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Effects of arsenite pretreatment on the acute toxicity of parathion

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Abstract

Parathion (PA) is a phosphorotioate pesticide requiring P-450-mediated oxidations to become activated to paraoxon. or to be metabolised to its less toxic metabolites. On the other hand. sodium arsenite [As(III)] markedly decreases total hepatic P-450 content and dependent monoxygenase activities. Our aim was to determine the effects of As(III) pretreatment on the acute toxicity of PA and its possible relationship with the effects of As(III) on P-450-dependent monooxygenase activities. Adult male Wistar rats were pretreated with As(III) (5.6 mg As(III)/kg, s.c.), and 24 h later given PA (5 to 20 mg/kg, per os). As(III) pretreatment increased the acute toxicity of PA. reducing 38% its median lethal dose (LD_{50}) from 11.68 to 7.21 mg PA/kg. In addition. As(III) pretreatment further decreased the inhibitory effect of PA on brain acetylcholinesterase activity, reducing 33% the median inhibitory dose (ID_{50}) from 3.44 to 2.31 mg PA/kg, whereas As(III) alone had no significant effects. As(III) decreased the P-450 content to 87% of control values, reduced EROD activity to 74% and BROD activity to 41%; PA produced no significant effects on these parameters, whereas the joint administration of As(III) + PA produced effects similar to those of As(III). PROD activity was reduced to 36% of control value by PA, whereas As(III) alone produced no significant effects. However, As(III) pretreatment apparently protected against the inhibition of CYP2B1-mediated PROD activity produced by PA. since PROD values were similar to those of control animals. Our results also indicated that the increase in PA toxicity caused by As(III) pretreatment, could also be related to the CYP2B2 isoform, since decreases in CYP2B2-dependent BROD activity were observed in both As(III) and As(III) + PA groups, but not in PA-treated animals. suggesting that CYP2B2 is involved in PA detoxification. Copyright © 1997 Elsevier Science Ireland Ltd.

Abbreciations: AChE, acetylcholinesterase: As(III), sodium arsenite; PA, parathion; PO, paraoxon; BROD, benzyloxyresorufin-*O*-dearylase; EROD, ethoxyresorufin-*O*-deethylase; PROD. pentoxyresorufin-*O*-dealkylase; P-450, cytochrome P450; CYP1A1. CYP2B1, CYP2B2, cytochrome P450 isoforms 1A1. 2B1 and 2B2, respectively: GSH. reduced glutathione; LPO, lipid peroxidation: TNB, 5-thio-2-nitrobenzoate.

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1. Introduction

Exposure to chemical mixtures often results in alterations of biological responses, as compared to those effects caused by exposure to individual chemicals (Murphy, 1983). Some of these responses result from the effects of chemicals on P-450 isozymes. Therefore, it is important to investigate those factors affecting P-450-dependent biotransformation. which could help in predicting toxic effects resulting from interactions between chemicals undergoing activation or deactivation.

Parathion (PA) is a phosphorotioate pesticide. requiring P-450-mediated oxidations to become activated by desulfuration to yield its phosphate ester, paraoxon (PO), which is a potent acetylcholinesterase (AChE) inhibitor, or to be metabolised through a dearylation reaction to form diethylphosphorothioate and p-nitrophenol. its less toxic metabolites; both oxidative pathways are believed to arise from the same phosphooxythiran intermediate (Neal, 1967; Kamataki et al., 1976). Numerous studies have shown that phosphorothioate bioactivation results in P-450 destruction (Halpert et al., 1980). Thus, P-450 isoforms not only participate in the metabolic activation of phosphorotioate pesticides but also are targets for reactive products that elicit enzyme destruction (Murray and Butler, 1995). Altered P-450-dependent monooxygenase activity 'due to enzyme depletion and/or induction by chemicals could result in decrements or enhancements in PA toxicity. depending upon whether oxidative degradation or activation predominates (Wilkinson and Denison, 1982; Chambers and Chambers. 1990). Thus, the relative ratio of biotransformation could be a major determinant for PA toxicity. Arsenic (As), an ubiquitous element present in the environment, causes histological and biochemical alterations in rodent and human liver (ATSDR, 1993). In rodents, cellular heme status is a major target of arsenite [As(III)] effects by virtue of its inhibitory effect on two enzymes of the heme synthesis pathway and by the concomitant increase in heme degradation (Cebrián et al., 1988). Therefore, As(III) markedly decreases total hepatic P450 content and P450-dependent monooxygenase activities (Albores et al., 1992). As and PA are two contaminants potentially found together in the environment and able to interact biologically, since As decreases the activity of P-450 isozymes and PA requires them for biotransformation. Consequently, the aim of this study was to determine the effects of As(III) pretreatment on the acute toxicity of PA and its possible relationship with the effects of As on P450-dependent monooxygenase activities.

