Toxicology 353–354 (2016) 11–20

Contents lists available at ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

Esterase detoxication of acetylcholinesterase inhibitors using human liver samples *in vitro*

Virginia C. Moser[a,*], Stephanie Padilla[b]

[a] Toxicity Assessment Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, US Environmental Protection Agency, Research Triangle Park, NC, USA
[b] Integrated Systems Toxicology Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, US Environmental Protection Agency, Research Triangle Park, NC, USA

ARTICLE INFO

Article history:
Received 4 February 2016
Received in revised form 31 March 2016
Accepted 21 April 2016
Available online 27 April 2016

Keywords:
Acetylcholinesterase inhibitor
PON1
Carboxylesterase
Organophosphate
Carbamate
In vitro
Human

ABSTRACT

Organophosphorus (OP) and *N*-methylcarbamate pesticides inhibit acetylcholinesterase (AChE), but differences in metabolism and detoxication can influence potency of these pesticides across and within species. Carboxylesterase (CaE) and A-esterase (paraoxonase, PON1) are considered factors underlying age-related sensitivity differences. We used an *in vitro* system to measure detoxication of AChE-inhibiting pesticides mediated via these esterases. Recombinant human AChE was used as a bioassay of inhibitor concentration following incubation with detoxifying tissue: liver plus Ca^{+2} (to stimulate PON1s, measuring activity of both esterases) or EGTA (to inhibit PON1s, thereby measuring CaE activity). AChE inhibitory concentrations of aldicarb, chlorpyrifos oxon, malaoxon, methamidophos, oxamyl, paraoxon, and methylparaoxon were incubated with liver homogenates from adult male rat or one of 20 commercially provided human (11–83 years of age) liver samples. Detoxication was defined as the difference in inhibition produced by the pesticide alone and inhibition measured in combination with liver plus Ca^{+2} or liver plus EGTA. Generally, rat liver produced more detoxication than did the human samples. There were large detoxication differences across human samples for some pesticides (especially malaoxon, chlorpyrifos oxon) but not for others (*e.g.*, aldicarb, methamidophos); for the most part these differences did not correlate with age or sex. Chlorpyrifos oxon was fully detoxified only in the presence of Ca^{+2} in both rat and human livers. Detoxication of paraoxon and methylparaoxon in rat liver was greater with Ca^{+2}, but humans showed less differentiation than rats between Ca^{+2} and EGTA conditions. This suggests the importance of PON1 detoxication for these three OPs in the rat, but mostly only for chlorpyrifos oxon in human samples. Malaoxon was detoxified similarly with Ca^{+2} or EGTA, and the differences across humans correlated with metabolism of *p*-nitrophenyl acetate, a substrate for CaEs. This suggests the importance of CaEs in malaoxon detoxication. Understanding these individual differences in detoxication can inform human variability in pesticide sensitivity.

Published by Elsevier Ireland Ltd.

1. Introduction

Organophosphorus (OP) and *N*-methyl carbamate pesticides inhibit acetylcholinesterase (AChE), blocking the hydrolysis of acetylcholine at cholinergic nerve terminals and leading to overstimulation of the cholinergic pathways and subsequent toxicity (reviewed in Fukuto 1990; Marrs, 1993). Concern has been raised regarding greater sensitivity to these pesticides in infants and children, and there are considerable differences across pesticides in terms of these age-related differences (National Research Council, 1993). Kinetic factors, particularly detoxication, have been proposed as a basis underlying these differences (Chambers et al., 1994).

