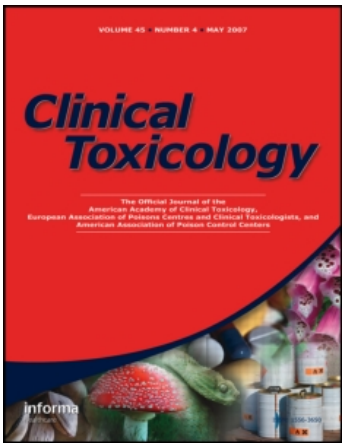

Clinical Toxicology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597279

Polymorphisms of Paraoxonase (PON1) and Their Significance in Clinical Toxicology of Organophosphates

Lucio G. Costa [a]; Toby B. Cole [b]; Clement E. Furlong [b]
[a] Department of Pharmacology and, Human Physiology, University of Bari, Bari, Italy [b] Departments of Genome Sciences and Medicine, Division of Medical Genetics, University of Washington, Seattle, Washington, USA

Online Publication Date: 01 March 2003

To cite this Article Costa, Lucio G., Cole, Toby B. and Furlong, Clement E.(2003)'Polymorphisms of Paraoxonase (PON1) and Their Significance in Clinical Toxicology of Organophosphates',Clinical Toxicology,41:1,37 — 45
To link to this Article: DOI: 10.1081/CLT-120018269
URL: http://dx.doi.org/10.1081/CLT-120018269

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

Journal of Toxicology
CLINICAL TOXICOLOGY
Vol. 41, No. 1, pp. 37–45, 2003

ARTICLE

Polymorphisms of Paraoxonase (PON1) and Their Significance in Clinical Toxicology of Organophosphates

Lucio G. Costa,[1,2,*] Toby B. Cole,[1,3] and Clement E. Furlong[3]

[1]Department of Environmental Health, University of Washington, Seattle, Washington, USA
[2]Department of Pharmacology and, Human Physiology, University of Bari, Bari, Italy
[3]Departments of Genome Sciences and Medicine, Division of Medical Genetics, University of Washington, Seattle, Washington, USA

ABSTRACT

Paraoxonase (PON1) is an HDL-associated enzyme capable of hydrolyzing multiple substrates, including several organophosphorous insecticides and nerve agents, oxidized lipids, and a number of drugs or pro-drugs. Several polymorphisms in the paraoxonase (PON1) gene have been described, which have been shown to affect either the catalytic efficiency of hydrolysis or the expression level of PON1. This review discusses the relevance of these polymorphisms for modulating sensitivity to organophosphorous compounds. Animal studies characterizing the PON1 polymorphisms have demonstrated the relevance of PON1 in modulating OP toxicity and have indicated the importance of an individual's PON1 status (i.e., genotype and phenotype taken together) rather than genotyping alone. Nevertheless, direct confirmation in humans of the relevance of PON1 status in conferring susceptibility to OP toxicity is still elusive. Recent studies examining the involvement of PON1 status in determining OP susceptibility of Gulf War veterans, sheep dippers, and individuals poisoned with chemical warfare agents represent a step in the right direction, but more studies are needed, with better documentation of both the level of exposure and the consequences of exposure.

INTRODUCTION

Adverse health effects from exposure to exogenous chemicals, including pesticides, depend on the intrinsic properties of the chemical, its dosage, and genetically determined variations in the host. Such variations, which often involve target proteins or biotransformation enzymes, have been studied for some time in relation

*Correspondence: Dr. Lucio G. Costa, Department of Environmental Health, University of Washington, 4225 Roosevelt Way NE, #100, Seattle, WA 98105-6099, USA; Fax: 206 685-4696; E-mail: lgcosta@u.washington.edu.

DOI: 10.1081/CLT-120018269
0731-3810 (Print); 1097-9875 (Online)

www.dekker.com

to differences in drug effectiveness or adverse drug reactions in the field of pharmacogenetics, and more recently as they relate to exposures to environmental agents, hence the term ecogenetics (1). Genetic polymorphisms may lead to enzyme variants with higher or lower activity and/or with different levels of expression. An example of the effects of such polymorphisms is represented by paraoxonase (PON1), a liver and plasma enzyme whose physiological role lies in its ability to metabolize oxidized lipids (2,3). PON1 is also involved in the metabolism of a number of drugs and pro-drugs (4), but its most studied role is in the metabolism of organophosphorus compounds (5). This brief review will focus on the polymorphisms of human PON1 and on their significance in modulating sensitivity to organophosphate toxicity.

