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Effect of Environmental Exposures to Lead and Cadmium on Human Lymphocytic Detoxifying Enzymes

S. J. D'Souza, L. M. Narurkar, M. V. Narurkar

Radiation Biology and Biochemistry Division, Bhabha Atomic Research Centre, Trombay, Bombay 400085, India

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Lead (Pb) is among the most toxic heavy elements in the atmosphere (Fergusson 1990). Aerosol lead enters the human blood stream by way of the respiratory tract and indirectly, by surface disposition in the alimentary tract followed by absorption (Fergusson 1990). Lead pollution is also known to occur through its presence in petrol, paint, glazed vessels and solder. Atmospheric lead pollution may be predominantly high around factories manufacturing Pb alloys (Schwartz and Levin 1991). Lead toxicity is associated with inhibition of α-aminolevulinic acid dehydrase (ALAD) activity, rise in the blood porphyrin, inhibition of ATPase in erythrocytes, decreased blood haemoglobin and anemia. Elevated lead concentrations in pregnant women have been shown to cause hypertension and birth defects (Rabinowitz 1988). Lead is also known to interact with other elements such as Fe, Zn, Ca and Cu in biological systems (Abdulla et al. 1979; Sandstead 1977). Cadmium (Cd) is not essential for human body. It enters the human environment as a contaminant. Human intake of Cd is chiefly through the food chain (about 400–500 μg/wk) (Fergusson 1990). Analysis of neuropsy material shows that smokers accumulate much more Cd than nonsmokers (WHO Report, 1972). Chronic Cd poisoning produces proteinurea and affects the proximal tubules of kidney, causing the formation of kidney stones (WHO Report, 1972). The reported hypertensive effect of Cd in man has been associated with high Cd/Zn ratio in kidney (Strehlow and Barltrop 1988). Studies on air pollution have shown that Cd concentration in air could be positively correlated with heart disease, hypertension and arteriosclerosis (Fergusson 1990). The present investigation was aimed at assessing the usefulness of human lymphocytic detoxicating enzyme activities and their ratios in an assessment of human health-risks during environmental exposures to Pb and Cd. The human

Correspondence to: S. J. D'Souza

subjects investigated comprised those exposed to highly contaminated lead and cadmium areas in the state of Maharashtra, India.

MATERIALS AND METHODS.

Histopaque 1077, 1-chloro-2,4-dinitrobenzene (CDNB), glutathione (reduced form), NADPH, cytochrome c and aminopyrine were purchased from Sigma Chemical Co., St. Louis, USA.

Blood samples 5-10 ml each, were withdrawn by vein puncture of human subjects from the sites of environmental exposures to Pb in a village near an alloy factory. For Cd exposure, blood of those individuals containing high Cd levels were provided by Dr. R.N. Khandekar of the Environmental Assessment Division, BARC. Blood samples were collected in tubes containing EDTA (1 mg/ml). Lymphocytes were isolated from whole blood by the method of Boyum (1968). NADPH cytochrome c reductase activity was determined as per William and Kamin (1962). Aminopyrine demethylase (AD) activity was assayed colorimetrically by the method of Mukkassa and Yang (1981). Glutathione S-transferase (GST) activity was assayed according to Habig et al. (1974). Protein was quantified according to Lowry et al. (1951) using bovine serum albumin as the standard.

RESULTS AND DISCUSSION.

The sample size in Pb contaminated areas is small because of frequent visits of medical teams to these areas for blood samples and consequent unwillingness on the part of uneducated villagers to part with their blood. Blood lead levels in the polluted area varied from 13.17 to 75.83 µg/dl. Five of the seven individuals showed significantly higher values (ranging from 38.58-75.83 µg/dl - mean 48.26 ± 9.18) than normal found in Bombay population whose general Pb level varies from 10.7 to 14.1 µg/dl - mean 12.4 ± 1.5 (Table 1). As evident from table 2, the detoxicating enzyme activities exhibited by the Pb-exposed individuals showed very low levels of NADPH cyt.c reductase activity ranging from 0.79 to 3.3 nmoles/min/mg protein (mean 2.18 ± 0.33) as compared to normal range of 4.1 to 12.25 nmoles/min/mg protein, (mean 6.76 ± 0.59) in healthy individuals. On the other hand, aminopyrine demethylase activity was found to be considerably higher than the normal average in these individuals, the range being 4.78 to 29.24 (mean 11.67 ± 3.15) nmoles/min/mg protein, as against the normal average of 6.73 ± 0.75 nmoles/min/mg protein. The phase II GST activity was extremely low in these individuals and ranged from 13-26 (mean 20.12 ± 1.5) nmoles per min per

Table 1. Levels of lead and cadmium in human whole blood*

Group	Lead	Cadmium
Normal	12.4 ± 1.5 (n = 24)	0.22 ± 0.006 (n = 30)
Exposed	48.26 ± 9.18* (n = 5)	0.42 ± 0.007* (n = 30)

* Analysis of Pb and Cd in human blood samples was carried out by Dr. R.N. Khandekar of the Environmental Assessment Division, BARC. Values expressed as µg/100 ml blood.