For many of these pesticides, interactions with esterases serve as detoxication pathways. Carboxylesterases (EC 3.1.1.1; CaE) bind stoichiometrically to and are inhibited by some OP and carbamate pesticides, thereby serving as a "sink" to remove active chemical from the system (Aldridge, 1993; Aldridge and Reiner, 1975; Junge and Krisch 1975; Satoh and Hosokawa, 1998, 2006). CaEs, a family of isozymes that vary across tissues, species, and substrate specificity, also hydrolyze other pesticides such as pyrethroids (Jokanović et al., 1996; Satoh and Hosokawa, 2006; Sogorb and Vilanova, 2002; Wheelock et al., 2005). Pharmacological influences

* Corresponding author. Toxicity Assessment Division (MD B105-04), 109 TW Alexander Dr US EPA, RTP, NC 27711, USA.
E-mail address: Moser.ginger@epa.gov (V.C. Moser).

http://dx.doi.org/10.1016/j.tox.2016.04.006
0300-483X/Published by Elsevier Ireland Ltd.

or genetic manipulations of CaE activity may influence the detoxication of specific AChE inhibitors and therefore impact their toxicity (Benke and Murphy, 1974; Satoh and Hosokawa, 1998).

Human CES1 (hCES1) and CES2 (hCES2) are major CaE isozymes in liver, with hCES1 being predominant (Ross and Crow, 2007; Ross et al., 2010): these enzymes are not present in human plasma (Li et al., 2005). Studies of the ontogeny of one or both of these isozymes in humans have consistently shown lower activity in the young. For example, across 104 liver microsomal samples, expression and hydrolytic activity of CaEs in fetal tissues were lowest, with tissues from children (0–10 years) also somewhat less active than adults (≥18 years old) (Yang et al., 2009). Within each of these age groups, inter-individual variability showed a similar correlation with age. Others have also reported low activity in tissues from humans at 1 year of age or younger (Pope et al., 2005; Zhu et al., 2009). CaE activity in humans is also influenced by genetics, nutrition, and other factors (Satoh and Hosokawa, 2006) that may impact their effectiveness.

A-esterases (EC 3.1.8.1) are aryldialkyl phosphatases that depend on divalent cations for activity and hydrolyze, but are not inhibited by, a number of pesticides (Aldridge, 1993; Sogorb and Vilanova, 2002; Vilanova and Sogorb, 1999; Walker, 1993; Walker and Mackness, 1987). The A-esterase that hydrolyzes paraoxon was originally termed paraoxonase (PON1), and it is still referred to as PON1 even though it has since been shown that it is not specific for paraoxon. While OPs are mostly detoxified by PON1, the paraoxonase gene family in humans (PON1, PON2, and PON3) also plays major roles in metabolism of oxidized lipids and cardiovascular health (Dragomir et al., 2005; Furlong et al., 2000, 2005; Mackness et al., 1998; Mackness and Mackness, 2015).

A-esterase activity in humans has been mostly documented in blood samples, although the enzyme is synthesized and secreted by liver (Mackness et al., 1998). Individual differences in PON1 are due to genetic variability of the enzyme isoforms and activity as well as age, drugs, and nutritional status (Costa et al., 2005b; Ginsberg et al., 2009; Schrader and Rimbach, 2011). Polymorphisms of PON1 that impact hydrolytic capacity for several OPs have been widely studied in humans (Eckerson et al., 1983; Furlong, 2007; Furlong et al., 2002), in terms of sensitivity to OP pesticides as well as general health conditions. Even within these polymorphic groupings, there is considerable variability in activity. Studies have shown that individuals with specific PON1 polymorphisms could be more or less sensitive to some or all pesticides (Costa et al., 2005a; Povey, 2010; You et al., 2013). Most studies show that PON1 activity is low in fetal and newborn tissue but reaches adult levels, as early as 1–2 yr (Augustinsson and Barr, 1963; Cole et al., 2003; Ecobichon and Stephens, 1973; Furlong et al., 2005; Holland et al., 2006; Mueller et al., 1983) and possibly as late as 7 years of age (Huen et al., 2009). In addition, regardless of PON1 status, individual variability is greater in developing infants and children than among adults (Chen et al., 2003; Furlong et al., 2002).