TOXICITY AND METABOLISM OF ORGANOPHOSPHORUS COMPOUNDS

A number of organophosphorus compounds (OPs) include toxic triesters of phosphoric acid. Approximately 80 OPs are currently used as insecticides, while other OPs have been used as drugs (e.g., trichlorfon for schistosomiasis or ecothiophate for glaucoma), or as nerve agents in chemical warfare (e.g., sarin or soman) (6). Exposure to OPs is associated with three distinct syndromes: a *cholinergic syndrome*, as a consequence of inhibition of acetylcholinesterase (AChE) and accumulation of acetylcholine at muscarinic and nicotinic receptors throughout the body; a still poorly defined *intermediate syndrome*, characterized by weakness of respiratory, neck, and proximal limb muscles, which occurs hours to days after the onset of severe cholinergic over-stimulation; and a *delayed polyneuropathy*, which is caused only by certain OPs, and can be described as dying-back axonopathy, whose primary event appears to be inhibition of another enzyme, neuropathy target esterase (NTE) (7). Only OPs with a P═O moiety can interact with AChE or NTE. Since most OP insecticides are organothiophosphates, they require metabolic activation to their corresponding oxygen analogs. Such activation is mediated by various isozymes of cytochrome P450, which are also involved in the detoxication of OPs. In addition, OPs can be detoxified hydrolytically by the action of A-esterases (8). One of these A-esterases is paraoxonase (PON1), which can hydrolyze the oxygen analogs of various commonly used OP insecticides and some nerve agents (9).

ROLE OF PARAOXONASE (PON1) IN ORGANOPHOSPHATE METABOLISM

Paraoxonase (PON1) is a high-density lipoprotein (HDL)-bound enzyme present primarily in plasma and liver. In vitro, PON1 can hydrolyze a number of organophosphate oxons such as chlorpyrifos oxon, diazoxon, paraoxon, as well as nerve agents such as sarin or soman, suggesting that it may play an important role in OP detoxication in vivo, and hence modulate their toxicity. Initial indirect evidence for such hypotheses came from cross-species comparisons. Birds, which have no or very low plasma PON1 activity, were more sensitive than rats to the toxicity of various OPs (10). In turn, rats were found to be more sensitive to the toxicity of OPs than rabbits, which have a seven-fold higher plasma PON1 activity (11). A more direct approach was provided by studies in which exogenous PON1 from rabbit serum was shown to protect rats from the toxicity of paraoxon (12). More recently, similar experiments utilized i.v. injections of PON1, purified to homogeneity from rabbit serum, which raised rat plasma PON1 activity toward paraoxon and chlorpyrifos oxon by 9- and 50-fold, respectively (13). When rats were challenged with either OP, significant protection (assessed by measuring inhibition of AChE in different tissues) was observed. Protection was more evident in the case of chlorpyrifos oxon and was also present when OP exposure occurred by the dermal route, which represents an important route of occupational exposure (13). Further experiments, which followed a similar protocol, extended these findings to mice (14). It was also found that by administering purified PON1 by the i.v. + i.m. route, serum PON1 levels could be increased for an extended period ($t1/2 > 30$ h) and that exogenous PON1 could also afford protection against the toxicity of chlorpyrifos, the parent compound used as an insecticide, when given before or even after (up to three hours) OP exposure (14,15).

OP toxicity has also been investigated in PON1 knockout ($PON1^{-/-}$) mice, which were produced by targeted disruption of exon 1 of the PON1 gene (16). $PON1^{-/-}$ mice have no plasma or liver hydrolytic activity toward paraoxon and diazoxon, and a very low level activity toward chlorpyrifos oxon (17). $PON1^{-/-}$ mice have dramatically increased sensitivity to chlorpyrifos oxon and diazoxon, and a slightly increased sensitivity to the respective parent compounds chlorpyrifos and diazinon (16,17). Surprisingly, $PON1^{-/-}$ mice did not show an increased sensitivity to paraoxon, the OP after which PON1 was named, despite a total lack of paraoxonase activity in plasma and liver (17).

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

Altogether, these animal studies provide evidence that PON1 plays a relevant role in modulating the toxicity of specific OPs. Indeed, increasing plasma PON1 activity by injection of purified PON1 or abolishing both plasma and liver activity, as in the $PON1^{-/-}$ mice, causes significant decreases or increases, respectively, in OP toxicity.

PARAOXONASE POLYMORPHISMS AND THEIR SIGNIFICANCE

Earlier studies measuring PON1 activity (utilizing either phenylacetate or paraoxon as substrates) in serum from human subjects of Caucasian origin revealed a bimodal or trimodal distribution (18,19). On the basis of enzymatic tests, humans could be divided into three serum PON1 phenotypes, with low, intermediate, and high activity. Studies in the early 1990s led to the purification, cloning, and sequencing of human PON1 (20–22). The PON1 cDNA encodes a protein of 355 amino acids, from which only the amino-terminal methionine residue is removed during secretion and maturation. Physical mapping placed the human PON1 gene on chromosome 7 q21-22 (23).

