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BRIEF COMMUNICATION

Genetic Variation in Paraoxonase Activity and Sensitivity to Diisopropylphosphofluoridate in Inbred Mice

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WEHNER, J. M., C. MURPHY-ERDOSH, A. SMOLEN AND T. N. SMOLEN. *Genetic variation in paraoxonase activity and sensitivity to diisopropylphosphofluoridate in inbred mice.* PHARMACOL BIOCHEM BEHAV **28**(2) 317–320, 1987.—The mechanism underlying genetic variation in the acute and chronic responses of mice to diisopropylphosphofluoridate (DFP) are unknown. We investigated whether variation in metabolism of organophosphates by A-esterase, as exemplified by the enzyme paraoxonase, was correlated to the degree of sensitivity to DFP in four inbred mouse strains. LD50s and plasma paraoxonase were measured in each strain. We observed genetic variation in both of these measures, but there was no significant correlation between the two measures. We conclude that plasma paraoxonase activity does not underlie genetic variation in sensitivity to the lethal effects of DFP in mice since it does not determine the degree of sensitivity or resistance to DFP.

DFP Genetic variation Inbred mice Paraoxonase

ORGANOPHOSPHATES such as diisopropylphosphofluoridate (DFP) inactivate acetylcholinesterase (AChE, EC 3.1.1.7) by irreversibly phosphorylating the catalytic site of the enzyme. The resulting accumulation of acetylcholine in cholinergic synapses causes stimulation of both muscarinic and nicotinic receptor-mediated pathways. The physiological signs of organophosphate intoxication include salivation, lacrimation, urination, defecation, tremors, convulsions, and ultimately, death.

We have been studying the genetic regulation of sensitivity to the toxic actions of DFP as indicated by LD50 values and a battery of behavioral and physiological tests using inbred mouse strains [22,23]. We have found that C57BL/6 mice are very sensitive to DFP, C3H mice are quite resistant to the acute effects of DFP, and DBA/2 mice are intermediate in sensitivity. This differential toxicity was not due to differences in brain AChE inhibition by DFP [23], which suggested that other factors, perhaps dispositional ones, could be important.

Organophosphates are detoxified primarily by type A esterases which are found in virtually all mammalian tissues and sera [3]. These A-esterases (or arylesterases) differ from choline esterases in that they do not have an activated serine in the active site and are not phosphorylated by the substrate. The A-esterases have traditionally been named for the substrate used to measure their activity and the literature contains references to DFPase [17], paraoxonase [2], somanase [7], and sarinase [1], among others. All of these enzymes require calcium in millimolar concentrations for optimal activity, and are inhibited by sulfhydryl reagents, such as *p*-hydroxymercuribenzoate. Recently, it has been reported that the arylesterase, paraoxonase and DFPase activities of human serum are due to a single calcium-requiring enzyme [20]. Similar cross reactivity by microbial arylesterases has also been reported [11]. Since the same enzyme (EC 3.1.1.2) is capable of catalyzing the hydrolysis of a wide range of ester substrates, the various names given to this enzyme should be considered synonomous. We shall refer to

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TABLE 1

PLASMA PARAOXONASE ACTIVITIES IN FOUR INBRED MOUSE STRAINS

Strain	Paraoxonase Activity[a] (nmole/min/mg protein) Females*	Males
BALB/cByJ	5.77 ± 0.43	4.59 ± 0.29
C3H/2Ibg	5.36 ± 0.53	4.53 ± 0.32
DBA/2Ibg	5.25 ± 0.16	4.23 ± 0.29
C57BL/6Ibg†	6.22 ± 0.45	5.44 ± 0.18

[a]Paraoxonase activity was determined in plasma as described under the Method section. Tabled values are the mean ± SEM of 5–8 individuals per group.

*Significantly greater than the males, $p<0.01$.

†Significantly greater than the DBA strain, $p<0.05$.

this enzyme by the name paraoxonase for the remainder of the text.