2. Materials and methods

2.1. Chemicals.

Parathion (*O*,*O*′-diethyl-*O*-*p*-nitrophenyl phosphorothionate) technical grade (99.1% purity) was a gift from Bayer de Mexico (Mexico). Sodium arsenite, glutathione, acetylthiocholine iodide, o-phtalaldehyde, thiobarbituric acid and 5.5'-dithiobis-2-nitrobenzoic acid were purchased from Sigma Chemical Co. (St. Louis, MO). Sodium dithionite was obtained from Merck (Darmstadt, Germany). Resorufin and ethoxy-. pentoxy- and benzyloxyresorufin, were purchased from Molecular Probes, Inc. (Eugene, OR). All other chemicals used were of the highest analytical grade available.

2.2. Animals

Male Wistar rats (180–220 g) bred in-house, were maintained in plastic cages with sawdust bedding, maintained at 21 ± 2°C and 50% relative humidity on a 12-h dark/light cycle (starting at 0700 h), and allowed free access to water and rat chow (Formulab Diet 5008, Purina Feeds Inc., St. Louis, MO).

2.3. Median lethal dose (LD_{50})

In order to determine the effects of As(III) on the toxicity of PA, rats were given a subcutaneous injection of sodium arsenite (5.6 mg As(III)/kg bw) or saline 24 h before administration per os of PA dissolved in corn oil (5 to 20 mg PA/kg bw). The number of deaths over 72 h was recorded and data evaluated by probit analysis; the values for LD_{16}, LD_{50} and LD_{84}, and their confidence limits were calculated according to Tallarida and Murray (1981).

2.4. Median inhibitory dose (ID_{50}) of *brain acetylcholinesterase (AChE) activity*

The ID_{50} was calculated following a treatment scheme similar to that described above, but rats were killed by cervical dislocation 24 h after PA administration. The brain was removed and rapidly frozen until enzyme activity was measured according to Ellman et al. (1961). using acetylthiocholine iodide and dithionitrobenzoic acid as substrates.

2.5. Hepatic *biochemical parameters*

In another set of experiments, the effects of a single dose of As(III) (5.6 mg As/kg bw), PA (3.8 mg/kg bw) or the joint administration of As(III) and PA were investigated. Four groups of rats were treated as follows: sodium arsenite plus corn oil (As(III) group), parathion plus saline (PA group), sodium arsenite plus parathion (As(III) + PA group), and controls receiving saline and/or corn oil. In all cases, PA was given 24 h after As(III) administration. Rats were killed by cervical dislocation 24 h after PA administration, livers were perfused with cold saline in situ. brain and the perfused liver were excised and stored at −70°C until analyzed. All ex vivo operations where performed on ice at 4°C.

2.5.1. P-450 *content* and *monooxygenase* activities

A piece of liver kept at −70°C was used for total P-450 content determined by the CO-dithionite method (Omura and Sato, 1964) and microsomal monooxygenase activities were determined fluorometrically, by measuring resorufin resulting from *O*-dealkylation of ethoxy-, pentoxy- and o-dearylation of benzyloxyresorufin (Burke and Mayer, 1983; Burke et al., 1985).