Two polymorphisms were observed in the PON1 coding sequence: a Gln (Q)/Arg (R)substitution at position 192, and a Leu (L)/Met (M) substitution at position 55 (23,24). PON1 192 and 55 genotypes have been established in several populations (25), utilizing a PCR method (23). The polymorphism at position 192 has been the most studied, with gene frequencies of $PON1_{Q192}$ ranging from 0.75 for Caucasian of northern European origin to 0.31 for some Asian populations (25). The L_{55} and R_{192} alleles are in strong disequilibrium, with approximately 98% of the R_{192} alleles having L at position 55 (25).

In addition to these two polymorphisms in the coding region of PON1, five polymorphisms have been found in the noncoding region of the PON1 gene (26–28). These are at positions −108 (C/T), −126 (G/C), −162 (A/G), −832 (G/A) and −909 (C/G). The most significant of these promoter region polymorphisms turned out to be that at position −108, which contributes 22.4% of the variation in PON1 expression, while the polymorphism at position − 162 contributes only 2.4% (29).

The coding region polymorphisms of PON1 have been investigated for effects on the catalytic efficiencies of hydrolysis of specific substrates. The L/M polymorphism at position 55 does not affect catalytic activity (23,24) but has been associated with variability of plasma levels, with $PON1_{M55}$ individuals having lower PON1 activity (29–31). This effect, however, seems to be related primarily to linkage disequilibrium with the −108 promoter polymorphism (25). The Q/R polymorphism at position 192, on the other hand, significantly affects the catalytic efficiency of PON1. Initial studies indicated that the $PON1_{R192}$ isozyme hydrolyzed paraoxon more readily than $PON1_{Q192}$ (23,24). Further studies indicated that this polymorphism was substrate-dependent, as the $PON1_{Q192}$ isoform was found to hydrolyze diazoxon, sarin, and soman more rapidly than $PON1_{R192}$ in vitro (32).

Because individuals homozygous for the $PON1_{Q192}$ allele appeared to have higher average diazoxonase activity than individuals homozygous for the $PON1_{R192}$ allele (32,33), it was hypothesized that the two isoforms would provide different degrees of protection against diazoxon when injected into $PON1^{-/-}$ mice. However, when either isoform was injected into $PON1^{-/-}$ mice at equivalent levels, there was no significant difference observed in the extent of protection from AchE inhibition by diazoxon (17). A major difference between the conditions of the in vitro PON1 diazoxonase assays and the in vivo experiments was the high concentration of NaCl (2 M) used in the in vitro assays. For population studies, the high salt conditions provide a better resolution of $PON1_{192}$ phenotypes/genotypes. However, the conditions of the in vitro assay do not reflect physiological conditions, where concentrations are approximately 150 mM Na^+ and 110 mM Cl^-. When the effect of NaCl on diazoxon hydrolysis by the two human PON1 isoforms was investigated in vitro, it was found that the $PON1_{Q192}$ isoform was stimulated by high NaCl concentrations, whereas the $PON1_{R192}$ isoform was inhibited by high salt (17). Under physiological conditions (150 mM NaCl) both $PON1_{192}$ isoforms exhibited similar catalytic efficiency of diazoxon hydrolysis, thus providing an explanation for the in vivo results in $PON1^{-/-}$ mice.

In the case of chlorpyrifos oxon, the $PON1_{R192}$ isoform had been shown to hydrolyze this compound at a slightly higher rate than the $PON1_{Q192}$ isoform (32,33). Unlike the situation for diazoxon, in vivo results were consistent with the initial in vitro findings. $PON1^{-/-}$ mice that received the $PON1_{R192}$ isoform had a 1.7-fold higher plasma chlorpyrifos oxonase activity than those that received the $PON1_{Q192}$ isoform, and $PON1_{R192}$ provided more protection against chlorpyrifos oxon toxicity (17). Furthermore, regardless of the NaCl concentration used in in vitro assays, $PON1_{R192}$ hydrolyzes chlorpyrifos oxon more efficiently than $PON1_{Q192}$ (17). Recent in vivo experiments in transgenic

mice expressing human $PON1_{R192}$ or $PON1_{Q192}$ on a mouse PON1 null background confirmed and expanded these findings. The two lines of transgenic mice express the same level of PON1 protein or mRNA, yet the $PON1_{R192}$ mice are significantly more resistant to chlorpyrifos oxon toxicity than the $PON1_{Q192}$ mice (Cole, Costa, and Furlong, unpublished observations).

