The effect of the human serum paraoxonase polymorphism is reversed with diazoxon, soman and sarin

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Many organophosphorus compounds (OPs) are potent cholinesterase inhibitors, accounting for their use as insecticides and, unfortunately, also as nerve agents. Each year there are approximately 3 million pesticide poisonings world-wide resulting in 220,00 deaths[1–2]. In 1990, there were 1.36 million kg of chlorpyrifos, 4.67 million kg of diazinon and 1.23 million kg of ethyl parathion manufactured in the USA (data supplied by the USEPA). In addition to exposure risks during pesticide manufacturing, distribution and use, there are risks associated with the major international effort aimed at destroying the arsenals of nerve agents, including soman and sarin. The United States has pledged to destroy approximately 25,000 tons of chemical agents by the end of the decade[3]. The high density lipoprotein (HDL)-associated enzyme paraoxonase (PON1) contributes significantly to the detoxication of several OPs (Fig. 1). The insecticides parathion, chlorpyrifos and diazinon are bioactivated to potent cholinesterase inhibitors[4] by cytochrome P-450 systems[5]. The resulting toxic oxon forms can be hydrolysed by PON1, which also hydrolyses the nerve agents soman and sarin[6] (Fig. 1). PON1 is polymorphic in human populations and different individuals also express widely different levels of this enzyme[7–9]. The Arg_{192} (R_{192}) PON1 isoform hydrolyses paraoxon rapidly, while the Gln_{192} (Q_{191}) isoform hydrolyses paraoxon slowly[6,10]. Both isoforms hydrolyse chlorpyrifos-oxon[8,9] and phenylacetate[6,7,9] at approximately the same rate. The role of PON1 in OP detoxication is physiologically significant[11–15]. Injected PON1 protects against OP poisoning in rodent model systems[12–15] and interspecies differences in PON1 activity correlate well with observed median lethal dose (LD_{50}) values[8,11,16]. We report here a simple enzyme analysis that provides a clear resolution of PON1 genotypes and phenotypes allowing for a reasonable assessment of an individual's probable susceptibility or resistance to a given OP, extending earlier studies on this system. We also show that the effect of the PON1 polymorphism is reversed for the hydrolysis of diazoxon, soman and especially sarin, thus changing the view of which PON1 isoform is considered to be protective.

In the course of evaluating the PON1 status of farm workers prior to pesticide exposure during the growing season, we also determined the rates of diazoxon hydrolysis. By plotting the activity distributions for the three substrates, chlorpyrifos oxon, phenylacetate and diazoxon, against the rates of paraoxon hydrolysis, we were able to clearly resolve individuals homozygous for the low-activity paraoxonase isoform (QQ individuals) from heterozygotes (QR individuals) (Fig. 2*a–c*). However, only the plot of diazoxon hydrolysis versus paraoxon hydrolysis (Fig. 2*c*) clearly resolved all three genotypes and at the same time provided important information about the level of enzyme expressed in a given individual. This two-dimensional enzyme analysis provides a complete assessment of an individual's PON1 status (genotype and phenotype). PON1 levels in a given individual are usually very stable over time[17].

One of the most interesting observations was the reversal of the effect of the PON1 activity polymorphism for diazoxon hydrolysis relative to paraoxon hydrolysis (Fig. 2*c*). RR homozygotes (high paraoxonase activity) had lower diazoxonase activities (mean=7948 U/l) than QQ homozygotes (mean=12,318 U/l). Average rates of diazoxon hydrolysis (10,619 U/l) were somewhat higher than the rates of chlorpyrifos oxon hydrolysis (8233 U/l), suggesting that on average, humans may be better able to detoxicate diazinon than chlorpyrifos or parathion.

We also observed an increased frequency for the R_{192} allele (0.41) in this Hispanic population compared with a frequency of 0.31 observed in populations of Northern European origin[18]. This results in approximately 16% of individuals of Hispanic origin being homozygous for the R_{192} PON1 isoform compared with only 9% of individuals of Northern European origin[8,18].

