*Biochemical Pharmacology*, Vol. 38, No. 3, pp. 529–534, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00
Pergamon Press plc

# ACETYLCHOLINESTERASE PROPHYLAXIS AGAINST ORGANOPHOSPHATE POISONING

## QUANTITATIVE CORRELATION BETWEEN PROTECTION AND BLOOD-ENZYME LEVEL IN MICE

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(*Received* 25 *April* 1988; *accepted* 19 *July* 1988)

**Abstract**—Fetal bovine serum actylcholinesterase (FBS-AChE, EC 3.1.1.7) was titrated, both *in vitro* and *in vivo*, with a highly toxic anti-ChE organophosphate, 7-(methylethoxyphosphinyloxy)-1-methylquinolinium iodie (MEPQ). Approximately 1:1 stoichiometry was obtained for the sequestration of MEPQ by FBS-AChE in mice. A quantitative, linear correlation was demonstrated between blood-AChE levels and the protection afforded by exogenously administered AChE in mice when challenged with anti-ChE MEPQ. The results presented in this report demonstrate that such prophylactic measures are indeed sufficient to protect animals against poisoning by as high as an $8 \times LD_{50}$ dose of organophosphate without the administration of any supportive drug. Despite the relatively large toxic dose, most of the mice that survived the challenge did not show any classical clinical signs of severe anti-ChE poisoning. MEPQ may be considered a suitable model compound for studying the quantitative aspects of the scavenger prophylactic approach described here.

Recently, administration of exogenous acetylcholinesterase (AChE,‖ EC 3.1.1.7) was shown to confer significant *in vivo* prophylaxis against organophosphate (OP) inhibitors of physiologically functional AChE [1]. This successful attempt to use large quantities of purified AChE from fetal bovine serum (FBS) as a scavenger against OPs in mice has substantiated the hypothesis that B-esterases could serve as prophylactic agents to prevent OP poisoning. Further, topical application of AChE-containing liposomes to the eyes of rabbits reversed diisopropylfluorophosphate (DFP)-induced miosis, suggesting a limited antidotal function for such B-esterases [2].

Effective treatment of poisoning by OP anti-ChE compounds appears to require combination therapy (e.g. antimuscarinic, reactivator and anticonvulsant drugs) [3]. In contrast, the use of AChE as a prophylactic agent possesses the considerable advantage of being a single drug, completely adequate for pretreatment against poisoning by anti-ChE. Therefore, it seems essential to study in detail the quantitative relationship between the blood-AChE level and the protective ratio conferred by pretreatment with AChE.

7- (Methylethoxyphosphinyloxy)- 1- methylquinolinium iodide (MEPQ) [4] was chosen to establish a meaningful basic correlation between blood-enzyme level and degree of protection against poisoning by anti-ChE OPs, for the following reasons: (a) A quaternary ammonium cation moiety on the MEPQ molecule will restrict its distribution to extracellular spaces [5, 6]. Similarly, the distribution of exogenously administered AChE is also assumed to be confined to the blood circulation, since it is unlikely that such a large molecule would cross the blood–brain barrier. Indeed, whole-body autoradiography experiments in mice administered butyrylcholinesterase intravenously show that this enzyme does not cross the blood–brain barrier [6]. Thus, it was inferred that, of all available anti-ChE drugs, MEPQ-type OPs would react preferentially with blood constituents and consequently enable one to determine the maximum availability of intravenously pre-administered AChE. Previously, OPs used in a study [1] tended to be lipid soluble and considered to be distributed in lipoid tissues immediately following their administration. (b) MEPQ inhibits AChE at an extremely high rate with a 1:1 stoichiometry [4], which assures rapid and complete inhibition of enzyme even at relatively low concentrations of both MEPQ and AChE. Thus, the high toxicity of MEPQ ($LD_{50}$ in mice, i.v. = 30 μg/kg or *ca.* 2.3 nmol/mouse) in conjunction with the high second-order bimolecular rate constant for the inhibition of FBS-AChE ($K_i = 2.44 \times 10^8\ M^{-1}\ min^{-1}$) permitted the use of relatively small quantities of AChE per mouse.