The $PON1_{R192}$ isoform hydrolyzes paraoxon better than the $PON1_{Q192}$ isoform at all NaCl concentrations (33). Yet neither isoform when injected into $PON1^{-/-}$ mice conferred protection against paraoxon toxicity (17). Furthermore, even hPON1R-Tg mice, transgenic mice expressing the human $PON1_{R192}$ gene in addition to the endogenous mouse PON1, showed a similar sensitivity as wild-type mice to paraoxon exposure (17). The surprising results obtained with paraoxon can be explained by additional experiments in which the catalytic efficiency of each purified human plasma $PON1_{192}$ isoform was tested under more physiological conditions (17). Though the efficiency of $PON1_{R192}$ was more than seven-times greater than that of the PON_{Q192} isoform, the catalytic efficiencies for hydrolysis of paraoxon by either $PON1_{192}$ isoform were very low compared with those for hydrolysis of diazoxon and chlorpyrifos oxon (Table 1). This confirms the hypothesis that PON1 is not efficient at hydrolyzing paraoxon at low concentrations, suggesting that metabolic enzymes other than PON1 are primarily responsible for detoxifying paraoxon in vivo (34,35). The better catalytic efficiency of $PON1_{R192}$ for chlorpyrifos oxon (Table 1) is in agreement with the previously discussed studies. Similarly, the higher rate of diazoxon hydrolysis by the $PON1_{Q192}$ isoform is compensated by the better affinity of the $PON1_{R192}$ isoform for diazoxon, resulting in nearly identical catalytic efficiencies (Table 1), again in agreement with the results of the in vivo studies.

Altogether, these in vitro and in vivo studies have clarified the relative role of the two $PON1_{192}$ isoforms in modulating the toxicity of certain OPs. As said earlier, a promoter region polymorphism affects the level of PON1 expression, which represents a second major determinant for determining the "PON1 status" of an individual (14,33). By plotting rates of diazoxon hydrolysis against paraoxon hydrolysis at high salt concentrations (2 M NaCl), an accurate inference of $PON1_{192}$ genotypes as well as PON1 activity levels for individuals can indeed be made (33,36). Results from animal studies with OPs, as well as from human studies on cardiovascular disease (36,37), do indeed indicate that PON1 status, comprised of both plasma PON1 levels and $PON1_{192}$ genotype, rather than simple $PON1_{192}$ genotyping, is the best descriptor of an individual's pharmacogenomic profile for PON1. Indeed, even within a group of individuals homozygous for the $PON1_{R192}$ or $PON1_{Q192}$ allele, enzyme activity can vary by at least 13-fold, depending on the substrate (32).

CLINICAL RELEVANCE OF PON1 POLYMORPHISMS

The early finding that human serum PON1 activity presented a bimodal distribution has long led to the hypothesis that low metabolizers may be more sensitive to the toxicity of OPs. The studies summarized have characterized the PON1 polymorphisms responsible for different catalytic activities and levels of expression, have demonstrated the relevance of PON1 in modulating OP toxicity in various animal models, and have indicated the importance of an individual's PON1 status. Nevertheless, direct confirmation in humans of the relevance of PON1 status in determining relative sensitivity to OP toxicity is still elusive. In recent years, however, three situations, in Japan, the Middle East, and the United Kingdom, have called for investigations in the role of PON1 in OP toxicity in humans.

On March 20, 1995 the subway system in Tokyo was subjected to a terrorist attack with sarin gas that left 12 people dead and over 5,000 injured (38,39). Sarin is metabolized by PON1, and in an in vitro assay, homozygotes for the $PON1_{Q192}$ allele were found to hydrolyze sarin approximately 10 times better then individuals homozygous for the $PON1_{R192}$ allele (32). In the Japanese population, the prevalence of the $PON1_{R192}$ genotype was found to be 0.66, compared with 0.25–0.30 for various Caucasian populations (25,40). Thus, Japanese individuals may have been more prone to sarin toxicity because of the low sarin hydrolyzing activity of $PON1_{R192}$ for sarin. However, among 10 of the victims of the Tokyo attack, 7 expressed the PON_{Q192} genotype, with 6 Q/R heterozygotes and 1 Q/Q homozygote (41). Thus, the genotype which confers high hydrolyzing activity toward sarin did not appear to provide protection from acute sarin poisoning. However, two issues should be considered: first, no evidence of the levels of PON1 were provided. In a Caucasian population, the range of sarinase activity ranged from 0 to 758 U/L among individuals with either the QQ or QR genotype (32). Second, exposure to sarin in these seven individuals was indeed massive, as it caused death either instantly or, with one exception, in less then 48 h (41). Such high-dose exposure would be expected to overcome any potential protection afforded by the $PON1_{Q192}$ genotype. Third, the catalytic efficiency of sarin hydrolysis by PON1 is

Table 1. Catalytic efficiency determines the in-vivo efficacy of PON1 for detoxifying OP compounds.