Following the March 1995 release of sarin in the Tokyo subway, we examined the effect of the PON1 polymorphism on soman and sarin hydrolysis, as PON1 is the only enzyme from humans known to hydrolyse the phosphorus-fluorine bond of these very toxic nerve agents[6]. It is clear that the effect of the polymorphism is reversed for both of these compounds, especially sarin (Fig. 2*d, e*). The mean value for sarin hydrolysis was only 38 U/l for the R_{192} homozygotes compared with 355 U/l for the Q_{192} homozygotes (Table 1). The ranges of values for hydrolysis of each of the PON1 substrates are also shown in Table 1.

These results help to explain the large individual differences in sensitivity to OP insecticides processed through the P-450/PON1 pathway or hydrolysed directly by PON1. As the dose response curves for OP toxicity are very steep[12], a small percentage difference in metabolic rate can represent a significant difference in OP sensitivity. In this light, it is important to note that we found in earlier studies that newborns have very low levels of PON1[19], leading to the prediction that newborns are probably significantly more sensitive than adults to OP compounds processed by PON1. Increased sensitivity to OPs has been observed in newborn rats[20,21].

In addition to playing a major role in OP detoxication, the PON1 polymorphism has been recently implicated in another important area of human health. Watson *et al.*[22] demonstrated that PON1 destroys biologically oxidized phospholipids, while other investigators have shown that the R_{192} allele represents a risk factor for coronary artery disease[23,24]. These studies suggest that the same considerations given to the determination of both PON1 genotype and phenotype (PON1 status) relative to OP sensitivity will also be important for studies on the role of PON1 in vascular disease.

These studies underline the importance of examining the effects of polymorphisms on each substrate or

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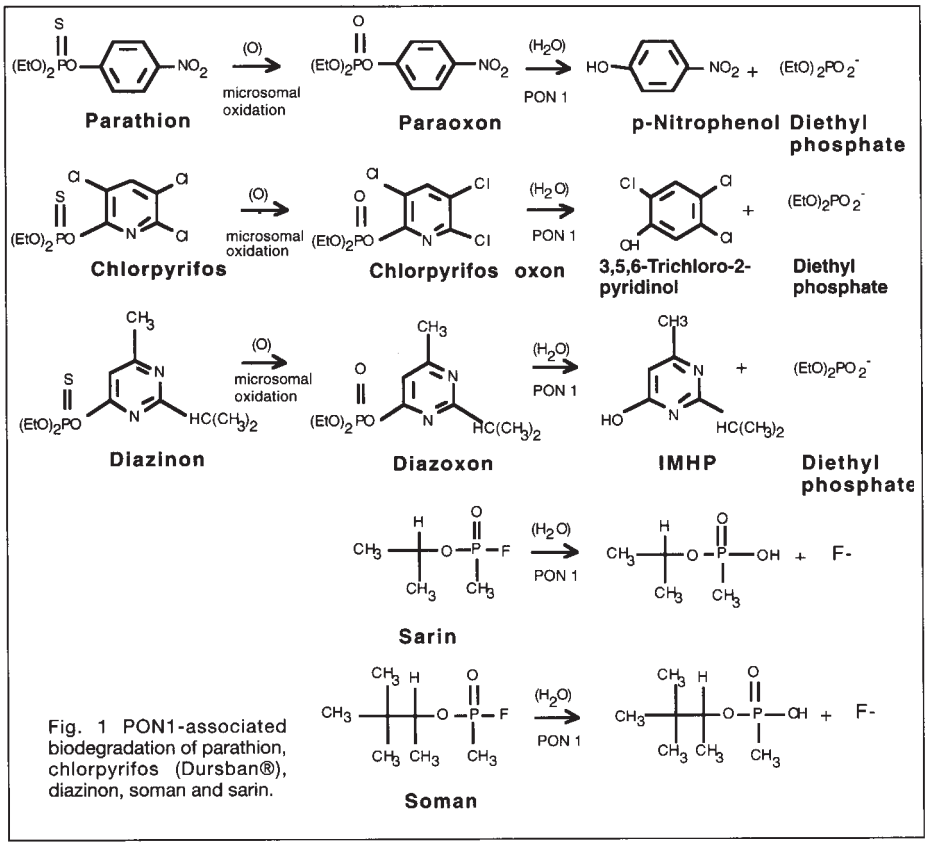