In this report we present the results of both *in*

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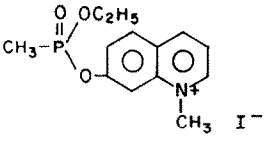
‖ Abbreviations: AChE, acetylcholinesterase; CBDP, 2(*O*-cresyl)4*H*:3:2-benzodioxaphosphorin-2-oxide; FBS, fetal bovine serum; MEPQ, 7-(methylethoxyphosphinyloxy)-1-methylquinolinium iodide; OP, organophosphate(s); and $TMB_4$, 1,1-trimethylene bis(4-hydroxyiminomethyl) dibromide.

*vitro* and *in vivo* titration of FBS-AChE with MEPQ and demonstrate approximately a 1:1 stoichiometry for the sequestering of MEPQ by FBS-AChE. It is further demonstrated that the protection afforded by FBS-AChE in mice was linearily correlated with the blood-AChE level, and that such prophylactic measures were indeed sufficient to protect animals against poisoning by several multiples of a toxic dose of MEPQ without the administration of any supportive drugs.

## MATERIALS AND METHODS



7-(methylethoxyphosphinyloxy)-1-methylquinolinium iodide (MEPQ)

7-(Methylethoxyphosphinyloxy)-1-methyl-quinolinium iodide (MEPQ) was prepared as previously described [4]. *S*-[2(*N,N,N*-Trimethylammonio)-ethyl]-*O,O*-diethylphosphorothiolate iodide (phospholine iodide) was obtained from the Ayerst laboratories (New York, NY) and *O,O*-diethyl-*O*-(*p*-nitrophenyl)phosphate (paraoxon) was purchased from Sigma (St. Louis, MO). 2-(*O*-cresyl) 4*H*:3:2-benzodioxaphosphorin-2-oxide (CBDP) and 1,1-trimethylene bis(4-hydroximinomethyl)dibromide ($TMB_4$) were prepared according to Eto *et al.* [7] and Poziomek *et al.* [8], respectively. Acetylcholinesterase from fetal-bovine serum (FBS-AChE, 11S) was purified as described before [9]. The specific activity of FBS-AChE was 400 ± 15 U/nmol of active-site with one mg of pure enzyme containing approximately 5600 units.

### *Animals*

Animals were cared for in accordance with the principles enunciated in the "Guide for Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

Adult male ICR mice weighing 28–33 g were used throughout this study.

*AChE assay.* FBS-AChE activity was determined spectrophotometrically by the method of Ellman *et al.* [10]. The assay mixture (3.2 ml) contained 0.8 mM acetylthiocholine iodide and 0.3 mM 5,5-dithiobis-(2-nitrobenzoic acid) in 50 mM phosphate buffer (pH 8.0). All measurements were performed at 25°.

*Stock solution of inhibitors.* Standard stock solutions of 0.1 to 1 mM MEPQ (in acetone), phospholine (in water) and paraoxon (in acetonitrile) were freshly prepared and kept at 4°. The concentration of the stock solutions of the inhibitors was routinely checked by absorption spectroscopy in the following manner: MEPQ, paraoxon and phospholine iodide were hydrolyzed completely in an alkaline solution (0.1 to 1.0 N NaOH). The corresponding leaving groups released from MEPQ and paraoxon were directly monitored at 406 and 400 nm, respectively, and concentration was determined from a calibration curve constructed for 1-methyl-7-hydroxyquinolinium iodide [4] and *p*-nitrophenol. The concentration of released leaving group from phospholine iodide (i.e. 2-[*N,N,N*-trimethylammonio]ethanthiol iodide) was determined by diluting the hydrolyzed inhibitor into a 0.3 mM solution of Ellman's reagent [10] in 10 mM phosphate buffer (pH 8.0) and using the molar extinction coefficient $E_{412} = 1.4 \times 10^4\ M^{-1}\ cm^{-1}$. The amount of free leaving groups in each stock solution (i.e. pre-hydrolyzed inhibitor), if present at all, was estimated as described above by replacing the NaOH solution with 10 mM phosphate buffer (pH 8.0).

*Kinetics of inhibition of FBS-AChE.* A standard stock solution of MEPQ (0.1 mM) was prepared in acetone and diluted at t = 0 into a solution of FBS-AChE (0.4 nM active site) in 10 mM phosphate buffer (pH 8.0) containing 0.01% BSA. At selected time intervals 50-μl samples were diluted into 3.2 ml of Ellman's assay reagent for measurement of enzyme activity.

