Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Toxicology Letters 169 (2007) 26–33
Toxicology Letters
www.elsevier.com/locate/toxlet

Zinc mediates normalization of hepatic drug metabolizing enzymes in chlorpyrifos-induced toxicity

Ajay Goel[a,b,*], Vijayta Dani[a], D.K. Dhawan[a,**]

[a] *Department of Biophysics, Panjab University, Sector 14, Chandigarh-160014, India*
[b] *Division of Gastroenterology, Department of Internal Medicine, Baylor University Medical Center, Dallas, TX, USA*

Received 8 June 2006; received in revised form 13 July 2006; accepted 13 July 2006
Available online 9 December 2006

Abstract

The present study investigated the protective effects of zinc in attenuating the altered activities of drug metabolizing enzymes in the livers of rats intoxicated with chlorpyrifos. Male Sprague–Dawley rats received oral chlorpyrifos treatment (at a dose level of 13.5 mg/kg body weight in corn oil every alternate day), zinc supplementation alone (at a dose level of 227 mg/l in drinking water), or combined chlorpyrifos plus zinc treatments for a total duration of 8 weeks. The effects of different treatments were studied on the specific activities of various drug metabolizing enzymes including cytochrome P_{450}, cytochrome b_5, NADPH cytochrome-*c*-reductase, NADH cytochrome-*c*-reductase, aminopyrene-*N*-demethylase (APD) and aromatic hydrocarbon hydroxylase (AHH). Additionally, serum zinc levels were also determined in each of the treatment groups at the end of the study. Chlorpyrifos treatment resulted in a significant decrease in the serum zinc concentrations. Analogous to these changes, we observed significant depression in the activities of majority of the drug metabolizing enzymes investigated in the present study, except for AHH, where the decrease in enzyme activity was not statistically significant. However, zinc treatment to chlorpyrifos treated animals effectively restored the depressed serum zinc levels to within normal limits. Similarly, co-administration of zinc to chlorpyrifos intoxicated animals normalized the enzymatic activities of cytochrome P_{450}, NADPH cytochrome-*c*-reductase and NADH cytochrome-*c*-reductase within normal range. Collectively, these findings suggest that zinc plays an important role in regulating the hepatic activities of drug metabolizing enzymes in chlorpyrifos intoxicated animals, although it remains to be determined whether such protective effects of zinc are regulated directly, or through some indirect mechanism.
© 2006 Elsevier Ireland Ltd. All rights reserved.

Keywords: Zinc; Chlorpyrifos; Organophosphorus; Drug metabolizing enzymes; Cytochrome P_{450}; Cytochrome b_5

1. Introduction

Organophosphorus insecticides (OPs) are used widely for a variety of agricultural and public health applications (Pope, 1999; Maroni et al., 2000). OPs produce a wide range of toxicity in mammals by inhibiting acetylcholinesterase (AChE), and the consequent accumulation of the neurotransmitter acetylcholine (ACh) in synaptic junctions leads to excessive stimulation of postsynaptic cells leading to cholinergic toxicity (Pope et al., 2005). Chlorpyrifos (*O*,*O*′-diethyl-3,5,6-trichloro-

* Corresponding authors at: Gastrointestinal Cancer Research Lab, Department of Internal Medicine, 250 Hoblitzelle, Baylor University Medical Center, Dallas, TX 75246, USA. Tel.: +1 214 820 2692; fax: +1 214 818 9292.
** Corresponding authors. Tel.: +91 172 2534121; fax: +91 172 2534118.
E-mail addresses: ajayg@baylorhealth.edu (A. Goel), dhawan@pu.ac.in (D.K. Dhawan).

0378-4274/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.toxlet.2006.07.342

2-pyridinyl-phosphorothioate) belongs to the phosphorothioate class of organophosphorus insecticides, and gets metabolically activated to its corresponding oxygen analog, oxon, in the liver (Sultatos and Murphy, 1983; Chambers and Chambers, 1989; Kousba et al., 2004). Chlorpyrifos-oxon potently binds to and inhibits AChE to elicit cholinergic toxicity. Bulk of the activation of chlorpyrifos occurs in liver and its detoxification takes place in liver and plasma. This biotransformation of organophosphorus insecticides is catalyzed by cytochrome P_{450} and associated enzymes, which are present in microsomal membranes of liver (Jokanovic, 2001). Additionally, the activation of organophosphorus compounds to corresponding oxons occurs through oxidative desulphuration, which is also mediated by the cytochrome P_{450} (Jokanovic, 2001). It has been shown that repeated doses of chlorpyrifos were able to cause significant hepatic atrophy (Miyazaki and Hodgson, 1972).