The level of paraoxonase activity may be an important mediator of organophosphate toxicity in mammals. Animal studies have indicated that LD50 values for certain organophosphates are inversely related to paraoxonase activity [1] and developmental studies have shown that animals are less sensitive to the lethal effects of organophosphates as paraoxonase activity increases [5,9]. Futhermore, species differences in susceptibility to organophosphate toxicity are well documented [4] and it has been suggested that these differences relate to the metabolism of organophosphates and to the intrinsic properties of specific AChEs [4,24]. Within certain species such as sheep, mutants lacking arylesterase activity are more sensitive to organophosphates than are animals with normal levels of activity [12].

In the human population, a genetic polymorphism which causes low levels of paraoxonase activity has been described [13]. The results of family studies suggest the presence of low and high allelic gene products controlled by a single autosomal locus [6, 8, 18, 19]. It has been proposed that this heterogeneity in the ability to metabolize organophosphates in humans may result in genetically regulated differences in sensitivity to organophosphate toxicity. However, this hypothesis cannot be tested in the human population because of the toxicity of organophosphorus compounds.

The cumulative data derived from both animal and human research would support the prediction that low paraoxonase activity would relate to increased sensitivity to the toxic effects of organophosphates. In the present study, genetic differences in sensitivity to DFP among inbred strains of mice were examined further by testing the hypothesis that differential sensitivity to DFP as measured by LD50 values might be regulated in part by metabolic inactivation of DFP by the enzyme paraoxonase.

METHOD

Animals

Male and female mice DBA/2Ibg, C57BL/6Ibg, C3H/2Ibg, and BALB/cByJ mice were used in this study. Mice were raised at the Institute for Behavioral Genetics at the University of Colorado. Mice of 60–90 days of age were kept on a 12 hour light cycle and allowed free access to food (Wayne Lab Blox) and water.

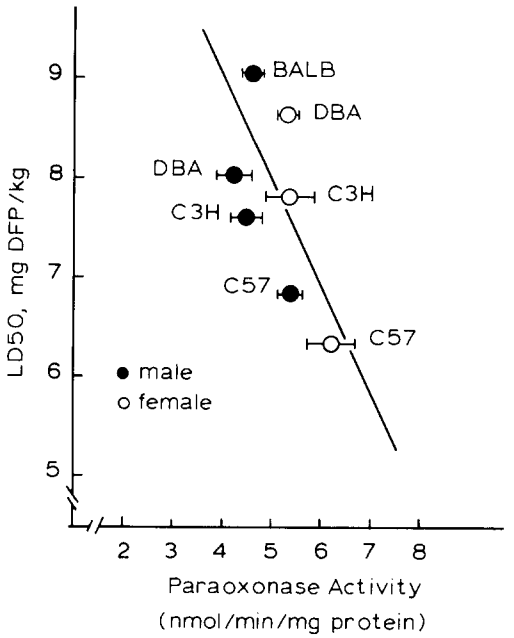

FIG. 1. Sensitivity to DFP as a function of paraoxonase activity in inbred mouse strains. Paraoxonase activity and LD50 values were determined as described under the Method section. The mean paraoxonase activity expressed as nmoles of product formed min^{-1} mg $protein^{-1}$ (n=5–8 per point) is plotted versus LD50 values (n=7–20 per point) for BALB, C3H, DBA, and C57BL mice. (Male ●, female ○, the LD50 was not determined for BALB females.)

LD50 Determinations

Diisopropylphosphofluoridate (Sigma) was administered in saline. Solutions were prepared fresh each day and used within one hour. DFP is stable in saline for several hours [10]. Mice were injected intraperitoneally with 0.01 ml per gram of body weight. Six doses (3–10 mg/kg) of DFP were used for each LD50 determination. Mice were kept 2 or 3 to a cage and observed for three hours. Time of injection and death was recorded. LD50 values were calculated from linear regression analysis of log dose vs. probit plots of the data [14].

Determination of Plasma Paraoxonase Activity

Mice were lightly anesthetized with halothane and approximately 300 μl of blood was collected from the retro-orbital sinus into heparinized microhematocrit tubes. The tubes were spun in an International clinical centrifuge for ten minutes at setting No. 6 and the plasma was removed.

