

Lysophosphatidylcholine hydrolases of human erythrocytes, lymphocytes, and brain: Sensitive targets of conserved specificity for organophosphorus delayed neurotoxicants

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Received 16 March 2007; revised 1 June 2007; accepted 20 June 2007

Available online 27 June 2007

Abstract

Brain neuropathy target esterase (NTE), associated with organophosphorus (OP)-induced delayed neuropathy, has the same OP inhibitor sensitivity and specificity profiles assayed in the classical way (paraoxon-resistant, mipafox-sensitive hydrolysis of phenyl valerate) or with lysophosphatidylcholine (LysoPC) as the substrate. Extending our earlier observation with mice, we now examine human erythrocyte, lymphocyte, and brain LysoPC hydrolases as possible sensitive targets for OP delayed neurotoxicants and insecticides. Inhibitor profiling of human erythrocytes and lymphocytes gave the surprising result of essentially the same pattern as with brain. Human erythrocyte LysoPC hydrolases are highly sensitive to OP delayed neurotoxicants, with *in vitro* IC₅₀ values of 0.13–85 nM for longer alkyl analogs, and poorly sensitive to the current OP insecticides. In agricultural workers, erythrocyte LysoPC hydrolyzing activities are similar for newborn children and their mothers and do not vary with paraoxonase status but have high intersample variation that limits their use as a biomarker. Mouse erythrocyte LysoPC hydrolase activity is also of low sensitivity *in vitro* and *in vivo* to the OP insecticides whereas the delayed neurotoxicant ethyl *n*-octylphosphonyl fluoride inhibits activity *in vivo* at 1–3 mg/kg. Overall, inhibition of blood LysoPC hydrolases is as good as inhibition of brain NTE as a predictor of OP inducers of delayed neuropathy. NTE and lysophospholipases (LysoPLAs) both hydrolyze LysoPC, yet they are in distinct enzyme families with no sequence homology and very different catalytic sites. The relative contributions of NTE and LysoPLAs to LysoPC hydrolysis and clearance from erythrocytes, lymphocytes, and brain remain to be defined.

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Keywords: Erythrocytes; Lysophosphatidylcholine; Lysophosphatidylcholine hydrolase; Neuropathy target esterase; Organophosphorus

Introduction

Organophosphorus (OP)-induced delayed neuropathy (OPIDN), with >70,000 human cases in the last 75 years, is associated with inhibition of neuropathy target esterase (NTE) and aging of the phosphorylated enzyme (Johnson and Glynn, 2001; Ehrlich and Jortner, 2001). NTE is normally assayed with brain membranes as the paraoxon-resistant and mipafox-sensitive portion of phenyl valerate (PV) hydrolysis (Johnson and Glynn, 2001). PV is an abiotic substrate modeled on a potent OP NTE inhibitor rather than an endogenous compound (Casida and Quistad, 2004). NTE also hydrolyzes lysophosphatidylcholine (LysoPC) (van Tienhoven et al., 2002; Quistad et al., 2003), yet belongs to a different enzyme family with a

Abbreviations: AChE, acetylcholinesterase; BChE, butyrylcholinesterase; CPO, chlorpyrifos oxon; DFP, diisopropyl fluorophosphate; DMSO, dimethyl sulfoxide; DP-CPO, di-*n*-pentyl analog of CPO; DP-dichlorvos, di-*n*-pentyl analog of dichlorvos; DSF, *n*-dodecanesulfonyl fluoride; EOPF, ethyl *n*-octylphosphonyl fluoride; IC₅₀, concentration inhibiting 50% of enzyme activity; IDFP, isopropyl *n*-dodecylfluorophosphonate; LysoPC, lysophosphatidylcholine; LysoPLA, lysophospholipase; NTE, neuropathy target esterase; OOS, trimethyl phosphorothiolate; OP, organophosphorus; OPIDN, organophosphorus-induced delayed neuropathy; OSF, *n*-octanesulfonyl fluoride; PON1, paraoxonase 1; PV, phenyl valerate; *R*- and *S*-OBDPO, *R*- and *S*-enantiomers of *n*-octyl benzodioxaphosphorin oxide; TBDPO, *o*-tolyl benzodioxaphosphorin oxide.