2.5.2. Determination of hepatic *glutathion* (GSH) *content* and lipid peroxidation (LPO)

The total content of GSH was measured in a fresh piece of tissue using a fluorometric method after reaction with o-phtalaldehyde (Hissin and Hilf. 1976) and LPO was determined by the thiobarbituric-reactive method (Buege and Aust, 1978).

Protein concentration was determined according to Lowry et al. (1951), using bovine serum albumin as standard.

3.6. Statistical analysis

The multiple range test of Student Newman-Keuls for statistical pairwise differences was used to assess differences between groups. The level of significance was set at $P \leq 0.05$.

3. Results

3.1. Effects of *As(III) pretreatment* on PA LD_{50} and *brain* AChE *activity*

Table 1 shows mortality data and the probit analysis of PA and As(III) + PA treatments, the values for LD_{16}, LD_{50} and LD_{84}, their 95% confidence limits and the slope values. As(III) pretreatment increased the acute toxicity of PA, since its LD_{50} value (11.68 mg PA/kg) was reduced 38% to 7.21 mg PA/kg and each of the combined doses of As(III) + PA was more toxic than those of PA alone. As expected, PA decreased brain AChE activity in a dose-dependent fashion, with an ID_{50} value of 3.44 mg/kg (Table 2). whereas As(III) alone had no significant effects, as compared to control animals (Table 3). However, As(III) pretreatment caused a further 33% decrease in the inhibitory effect of PA, decreasing the ID_{50} from 3.44 to 2.31 mg PA/kg (Table 2).

Table 1
Effects of arsenic pretreatment on the lethality of parathion.

Log dose PA (mg/kg)	PA			As(III) + PA		
	Observed effect	Probit	Expected effect (%)	Observed effect	Probit	Expected effect (%)
0.7	–	–	–	015	4.25	22.7
0.8	0/6	3.52	6.9	3/6	4.72	39.2
0.9	3/11	4.07	17.6	9/12	5.20	57.8
1.0	1/12	4.63	35.3	4/6	5.67	74.9
1.1	10/12	5.18	57.2	11 12	6.14	87.4
1.2	5/6	5.73	76.8	6/6	6.62	94.7
1.3	5/6	6.29	90.1	6/6	7.09	98.2
LD_{16}		7.73 (7.47–7.99)			4.44 (4.16–4.74)	
LD_{50}		11.68 (9.77–14.00)			7.21* (5.62–9.25)	
LD_{84}		17.7 (17.10–18.30)			11.7 (11.0–12.50)	
Slope		5.54			4.73	

Single doses per os of PA were given to male rats (6–12 animals/dose) pretreated 24 h before with saline or sodium arsenite (5.6 mg/kg bw). LD_{16}, LD_{50} and LD_{84} values and their confidence intervals (in parenthesis) are expressed in mgPA/kg bw.
Data were evaluated by probit analysis.
*Statistical significance was set at $P < 0.05$.

3.2. *Effects of As(III)* and *PA* on some hepatic biochemical parameters

As(III) administration decreased the P-450 content to 87% of control values, reduced EROD activity to 74% and BROD activity to 41%; PA produced no significant effects on these parameters, whereas the joint administration of As(III) + PA produced effects similar to those caused by As(III) alone. PROD activity was reduced to 36% of control value by PA, whereas As(III) produced no significant effects. However, As(III) pretreatment apparently protected against the inhibition of PROD activity produced by PA, since PROD value were similar to those of control animals (Table 3). None of the treatments significantly affected the hepatic LPO or the GSH content at the time of analysis (data not shown).