Previous reports from our laboratory have also demonstrated the hepatotoxic actions of chlorpyrifos, wherein, the profile of liver marker enzymes, antioxidant enzymes and essential trace elements were found to be adversely affected (Goel et al., 2000, 2005, 2006; Goel and Dhawan, 2001). In view of the vital role of hepatic biotransformation of chlorpyrifos in modulating its acute mammalian toxicity, a better understanding of the enzymatic-regulators will allow designing appropriate preventative regimens to mitigate the mammalian toxicity of this class of insecticides. However, not much effort has been made in this direction, and there is a potential need for identification of new preventive compounds targeted against occupational insecticide exposures, that may offer significant protection but least toxicity of their own.

In this regard, the present study explored the possible protection afforded by zinc, which is known to possess catalytic and structural roles in over 300 metalloenzymes, as well as regulatory roles in diverse cellular processes such as signaling transduction and gene expression (Prasad, 1983). Zinc (Zn) is an essential trace element, is relatively nontoxic and is integral to several key functions in human metabolism (Fang et al., 2002; Daniel and Tom Dieck, 2004). Not only has Zn been identified as a component of key enzymes and regulatory proteins, it was recently suggested that the preventive effects of zinc may partly be mediated through increase in cytochrome P_{450} enzymes in subjects with alcoholic liver disease (Zhou et al., 2005; Kang and Zhou, 2005). Although the precise biological explanation for zinc-mediated protective effects is not certain, nonetheless, there is sufficient evidence that zinc supplementation provides significant hepatoprotection under different toxic conditions (Cagen and Klaassen, 1979; Cabre et al., 1999). In this regard, studies from our laboratory have also indicated the efficacy of zinc in regulating the liver functions in various animal models of increased oxidative stress (Goel et al., 2000, 2005, 2006; Sidhu et al., 2005). However, there is still a clear lack of understanding whether the toxic effects of chlorpyrifos are mediated through drug metabolizing enzymes, and further if zinc may have any preventive role in such toxic conditions.

Thus, in this study we sought to investigate the protective potential of zinc on drug metabolizing enzymes in the liver of animals, which were exposed to long-term toxicity with chlorpyrifos. Herein, we demonstrate that the liver activities of key drug metabolizing enzymes are markedly altered following chlorpyrifos intoxication. However, co-administration of zinc to these animals significantly normalized these enzyme activities indicating the protective role for zinc in chlorpyrifos-induced toxicity.

2. Materials and methods

2.1. Grouping of animals

Male Sprague–Dawley rats weighing 145 ± 20 g were procured from the Central Animal House, Punjab University, Chandigarh, and were maintained on the standard laboratory feed and water ad libitum. Animals were segregated into four different groups (Goel et al., 2006). Briefly, Group 1 (G-1) animals served as normal controls and 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 (Goel et al., 2005, 2006). Animals in Group 3 (G-3) served as normal zinc controls for the Group 4 (G-4) animals, and were supplemented with zinc in the form of $ZnSO_4 \cdot 7H_2O$, at a dose level of 227 mg/l added to their drinking water (Dhawan and Goel, 1994; Goel et al., 2006). All the treatments continued for a period of 8 weeks.

2.2. Biochemical estimations

The livers were removed and homogenized in 100 mM potassium phosphate buffer (pH 7.5) containing 0.15 M KCl to obtain 10% homogenate, using a mechanically driven Teflon fitted Potter Elvejhem homogenizer. The tissue homogenates were further processed at predetermined centrifugation speeds and time intervals to obtain different sub-cellular fractions for the estimation of various biochemical parameters.

2.2.1. Protein

Protein assay was done by standard methods (Lowry et al., 1951). Briefly, the protein lysates were appropriately diluted

and the protein contents were determined by using Folin's reagent as detailed previously (Goel et al., 2006).

2.2.2. Drug metabolizing enzymes

The livers were removed and homogenized in 100 mM potassium phosphate buffer (pH 7.5) containing 0.15 M KCl to obtain 25% homogenate, using a mechanically driven Teflon fitted Potter Elvejhem homogenizer. The homogenates were centrifuged at 10,000 × *g* for 20 min. The 10,000 × *g* supernatant was thereafter centrifuged again at 100,000 × *g* for another 60 min. The microsomal pellet thus obtained was suspended in 100 mM potassium phosphate buffer containing 20% glycerol (v/v). Aliquots of microsomal fractions were stored at −20 °C till further use.