In vitro *titration of FBS-AChE against MEPQ, paraoxon and phospholine.* The titration was performed in accordance with the residual activity method [4]. To 0.01 to 1 μM AChE active site (in 10 mM phosphate buffer, pH 8.0, containing 0.01% BSA), various amounts of inhibitor solution (0.15 to 1.2 times enzyme equivalents) were added and incubated at 25°. The inhibition was allowed to proceed to completion, and the residual AChE activity was assayed by diluting 10–25 μl of the inhibition mixture into Ellman's assay reagent as described above. Percent residual enzyme activity was plotted against the number of equivalents of MEPQ, phospholine iodide or paraoxon.

In vivo *titration of FBS-AChE.* Mice were administered intraveneously 0.2 ml solution of FBS-AChE (10 mM phosphate-saline, pH 7.6) containing 1000–2600 enzyme units (2.5 to 6.5 nmol of active sites). Approximately 2–3 hr following an i.v. bolus administration of AChE, the mice were challenged with an i.v. bolus injection of 2 nmol of MEPQ in 0.15 to 0.2 ml saline. Three to four injections of MEPQ were used in each mouse at 15- to 20-min intervals. Heparinized blood samples (5–10 μl) were drawn from either the eye sinus or by tail vein nick, diluted 10- to 20-fold into distilled water and assayed for AChE activity as described above. These blood samples were drawn 2 min prior to each administration of MEPQ. Residual activity of FBS-AChE was plotted against the cumulative dose of MEPQ administrered to the individual mouse.

In vitro *hydrolysis of MEPQ in mice serum.* Heparinized blood samples pooled from three to four mice, either control or AChE-treated, were centrifuged and serum was separated from erythrocytes. Serum was further diluted 1:10 into 10 mM phosphate buffer, pH 8.0. To each of these serum samples MEPQ was added (final concentration, 1 μM), and the samples were incubated at 25°. At selected time intervals, 10 μl of serum-MEPQ sample was diluted into 1 ml of $1.2–1.6 \times 10^{-8}$ M FBS-AChE in 10 mM phosphate buffer, pH 8.0. The inhibition of enzyme

activity by MEPQ was allowed to reach completion and the residual concentration of MEPQ was determined from a calibration curve constructed as described before in the *in vitro* titration of AChE.

*Determination of protective ratio conferred by AChE against MEPQ.* Six groups of mice (twelve mice per group) were administered various amounts of FBS-AChE by i.v. injections (0 to 6.5 nmol each). Ten to fifteen minutes later, blood samples were drawn for AChE determination, followed by a single i.v. bolus injection of 0.10 to 0.15 ml MEPQ in saline. Mortality was determined up to 24 hr post-intoxication, and the median lethal dose ($LD_{50}$) was calculated by the method of Weil [11]. No additional supporting therapy was given to the mice throughout this study. The mice had access to food and water *ad lib.* before and after administration of both AChE and MEPQ.

In-vivo *reactivation of MEPQ-inhibited FBS-AChE.* Reactivation of MEPQ-inhibited FBS-AChE in mice previously challenged with MEPQ was determined 90 min following i.m. injection of 12.5 mg/kg of the powerful reactivator $TMB_4$ [12]. To estimate the maximum amount of enzyme activity that can be reactivated, blood samples were incubated further for 16 hr at 25° with 1 mM $TMB_4$ in 10 mM phosphate buffer (pH 8.0) and reassayed for AChE activity. Non-enzymic hydrolysis of the substrate acetylthiocholine was subtracted automatically by addition of $TMB_4$ to the blank cuvette mixture which contained all assay components except AChE.