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catalytic site unique from the well-characterized lysophospholipase (LysoPLA) I (Wang and Dennis, 1999). Mouse brain homogenate assayed as either PV or LysoPC hydrolysis has similar reduced activity in *nfe* +/- vs. wild type mice and the same OP sensitivity and specificity profiles (Quistad and Casida, 2004). LysoPC hydrolytic activity is therefore a more mechanism-based approach to OPIDN assayed directly or as the paraoxon-resistant and mipafox-sensitive portion (Quistad et al., 2003) (Fig. 1). Lymphocyte NTE inhibition assayed with PV has been proposed to monitor exposure to insecticides and OPIDN agents (Lotti et al., 1986), but this approach has limited value due to poor enzyme stability and reproducibility (Lotti, 1992; Mutch et al., 1992). Erythrocyte and lymphocyte LysoPC hydrolases are possible alternative biomarkers for OP delayed neurotoxicant exposure.

The monitoring of human exposure to OP insecticides relies on inhibition of erythrocyte acetylcholinesterase (AChE) or plasma butyrylcholinesterase (BChE) activity (Ballantyne and Salem, 2006) or analysis of blood and urine for the OPs or their metabolites. OP pesticides are of special concern for pregnant women, infants, and children (National Research Council, 1993; Neri et al., 2006). Plasma levels of chlorpyrifos among women in New York City are associated with low birth weight and length and adverse effects on neurodevelopment (Perera et al., 2003; Rauh et al., 2006). Maternal urinary dialkyl phosphate metabolite levels in an agricultural population are correlated with shortened gestational length, a higher number of abnormal reflexes in neonates, and poorer neurodevelopment in young children (Eskenazi et al., 2004, 2007; Young et al., 2005). Paraoxonase 1 (PON1) is an OP-hydrolyzing enzyme with high activity for detoxifying chlorpyrifos oxon (CPO) among many OPs (Geldmacher-von Mallinckrodt and Diepgen, 1988; Furlong et al., 1988). PON1 levels in children are often lowest at birth and can take up to 24 months or longer to reach adult levels (Augustinsson and Barr, 1963; Cole et al., 2003). The *PON1*₁₉₂ genotype may be a factor in OP detoxification and therefore sensitivity (Furlong et al., 2006; Holland et al., 2006).

This study considers the LysoPC hydrolases as sensitive targets for OP delayed neurotoxicants, insecticides, and other inhibitors. The first goal is to characterize the LysoPC hydrolases of human erythrocytes, lymphocytes, and brain and profile their inhibitor sensitivities. The second is to determine the potency of OP neurotoxicants and insecticides for inhibition of human erythrocyte LysoPC hydrolases *in vitro* and mouse erythrocyte activity *in vivo*. Finally, factors affecting

human erythrocyte LysoPC hydrolases are evaluated including *PON1* genotype using both newborn children and mothers. We conclude that although blood LysoPC hydrolases are poorly sensitive to current OP insecticides, they are as good as brain NTE as a predictor of OP inducers of delayed neuropathy.

Materials and methods

Chemicals. The OPs and other inhibitors were selected for major differences in structure and potency thereby serving as a suitable set to examine the inhibitor specificity and binding pocket conformation of the serine hydrolases (Casida and Quistad, 2004). Alternatively, they were widely used insecticides in Monterey County, California (California DPR, 2002), the study area described below for human environmental exposure. Insecticides are referred to by their common names (Tomlin, 2003) or are identified in the abbreviations and table footnotes. ChemService (West Chester, PA) was the source of the seven widely used OP insecticides (acephate, chlorpyrifos, diazinon, dimethoate, malathion, methamidophos, and oxydemeton-methyl) and activated metabolites of some of these compounds (CPO, diazoxon, malaosxon, and *o*-methoate). The other candidate inhibitors were obtained from commercial sources or synthesized in the Environmental Chemistry and Toxicology Laboratory.