4. Discussion

Since xenobiotics may reciprocally affect their metabolism by altering their activation and/or detoxification, the resulting effect might be different from that typically expected (Murphy, 1983). This study examined the effects of As(III) pretreatment on PA toxicity and its relationship with the effects of As(III) on the P-450 biotransformation system. Our results showed a statistically significant decrease in the LD_{50} value and an increased inhibition of brain AChE in As(III)-pretreated rats, as compared to those treated with PA alone; these effects were related to alterations in P-450-dependent enzyme activities probably involved in PA metabolism. It has been reported that induction of P-450 by xenobiotics, such as halogenated benzenes, benzo[*a*]pyrene, phenobarbital and 3-methylcholanthrene has a protective effect against PA acute toxicity, increasing the LD_{50} and decreasing the inhibitory effect of the pesticide on brain AchE (Villeneuve et al., 1970; Townsend and Carlson, 1981; Purshottam and Srivastava, 1987). On the other hand, organic mercury is known to potentiate the toxicity of PA by reducing the LD_{50} and increasing the inhibitory effect of the pesticide on brain and plasma AChE (Dieter and Ludke, 1975); in contrast, lead exposure, which affects P-450- dependent monooxygenase systems, did not significantly'

Table 2
Effects of arsenic pretreatment on the inhibitory effect of parathion on brain acetylcholinesterase activity

Log dose PA (mg/kg)	PA brain AChE activity		As(III)+PA brain AChE activity	
	(nmol TNB/min·mg protein)	% Inhibition	(nmol TNB/min·mg protein)	% Inhibition
0.0	32.8 ± 4.1	–	34.0 ± 2.2	–
0.3	19.5 ± 5.3	40.5	18.1 ± 0.8	46.6
0.5	16.6 ± 5.6	49.4	15.6 ± 1.7	54.1
0.7	12.7 ± 2.0	61.2	8.4 ± 1.9	75.3
0.9	9.7 ± 3.7	70.4	8.0 ± 2.9	76.6
ID_{50}	3.44 (2.88–4.11)		2.31 (1.09–4.86)	

Enzyme activity is expressed as mean ± S.D. Values of ID_{50} and their confidence intervals (in parenthesis) are expressed in mgPA/kg bw. As(III) (5.6 mg kg bw) was given 14 h before PA administration. At least four animals were used for each group of treatment.

alter malathion metabolism (Abd-Elraof et al., 1981). Little is known on the mechanisms and effects of metallic or metalloid compounds on AChE activity. Although it has been reported that As(III) in concentrations above 10^{-4} M inhibits the release of acetylcholine, choline acetyltransferase and AChE in vitro (Kobayashi et al., 1987), no inhibition of brain AChE was observed in our As(III) treated rats, suggesting that the effects of As(III) on PA toxicity here reported were not related to inhibition of AChE by As(III).

A possible explanation for the increase in PA toxicity caused by As(III) is related to the finding that As(III) pretreatment prevents the inhibitory effect of PA on CYP2B1-mediated PROD activity. CYP2B1 is involved in the activation of PA to PO and later inactivated by a suicide mechanism; the covalent binding of the released sulfur-containing portion of PA to CYP2B1 leads to cross-linking and aggregation of proteins and consequently, to a concentration-dependent loss of enzymatic activity (Kamataki and Neal, 1976; Halpert et al., 1980; Murray and Butler, 1995). Since As(III) has a high affinity for sulfur-containing molecules (Webb, 1966), we suggest that As(III) interacts with CYP2B1 cysteine residues preventing the formation of hydrodisulfide bonds between the sulfur atom of PA and P-450. This contention is supported by previous reports showing that the addition of dithiothreitol and GSH did not significantly reactivated the enzyme activity of a P-450 reconstituted system (Halpert et al., 1980), probably because addition of these reducing agents was performed after PA incubation. These findings illustrate the need for further research on the direct effects of As(III) on CYP2B1.