## RESULTS

In vitro *titration of FBS-AChE.* The biomolecular rate constant for the inhibition of FBS-AChE by MEPQ in phosphate buffer, pH 8.0, at 25° was found to be 2.44 ($\pm$0.38) $\times 10^8\,M^{-1}\,min^{-1}$. Under the experimental conditions employed, the rate of inhibition was first order and linearly related to the inhibitor concentration (not shown). Because of the high rate of inhibition of FBS-AChE by MEPQ, low concentrations (*ca.* 1 nM) could be determined by the method of residual activity, using MEPQ as a titrant. When the same enzyme was treated with increasing concentrations of either MEPQ or the non-chiral inhibitors paraoxon and phospholine, essentially similar titration curves were obtained (Fig. 1). It was demonstrated previously that stoichiometric amounts (1:1) of MEPQ react with eel AChE [4]. The present result establishes the same relationship of MEPQ with FBS-AChE. Thus, both enantiomers of MEPQ displayed a similar rate of inhibition towards FBS-AChE.

*Stability of MEPQ in mice serum.* Since >95% of MEPQ (1 $\mu$M) was hydrolyzed by mouse serum in less than 1 min, the loss of anti-cholinesterase activity of MEPQ was monitored in serum after dilution (2–10 times) in 50 mM phosphate buffer (pH 8.0) at 25°. The rate of MEPQ hydrolysis could be correlated with the extent of serum dilution in phosphate buffer (not shown). Figure 2A represents the time course of the hydrolysis of MEPQ in control serum and serum obtained from mice pretreated with various amounts of FBS-AChE. The time course for MEPQ disappearance in serum of AChE-treated mice after

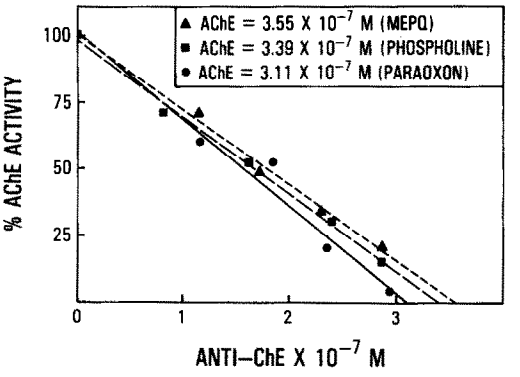



Fig. 1. *In vitro* titration of FBS-AChE with anti-cholinesterase drugs. To the same amount of AChE (137 units/ml in 2 mM phosphate, pH 8.0, 25°), increasing amounts of anti-ChE drug were added and incubated until completion of the inhibition reaction (MEPQ, 30 min; phospholine, 2 hr; paraoxon, 20 hr). The inset figures describe the molar concentration of the three inhibitors required for 100% inhibition of enzyme activity.

the subtraction of the control serum activity (Fig. 2B) suggests that serum-AChE concentration ranging between 0.095 and 0.27 $\mu$M (39–110 units/ml) completed the sequestration of stoichiometric amounts of MEPQ in less than 25 sec, as would be anticipated from the bimolecular second-order rate constant for the inhibition of the enzyme by MEPQ ($2.44 \times 10^8\,M^{-1}\,min^{-1}$) and the initial concentration of the reactants (0.1 to 0.3 $\mu$M).

In vivo *titration of FBS-AChE by MEPQ.* To determine the optimal conditions for *in vivo* titration of FBS-AChE by MEPQ, 0.15 to 0.2 ml FBS-AChE solution was administered either i.v. or i.p. to mice, and blood-enzyme levels were monitored over a

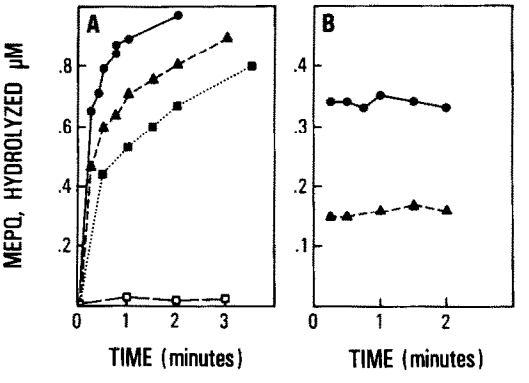



Fig. 2. *In vitro* hydrolysis of MEPQ in mouse serum obtained from animals pretreated with AChE. At t = 0, MEPQ (1 $\mu$M) was added to each serum sample previously diluted 1:10 in 10 mM phosphate, pH 8.0. The residual concentration of MEPQ is plotted against time of incubation. Panel A: (■——■) diluted serum from untreated animals; (▲——▲) diluted serum containing 39 units/ml of AChE; (●——●) diluted serum containing 110 units/ml; and (□——□) 10 mM phosphate, pH 8.0 (buffer control). Panel B: Presents the same data as in panel A after the values of AChE-untreated animals were subtracted from the experimental results obtained for serum of AChE-treated mice.