2.2.2.1. Cytochrome P_{450}. Cytochrome P_{450} was measured in the microsomal fractions as described previously (Omura and Sato, 1964a). Briefly, carbon monoxide was bubbled through diluted microsomes and the sample was transferred to the reference and sample cuvettes. Subsequently, a pinch of sodium dithionite was added only to the sample cuvette and mixed thoroughly. The carbon monoxide difference spectrum of dithionite reduced microsomes was recorded from 400 to 500 nm on a Shimadzu 160 A double beam spectrometer. Molar extinction coefficient of 91 mM^{-1} cm^{-1} was used to calculate the amount of cytochrome P_{450}, which is expressed as nmol/mg protein.

2.2.2.2. Cytochrome b_5. Cytochrome b_5 was measured by recording the difference spectrum of NADH reduced microsomes versus air saturated microsomes from 409 to 424 nm on a double beam spectrometer as described previously (Omura and Sato, 1964b). The molar extinction coefficient of the difference spectrum of cytochrome b_5 utilized was 185 mM^{-1} cm^{-1}. The amount of cytochrome b_5 was expressed as nmol cytochrome b_5/mg protein.

2.2.2.3. NADPH cytochrome-c-reductase. The assay was carried out by the method of Yasukochi and Masters (1976). The assay mixture contained 300 mM potassium phosphate buffer (pH 7.7), 0.1 mM NADPH, 0.1 mM EDTA, 36 μM cytochrome *c* (Type III) and the appropriate amount of microsomal enzyme. For each set of reference tube, a blank tube lacking NADPH was run simultaneously. The reduction of cytochrome *c* was monitored by recording the increase in absorbance at 550 nm, and the enzymatic activity was expressed as nmol cytochrome *c* reduced/min/mg protein.

2.2.2.4. NADH cytochrome-c-reductase. The assay was carried out by the methods published previously (Yasukochi and Masters, 1976). The reaction conditions were very similar to that of NADPH cytochrome-*c*-reductase assay. However, instead of using NADPH as a substrate, equivalent amounts of NADH were added to the reaction mixture and absorbance due to the reduction of cytochrome *c* by NADH was measured at 550 nm. The specific enzyme activity was expressed as nmol of cytochrome *c* reduced/min/mg protein.

2.2.2.5. Aminopyrene-N-demethylase (APD). For the determination of aminopyrene-*N*-demethylase activity, the assay mixture contained 50 mM Tris–HCl buffer (pH 7.5), 2.5 mM NADPH, 0.5 mM aminopyrene and appropriate amount of microsomes (Nash, 1953). The reaction was carried out in 25 ml Erlenmeyer flasks, which were shaken at 37 °C for 15 min during the time of the incubation with the substrate. The blanks lacking NADPH were also run simultaneously. The reaction was stopped by the addition of zinc sulfate, saturated solutions of barium hydroxide and sodium borate. During this reaction, aminopyrene gets converted to formaldehyde by oxidative demethylation and the formaldehyde so formed was allowed to react with 2.0 ml of NASH reagent (solution of acetylacetone, acetic acid and ammonium salt) to form a yellow colored complex. The color developed was measured at 415 nm and the enzyme activity was expressed as nmol formaldehyde formed/min/mg protein.

2.2.2.6. Aromatic hydrocarbon hydroxylase (AHH). The activity of microsomal AHH was assayed according to the method of Nebert and Gelboin (1968) with few modifications done by Yang et al. (1978). This enzyme catalyzes the hydroxylation of substrate benzo(a)pyrene to hydroxy benzo(a)pyrene which was measured spectroflourimetrically. In brief, the assay mixture contained 50 mM Tris–HCl buffer (pH 7.4), 0.5 μmol NADPH, 5.0 μmol $MgCl_2$ and 50 mg tissue equivalents of the enzyme source in a final reaction volume of 1.0 ml. The reaction mixture was pre-incubated at 37 °C for 2 min and finally the reaction was initiated by the addition of 80 nmol of 3,4-benzo(a)pyrene in 25 μl acetone. The reaction was stopped after 10 min by addition of 4.0 ml of chilled acetone:hexane (1:3) mixture. The contents of the mixture were vortexed for 10 min. Following centrifugation at 3000 × *g* for 10 min, 2.0 ml aliquot of the organic phase was extracted with 4.0 ml of 1 N NaOH. Contents of the phenolic metabolites were measured on a fluorimeter at an activation wavelength of 396 nm and an emission wavelength of 522 nm. The activity of AHH was expressed as units of fluorescence/min/mg protein.

2.3. Zinc estimation

2.3.1. Preparation of serum

The blood samples were allowed to clot at room temperature. The clotted blood samples were centrifuged at 1500 rpm for 10–15 min to separate the clot, and the estimation of various elements was carried out in the supernatant.