Fig. 1 PON1-associated biodegradation of parathion, chlorpyrifos (Dursban®), diazinon, soman and sarin.

inhibitor of physiological importance. A single amino acid mutation in acetylcholinesterase has been demonstrated to cause a reversal in sensitivities of leaf hoppers to specific OP insecticides[25]. Reversal of sensitivity to inhibitors by single amino acid changes have also been observed in plant[26] and viral[27] systems. The effect of the PON1 polymorphism on sarin hydrolysis illustrates how dramatic the reversal of the effect of an enzyme polymorphism can be.

Methods

Human subjects. Plasma (heparin) from 92 individuals of Hispanic origin were drawn via venipuncture with informed consent.

Enzyme assays. Hydrolysis rates of paraoxon[8], phenylacetate[28] and chlorpyrifos oxon (CPO)[8] were determined as described. Rates of diazoxon hydrolysis were determined by a continuous spectrophotometric assay developed in our laboratory (R.J.R. and C.E.F., manuscript in preparation) based on published

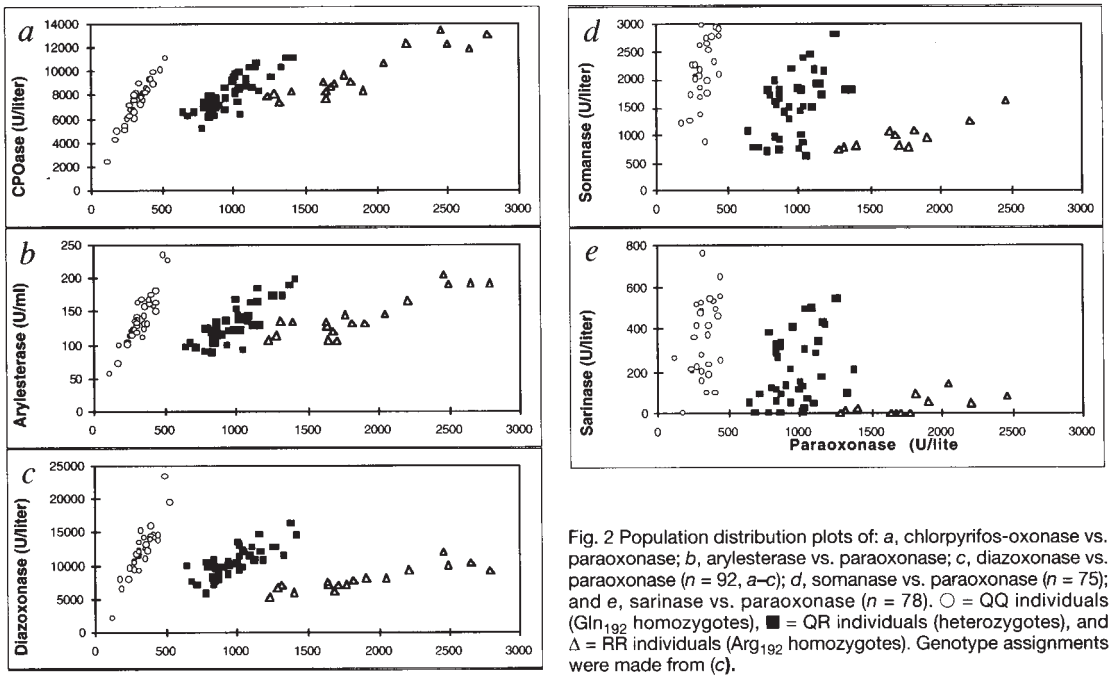

Fig. 2 Population distribution plots of: *a*, chlorpyrifos-oxonase vs. paraoxonase; *b*, arylesterase vs. paraoxonase; *c*, diazoxonase vs. paraoxonase (n = 92, *a*–*c*); *d*, somanase vs. paraoxonase (n = 75); and *e*, sarinase vs. paraoxonase (n = 78). ○ = QQ individuals (Gln_{192} homozygotes), ■ = QR individuals (heterozygotes), and Δ = RR individuals (Arg_{192} homozygotes). Genotype assignments were made from (*c*).

Table 1 Ranges of PON substrate activities in human serum

	Diazoxonase[a] (U/L)		Sarinase[a] (U/L)		Somanase[a] (U/L)	
	Range	Mean ± S.D.	Range	Mean ± S.D.	Range	Mean ± S.D.
All	2174–23316[b]	10619 ± 3207	0–758[c]	230 ± 191	616–2982[d]	1658 ± 660
QQ	2174–23316[e]	12318 ± 3748	0–758[f]	355 ± 183	870–2982[g]	2143 ± 576