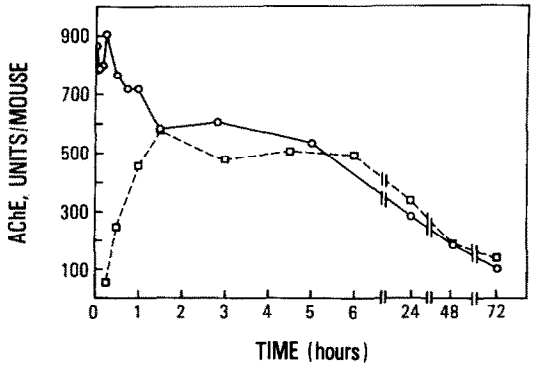



Fig. 3. Average whole blood–AChE levels following i.v. or i.p. administration of FBS-AChE. Key: (○——○) mice administered 920 units/mouse by i.v. route (N = 2), and (□——□) mice administered 760 units/mouse by i.p. route (N = 3). Variations among individual animals administered the same amount of AChE was <15%. Blood volume was assumed to be 7.5% of body weight.

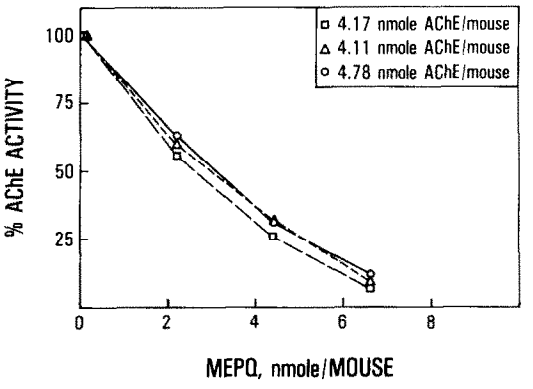



Fig. 4. *In vivo* titration of blood-AChE in mice pretreated i.v. with FBS-AChE. Consecutive injections were administered at 15-min time intervals, followed by assay for blood-AChE residual activity. Representative results shown are for three individual mice with initial blood-AChE levels as depicted in the inset. Titrations were initiated 2–3 hr after the administration of FBS-AChE, in accordance with the time profiles of blood-AChE levels shown in Fig. 3.

period of 72 hr. The same enzyme levels were obtained whether blood samples were drawn from eye sinus or tail vein. The results in Fig. 3 suggest that blood-AChE concentration is maintained essentially at a constant level during a 2- to 4-hr period after i.v. injection of 920 units/mouse of FBS-AChE. Similar profiles were observed in mice receiving 4000 units/mouse (not shown). Significant blood-AChE activity was observed even 72 hr after injection. When FBS-AChE was administered i.p., peak level occurred within 90 min, and the clearance of AChE activity was similar to the profile observed after i.v. injection. The variations in blood-AChE level among individual animals treated with the same amount of AChE per dose was less than 15%, irrespective of the route of administration. To determine stoichiometry *in vivo* of MEPQ scavenging by FBS-AChE, MEPQ was injected i.v. within a time period of 2–4 hr after i.v. administration of AChE so as to minimize errors that may stem from normal clearance of AChE (see Fig. 3). The results, shown in Fig. 4, indicate an essentially linear relationship between AChE residual activity in mouse blood and MEPQ dose; however, in all cases studied it appeared that complete inhibition of the activity of the exogenous esterase required the addition of approximately 2 to 2.5 nmol of MEPQ per mouse over the calculated dose which was based on the initial blood-enzyme level and assuming a 1:1 stoichiometry (Fig. 4) (blood volume was assumed to be 7.5% of body weight). It should be noted that the $LD_{50}$ of MEPQ in untreated mice (wt = 30 g) was approximately 2.3 nmol/mouse (see Table 1).