2.3.2. Determination of zinc levels

Determination of zinc was carried out by the method of Evenson and Anderson (1975) using atomic absorption spectrophotometer. The serum samples were diluted ten times with 10 mM nitric acid and the zinc-standards (procured from Sigma Chemical Co., St. Louis, MO, USA) were also run simultaneously.

Table 1
Alterations in the body weights of the animals following zinc treatment to chlorpyrifos intoxicated rats

GROUPS	Body weight
G-1 Normal control	245.00 ± 16.43
G-2 Chlorpyrifos	221.25 ± 42.33[a]
G-3 Zinc	253.24 ± 33.40
G-4 Chlorpyrifos + Zinc	232.88 ± 32.70

Values are expressed as mean ± S.D. Values are expressed as mean ± S.D. by Newman–Keuls test; the statistical significance between different treatment groups was calculated by Newman–Keuls test; a minimum of 8–10 animals were used for each independent analyses.
[a] $P < 0.05$ in comparison to G-1.

2.4. Statistical analysis

The statistical significance of the data has been determined using a multiple post hoc test (Student-Newman–Keuls) with 5% differences considered significant. Prior to using Newman–Keuls test, the data were analyzed using a one-way analysis of variance (ANOVA). The results are represented as mean ± S.D. A minimum of seven animals were included for statistical analysis in each group, and the significance is given as $p < 0.05$, $p < 0.01$ and $p < 0.001$.

3. Results

All the results of various treatment groups have been compared with their normal controls. However, results from zinc + chlorpyrifos treated group (Group 4) have also been compared with the results of the chlorpyrifos treated group (Group 2).

3.1. Body weights

The variations in the body weights of the animals subjected to different treatments are shown in Table 1. During the course of present investigations, it was observed that the body weights of the normal control animals and zinc treated controls increased progressively throughout the study, and at the end of the study net body weight gains of 67.8% and 69.79%, respectively, were recorded for these two groups of animals in comparison to their initial weights. However, the net body weight gain of the animals intoxicated with chlorpyrifos was markedly less and was of the order of 36.41% only, when compared to the untreated controls ($P < 0.05$). Zinc treatment to the chlorpyrifos intoxicated animals also resulted in significant net body weight gain (48.8%), but was less when compared to the normal controls.

3.2. Drug metabolizing enzymes

Hepatic microsomal cytochrome P_{450} and cytochrome b_5 enzyme activities were found to be significantly inhibited ($P < 0.001$ for P_{450}; and $P < 0.05$ for b_5) in chlorpyrifos treated animals, in comparison to untreated normal controls (Table 2). No significant changes in the enzyme activities were observed for zinc control animals (G-3). However, zinc treatment to chlorpyrifos-treated animals reversed the decreased cytochrome P_{450} activities significantly, although a relatively less significant decrease ($P < 0.05$) was still evident in this enzymatic activity in these animals. On the contrary, complete normalization of cytochrome b_5 activity was observed in zinc + chlorpyrifos treated group, when compared to normal controls.

Similarly, as shown in Table 2, chlorpyrifos treatment resulted in a significant decrease in microsomal NADPH cytochrome-*c*-reductase, NADH cytochrome-*c*-reductase and APD activities after 8 weeks of treatment. Furthermore, a marginal decrease was observed in the levels of AHH following chlorpyrifos treatment, but this was not statistically significant. Interestingly, zinc supplementation to chlorpyrifos treated animals brought back the already depressed levels of NADPH cytochrome-*c*-reductase and NADH cytochrome-*c*-reductase, to within normal limits. However, similar treatment with zinc to chlorpyrifos intoxicated animals did not affect the microsomal APD activity in these animals, when compared to the untreated controls.

3.3. Elemental estimation

Serum zinc levels were observed to be lowered to statistically significant limits ($P < 0.01$) after 8 weeks of chlorpyrifos treatment (Table 3). However, serum zinc concentrations were found to be within normal limits following zinc supplementation in the zinc treated animals (G-3), as well as the combined zinc + chlorpyrifos treatment group (G-4) when compared to the controls.