Paraoxonase activity was measured using a modification of the method of Eckerson *et al.* [8] with 1.0 mM paraoxon in a total volume of 1.0 ml. Hydrolysis of paraoxon was monitored by recording the production of 4-nitrophenol at 412 nm in 50 mM Tris-glycine buffer, pH 7.4 (adjusted at 37°), containing 1.0 mM $CaCl_2$. Enzyme (20 μl of plasma) was added to start the reaction and the increase in absorbance at 412 nm was recorded. The amount of 4-nitrophenol formed was calculated from the extinction coefficient, 12,800 $M^{-1}cm^{-1}$, at pH 7.4. Blanks contained no enzyme. Paraoxonase activity is expressed as nmoles of product formed $min^{-1}mg\ protein^{-1}$. Protein was determined by the Lowry method using bovine serum albumin as standard [15]. The results of preliminary

experiments indicated that the pH optimum for mouse plasma paraoxonase activity was 7.4, and that the assay was linear as a function of protein and time.

Data Analysis

Potential correlations between variables were analyzed by linear regression analysis of means for each parameter of the respective populations. Strain and sex differences in paraoxonase activity were analyzed by a two-way ANOVA followed by post-hoc Tukey's B-test.

RESULTS AND DISCUSSION

Table 1 presents the plasma paraoxonase activity in males and females of the four inbred strains of mice. Two-way analysis of variance of these data indicated a significant effect of strain, $F(3,47)=2.96$, $p<0.05$, and a significant effect of sex, $F(1,47)=12.83$, $p<0.01$. The two-way interaction term was not significant. Analysis of strain differences by Tukey's B-test indicated that C57BL mice were significantly different from DBA mice in paraoxonase activity ($p<0.05$). No other strain differences in paraoxonase proved to be significant. The significant sex difference was due to consistently higher paraoxonase activity in the females.

Figure 1 shows a plot of LD50 values versus paraoxonase activity. The inbred strains of mice differed in sensitivity to DFP as evidenced by susceptibility to its lethal effects. The most resistant strain was BALB/cByJ and the most sensitive strain C57BL. A negative correlation was observed between sensitivity as determined by LD50 and the detoxification of DFP by paraoxonase ($r=-.66$, n.s.).

These data do not support the hypothesis that genetic sensitivity to the lethal effects of DFP, and potentially other organophosphates, is determined by the level of paraoxonase activity. Rather, it appears that increased paraoxonase activity was of little value in protecting the organism against the lethal effects of DFP since the most sensitive strain, C57BL, had the highest paraoxonase activity. This observation is supported by recent evidence which suggests that liver somanase provides little protective effect against soman poisoning [7]. Our previous studies have indicated that neither the level of brain AChE activity, nor the time course and extent of enzyme inhibition are sufficiently different to account for variable sensitivity to organophosphates among these inbred strains [22]. Futhermore, the time course of recovery of AChE activity in the brain after acute DFP treatment is not different among the inbred mouse strains [23].

It is apparent, then, that neither the primary metabolism of DFP by the enzyme paraoxonase, nor the intrinsic properties of brain AChE can explain the genetic differences in sensitivity to the acute effects of DFP in mice. The half-life of free DFP in blood is very brief. A recent study reported that following IV administration of radiolabelled DFP, plasma radioactivity fell 75% by 15 sec and 97% by one min [16]. Since DFP binds quickly and irreversibly to AChE, the peak concentration of DFP may not be sufficiently reduced by paraoxonase before it produces its toxic actions by inhibiting AChE. Studies by Clement [7] have described a role for plasma aliesterase in the detoxification of soman, and although it is not known if aliesterase can metabolize DFP, it may be possible that this enzyme could account for a portion of the differential toxicity among mouse strains.

In summary, genetic variation in paraoxonase activity and genetic variation in sensitivity to DFP are not correlated, since relatively high levels of paraoxonase activity are not related to the ability of mice to withstand the lethal effects of DFP. It appears then, that other factors, such as intrinsic peripheral and central nervous system sensitivity or alternate routes of DFP metabolism, must be explored to understand genetic variation in lethality to organophosphates.

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