*Protective ratio against MEPQ.* Table 1 summarizes the results of the determination of i.v. $LD_{50}$ values of MEPQ in AChE-pretreated mice, as compared to the toxicity of MEPQ in unprotected animals. For all cases studied, the initial amount of AChE recovered in mouse blood immediately after i.v. injection was in excellent agreement with the

Table 1. Protective-radio conferred by AChE against MEPQ poisoning*

| AChE† units/mouse | $LD_{50}$‡ (i.v.) μg/kg | Protective ratio§ | |
|---|---|---|---|
| | | Observed | Calculated‖ |
| Unprotected mice | 30 (28–33)¶ | 1.0 | — |
| 474 ± 7 (1.2) | 46 (38–56) | 1.5 | 1.5 |
| 808 ± 23 (2.0) | 60 (53–69) | 2.0 | 1.9 |
| 1387 ± 19 (3.5) | 80 (66–96) | 2.7 | 2.5 |
| 2246 ± 46 (5.6) | 121 (111–132) | 4.0 | 3.5 |
| 2606 ± 48 (6.5) | 124 (108–141) | 4.1 | 3.9 |

* MEPQ was administered (i.v.) 15 min following i.v. injection of AChE.

† Average ± SEM blood-AChE activity prior to MEPQ injection; N = 12. The figures in parentheses are nmol/mouse (assuming 1 nmol = 400 units).

‡ 95% confidence limits.

§ $LD_{50}$ in treated mice divided by $LD_{50}$ in untreated mice.

‖ Calculated on the assumption of 1:1 stoichiometry of MEPQ sequestering by AChE and an average $LD_{50}$ of 2.27 nmol MEPQ per mouse.

¶ MEPQ toxicity in CBDP-treated mice: $LD_{50}$ = 9.3 (8.2–10.4) μg/kg (determined 90–120 min following an i.m. injection of 5 mg/kg CBDP).

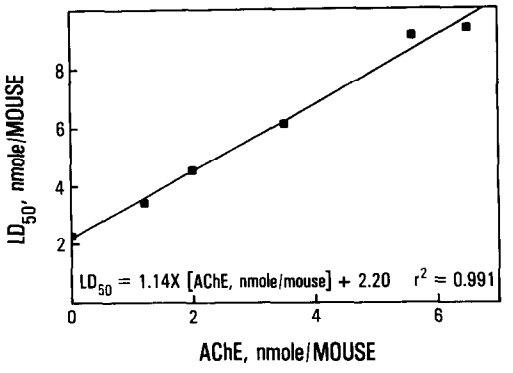



Fig. 5. Correlation between i.v. $LD_{50}$ of MEPQ and blood-AChE levels expressed in nmol/mouse. Mice were challenged with MEPQ 15 min following i.v. administration of FBS-AChE. Each dose group contained twelve mice. Line equation was calculated by the method of linear regression analysis. The $LD_{50}$ of MEPQ in unprotected mice was found to be 2.27 nmol/mouse weighing 30 g and assuming blood volume of 7.5% of body weight.

administered dose. The calculated protective ratio, which was based on the assumption that AChE protected mice by a 1:1 stoichiometry, was found to be in reasonable agreement with the observed protective ratio ($LD_{50}$ in protected animals divided by the $LD_{50}$ in unprotected animals). Figure 5 demonstrates a strong correlation between MEPQ toxicity expressed in nanomoles per mouse and blood FBS-AChE level depicted in nanomoles of active site per mouse.

Since mouse serum hydrolyzed MEPQ extremely rapidly, it was interesting to test the potentiation of MEPQ toxicity following the administration of a plasma-aliesterase inhibitor such as CBDP [13, 14]. Intramuscular pretreatment of mice with 5 mg/kg CBDP increased the toxicity of MEPQ by 3.3-fold, thereby indicating that aliesterase might be involved in the detoxification mechanism of MEPQ in mice (see Table 1 footnote ¶).

In a separate experiment, four mice were administered approximately 7000 units/mouse (i.v.) FBS-AChE followed by an i.v. challenge of $8 \times LD_{50}$ of MEPQ: three animals survived. It should be noted that all animals tested in this study appeared to tolerate high concentrations of AChE in their circulation. Furthermore, despite the large toxic dose of MEPQ, most of the mice that survived the challenge did not show any clinical signs of anti-ChE poisoning, even when the MEPQ does was as high as $8 \times LD_{50}$.