4. Discussion

The present study investigates the effects of chlorpyrifos intoxication, on the drug metabolizing enzymes, which play an important role in the bio-activation and metabolism of chlorpyrifos in the liver. An additional aim of this study was also to determine whether zinc may mitigate the toxicity of chlorpyrifos by altering the activities of some or all of these drug metabolizing enzymes, thus providing a rationale for the protective

Table 2
Effect of zinc on the hepatic drug metabolism enzymes in rats subjected to chlorpyrifos and zinc treatments

Groups	Cytochrome P_{450} (nmol/mg protein)	Cytochrome b_5 (nmol/mg protein)	NADPH cytochrome-*c*-reductase	NADH cytochrome-*c*-reductase	Aminopyrine-*N*-demethylase	Arylhydrocarbon hydroxylase
G-1 Normal control	0.136 ± 0.010	0.182 ± 0.027	69.58 ± 10.12	757.42 ± 134.96	2.81 ± 0.37	11.54 ± 2.10
G-2 Chlorpyrifos	0.063 ± 0.023[c]	0.142 ± 0.031[a]	48.55 ± 8.54[c]	606.26 ± 140.53[a]	2.04 ± 0.23[c]	10.76 ± 1.96
G-3 Zinc	0.119 ± 0.031	0.199 ± 0.028	60.70 ± 14.13	698.26 ± 176.71	2.83 ± 0.481	10.95 ± 2.221
G-4 Chlorpyrifos + Zinc	0.116 ± 0.025[a,z]	0.162 ± 0.028	59.98 ± 11.07[x]	785.75 ± 109.33[y]	2.07 ± 0.40[b]	10.83 ± 2.38

The activities of the various enzymes are expressed as follows; NADPH cytochrome-*c*-reductase and NADH cytochrome-*c*-reductase (nmol cyt-*c* reduced/min/mg protein); aminopyrine-*N*-demethylase (nmol formaldehyde formed/min/mg protein); arylhydrocarbon hydroxylase (fluorescent units/min/mg protein). Values are expressed as mean ± S.D.; the statistical significance between different treatment groups was calculated by Newman–Keuls test; at least six to eight animals were used for each independent analyses.
[a] $P<0.05$ in comparison to G-1.
[b] $P<0.01$ in comparison to G-1.
[c] $P<0.001$ in comparison to G-1.
[x] $P<0.05$ in comparison between G-2 and G-4.
[y] $P<0.01$ in comparison between G-2 and G-4.
[z] $P<0.001$ in comparison between G-2 and G-4.

Table 3
Effect of zinc on serum zinc levels in rats subjected to chlorpyrifos treatment

GROUPS	Zinc (μg/ml of serum)
G-1 Normal control	4.88 ± 0.72
G-2 Chlorpyrifos	3.91 ± 0.63[a]
G-3 Zinc	4.94 ± 0.79
G-4 Chlorpyrifos + Zinc	4.29 ± 0.47

Values are expressed as mean ± S.D. by Newman–Keuls test; the statistical significance between different treatment groups was calculated by Newman–Keuls test; a minimum of eight animals were used for each independent analyses.
[a] $P<0.01$ in comparison to G-1.

efficacy of this metal ion. Here, we report that the hepatic microsomal activities of most of the drug metabolizing enzymes were significantly depressed following chlorpyrifos intoxication, however, simultaneous zinc co-administration to these animals was able to restore these enzyme activities to within normal limits, suggesting its protective potential.

In this study, we observed that the net body weight gain of the animals intoxicated with chlorpyrifos was markedly less as compared to the normal controls, which is consistent with the existing literature (Abou-Donia, 1981; Rahman et al., 1990). Additionally, in our study, we did not observe any appreciable change in the diet consumption of chlorpyrifos intoxicated rats, suggesting an overall increased degeneration of lipids and proteins as a result of the direct effects of the organophosphate. Zinc treatment to chlorpyrifos intoxicated animals helped in a better net body weight gain, which was attributed to its ability to reduce collagen accumulation in liver in such stress conditions (Dhawan and Goel, 1994).

Cytochrome P_{450} enzyme system plays an important role in the bio-activation of chlorpyrifos, and is central to the toxic manifestation of OP insecticides. We observed an overall significant inhibition in the activity of the microsomal cytochrome P_{450} and cytochrome b_5 following chlorpyrifos intoxication, which is in line with the observations made with chlorpyrifos and other organophosphorus insecticides (Sultatos, 1991; Barron et al., 1991; Ma and Chambers, 1994). We also observed significant depression in the activity of both NADPH cytochrome-*c*-reductase and NADH cytochrome-*c*-reductase following 8 weeks of chlorpyrifos intoxication. NADPH cytochrome-*c*-reductase, a flavoprotein present in endoplasmic reticulum is thought to be involved in the oxidation of various drugs, steroids and other chemicals (Masters, 2005). Analogous results for depressed NADPH cytochrome-