QR	5903–1627[h]	10426 ± 2302	0–541[i]	198 ± 161	616–2815[j]	1518 ± 558
RR	5400–11193[k]	7948 ± 1712	0–144[l]	38 ± 47	754–1616[m]	992 ± 263

	Paraoxonase[a] (U/L)		CPOase[a] (U/L)		Arylesterase[a] (U/ml)	
	Range	Mean ± S.D.	Range	Mean ± S.D.	Range	Mean ± S.D.
All	121–2786[b]	924 ± 603	2415–13540[b]	8233 ± 1908	57–235[b]	136 ± 32
QQ	121–532[e]	328 ± 79	2415–11101[e]	7484 ± 1840	57–235[e]	138 ± 37
QR	653–1418[h]	977 ± 171	5134–11160[h]	8152 ± 1519	88–198[h]	131 ± 28
RR	1237–2786[k]	1769 ± 354	7480–13540[k]	9794 ± 2001	106–205[k]	145 ± 32

[a]Assays are described in Methods. [b]n = 92, [c]n = 78, [d]n = 75, [e]n = 33, [f]n = 28, [g]n = 26, [h]n = 41, [i]n = 38, [j]n = 38, [k]n = 18, [l]n = 12, [m]n = 11. All = all individuals in study, QQ = Gln_{191} homozygotes, QR = heterozygotes, RR = Arg_{191} homozygotes.

spectral data[29,30]. The incubation mixtures contained 0.1 M Tris-HCl, pH 8.5, 2.0 M NaCl, 2.0 mM $CaCl_2$, 500 μM diazoxon, and 5 μl of plasma in a volume of 1 ml at 24 °C. Appearance of 2-isopropyl-4-methyl-6-hydroxy pyrimidine (IMHP) was continuously monitored at 270 nm in a Beckman DU-70 spectrophotometer. The reaction was initiated by addition of plasma.

Hydrolysis rates of sarin and soman were determined at the USAMRICD Facility with a titrimetric procedure, using a Radiometer TTT80 pH-stat and an ABU80 autoburette. 3 ml of 1 mM soman or sarin in 1.0 M NaCl with 2.0 mM $CaCl_2$ were added to a temperature-controlled reaction vessel fitted with a capillary delivery tube from the autoburette, and the background hydrolysis rate was monitored for several minutes. Then, 50 μl of plasma were added and the resulting hydrolysis rate monitored at 25 °C. The background rate was subtracted from the sample hydrolysis rate. All samples were measured in triplicate.

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