Tissues. Whole human blood from seven subjects and lymphocytes (isolated by aphaeresis using a leukopac filter) from three subjects were obtained from AllCells LLC (Emeryville, CA). Mouse blood from Swiss-Webster males was obtained by cardiac puncture and collected in heparin. Erythrocytes were separated from whole blood by centrifugation and washed with three volumes of isotonic saline. To determine the contribution of blood fractions to LysoPC hydrolase activity, human whole blood was layered with Ficoll and centrifuged to obtain plasma, lymphocyte, and erythrocyte portions. To minimize loss of LysoPC hydrolase activity, erythrocytes and lymphocytes were aliquoted and kept at -80°C until use. Storage of erythrocytes for 3 months at -80°C or at liquid nitrogen temperature preserved 75% of the original LysoPC hydrolase activity, compared to 40% at -20°C . The activity loss was $>50\%$ within 48 h at 4°C . An internal standard erythrocyte sample was used to correct for variability in assays on separate days (4.1 ± 1.1 pmol/min/mg protein, $n=28$). Human brain was obtained from a medical examiner's office (Cole et al., 1984) and samples from the frontal cortex were used for assays.

Human erythrocytes were obtained from 25 randomly selected pregnant women (at approximately 26 weeks gestation) and their newborns (cord blood) who participated in the "Center for the Health Assessment of Mothers and Children of Salinas" (CHAMACOS) project in Monterey County, California, a longitudinal birth cohort study to evaluate the effects of pesticides and other environmental exposures on the health of children and pregnant women (Eskenazi et al., 1999). After the samples were divided into newborn and mother groups and according to *PON1*₁₉₂ genotype (Q/Q slow metabolizers; Q/R; R/R fast metabolizers) (Holland et al., 2006), 100 μl of erythrocytes from each subject was pooled. Written informed consent was obtained from all women in the study and protocols were approved by the Institutional Review Board at the University of California, Berkeley.

Mouse studies. Mice (three per compound) were treated ip with the seven widely used insecticides at the highest non-lethal dose or with ethyl

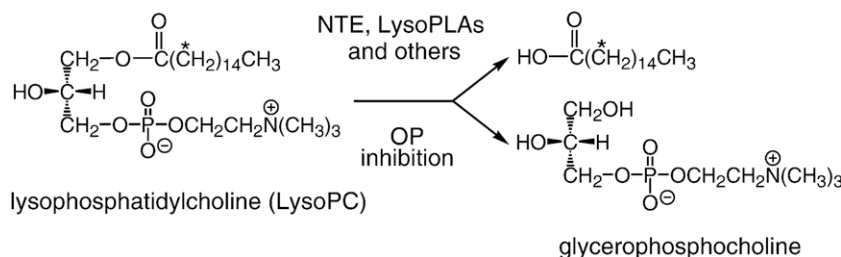


Fig. 1. OP-sensitive LysoPC hydrolases assayed with [^{14}C -palmitoyl]LysoPC.

n-octylphosphonyl fluoride (EOPF) at 1 and 3 mg/kg and sacrificed 4 h later. EOPF is the most commonly used OP delayed neurotoxicant in recent studies (Wu and Casida, 1996). Erythrocytes were obtained as above and brains were removed and frozen at -80°C until analyzed.

Enzyme activity assays. LysoPC hydrolases were assayed with [^{14}C -palmitoyl]LysoPC (Amersham, 56 mCi/mmol, 99% radiochemical purity) by a specific partitioning method (Fig. 1). Brain was homogenized and lymphocytes were sonicated in assay buffer (1 mM CaCl_2 , 0.01% Triton-X100, 100 mM Tris-HCl, pH 8.0, 25 $^{\circ}\text{C}$). Erythrocytes were sonicated without prior dilution. In one study, the brain homogenate and sonicated erythrocytes were centrifuged at $100,000\times g$ for 1 h at 4 $^{\circ}\text{C}$ to obtain membrane and cytosol fractions. Protein was quantitated (Bradford, 1976) and an amount within the linear range for protein level-percent hydrolysis was used. The tissue preparation in assay buffer (490 μl) was treated with the test OP in dimethyl sulfoxide (DMSO) (5 μl) and after 15 min at room temperature with [^{14}C]LysoPC (70,000 dpm) in DMSO (5 μl). The reaction in a 7.4-ml vial was then incubated at 37 $^{\circ}\text{C}$ for 15 min. The [^{14}C]palmitic acid hydrolysis product was separated from unhydrolyzed [^{14}C]LysoPC by a modified Dole extraction method (Zhang et al., 1991) with hexane substituted for heptane. A solution (2.5 ml) of isopropanol:hexane:1 N sulfuric acid (20:5:1) was added to stop the reaction. After introduction of ~ 100 mg silica gel, the vial was vortexed for 10 s. Hexane (1.5 ml) and water (1.5 ml) were added followed by vortexing for 15 s. After 2–5 min for phase separation, most of the top organic layer containing the [^{14}C] palmitic acid was removed (1.0 ml) and the ^{14}C content was determined in a scintillation counter. A control sample was assayed with isopropyl *n*-dodecylfluorophosphonate (IDFP) (100 μM , 15 min preincubation) to completely block the LysoPC hydrolase activity and this value, averaging 7% of the total, was subtracted to obtain the specific enzymatic hydrolysis. All assays were done in triplicates. LysoPC hydrolase activity is reported as pmol [^{14}C]LysoPC hydrolysis per min per mg protein.