c-reductase activity following parathion intoxication in a reconstituted in vitro mixed function oxidase system were also observed. It was suggested that the sulphur released during desulfuration of an organophosphorus insecticide may bind not only to the cytochrome P_{450} but also to NADH cytochrome-*c*-reductase, culminating in the overall reduced activity of this monooxygenase (Kamataki and Neal, 1976). Alternatively, NADPH cytochrome-*c*-reductase is also involved in initiating the process of NADPH-dependent lipid peroxidation in the microsomal membranes, and increased lipid-peroxidation as a result of chlorpyrifos intoxication may also be the cause of depressed enzymatic activity (Sevanian et al., 1990). This explanation is particularly of interest since findings from our group have previously reported a marked elevation in NADPH-dependent lipid peroxidation in microsomes following chlorpyrifos treatment (Goel et al., 2005). The observed decrease in the cytochrome b_5 and subsequent concomitant decrease in NADH cytochrome-*c*-reductase with chlorpyrifos intoxication could also be explained on a similar basis, as cytochrome b_5 potentiates the catalytic desaturation of fatty acids (Hildebrandt and Estabrook, 1971).

A significant inhibition of microsomal APD activity was observed in chlorpyrifos intoxicated animals, although no change was discernible in the levels of AHH. The present study, therefore indicates that chlorpyrifos is a phenobarbitone (PB) type of inducer rather than a mixed type. These findings seem logical as it is not surprising to observe a considerable inconsistency in the relative effectiveness of cytochrome P_{450} isoenzymes to activate different pesticides (Levi and Hodgson, 1985). Although to the best of our knowledge ours is the first study to report such effects with chlorpyrifos, similar inhibition in APD activity has been observed with sub-acute intoxications with parathion, malathion and phosalone (Bulusu and Chakravarty, 1988).

Interestingly, we observed that co-administration of zinc to chlorpyrifos intoxicated animals reverted most of these altered enzyme levels to within normal limits. The most simplistic explanation for these observed effects is that zinc regulates the hepatic microsomal drug metabolism, as well as the related oxidation of NADPH in the setting of chlorpyrifos intoxication. Zinc activates microsomal pyrophosphatase II, a zinc containing enzyme that is capable of metabolizing both NADPH and NADH (Zyryanov et al., 2004). Zinc is known to complex with NADPH, and it is suggested that regulation of drug metabolism by zinc might be due to this complex formation (Ludwig et al., 1980). It has also been hypothesized that zinc by binding to reductase, changes its oxidation-reduction potential, thus impacting the regulation of the cytochrome P_{450} levels (Ludwig et al., 1980; Zhou et al., 2005). Additionally, it is known that majority of zinc in liver exists bound to zinc-metallothionein (MT) and this trace metal may be readily available to the hepatic cytochromes (Bremner and Davies, 1975; Eaton et al., 1980). Alternatively, it can be hypothesized that the increase in Zn turnover in concert with the synthesis of metallothionein might be pivotal behind these effects; however, further studies are still required in this context. It has been shown that liver microsomes from zinc deficient rats generate more NADPH and aminopyrene-dependent H_2O_2 due to simultaneously inhibited APD activity in these animals (Hammermueller et al., 1987). Although MT synthesis is known to be induced in response to various pathological and physiological agents, however, we have previously reported decrease in MT content following chlorpyrifos intoxication, and ascribed it to the zinc deficient state in the livers of these animals (Goel et al., 2000, 2006). Abnormalities in the zinc metabolism leading to its deficiency are generally attributed to factors such as malabsorption, malnutrition, decreased intestinal zinc binding factors or the increased excretion of the zinc via the gastrointestinal tract or via urine (McClain and Su, 1983). In biochemical systems, the antioxidant properties of zinc have been clearly demonstrated and, for the most part, appear to be independent of zinc metalloenzyme activity. Therefore, our observed decreased serum zinc can also be attributed to its excessive utilization as an antioxidant.

In conclusion, these data suggest that zinc supplementation is protective in the animals subjected to chlorpyrifos intoxication, as it markedly helps regulating the activities of key drug metabolizing enzymes in conditions of chlorpyrifos toxicity. Although zinc-induced metallothionein levels and its antioxidant effects may be central to its biological protective role, further studies are warranted to ascertain the precise mechanisms of its action.

References

Abou-Donia, M.B., 1981. Organophosphorus ester-induced delayed neurotoxicity. Annu. Rev. Pharmacol. Toxicol. 21, 511–548.

Barron, M.G., Plakas, S.M., Wilga, P.C., 1991. Chlorpyrifos pharmacokinetics and metabolism following intravascular and dietary administration in channel catfish. Toxicol. Appl. Pharmacol. 108 (3), 474–482.

Bremner, I., Davies, N.T., 1975. The induction of metallothionein in rat liver by zinc injection and restriction of food intake. Biochem. J. 149 (3), 733–738.