	Paraoxon				Diazoxon				Chlorpyrifos-oxon			
	$PON1^{+/+}$	$PON1^{-/-}$	192Q	192R	$PON1^{+/+}$	$PON1^{-/-}$	192Q	192R	$PON1^{+/+}$	$PON1^{-/-}$	192Q	192R
[a]Brain ChE activity (% of control)	77 ± 15	53 ± 13	41 ± 9	34 ± 11	102 ± 5	20 ± 7	68 ± 22	81 ± 17	n.d. at 2 mg/kg?	14 ± 1	28 ± 11	87 ± 13
[a]Diaphragm ChE activity (% of control)	75 ± 18	57 ± 7	54 ± 8	57 ± 12	55 ± 4	22 ± 7	47 ± 13	56 ± 13	n.d. at 2 mg/kg?	19 ± 3	47 ± 5	77 ± 15
[b]Km (mM)	—	—	0.81	0.52	—	—	2.98	1.02	—	—	0.54	0.25
[b]Vmax (units/mg)	—	—	0.57	3.26	—	—	222	79	—	—	82	64
[b]Catalytic efficiency (Vmax/Km)	—	—	0.71	6.27	—	—	75	77	—	—	152	256

[a] Toxicity (ChE inhibition) associated with dermal exposure to OP compounds (0.3 mg/kg, paraoxon; 1 mg/kg, diazoxon; 2 mg/kg, chlorpyrifos-oxon) of wild-type ($PON1^{+/+}$) and PON1 knockout ($PON1^{-/-}$) mice injected with either purified human $PON1_{192Q}$ (192Q) or $PON1_{192R}$ (192R) 4 hr prior to exposure. Data are expressed as percent ± SEM of ChE values for control mice receiving acetone alone.

[b] Paraoxonase, diazoxonase, and chlorpyrifos-oxonase activities were determined in vitro (in 0.15 M NaCl), using purified human plasma $PON1_{192Q}$ or $PON1_{192R}$ isoforms (17).

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

low, and is probably similar to the case of paraoxon hydrolysis, where one $PON1_{192}$ isoform hydrolyzes sarin with better efficiency, but still not efficiently enough to provide protection.

Among the almost 700,000 U.S. troops that were deployed to the Persian Gulf area in 1990–1991, a large number of veterans have had a range of unexplained illnesses including chronic fatigue, muscle and joint pain, loss of concentration, forgetfulness, and headache (42). Men and women who served in the Gulf War theater were potentially exposed to a wide range of biological and chemical agents, including sand, smoke from oil well fires, solvents, petroleum fuels, pyridostigmine bromide, depleted uranium, anthrax and botulinum toxoid vaccinations, insecticides, and nerve agents (42). A recent study investigated PON1 genotypes and plasma enzyme activity in a group of 25 ill Gulf War veterans and 20 controls (43). $PON1_{R192}$ homozygotes or $PON1_{Q/R192}$ heterozygotes were reported to be more likely to have neurologic symptom complexes than were individuals homozygous for $PON1_{Q102}$ (43). In addition, low activity of the plasma $PON1_{Q192}$ isoform appear to correlate with illness better than the PON1 genotype or the activity levels of the $PON1_{R192}$ isoform (43). This single study raises the possibility that the $PON1_{R192}$ genotype (low sarin-hydrolyzing activity) may represent a risk factor for illness in Gulf War veterans. However, because of the very small size of the study, such findings necessitate further confirmation in a larger population (44). Furthermore, PON1, as well as PON2 and PON3, appear to play a major role in protecting against oxidative stress. Thus, this role of the PON proteins should also be considered.

In a similar study, PON1 activity, concentration, and genotype were determined in a group of 152 Gulf War veterans from the United Kingdom who self-reported the presence of symptoms associated with the Gulf War syndrome (45). In Gulf War veterans, plasma paraoxon-hydrolyzing activity was 50% less than in a control group. PON1 concentration, measured by an ELISA assay, was slightly (14%) but significantly reduced, while plasma diazoxonase activity did not differ between the two groups. The low activity and the lower serum concentration were independent of the PON1 genotype in the veterans (45). Thus, the results of this study were different from those of Haley et al. (43). Though in both cases a reduced plasma paraoxonase activity was found, in one case it was attributed to an over-representation of the low-activity PON1 isozyme (43), whereas in the other it was common to all PON1 genotypes (45). Though the latter study suggests that this group of veterans may have a decreased capacity to hydrolyze some OP insecticides, such as paraoxon or chlorpyrifos oxon, its significance is hampered by the lack of information on the extent of exposure to such compounds among the veterans.

