Role of zinc in mitigating the toxic effects of chlorpyrifos on hematological alterations and electron microscopic observations in rat blood

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Abstract

The present study determined the protective potential of zinc in attenuating the toxicity induced by chlorpyrifos in rat blood. Male Sparque Dawley (SD) rats received either oral chlorpyrifos (13.5 mg/kg body weight) treatment every alternate day, zinc alone (227 mg/l in drinking water) or combined chlorpyrifos plus zinc treatment for a total duration of 8 weeks. The effects of different treatments were studied on various parameters in rat blood including haemoglobin (Hb) levels, total leukocyte count (TLC), differential leukocyte count (DLC), zinc protoporphyrins (ZPP), serum trace elemental concentrations and Scanning Electron Microscopic (SEM) observation of the blood cells. Chlorpyrifos treatment to normal control animals resulted in a significant decrease in TLC and ZPP concentration after 4 and 8 weeks. Chlorpyrifos treated animals also showed significant neutrophilia and lymphopenia after 8 weeks of toxicity. In addition, a significant decrease in serum zinc and iron concentrations were observed following chlorpyrifos intoxication, however, these animals responded with increased serum copper levels following the toxic treatment with this organophosphate. SEM studies of the red blood cells from chlorpyrifos treated animals indicated marked alterations in the topographical morphology of the various cell types, with the prominent feature being common anisocytosis of the erythrocytes. Oral zinc treatment to the chlorpyrifos treated animals significantly improved the total leukocyte, neutrophil and lymphocyte counts, as well as the otherwise reduced concentrations of ZPP and the levels of various serum trace elements. Protective effects of zinc were also evident in the electron microscopic observations where most blood cell types depicted reverted to a close to the normal appearance. Based upon these data, the present study is first of its kind and suggests that zinc treatment considerably attenuates chlorpyrifos induced toxicity induced in restoring the altered hematological indices and morphological changes.

Introduction

Chlorpyrifos is a broad-spectrum organophosphate insecticide and is extensively being used to control agricultural pests, disease vectors and is preferred to chlorinated hydrocarbons for field applications because of its quick action, relatively shorter half-life and poor-accumulation in the food web (Kwong 2002). Chlorpyrifos manifests its mammalian toxicity through activation to its corresponding oxygen analog (chlorpyrifos-oxon), which in turn is responsible for the inhibition of the acetylcholinesterase (AChE) enzyme leading to neuropathy. Besides, the moderate to severe toxicity of chlorpyrifos in non-neuronal tissues in many mammalian species has also been attributed to the hydrolytic detoxification of the chlorpyrifos-oxon by a group of hepatic arylesterases (Costa
et al. 1990; Li et al. 1993). Previous reports from our laboratory have clearly indicated the adverse effects of chlorpyrifos intoxication on the profile of liver marker enzymes, antioxidant enzyme system and the hepatic levels of essential trace elements (Goel et al. 2000, 2005; Goel & Dhawan 2001). More specifically, in a recent study, Singh et al. (2004) reported a significant increase in lipid-peroxidation levels in rat erythrocytes following chlorpyrifos treatment. Although the primary toxicological target for chlorpyrifos is nervous system, it is very common to observe toxic manifestations of these compounds in other organs including hematological system. Curiously, besides the well-known effects of organophosphates on brain and some reports for their hepatic toxicity, there is limited evidence that these insecticides may have adverse effects on hematological profiles (Gibel & Lohs 1975; Mandal et al. 1986). Despite these sporadic reports, till date there is no clear information and understanding whether or not organophosphates in general, and chlorpyrifos in particular, mediates its toxic effects through alterations in the hematological indices in the laboratory animals.

Another challenging aspect of organophosphorus insecticide toxicity is lack of adequate preventative strategies that are safe and non-toxic for occupationally exposed human population. Various chemical compounds have been attempted for such preventative interventions, but without much success. More recently, much attention is being focused on the possible role of essential trace elements in providing the necessary preventive efficacy with least toxicity and side-effects (Xiu 1996; Kang & Zhou 2005; Zhou et al. 2005). In this context, zinc, a key constituent or cofactor of over 300 mammalian proteins, is intensively being studied for its protective efficacy in various models of animal toxicity (Joshi et al. 2004; Zhou et al. 2005). A number of studies have strongly suggested zinc to be a beneficial agent in mitigating the damage arising in the setting of increased oxidative stress (Cagen & Klaassen 1979; Cabre et al. 1999; Zhou et al. 2005). Data from our laboratory are in support of these observations and we have also demonstrated the protective effects of zinc in regulating the hepatic function in various models of hepatotoxicity (Dhawan & Goel 1994, 1995; Sidhu et al. 2004a, b).

Therefore, due to the paucity of information on the toxic manifestations of chlorpyrifos on hematological parameters, and the lack of information on the role of zinc in such conditions, it was of our interest in the present study to investigate whether zinc would ameliorate the damage inflicted on the blood cells of the rats intoxicated with chlorpyrifos. Here, we demonstrate that oral zinc treatment to the chlorpyrifos treated animals significantly improved altered hematological indices, serum trace elemental concentrations and morphological changes in blood cells. Collectively, these data suggest that zinc treatment can be potentially be considered as an intervention in human subjects with accidental exposures to acute doses of chlorpyrifos and related organophosphates.

**Materials and methods**

**Evaluation of chlorpyrifos purity**

Before the initiation of various treatments, the purity of chlorpyrifos procured from Montari Agro Industries, Bombay, India was evaluated using a VG70S-11-250J + Gas Chromatographic-Mass Spectrometer (GC-MS) at the Regional Sophisticated Centre, Panjab University, India.

**Experimental design**

**Grouping of animals**

Male Sparque Dawley (SD) rats weighing 145±20 g were procured from the Central Animal House, Punjab University, Chandigarh. The animals were housed in polypropylene cages in the departmental animal house under hygienic conditions and were acclimatized for at least 1 week before prior to different treatments. The animals were maintained on the standard laboratory feed and water *ad libitum*, throughout the period of experimentation.

Animals were segregated into four different groups. Animals in Group 1 (G-1) served as normal controls and were fed only with normal diet and water *ad libitum*. Group 2 (G-2) animals were given an oral chlorpyrifos treatment at a dose level of 13.5 mg/kg body weight (oral LD$_{50}$: 135/kg body weight) in corn oil, every alternate day for a duration of 8 weeks. Animals in Group 3 (G-3)...