Mouse brain NTE activity assays were done as described above for LysoPC hydrolases, with one additional step. Mipaflox (50 μM final concentration) was added in 50 mM tris-citrate buffer (pH 6.0) followed by a 20-min incubation at 25 $^{\circ}\text{C}$ before [^{14}C]LysoPC was added. In contrast to assays in which NTE is measured using PV as the substrate, there is no inhibition by paraoxon when LysoPC is the substrate. Thus, in this study mipaflox was added but not paraoxon to determine NTE activity.

IC_{50} values and statistical analysis. OP sensitivity is expressed as the IC_{50} value (concentration which inhibits 50% of enzymatic activity) with the standard deviation and number of replicates. The IC_{50} s were used to compare different compounds and enzyme sources to determine the specificity of the binding pocket rather than as indicators of toxicity. Correlation plots were made by linear regression using SigmaPlot 8.0. Activity values (means \pm SD) also allowed comparison of the differential activity between selected groups (newborns vs. mothers and PON1 genotype pools) using the Student's *t*-test to evaluate significant differences. In dose-response considerations, the alpha value for significance was adjusted for Bonferroni inequality to account for multiple hypothesis testing.

Results

Activity and distribution

The LysoPC hydrolase activities (pmol/min/mg protein, mean \pm SD) observed for human erythrocytes averaged 21 ± 25 , range 4–75 ($n=7$ subjects), for lymphocytes 135 ± 28 , range 110–165 ($n=3$ subjects), and for brain 56 ± 8 (single subject, variability of 23 analyses), in each case with good linearity for protein level or incubation time vs. substrate hydrolysis (Fig. 2). Mouse erythrocyte LysoPC hydrolytic activity was considerably lower (0.51 ± 0.05 pmol/min/mg, $n=5$ subjects) than that of human erythrocytes. The LysoPC hydrolase activity of human erythrocytes was predominantly (98 \pm 2%) in the cytosol, of human brain was mostly in the membrane fraction (96 \pm 7%), and of human blood was almost entirely (>99%) in the erythrocyte fraction, with the lymphocyte and plasma portions contributing <1% of the total activity.

OP sensitivity and specificity profiles

Erythrocyte, lymphocyte, and brain LysoPC hydrolases are ultra sensitive to *S*-OBDPO, IDFP, and EOPF (IC_{50} s 0.13–0.93 nM), highly sensitive to DP-dichlorvos, DP-CPO and *R*-OBDPO (IC_{50} s 3.2–85 nM), moderately sensitive to CPO, TBDPO, DSF, DFP, and OSF (IC_{50} s 210–10,500 nM), and somewhat less sensitive to phenyl benzylcarbamate (Table 1). The other candidate inhibitors (compounds 13–15) are generally less potent. Using data for the 12 compounds with discrete IC_{50} numbers for all enzyme sources, the r^2 values (0.93–0.97) indicate a conserved inhibitor specificity or binding site conformation (Fig. 3). This is considered to be preliminary evidence that the erythrocyte, lymphocyte, and brain enzyme(s) are very similar to each other.