In recent years, the possible relationship between exposure of sheep dippers to OPs and chronic central and/or peripheral nervous system abnormalities has been investigated (46). Since one of the often-used OPs is diazinon, Cherry et al. (47) sought to investigate whether genetic polymorphisms of PON1 would play a role in such ill-health effects. Cases (175) were recruited among individuals who were ill and who believed this was because of exposure to sheep dip; the referent group consisted of 234 individuals that had also used diazinon in sheep dipping, but did not complain of any illness. The allele frequency of the $PON1_{R192}$ polymorphism was 0.35 in cases versus 0.23 in referents, and diazoxonase activity was lower in cases than referents (47). The authors of this study concluded that these findings suggest that OPs may have contributed to the ill health of people who dip sheep. However, it should be noted that the enzymatic assay was carried out at high NaCl concentration; thus, for the reasons discussed earlier, these results do not fully demonstrate that PON1 status is a key factor in a potential higher sensitivity to diazinon toxicity. Future studies may want to make use of the two-substrate assay (with diazoxon and paraoxon as substrates) to clearly infer PON1 genotypes and phenotypes, as well as a determination of rates of phenylacetate hydrolysis to quantify PON1 levels among the different $PON1_{192}$ genotypes, because the rates of phenylacetate hydrolysis do not significantly differ between the two $PON1_{192}$ isoforms.

Finally, a recent study in Turkey examined serum paraoxonase activity, PON1 concentration and PON1 55 and 192 polymorphisms in a group of 28 patients acutely poisoned with various OPs (48). The percentage of PON1Q192 homozygotes was higher among patients (86%) than controls (59%); however, no significant associations were found between the severity of symptoms upon admittance or time in hospital with PON1 activity, concentration or L55M or Q192R polymorphisms (48). Serum paraoxonase activity was 30% lower in poisoned patients than controls and was back to control levels after five months. As the concentration of PON1 did not differ between groups, the authors suggested that OPs may inactivate PON1 directly or, more likely, that the reduced activity was the result of an artifact due to in vitro competition by the intoxicating OP (48).

These studies in human populations, albeit with several shortcomings, are nevertheless of interest, as they

try to address the issue of whether an individual's PON1 status may confer protection or increased sensitivity to the toxicity of specific OPs. Clearly, more studies are needed, where better indications of the level and nature (i.e., the specific OP involved) of exposure and the consequences of exposure are documented.

CONCLUSIONS

The animal studies summarized in this review provide evidence that PON1 plays a relevant role in the metabolism of certain OPs and modulates their acute toxicity. Of interest is that the toxicity of paraoxon, the OP after which PON1 was named, is not significantly influenced by PON1. Careful in vitro studies carried out under physiological conditions, together with in vivo studies in various lines of PON1 transgenic mice, have been shown to be extremely useful in dissecting the functional significance of the PON1 polymorphisms.

Polymorphisms in the PON1 gene influence both the quantity and the quality of PON1 (i.e., PON1 status). Strong evidence indicates that PON1 levels and, in some cases, the Q192R polymorphism, determine the efficiency with which an individual will detoxify a specific OP. However, direct proof of this in human populations is still lacking and will require carefully conducted studies where PON1 status is correlated with the degree of exposure and with signs and symptoms of toxicity.

Animal studies have also pointed out the potential therapeutic use of PON1 in treating individuals for exposures to OP insecticides or nerve agents. Engineering recombinant PON1 variants with high catalytic efficiencies toward specific compounds may indeed prove to be a useful addition to the treatment of OP intoxication.

ACKNOWLEDGMENTS

Research by the authors was supported by grants ES04696, P30 ES07033, U19 ES11387, ES09883, ES09601/EPA-R826886, and T32 AG00057 (TBC).