Bulusu, S., Chakravarty, I., 1988. Profile of drug metabolizing enzymes in rats treated with parathion, malathion, and phosalone under various conditions of protein energy malnutrition. Bull. Environ. Contam. Toxicol. 40 (1), 110–118.

Cabre, M., Ferre, N., Folch, J., Paternain, J.L., Hernandez, M., del Castillo, D., Joven, J., Camps, J., 1999. Inhibition of hepatic cell nuclear DNA fragmentation by zinc in carbon tetrachloride-treated rats. J. Hepatol. 31 (2), 228–234.

Cagen, S.Z., Klaassen, C.D., 1979. Protection of carbon tetrachloride-induced hepatotoxicity by zinc: role of metallothionein. Toxicol. Appl. Pharmacol. 51 (1), 107–116.

Chambers, J.E., Chambers, H.W., 1989. Oxidative desulfuration of chlorpyrifos, chlorpyrifos-methyl, and leptophos by rat brain and liver. J. Biochem. Toxicol. 4 (3), 201–203.

Daniel, H., Tom Dieck, H., 2004. Nutrient-gene interactions: a single nutrient and hundreds of target genes. Biol. Chem. 385 (7), 571–583.

Dhawan, D., Goel, A., 1994. Protective role of zinc on rat liver function in long-term toxicity induced by carbontetrachloride. J. Trace Elem. Exp. Med. 7, 1–9.

Eaton, D.L., Stacey, N.H., Wong, K.L., Klaassen, C.D., 1980. Dose-response effects of various metal ions on rat liver metallothionein, glutathione, heme oxygenase, and cytochrome P-450. Toxicol. Appl. Pharmacol. 55 (2), 393–402.

Evenson, M.A., Anderson Jr., C.T., 1975. Ultramicro analysis for copper, cadmium, and zinc in human liver tissue by use of atomic absorption spectrophotometry and the heated graphite tube atomizer. Clin. Chem. 21 (4), 537–543.

Fang, Y.Z., Yang, S., Wu, G., 2002. Free radicals, antioxidants, and nutrition. Nutrition 18 (10), 872–879.

Goel, A., Dhawan, D.K., 2001. Zinc supplementation prevents liver injury in chlorpyrifos-treated rats. Biol. Trace Elem. Res. 82 (1–3), 185–200.

Goel, A., Chauhan, D.P., Dhawan, D.K., 2000. Protective effects of zinc in chlorpyrifos induced hepatotoxicity: a biochemical and trace elemental study. Biol. Trace Elem. Res. 74 (2), 171–183.

Goel, A., Dani, V., Dhawan, D.K., 2005. Protective effects of zinc on lipid peroxidation, antioxidant enzymes and hepatic histoarchitecture in chlorpyrifos-induced toxicity. Chem. Biol. Interact. 156 (2–3), 131–140.

Goel, A., Dani, V., Dhawan, D.K., 2006. Chlorpyrifos-induced alterations in the activities of carbohydrate metabolizing enzymes in rat liver: the role of zinc. Toxicol. Lett. 163 (3), 235–241.

Hammermueller, J.D., Bray, T.M., Bettger, W.J., 1987. Effect of zinc and copper deficiency on microsomal NADPH-dependent active oxygen generation in rat lung and liver. J. Nutr. 117 (5), 894–901.

Hildebrandt, A., Estabrook, R.W., 1971. Evidence for the participation of cytochrome b5 in hepatic microsomal mixed-function oxidation reactions. Arch. Biochem. Biophys. 143 (1), 66–79.

Jokanovic, M., 2001. Biotransformation of organophosphorus compounds. Toxicology 166 (3), 139–160.

Kamataki, T., Neal, R.A., 1976. Metabolism of diethyl *p*-nitrophenyl phosphorothionate (parathion) by a reconstituted mixed-funcion oxidase enzyme system: studies of the covalent binding of the sulfur atom. Mol. Pharmacol. 12 (6), 933–944.

Kang, Y.J., Zhou, Z., 2005. Zinc prevention and treatment of alcoholic liver disease. Mol. Aspects Med. 26 (4–5), 391–404.

Kousba, A.A., Sultatos, L.G., Poet, T.S., Timchalk, C., 2004. Comparison of chlorpyrifos-oxon and paraoxon acetylcholinesterase inhibition dynamics: potential role of a peripheral binding site. Toxicol. Sci. 80 (2), 239–248.

Levi, P.E., Hodgson, E., 1985. Oxidation of pesticides by purified cytochrome P-450 isozymes from mouse liver. Toxicol. Lett. 24 (2–3), 221–228.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193 (1), 265–275.