Delayed neurotoxicant inhibitors

EOPF was used to determine the relative *in vivo* sensitivity of erythrocyte and brain LysoPC hydrolase and brain NTE activities at 4 h after ip treatment. EOPF at 1 and 3 mg/kg caused dose-dependent reduction in erythrocyte LysoPC hydrolase activity closely paralleling the brain LysoPC hydrolase and NTE

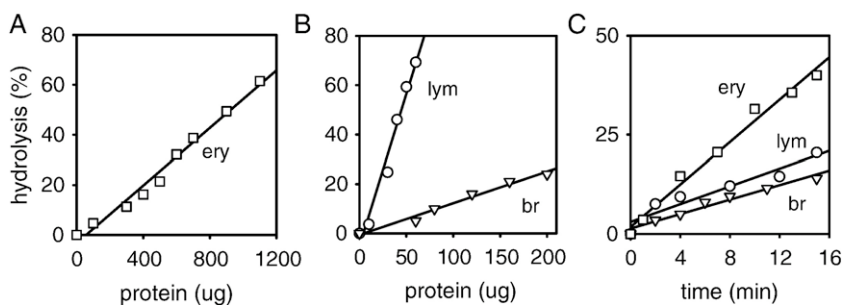


Fig. 2. [^{14}C]LysoPC hydrolytic activities of human erythrocytes (ery), lymphocytes (lym), and brain (br). (A and B) Dependence on protein level in assays for 15 min. (C) Dependence on time with protein levels (μg) of 700 for erythrocytes, 25 for lymphocytes, and 80 for brain.

Table 1
Inhibitor sensitivity and specificity profiles *in vitro* for human erythrocyte, lymphocyte, and brain LysoPC hydrolases

Compounds ^a	IC ₅₀ ±SD (nM) (n=3)		
	Erythrocytes	Lymphocytes	Brain
1 S-OBDO	0.25±0.07	0.19±0.00	0.71±0.09
2 IDFP	0.31±0.03	0.13±0.00	0.72±0.18
3 EOPF	0.39±0.06	0.93±0.08	0.48±0.03
4 DP-dichlorvos	3.2±0.4	4.2±0.8	41±5.3
5 DP-CPO	24±5	5.2±0.8	85±22
6 R-OBDO	23±6	16±3	62±9
7 CPO	230±53	210±30	230±9
8 TBDPO	640±92	510±180	813±70
9 DSF	800±170	220±20	1800±80
10 DFP	3100±460	10,500±3000	9600±2800
11 OSF	3600±530	2800±500	4300±600
12 Phenyl benzylcarbamate	21,000±5200	13,000±600	66,000±17,000
13 Mipaflox	<50,000 (65±5) ^b	<50,000 (62±2) ^b	>50,000 (40±5) ^b
14 Paraoxon	>40,000 (32±3) ^b	<40,000 (77±1) ^b	>40,000 (22±10) ^b
15 Phenylmethanesulfonyl fluoride	>100,000 (17±13) ^b	>100,000 (26±2) ^b	>100,000 (23±2) ^b

^a Three types of compounds are represented: 1–8, 10, 13, and 14 are OPs; 12 is a carbamate; 9, 11, and 15 are sulfonyl fluorides. See the abbreviations for compound identifications.

^b Inhibition (%) at indicated concentration.

diminished activities (Fig. 4). At 1 mg/kg, erythrocyte LysoPC hydrolase activity was inhibited 59% while brain LysoPC hydrolase and NTE activities were inhibited 18 and 55%, respectively. At 3 mg/kg, erythrocyte inhibition was 84%, while brain LysoPC hydrolase and NTE activities were inhibited 88 and 99%, respectively. Mice 4 h post-treatment with EOPF at 1 mg/kg appeared normal without significant hyperactivity measured as vertical rearing and distance traveled (data not shown).