(n) = Number of individuals.

* Significantly different from control value at $P < 0.001$.

Table 2. Lymphocytic detoxicating enzyme activities in human subjects during environmental exposure to lead and cadmium

Exposure	Enzyme activities*			Ratio of AD/GST
	NADPH cyt.c reductase	AD	GST	
Nil Controls (n=24)	6.76 ± 0.59	6.73 ± 0.75	62.4 ± 3.7	0.112 ± 0.009
Lead (n=7)	2.18 ± 0.33*	11.67 ± 3.15*	20.12 ± 0.5**	0.629 ± 0.19**
Cadmium (n=20)	Not done	110.22 ± 10.09*	24.35 ± 3.2*	6.065 ± 1.2**

* Unit of activity expressed as nmoles product/min/mg protein mean ± SEM.

(n) = Number of individuals.

Significantly different from control value at $P < 0.01$*; $P < 0.001$**.

mg protein which was much lower than the normal average of 62.4 ± 3.7 units (Table 2), and even lesser than the lowest normal range of 30-50 units - mean 40.35 ± 2.9 (Narurkar et al. 1988).

Exposure to Pb has been shown to reduce resistance and aggravate bacterial and viral infection in animals (Nakaguwa 1991). Pb as a metallic pollutant in environment has been recognized to affect microsomal mixed function oxidase system through depletion of cytochrome P-450 (Borella and Giardino 1991). In addition, the phase II GST is also adversely affected probably because of the low content of GSH (Nakaguwa 1991). A genetic difference in susceptibility to immunomodulation by lead explained the variability of its immunotoxicity in animal species (Borella & Giardino 1991). In the present study at least two out of the seven individuals were found to be suffering from leprosy and the rest were in poor health needing medical check up for respiratory ailments. It may be interesting to note that a very low activity of GST was also observed in the untreated leprosy patients in an earlier study (Narurkar et al. 1988). High levels of demethylase activity are likely to increase the carcinogenic potential of environmental carcinogens, if not conjugated and detoxified by the appropriate levels of phase II enzymes. The imbalance in the ratio of AD/GST enzyme activities due to environmental pollution may have important implications in the health disorders including susceptibility to environmental carcinogens. The results in this study did not appear to be due to lead poisoning though there were definite indications of air, soil and grass contamination with lead (*Khandekar et al. BARC Report 1991*). The imbalance of AD/GST ratio could be due to heavy metal exposure from among several other parameters.

The Cd levels in blood samples of the Cd-exposed human subjects were in the range of 0.38-0.46 µg/dl - mean 0.42 ± 0.007 which was double the normal range of 0.19-0.25 µg/dl - mean 0.22 ± 0.006 in Bombay (Table 1). It is evident from table 2 that AD activity is 6-7 times more in the Cd-exposed subjects in comparison with the control subjects. On the other hand, the GST activity is about 3 times less than that in the controls resulting in a tremendous increase in the AD/GST ratio (6.065), the normal ratio being 0.112. The carcinogenic effect of Cd has been established in animals. Although the situation is less clear in human beings, a relationship has been shown between Cd exposure in industry and the incidence of prostatic cancer (Sunderman 1977). Cadmium sulphide was suggested to be mutagenic by Friberg et al. (1974). Even if Cd is not directly involved in carcinogenesis

in human subjects, the highly increased AD/GST ratio suggests that Cd-exposed persons could be more susceptible to other environmental carcinogens. One of the toxic effects of Cd has been shown to be the development of Itai-Itai disease which is marked by softening of bones (osteomalacia) produced by a deficiency of vitamin D (Fergusson 1990). Cd has been implicated in adversely affecting the metabolism of Ca, PO_4 and vitamin D in the body. Cadmium is also known to create deficiency of copper and iron as well as to compete with zinc for the binding site on the enzyme causing its inactivation (Fergusson 1990). It is apparent, therefore, that although cadmium is not directly implicated in the changes in detoxicating enzymes, the resultant metabolic alterations probably are responsible for the lymphocytic enzyme changes and ratios indicative of serious health hazards to the exposed subjects.

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