Our results also indicated that the increase in PA toxicity caused by As(III) pre-treatment, could be related to effects on the CYP2B2 isoform. since decreases in CYP2B2-dependent BROD activity were observed in both As(III) and As(III)+PA groups, but not in PA-treated animals. suggesting a role for CYP2B2 in PA detoxification. This PA degradative pathway to yield p-nitrophenol and diethyl phosphorotioate requires molecular oxygen and NADPH, implicating a monooxygenase mechanism (Neal, 1967), but no information is available on the P-450 isoform involved. Our results confirm earlier studies (Albores et al., 1992; Falkner et al., 1993) showing that P-450-dependent monooxygenase activities are differentially inhibited by As(III). In order to explain these isoform specific effects of As(III), Falkner et al. (1993) have postulated that some P-450 isoforms are resistant to heme destruction by heme oxigenase, a heat-shock protein induced by As(III) (Sardana et al., 1981; Cebrian et al.. 1988), which catalyze the removal of heme depending on the molecular configuration of the P-450 isoforms (Kutty et al., 1988).

Besides alterations in hepatic P-450 content, other mechanisms may be involved in the increase in PA toxicity caused by As(III) pretreatment: (i) inhibition of class A esterases, particularly paraoxonase, which hydrolyze PO to *p*-nitrophenol. since correlative data in several animal spe-

Table 3
Effects of arsenic, parathion and arsenic plus parathion on brain acetylcholinesterase. and hepatic cytochrome P450 content and related enzyme activities in rats

Group	AChE[1]	P-450[2]	EROD[3]	PROD[4]	BROD[5]
Control	18.0 ± 1.29'''	0.83 ± 0.09''	36.0 ± 4.29''	2.22 ± 0.16''	28.4 ± 0.69[a]A
As(III)	18.5 ± 1.59''	0.67 ± 0.04'	26.8 ± 2.55'	2.05 ± 0.52''	11.7 ± 1.12[b]
PA	7.79 ± 1.62[b]	0.75 ± 0.05''	32.8 ± 1.41''	0.79 ± 1.21[a]	29.4 ± 3.73''
As(III) + PA	4.37 ± 1.81[c]	0.63 ± 0.10[b]	38.7 ± 6.36''	2.44 ± 1.05[a]	12.6 ± 1.64'

Values are expressed as mean ± S.D. (five rats group). A subcutaneous dose of As(III) (5.6mg kg bw) and a dose per os of PA (3.8 mg/kg bw) were administered as described in Material and methods (Section 2). Biochemical assays were performed 48 h after As(III) administration.
*Values within a column not followed by the same letter (a,b,c) are significantly different by Student's Newman-Keuls' test ($P \leq 0.05$).
[1]nmol TNB/min mg protein
[2]nmol P-450/mg protein
[3–5]pmol resorufin/min mg protein.

cies suggest that high serum paraoxonase levels provide protection against PA poisoning (Furlong et al.. 1991). Thus. it is possible that As(III) could target paraoxonase by its affinity to SH-containing macromolecules (Webb. 1966), since an SH bearing amino acid appears to be involved at the active site of the enzyme (Walker and Mackness. 1983); however, no information is available about As(III) interactions with this enzyme. Therefore. the role of As-paraoxonase interaction in the expression of PA toxicity remains to be elucidated. (ii) The non-specific binding of PO and PA to albumin might influence the distribution. biotransformation, and consequently the degree of toxicity of the insecticide (Sultatos et al., 1984). Although the reversible binding of PA or PO to albumin may play an important role in toxicity. 'data about the interaction between As and albumin have not been reported. The effects of As(III) on AChE inhibition produced by low doses of PA were not as marked as those observed at higher doses, suggesting that the main As(III) targets were detoxification pathways important at higher doses of PA.

In summary, our results showed that pretreatment with As(III) increases PA toxicity. Possible explanations include: (i) an interaction of As(III) with CYP2B1, involved in PA activation to PO, protecting the isoform from the suicide inactivation caused during PA oxidation, and (ii) an inhibition of CYP2B2, which is probably involved in the detoxification of PA. Therefore, our data suggest that As(III) increases PA toxicity by affecting both its activation and detoxification pathways. Further studies on the mechanisms of interaction of these two substances will help to predict potential effects of exposure.

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