OP insecticide inhibitors

Seven of the OPs (or their bioactivated metabolites) were compared for inhibition of LysoPC hydrolases *in vitro* and *in vivo*. The most potent inhibitor was CPO with IC₅₀ values of 210–900 nM while diazoxon and oxydemeton-methyl were much less potent (Table 2). Interestingly, the mouse enzyme preparations appeared to be less sensitive than the human enzymes to CPO, diazoxon, and oxydemeton-methyl (IC₅₀s 900

vs. 230 nM, >100,000 vs. 42,000 nM, and >100,000 vs. 68,000 nM, respectively). The remaining insecticides and metabolites had IC₅₀ values over 100 μM. A similar sensitivity comparison was made for *in vivo* inhibition of mouse erythrocyte LysoPC hydrolases at 4 h post-treatment (Table 3). At the highest non-lethal dose, acephate and diazinon showed the greatest inhibition (24–28%), while dimethoate and chlorpyrifos were less potent with 14–16% inhibition. The remaining compounds did not inhibit LysoPC hydrolases at the test doses.

Activity and OP sensitivity of erythrocytes from agricultural workers

Some enzymes are not fully functional at birth, so possible differences were considered in erythrocyte LysoPC hydrolases

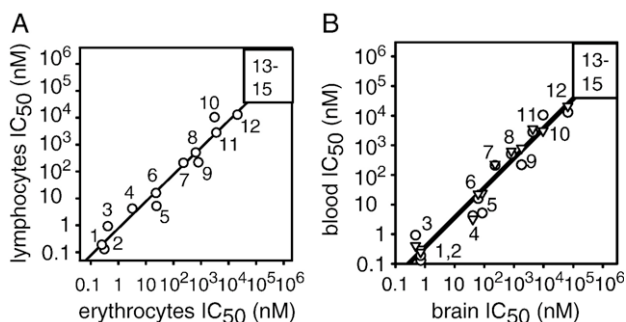


Fig. 3. Correlation of OP sensitivity and specificity profiles for human erythrocyte, lymphocyte, and brain LysoPC hydrolases. (A) Erythrocytes compared with lymphocytes. (B) Brain compared with erythrocytes (∇) and lymphocytes (○). The data plotted are from Table 1. Correlation coefficients for compounds 1–12 with discrete IC₅₀ values: (A) $r^2=0.96$; (B) erythrocytes $r^2=0.97$ and lymphocytes $r^2=0.93$.

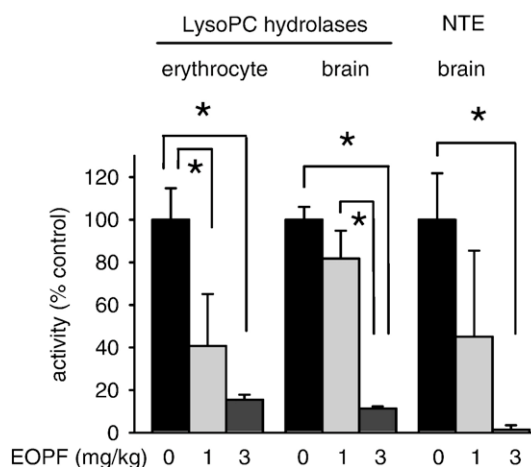


Fig. 4. Effect of EOPF on mouse erythrocyte and brain LysoPC hydrolase activities and brain NTE activity assayed with [¹⁴C]LysoPC 4 h after ip treatment. The mipaflox-sensitive brain NTE activity represents 12±4% (n=4) of the total LysoPC hydrolase activity. * $p<0.017$, adjusted for Bonferroni inequality.

Table 2
OP insecticide or activated metabolite potencies for *in vitro* inhibition of human and mouse erythrocyte and lymphocyte LysoPC hydrolases

Compound	IC ₅₀ ±SD (nM) (n=3) ^a		
	Human		Mouse
	Lymphocyte	Erythrocyte	Erythrocyte
CPO	210±30	230±53	900±330
Diazoxon	67,000±2300	42,000±3000	>100,000 (37±3) ^b
Oxydemeton-methyl	>100,000 (5±4) ^b	68,000±6000	>100,000 (15±27) ^b

^a OPs with IC₅₀s>100,000 nM for all three assay systems were acephate, malaoxon, methamidophos, and *o*-methoate.