Ludwig, J.C., Misiorowski, R.L., Chvapil, M., Seymour, M.D., 1980. Interaction of zinc ions with electron carrying coenzymes NADPH and NADH. Chem. Biol. Interact. 30 (1), 25–34.

Ma, T., Chambers, J.E., 1994. Kinetic parameters of desulfuration and dearylation of parathion and chlorpyrifos by rat liver microsomes. Food Chem. Toxicol. 32 (8), 763–767.

Maroni, M., Colosio, C., Ferioli, A., Fait, A., 2000. Biological monitoring of pesticide exposure: a review. Introduction. Toxicology 143 (1), 1–118.

Masters, B.S., 2005. The journey from NADPH-cytochrome P450 oxidoreductase to nitric oxide synthases. Biochem. Biophys. Res. Commun. 338 (1), 507–519.

McClain, C.J., Su, L.C., 1983. Zinc deficiency in the alcoholic: a review. Alcohol Clin. Exp. Res. 7 (1), 5–10.

Miyazaki, S., Hodgson, G.C., 1972. Chronic toxicity of dursban and its metabolite, 3,5,6-trichloro-2-pyridinol in chickens. Toxicol. Appl. Pharmacol. 23 (3), 391–398.

Nash, T., 1953. The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. Biochem. J. 55 (3), 416–421.

Nebert, D.W., Gelboin, H.V., 1968. Substrate-inducible microsomal aryl hydroxylase in mammalian cell culture. I. Assay and properties of induced enzyme. J. Biol. Chem. 243 (23), 6242–6249.

Omura, T., Sato, R., 1964a. The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. J. Biol. Chem. 239, 2370–2378.

Omura, T., Sato, R., 1964b. The carbon monoxide-binding pigment of liver microsomes. II. Solubilization, purification, and properties. J. Biol. Chem. 239, 2379–2385.

Pope, C.N., 1999. Organophosphorus pesticides: do they all have the same mechanism of toxicity? J. Toxicol. Environ. Health B Crit. Rev. 2 (2), 161–181.

Pope, C.N., Karanth, S., Liu, J., Yan, B., 2005. Comparative carboxylesterase activities in infant and adult liver and their in vitro sensitivity to chlorpyrifos oxon. Regul. Toxicol. Pharmacol. 42 (1), 64–69.

Prasad, A.S., 1983. Clinical, biochemical and nutritional spectrum of zinc deficiency in human subjects: an update. Nutr. Rev. 41 (7), 197–208.

Rahman, M.F., Siddiqui, M.K., Mahboob, M., Mustafa, M., 1990. Haematological and hepatotoxic effects of isoprocarb in chicken. J. Appl. Toxicol. 10 (3), 187–192.

Sevanian, A., Nordenbrand, K., Kim, E., Ernster, L., Hochstein, P., 1990. Microsomal lipid peroxidation: the role of NADPH—cytochrome P450 reductase and cytochrome P450. Free Radic. Biol. Med. 8 (2), 145–152.

Sidhu, P., Garg, M.L., Dhawan, D.K., 2005. Protective effects of zinc on oxidative stress enzymes in liver of protein-deficient rats. Drug Chem. Toxicol. 28 (2), 211–230.

Sultatos, L.G., 1991. Metabolic activation of the organophosphorus insecticides chlorpyrifos and fenitrothion by perfused rat liver. Toxicology 68 (1), 1–9.

Sultatos, L.G., Murphy, S.D., 1983. Hepatic microsomal detoxification of the organophosphates paraoxon and chlorpyrifos oxon in the mouse. Drug Metab. Dispos. 11 (3), 232–238.

Yang, C.S., Strickhart, F.S., Kicha, L.P., 1978. Analysis of the aryl hydrocarbon hydroxylase assay. Biochem. Pharmacol. 27 (19), 2321–2326.

Yasukochi, Y., Masters, B.S., 1976. Some properties of a detergent-solubilized NADPH-cytochrome c(cytochrome P-450) reductase purified by biospecific affinity chromatography. J. Biol. Chem. 251 (17), 5337–5344.
Zhou, Z., Wang, L., Song, Z., Saari, J.T., McClain, C.J., Kang, Y.J., 2005. Zinc supplementation prevents alcoholic liver injury in mice through attenuation of oxidative stress. Am. J. Pathol. 166 (6), 1681–1690.
Zyryanov, A.B., Tammenkoski, M., Salminen, A., Kolomiytseva, G.Y., Fabrichniy, I.P., Goldman, A., Lahti, R., Baykov, A.A., 2004. Site-specific effects of zinc on the activity of family II pyrophosphatase. Biochemistry 43 (45), 14395–14402.