REFERENCES

1. Costa LG. The emerging field of ecogenetics. NeuroToxicology 2000; 21:85–89.
2. Mackness MI, Durrington PN, Mackness B. The role of paraoxonase in lipid metabolism. In: Costa LG, Furlong CE, eds. Paraoxonase (PON1) in Health and Disease: Basic and Clinical Aspects. Norwell, MA: Kluwer Academic Publishers, 2002:79–92.
3. Shih DM, Reddy S, Lusis AJ. CHD and atherosclerosis: human epidemiological studies and transgenic mouse models. In: Costa LG, Furlong CE, eds. Paraoxonase (PON1) in Health and Disease: Basic and Clinical Aspects. Norwell, MA: Kluwer Academic Publishers, 2002:93–123.
4. Furlong CE, Cole TB, Jarvik GP, Costa LG. Pharmacogenomic considerations of the paraoxonase polymorphisms. Pharmacogenomics 2002; 3:1–8.
5. La Du BN. Historical considerations. In: Costa LG, Furlong CE, eds. Paraoxonase (PON1) in Health and Disease: Basic and Clinical Aspects. Norwell, MA: Kluwer Academic Publishers, 2002:1–25.
6. Lotti M. Organophosphorus compounds. In: Spencer PS, Schaumburg H, Ludolph AC, eds. Experimental and Clinical Neurotoxicology. Oxford: Oxford University Press, 2000:898–925.
7. Lotti M. Low level exposures to organophosphorus esters and peripheral nerve function. Muscle Nerve 2002; 23:492–504.
8. Sogorb MA, Vilanova E. Enzyme involved in the detoxification of organophosphorus, carbamate and pyrethroid insecticides through hydrolysis. Toxicol Lett 2002; 128:215–228.
9. Geldmacher-von Mallinckrodt M, Diepgen TL. The human serum paraoxonase: polymorphism and specificity. Toxicol Environ Chem 1988; 18:79–196.
10. Brealey CB, Walker CH, Baldwin BC. A-esterase activities in relation to the differential toxicity of pirimiphos-methyl to birds and mammals. Pestic Sci 1980; 11:546–554.
11. Costa LG, Richter RJ, Murphy SD, Omenn GS, Motulsky AG, Furlong CE. Species differences in serum paraoxonase correlate with sensitivity to paraoxon toxicity. In: Costa LG, Galli CL, Murphy SD, eds. Toxicology of Pesticides: Experimental, Clinical and Regulatory Perspectives. Heidelberg: Springer-Verlag, 1987:262–266.
12. Main AR. The role of A-esterase in the acute toxicity of paraoxon, TEPP and parathion. Can J Biochem Physiol 1956; 34:197–216.
13. Costa LG, McDonald BE, Murphy SD, Richter RJ, Motulsky AG, Furlong CE. Serum paraoxonase and its influence on paraoxon and chlorpyrifos oxon toxicity in rats. Toxicol Appl Pharmacol 1990; 103:66–76.
14. Li WF, Costa LG, Furlong CE. Serum paraoxonase status: a major factor in determining resistance to organophosphates. J Toxicol Environ Health 1993; 40:337–346.
15. Li WG, Furlong CE, Costa LG. Paraoxonase protects against chlorpyrifos toxicity in mice. Toxicol Lett 1995; 76:219–226.
16. Shih DM, Gu L, Xia YR, Navab M, Li WF, Hama S, Castellani LW, Furlong CE, Costa LG, Fogelman AM, Lusis AJ. Mice lacking serum paraoxonase are susceptible

to organophosphate toxicity and atherosclerosis. Nature 1998; 394:284–287.