^b Inhibition (%) at indicated concentration.

between children and adults. Newborn children and their mothers were examined in two independent experiments using pooled erythrocyte samples to minimize intersample differences. The first experiment ($n=16$) indicated that newborns had 60% of the activity of their mothers, while a second larger experiment ($n=31$) showed similar LysoPC hydrolase activities (7.9 ± 1.0 and 7.3 ± 1.7 pmol/min/mg for newborns and mothers, respectively; $p>0.5$). PON1 activity is indirectly a potential source of variability since by possibly detoxifying OPs it may reduce their effectiveness as inhibitors of LysoPC hydrolases in erythrocytes. If true, we expected that the *PON1*₁₉₂ QQ subjects, especially newborns, would show lower levels of LysoPC hydrolase activity compared with the normal *PON1*₁₉₂ RR due to the greater availability of OPs in their blood. However, although the data are not shown, the *PON1*₁₉₂ QQ and *PON1*₁₉₂ RR groups showed no significant difference in erythrocyte LysoPC hydrolase activities in both mothers and newborns. The erythrocytes from pooled samples of newborns and their mothers were compared for sensitivity to five OPs of varying potency (Table 4). There was no significant difference in the IC₅₀ values for IDFP, CPO, OOS, diazoxon, or oxydemeton-methyl (p values between 0.09 and 0.87). Finally, we investigated the possibility that *PON1*₁₉₂ genotype could influence the availability of OPs to inhibit other secondary targets. To test this hypothesis, the LysoPC hydrolase activity of erythrocyte pools created according to the *PON1* genotype was tested for sensitivity to 200 nM CPO but no difference was found in three experiments (percent inhibition±SD), i.e. 53 ± 11 and 59 ± 7 for *PON1*₁₉₂ QQ and

Table 3
OP insecticide *in vivo* inhibition of mouse erythrocyte LysoPC hydrolases and AChE at 4 h post-treatment

Compound	Inhibition (%)		
	Dose (mg/kg)	LysoPC hydrolases	AChE ^a
Acephate	30	28±7	59±12
Diazinon	30	24±27	85±4
Dimethoate	100	16±7	74±16
Chlorpyrifos	30	14±15	93±7
Malathion	30	0±0	2±2
Methamidophos	3	1±2	64±12
Oxydemeton-methyl	10	0±0	72±2

^a Quistad et al. (2005).

Table 4
OP sensitivities of erythrocyte LysoPC hydrolases of newborn children and their mothers

Compounds ^a	IC ₅₀ ±SD (nM) (n=3)	
	Children	Mothers
IDFP	0.055±0.006	0.048±0.002
CPO	190±30	170±10
OOS	15,700±2300	16,200±3500
Diazoxon	29,700±6000	40,300±5500
Oxydemeton-methyl	>100,000 (18±4) ^b	>100,000 (1) ^b

^a See the abbreviations for compound identifications.

^b Inhibition (%) at indicated concentration.

RR newborn pools, respectively, and 52 ± 4 and 62 ± 8 for *PON1*₁₉₂ QQ and RR mothers.

Discussion

OP sensitivity and specificity profiles

The LysoPC hydrolases of human erythrocytes, lymphocytes, and brain are observed here to be surprisingly similar in OP sensitivity and specificity. All three activities are extremely sensitive to OPs with long alkyl chains (IC₅₀ values <1 nM for *S*-OBDPO, IDFP and EOPF) but less sensitive to widely used OP insecticides.

Delayed neurotoxicant inhibitors

Two of the three most potent inhibitors of LysoPC hydrolases (*S*-OBDPO and EOPF, Table 1) are delayed neurotoxicants in mice and hens (Wu and Casida, 1996). Human erythrocyte LysoPC hydrolase activity in this study is >300-fold more sensitive than mouse brain AChE to two OP NTE inhibitors (IDFP and EOPF) (Casida and Quistad, 2004). Thus, inhibition of blood LysoPC hydrolases is a candidate marker of OP-delayed neurotoxicants. However, high intersample variation of human erythrocyte LysoPC hydrolase activities would necessitate a pre-exposure baseline for each individual. The lipid profile of erythrocytes is influenced by dietary intake (Popp-Snijders et al., 1986), and the impact of diet on erythrocyte LysoPC hydrolases is unexplored.

