	SE	0.87	0.64	0.64
	U	3.95	2.57	

U: Wilcoxon-Mann-Whitney test between exposed workers and control subjects.

The level of exposure to the different pollutants in the present study was assessed using the biological indices of exposure. PbB and HgU levels are reported in Table 2. These parameters are recommended in literature to be used in the biological monitoring of exposure to lead and mercury (Symansky and Greeson, 2002). The median levels of PbB and HgU among exposed workers were found to be 35.00 ± 0.87 µg/dl and 100.00 ± 2.89 µg/g creatinine respectively. These values were significantly higher than those of the control subjects, confirming that actual exposure to each pollutant does exist. The level of exposure to lead may be considered as acceptable when compared to the biological limit value (BLV) of 60 µg/dl. However, concerning workers exposed to mercury, they can be classified as "highly exposed workers", when we put into consideration the 50 µg/g creatinine biological limit value for exposure to mercury (Rappaport, 1995).

Table 2. Biological indices of exposure among the different exposure groups and the control subjects (Median + S.E. of Median).

Biological indices of exposure		Exposed groups		Controls n = 30
		Lead n = 27	Mercury n = 24	
Lead in blood (µg/dl)	Median	35.00	–	13.00
	SE	0.87	–	0.58
	U	42.05**	–	–
Mercury in urine (µg/g creatinine)	Median	–	100.00	1.90
	SE	–	2.89	0.14
	U	–	39.42**	

U: Wilcoxon-Mann-Whitney test between exposed workers and control subjects.
*$p \leq 0.05$ **$p \leq 0.01$

The neuroimmunotoxic effects, as manifested by the IgG and IgM auto-antibodies against the neurotypic and gliotypic proteins, of occupational exposure to Pb and Hg are presented in Table 3.

Table 3. IgG and IgM autoantibodies against neurotypic and glyotypic proteins among lead and mercury exposed workers and their controls (positive titers: %, median ± SE: ng/ml).

Immunoautoantibodies		Exposed groups		Controls n = 30
		Lead n = 27	Mercury n = 24	
Anti-NF68				
IgG	Positive titers	59.26**	66.67**	16.67
	Median ± SE	5.60 ± 0.95	5.60 ± 0.20	4.50 ± 2.48
IgM	Positive titers	14.81	16.67	10.00
	Median ± SE	2.05 ± 0.45	0.98 ± 1.89	0.90 ± 0.72
Anti-NF160				
IgG	Positive titers	40.74	20.83	20.00
	Median + SE	0.60 ± 0.11	0.20 ± 0.96	0.55 ± 0.12
IgM	Positive titers	14.80	16.67	16.67
	Median ± SE	4.35 ± 1.13	3.55 ± 1.93	2.40 ± 1.30
Anti-NF200				
IgG	Positive titers	22.22	25.00	10.00
	Median ± SE	53.20 ± 29.80*	50.10 ± 32.82*	0.30 ± 0.18
IgM	Positive titers	44.44**	50.00**	10.00
	Median ± SE	18.85 ± 3.78**	19.62 ± 2.65**	0.24 ± 0.18
Anti-GFAP				
IgG	Positive titers	81.48**	83.33**	20.00
	Median ± SE	0.20 ± 0.24	0.23 ± 0.19	0.20 ± 0.01
IgM	Positive titers	85.19**	83.33**	13.33
	Median ± SE	32.00 ± 25.98*	32.00 ± 28.69	0.63 ± 0.01
Anti-MBP				
IgG	Positive titers	14.80	12.50	10.00
	Median ± SE	0.04 + 0.01	0.62 ± 1.19	0.06 ± 0.02
IgM	Positive titers	77.78**	75.00**	20.00
	Median ± SE	1.58 ± 3.40**	0.63 ± 2.57	0.08 ± 0.03

*significant difference between exposed and controls at $p < 0.05$.
** significant difference between exposed and controls at $p < 0.01$.

Pb and Hg exposed workers showed significantly higher percentage ($p \leq 0.5$, 0.01) of positive titers than controls in the IgG type of anti-NF68 and anti-GFAP and the IgM type of anti-NF200, anti-GFAP and anti-MBP. Also, both exposures showed to raise the levels of IgG type of anti-NF200 and the IgM type of anti-NF200, anti-GFAP and anti-MBP. Anti-NF160 has not been affected by neither Pb nor Hg exposure.