In vivo *reactivation.* Five mice treated with an average enzyme dose of 2246 ± 46 (SE) units/mouse were challenged with $3.5–4.0 \times LD_{50}$ of MEPQ. Two hours after MEPQ injection, the blood AChE level decreased from an average initial concentration of 937 ± 18 units/ml to 85 ± 1 units/ml, mainly due to inhibition of enzyme by MEPQ and partially as a result of clearance. Indeed, $TMB_4$ (12.5 mg/kg, i.m.) administered 2 hr after the injection of MEPQ increased the average enzyme level from 85 to 302 ± 18 units/ml within 90 min. The maximum reactivatable enzyme, in blood drawn 90 min after the administration of $TMB_4$, was determined after further incubation of the blood sample at 25° for 16 hr in the presence of 1 mM $TMB_4$. The results of the reactivation studies for each individual mouse are summarized in Table 2. Three and one-half hours after the i.v. injection of FBS-AChE, the level of enzyme activity (as determined after completion of reactivation *in vitro*) was found to be 536 ± 20 units/ml, as would be predicted from the blood-enzyme clearance studies shown in Fig. 3. Thus, the administration of MEPQ did not appear to affect the clearance of AChE. The degree of *in vivo* reactivation, observed 90 min following $TMB_4$ injection, did not exceed an average level of 56%, due either to the rapid elimination of $TMB_4$ from the circulation or to the differences in the rate of reactivation of enzyme inhibition by the two optical isomers of MEPQ or both. It should be noted that, under *in vitro* conditions, reactivation of MEPQ-inhibited FBS-AChE was >95% (not shown).

Table 2. *In vivo* reactivation of AChE by $TMB_4$ in mice challenged with MEPQ*

| Mouse | Initial AChE level† (units/ml) | AChE level before $TMB_4$‡ (units/ml) | AChE level after $TMB_4$ treatment§ (units/ml) | *In vivo* reactivation‖ (%) |
|---|---|---|---|---|
| 1 | 964 | 90 | 328 | 57 (575) |
| 2 | 881 | 96 | 296 | 51 (580) |
| 3 | 930 | 78 | 324 | 59 (549) |
| 4 | 980 | 48 | 235 | 46 (511) |
| 5 | 921 | 114 | 330 | 70 (471) |

* Mice were administered i.v. an average dose of 2246 ± 46 units AChE per individual mouse; 2–10 min later an i.v. injection of $3.5–4.0 \times LD_{50}$ MEPQ was administered.

† AChE in units per ml whole blood 2–10 min following AChE injection.

‡ Assayed 1.5–2 hr after the administration of MEPQ. $TMB_4$ was administered i.m. at a dose of 12.5 mg/kg.

§ Assayed 90 min following the administration of $TMB_4$.

‖ Percent reactivation relates to maximum reactivatable AChE of each individual blood sample after 16 hr of incubation at 25° with 1 mM $TMB_4$. Figures in parentheses are maximum activity of FBS-AChE in units/ml.

## DISCUSSION

The results presented here demonstrate that both *in vivo* and *in vitro* 1 mole of FBS-AChE active site can scavenge or neutralize 1 mole of MEPQ. This 1:1 stoichiometric relationship is further substantiated by data showing a linear correlation between i.v. $LD_{50}$ of MEPQ and the blood-enzyme level of mice pretreated with FBS-AChE. In a previous publication [1], it was reported that i.p. pretreatment of mice with FBS-AChE conferred a protective ratio of 3.6 from ethyl-*S*-2-diisopropylaminoethylphosphonothiolate (VX) administered intramuscularly. Despite the different protocol used, both papers clearly demonstrate that exogeneous AChE is an effective prophylactic agent and scavenger with respect to various OPs. The results herein further demonstrate that a single dose of AChE, as a pretreatment, was adequate to completely protect animals against a dose in excess of $5 \times LD_{50}$ of an extremely toxic anti-ChE compound such as MEPQ. Both the level of protection achieved and the stoichiometry are unique: only complex drug mixtures have hitherto achieved or exceeded this level of protection, provided that post-treatment supportive drugs were administered following the challenge. Of particular importance is the observation that the animals that survived a challenge dose as high as $8 \times LD_{50}$ of MEPQ showed, if any, only minor symptoms of OP poisoning (slight tremors). Furthermore, these animals when treated (90 min after the challenge by MEPQ) intramuscularly with $TMB_4$ (12.5 mg/kg) survived a second challenge of $4 \times LD_{50}$ of MEPQ administered 90 min after $TMB_4$ treatment.