OP insecticide inhibitors

The traditional enzymes for OP exposure monitoring are erythrocyte AChE and to some extent plasma BChE. However, each serine hydrolase has a unique inhibitor profile and is worthy of consideration for monitoring exposure to complex mixtures of pesticides and neurotoxicants (Quistad et al., 2006). For example, erythrocyte acylpeptide hydrolase is a sensitive marker for exposure to some OP pesticides and metabolites (Richards et al., 2000) and relative to erythrocyte LysoPC hydrolases is 11-fold more sensitive to CPO (with an IC₅₀ of 21 nM), 45-fold to diazoxon, and >16-fold to acephate (Quistad et al., 2005). The most potent insecticide metabolite inhibitor of human blood LysoPC hydrolases is CPO, with an IC₅₀ of

210 nM for lymphocytes and 230 nM for erythrocytes. The other insecticides or metabolites tested were less potent or completely inactive at 100 μ M. OP compounds administered to mice were generally weak *in vivo* inhibitors of erythrocyte LysoPC hydrolases, i.e. at the highest non-lethal doses, acephate and diazinon inhibited about one quarter of the total activity and the other compounds showed less inhibition. These *in vivo* data suggest that even at large doses of OPs, erythrocyte LysoPC hydrolases are not likely to be appreciably inhibited. Erythrocyte AChE was a more sensitive indicator of OP insecticide exposure (59–93% inhibition with the exclusion of malathion, Table 3) (Quistad et al., 2005). Most of the acute toxicity is due to inhibition of AChE, while LysoPC hydrolases do not appear to be involved. Thus, erythrocyte LysoPC hydrolases are very sensitive biomarkers for OP delayed neurotoxicants but AChE is better for the commonly-used OP insecticides.

LysoPLAs and NTE regulate LysoPC levels

LysoPC-hydrolyzing enzymes, particularly the LysoPLAs, are the major route by which LysoPC levels are controlled (Zhang et al., 1991; Ross and Kish, 1994; Wang and Dennis, 1999). LysoPLA activity is reported in brain (Ross and Kish, 1994; Quistad et al., 2003) and erythrocytes (Mulder et al., 1965), and the current study describes LysoPC hydrolase activity in lymphocytes as well. NTE is a contributor to brain LysoPC hydrolyzing activity (Quistad and Casida, 2004). NTE is found in lymphocytes (Lotti et al., 1986), but the blood enzymes responsible for LysoPC hydrolytic activity are not identified. Although LysoPLAs and NTE hydrolyze the same LysoPC substrate, they are not homologous enzymes. The catalytic site of LysoPLA I is a typical α/β Ser–Asp–His triad (Wang et al., 1997; Devedjiev et al., 2000), while that of the modeled NTE patatin domain consists of a Ser–Asp dyad (Wijeyesakere et al., 2007), also present in the active sites of the patatin protein and phospholipase A₂ (Dessen et al., 1999; Rydel et al., 2003). LysoPLAs and other phospholipases regulate phospholipid levels to maintain membrane homeostasis, flexibility, and permeability (Selle et al., 1993; Wang and Dennis, 1999), and NTE apparently performs the same function relative to LysoPC. This is critical to cell maintenance, as excess LysoPC leads to shape change and lysis in erythrocytes (Shohet and Beutler, 1983) and is associated with several diseases such as atherosclerosis, inflammation, and hyperlipidemia (Wang and Dennis, 1999). Both NTE and LysoPLAs are involved in LysoPC hydrolysis and their inhibition may contribute to the delayed effects of some OP toxicants.

Acknowledgments

This work was supported by Grants 2 P01 ES09605 from the National Institute of Environmental Health Sciences (NIEHS) and US EPA (to B.E. and N.H.) and by Grant R01 ES08762 (to J.E.C.) from the NIEHS, NIH, and its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIEHS, NIH. We thank Gary Quistad,

Daniel Nomura, and Kevin Ford of the Environmental Chemistry and Toxicology Laboratory for advice and assistance. We acknowledge the help of Kim Harley and Asa Bradman on the CHAMACOS project and the participation of the CHAMACOS families and staff. We are also grateful to Karen Huen and Nishat Shaikh of the Children's Environmental Health Laboratory for their help with statistical analysis and biological samples. The authors declare that there are no conflicts of interest.

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