The relative role of esterase (both CaE and PON1) detoxication differs across OPs and carbamates, which influences the total metabolic profile and subsequent toxicity of these pesticides. Knowledge of such differences can inform predictions of human variability in susceptibility or sensitivity. The majority of studies on CaEs and PON1 have focused on detoxication of only a handful of OPs (*e.g.*, chlorpyrifos, parathion, methylparathion), whereas such information is lacking for many other AChE-inhibiting pesticides. In earlier studies, we used a simple *in vitro* system to evaluate esterase detoxication of 16 OP and carbamate pesticides (Moser and Padilla, 2011; Padilla et al., 2000, 2004). This system indirectly measures the disappearance of the chemical via incubation with liver tissue, using inhibition of AChE as a bioassay of pesticide concentration. We reported, using liver tissue from adult and neonatal rats, that chemicals that show greater sensitivity (*i.e.*, greater AChE inhibition) in the young rat *in vivo* also show considerably less detoxication in the presence of neonatal liver (Padilla et al., 2000, 2004). We also showed large differences across chemicals in terms of overall detoxication and relative esterase contribution (Moser and Padilla, 2011). Here we test a subset of those chemicals using commercially obtained human liver samples to evaluate rat and human differences as well as individual variability in detoxication.

2. Methods

2.1. Chemicals

AChE inhibitors were obtained from Chem-Serve Inc (West Chester, PA): chlorpyrifos oxon (CAS 5598-15-2, 99.1% purity), malaoxon (CAS 1634-78-2, 95% purity), methamidophos (CAS 10265-92-6, 98% purity), methylparaoxon (CAS 950-35-6, 98.3% purity), ethyl paraoxon (CAS 311-45-5, 98.7% purity), aldicarb (CAS 116-06-3, 99.3% purity), and oxamyl (CAS 23135-22-0, 99% purity). All pesticides were dissolved in methanol (99.9% pure, HPLC grade) at the appropriate concentrations, added to the microtiter plate, and then the methanol was placed under a hood for 15 min to allow evaporation. Human recombinant AChE (#C1682) and all other chemicals (analytical grade) were obtained from Sigma-Aldrich (St. Louis, MO). Buffers were titrated to the desired pH at room temperature.

2.2. Liver samples

Rat liver samples were obtained from five adult male Long-Evans rats (Charles River Laboratory, Raleigh, NC). Rats were housed on hardwood chip bedding, provided free access to feed and filtered water for a week after receipt, and then euthanized with CO_2. All procedures and husbandry were approved by the NHEERL Animal Care and Use Committee. Livers were combined and homogenized at 1:50 (w/v) in Tris buffer (20 mM, pH 8) on ice, aliquoted into single-use tubes, and frozen at −80 °C.

Twenty human liver samples from volunteer donors were obtained from Cellzdirect (Morrisville, NC). This study was reviewed by the US EPA Human Subjects Research Board and

Table 1
Characteristics of human liver samples.

Sample	Age	Sex	Race	BMI
H1	11	F	C	25.6
H2	24	F	C	24.1
H3	52	F	C	26.2
H4	47	F	C	19.0
H5	75	F	C	29.9
H6	11	M	C	24.5
H7	23	M	H	27.8
H8	24	M	AA	29.7
H9	51	M	C	23.3
H10	77	M	C	25.7
H11	83	F	C	19.7
H12	34	F	H	32.8
H13	16	M	H	23.0
H14	51	F	C	27.6
H15	25	M	C	40.8
H16	59	M	C	28.9
H17	36	M	C	31.2
H18	61	M	C	24.5
H19	47	M	C	23.0
H20	59	F	C	33.1

BMI = body mass index, based on weight and height. M = male, F = female.
C = Caucasian, H = Hispanic, AA = African American.

Download English Version:

https://daneshyari.com/en/article/2595451

Download Persian Version:

https://daneshyari.com/article/2595451

Daneshyari.com