17. Li WF, Costa LG, Richter RJ, Hagen T, Shih DM, Tward A, Lusis AJ, Furlong CE. Catalytic efficiency determines the in vivo efficacy of PON1 for detoxifying organophosphates. Pharmacogenetics 2000; 10:1–13.
18. Eckerson HW, White CM, La Du BN. The human serum paraoxonase/arylesterase polymorphism. Am J Hum Genet 1983; 35:1126–1138.
19. Mueller RF, Hornung S, Furlong CE, Anderson J, Giblett ER, Motulsky AG. Plasma paraoxonase polymorphism: a new enzyme assay, population, family, biochemical and linkage studies. Am J Hum Genet 1983; 35:393–408.
20. Gan KN, Smolen Al, Eckerson HW, La Du BN. Purification of human serum paraoxonase/arylesterase. Evidence for one esterase catalyzing both activities. Drug Metab Dispos 1991; 19:100–106.
21. Furlong CE, Richter RJ, Chapline C, Crabb JW. Purification of rabbit and human serum paraoxonase. Biochemistry 1991; 30:10133–10140.
22. Hassett C, Richter RJ, Humbert R, Chapline C, Crabb JW, Omiecinski CJ, Furlong CE. Characterization of DNA clones encoding rabbit and human serum paraoxonase: the mature protein retains its signal sequence. Biochemistry 1991; 30:10141–10149.
23. Humbert R, Adler DA, Disteche CM, Hassett C, Omiecinski CJ, Furlong CE. The molecular basis of the human serum paraoxonase activity polymorphism. Nat Genet 1993; 3:36–73–76.
24. Adkins S, Gan KN, Mody M, La Du BN. Molecular basis for the polymorphic forms of human serum paraoxonase/-arylesterase: glutamine or arginine at position 191, for the respective A or B allozymes. Am J Hum Genet 1993; 53:598–608.
25. Brophy VH, Jarvik GP, Furlong CE. PON1 polymorphisms. In: Costa LG, Furlong CE, eds. Paraoxonase (PON1) in Health and Disease: Basic and Clinical Aspects. Norwell, MA: Kluwer Academic Publishers, 2002:53–77.
26. Brophy VM, Hastings MD, Clendenning JB, Richter RJ, Jarvik GP, Furlong CE. Polymorphisms in the human paraoxonase (PON1) promoter. Pharmacogenetics 2001a; 11:64–77–84.
27. Leviev I, James RW. Promoter polymorphisms of human paraoxonase PON1 gene and serum paraoxonase activities and concentrations. Arterioscler Thromb Vasc Biol 2000; 20:516–521.
28. Suehiro T, Nakamura T, Inove M, Shiinoki T, Ikeda Y, Kumon Y, Shindo M, Tanaka H, Hashimoto K. A polymorphism upstream from the human paraoxonase (PON1) gene and its association with PON1 expression. Atherosclerosis 2000; 150:295–298.
29. Brophy VH, Jampsa RL, Clendenning JB, McKinstry LA, Furlong CE. Effects of 5′ regulatory-region polymorphisms on paraoxonase-gene (PON1) expression. Am J Hum Genet 2001b; 68:1428–1436.
30. Blatter Garin MC, James RW, Dussoix P, Blanche M, Passa P, Froguel P, Ruiz J. Paraoxonase polymorphism Met-Leu 54 is associated with modified serum concentrations of the enzyme. A possible link between the paraoxonase gene and increased risk of cardiovascular disease in diabetes. J Clin Investig 1997; 99:62–66.
31. Mackness B, Mackness MI, Arrol T, Turkie W, Durrington PN. Effect of the human serum paraoxonase 55 and 192 genetic polymorphisms on the protection by high density lipoprotein against low density lipoprotein oxidative modifications. FEBS Lett 1998; 423:57–60.
32. Davies HG, Richter RJ, Keifer M, Broomfield CA, Sowalla J, Furlong CE. The effect of the human serum paraoxonase polymorphism is reversed with diazoxon, soman and sarin. Nat Genet 1996; 14:334–336.
33. Richter RJ, Furlong CE. Determination of paraoxonase (PON1) status require more then genotyping. Pharmacogenetics 1999; 9:745–753.
34. Chambers JE, Ma T, Boone JS, Chambers HW. Role of detoxication pathways in acute toxicity levels of phosphorothioate insecticides in the rat. Life Sci 1994; 54:1357–1364.
35. Pond AL, Chambers HW, Chambers JE. Organophosphate detoxication potential of various rat tissues via A-esterases and aliesterase activities. Toxicol Lett 1995; 78:245–252.
36. Jarvik GP, Rozek LS, Brophy VH, Hatsukami TS, Richter RJ, Schellenberg GD, Furlong CE. Paraoxonase (PON1) phenotype is a better predictor of vascular disease than is $PON1_{192}$ or $PON1_{55}$ genotype. Arterioscler Thromb Vasc Biol 2000; 20:2441–2447.
37. Mackness B, Davies GK, Turkie W, Lee E, Roberts DH, Mill E, Roberts C, Durrington PN, Mackness MI. Paraoxonase status in coronary heart disease. Are activity and concentration more important than genotype? Arterioscler Thromb Vasc Biol 2001; 21:1461–1467.
38. Suzuki T, Morito H, Ono K, Mackawa K, Nagai R, Yazaki Y. Sarin poisoning in Tokyo subway. Lancet 1995; 345:980–981.
39. Nagao M, Takatori T, Matsuda Y, Nakajima M, Iwase H, Iwadate K. Definitive evidence for the acute sarin poisoning diagnosis in the Tokyo subway. Toxicol Appl Pharmacol 1997; 144:198–203.
40. Yamasaki Y, Sakamoto K, Watada H, Kajimoto Y, Hori M. The Arg_{192} isoform of paraoxonase with low sarin-hydrolyzing activity is dominant in the Japanese. Hum Genet 1997; 101:67–68.
41. Yamada Y, Takatori T, Nagao M, Iwase H, Kurada N, Yanagida J, Shinozuka T. Expression of paraoxonase isoform did not confer protection from acute sarin poisoning in the Tokyo subway terrorist attack. Int J Leg Med 2001; 115:82–84.

42. Institute of Medicine, Gulf War and Health. Vol. 1. Depleted Uranium, Pyridostigmine Bromide, Sarin, Vaccines. Washington, DC: National Academy Press, 2000; p. 408.
43. Haley RW, Billecke S, La Du BN. Association of low PON1 type Q (type A) arylesterase activity with neurologic symptom complexes in Gulf War veterans. Toxicol Appl Pharmacol 2000; 157:227–233.
44. Furlong CE. PON1 status and neurologic symptom complexes in Gulf War Veterans. Gen Res 2000; 10:153–155.
45. Mackness B, Durrington PN, Mackness MI. Low paraoxonase in Persian gulf War veterans self-reporting Gulf War Syndrome. Biochem Biophys Res Commun 2000; 276:729–733.
46. Pilkington A, Buchanan D, Jamal GA, Gillham R, Hansen S, Kidd M, Hurley JF, Soutar CA. An epidemiological study of the relations between exposure to organophosphate pesticides and indices of chronic peripheral neuropathy and neuropsychological abnormalities in sheep farmers and dippers. Occup Environ Med 2001; 58:702–710.
47. Cherry N, Mackness M, Durrington P, Povey A, Dippnal M, Smith T, Mackness B. Paraoxonase (PON1) polymorphisms in farmers attributing ill health to sheep dip. Lancet 2002; 359:763–764.
48. Sozmen EY, Mackness B, Sozmen B, Durrington P, Girgin FK, Aslan L, Mackness M. Effect of organophosphate intoxication on human serum paraoxonase. Hum Exp Toxicol 2002; 21:247–252.