The detection and quantification of autoantibodies against nervous system specific proteins in sera of individuals exposed to neurotoxicants afford the opportunity to detect nervous system insult prior to the development of overt neurotoxicity (Evans, 1995; El-Fawal, *et al*., 1996; Rowley and Monestier, 2005).

For some autoantibodies (anti-MBP) only IgM titers were predominant, for others (anti-NF68) only IgG were predominant, while anti-GFAP showed increased levels for both IgG and IgM titers. IgM antibody is the isotype associated with primary antigen challenge and recent exposure, while IgG antibody is the isotype associated with secondary antigen challenge and persistent pathology. Consequently, the occurrence of both IgG and IgM together means secondary antigen challenge and persistent pathology with continuous degeneration. However, the occurrence of IgG only without IgM means switching from primary to secondary antigen challenge (Evans, *et al*., 1994; Hart and Fabry, 1995).

From the results of the present study, it can be deduced that the neurotoxic effects of occupational exposure to lead and mercury can be manifested through persistent axonal insult (increased anti-NF), CNS and astrocyte involvement (increased anti-GFAP) and secondary demyelination (increased anti-MBP). This goes in full agreement with the works of Kilburn and Warshaw (1994), Bigazzi (1996), El-Fawal (1996) and El-Fawal, *et al*. (1999).

The precipitation of autoimmunity (formation of autoantibodies) found in the present study might be referred to the alteration of the antigen (neuroprotein) immunogenicity by the heavy metals in question. For example, binding metal cations to MBP molecules in a charge neutralization process may produce a larger more immunogenic antigen, and the reaction of metals with the methionine and cysteine residues of NFs and GFAP may unmask epitopes on the autoantigen rendering it immunogenic or more immunogenic (Waterman, 1996). Numerous studies have indicated that these autoantibodies may not simply represent an epiphenomenon indicative of tissue damage. They do not interact with surface antigens but may penetrate normal cells, including neurons, to produce degeneration and apoptosis (Ogawa, *et al*., 1995; Alarcon-Segovia, *et al.*, 1996; DeFeo & El-Fawal, 1998; Ruiz-Arguelles and Alarcon-Segovia, 1998).

It can be concluded that autoantibody detection may be considered as a means of monitoring chemical-induced neuropathogenesis, and may as well be used as a tool for screening potential neurotoxicity by agencies concerned with risk assessment and chemicals regulations.

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التأثيرات العصبية – المناعية السامة للتعرض المهني لكل من الرصاص والزئبق

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الولايات المتحدة الأمريكية

المستخلص. مازال التعرض المهني للرصاص والزئبق يمثل تهديداً لصحة العمال مما يجعل الكشف المبكر عن تأثيراتهما العصبية السامة مسألة جديرة بالاهتمام. وقد تكون لمستويات الأجسام المضادة الذاتية لبروتينات الجهاز العصبي أهمية في هذا الصدد. وقد أجريت هذه الدراسة بهدف الكشف عن السمية العصبية تحت السريرية والتي يسببها التعرض المهني للرصاص (عدد: ٢٧) والزئبق (عدد: ٢٤) باستخدام مستويات الأجسام المضادة الذاتية لبروتينات الجهاز العصبي بالدم. وكانت الأجسام المضادة الذاتية المستخدمة عبارة عن نوعي الجلوبيولينات المناعية IgG و IgM للخيوط العصبية ٦٨ و١٦٠ و٢٠٠ والبروتين القاعدي للنخاعين والبروتين الحامضي لليفيات الدبقية. وبالمقارنة مع العينة الضابطة (عدد: ٣٠) وجد زيادة في العيار الإيجابي لدى المجموعتين المعرضتين في كل من مضادات الخيوط العصبية ٦٨ (IgG) و٢٠٠ (IgM) ومضاد البروتين الحامضي لليفيات الدبقية (IgM و IgG) ومضاد البروتين القاعدي للنخاعين (IgM)، وقد تم أيضا التحصل على قيم أعلى للوسيط لكل من مضاد الخيوط العصبية ٢٠٠ (IgM و IgG) ومضاد البروتين الحامضي لليفيات الدبقية ((IgMومضاد البروتين القاعدي النخاعيني (IgM). هذا ولم يتأثر مضاد الخيوط العصبية ١٦٠ بالتعرض. وتوصي الدراسة بتقدير تركيز الأجسام المضادة العصبية الذاتية في المصل أثناء الرصد الحيوي للتعرض لكل من الرصاص والزئبق.