We have been able to demonstrate a quantitative relationship between blood-AChE levels and the protection afforded against MEPQ challenges because MEPQ possesses several unique properties. These properties include a high biomolecular rate constant for the inhibition of AChE, a high toxicity in mice, and a limited distribution in peripheral tissues. These properties suggest that MEPQ may be considered a suitable model compound for studying the quantitive aspects of the scavenger approach described here. However, the preliminary results we report with CBDP indicate that MEPQ is partially detoxified in mouse blood by aliesterases, since mice pretreated with CBDP were 3.3 times more sensitive to MEPQ than naive mice. The effect of CBDP is similar to that obtained with methylphosphonofluoridates such as soman [13, 14], and for this reason further study in animals having aliesterase free blood may be of interest. Nonetheless, the present protocol can be extended to comparative evaluation of other enzymes, e.g. human AChE or BuChE, as scavengers of toxic OP esters. It should be noted that both previous [1] and present papers in this series utilized oximes in some experiments to extend AChE protection against OPs, and this use suggests other experimental approaches and treatment regimens.

Experiments are underway to evaluate the antidotal efficacy of FBS-AChE against high dose challenges of lipid soluble anti-ChE poisons. The data presented here should be utilized as basic quantitative information for the interpretation of results from experiments now in progress.

*Acknowledgements*—This work was done while one of the authors, Y. Ashani, held a National Research Council-WRAIR Research Associateship.

## REFERENCES

1. Wolfe AD, Rush RS, Doctor BP, Koplovitz I and Jones D, Acetylcholinesterase prophylaxis against organophosphate toxicity. *Fundam Appl Toxicol* **9**: 266–270, 1987.
2. Shek PN and Barber RF, Liposomes are effective carriers for the ocular delivery of prophylactics. *Biochim Biophys Acta* **902**: 229–236, 1987.
3. Gray AP, Design and structure–activity relationships of antidotes to organophosphorus anticholinesterase agents. *Drug Metab Rev* **15**: 557–589, 1984.
4. Levy D and Ashani Y, Synthesis and *in vitro* properties of a powerful quaternary methylphosphonate inhibitor of acetylcholinesterase. *Biochem Pharmacol* **35**: 1079–1085, 1986.
5. Ashani Y, Levy D, Heyman E, Grunfeld Y, Kadar T and Spiegelstein M, A quarternary anticholinesterase probe for determining the integrity of the blood–brain barrier. *Life Sci* **36**: 493–497, 1985.
6. Balan A, Barness I, Simon G, Levy D and Ashani Y, Tritium labeling of a powerful methylphosphonate inhibitor of cholinesterase: synthesis and biological applications. *Anal Biochem* **169**: 95–103, 1988.
7. Eto M, Casida JE and Eto T, Hydroxylation and cyclization reactions involved in the metabolism of tri-*O*-cresyl phosphate. *Biochem Pharmacol* **11**: 337–352, 1962.
8. Poziomek EJ, Hackley BE and Steinberg M, Pyridinium aldoximes. *J Org Chem* **23**: 714–717, 1958.
9. De La Hoz D, Doctor BP, Ralston JS, Rush RS and Wolfe AD, A simplified procedure for the purification of large quantities of mammalian acetylcholinesterase. *Life Sci* **39**: 195–199, 1986.
10. Ellman GL, Courtney KD, Andres V Jr and Featherstone RM, A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol* **7**: 88–95, 1961.
11. Weil CS, Tables for convenient calculation of medium effective dose ($LD_{50}$ or $ED_{50}$) and instructions in their use. *Biometrics* **8**: 249–263, 1952.
12. Hobbiger F, O'Sullivan DG and Sadler PW, New potent reactivators of acetylcholinesterase inhibited by tetraethyl pyrophosphate. *Nature* **182**: 1498–1499, 1958.
13. Boskovic B, Influence of CBDP on organophosphate poisoning and its therapy. *Arch Toxicol* **42**: 207–216, 1979.
14. Clement JG, Importance of aliesterase as a detoxification mechanism for soman in mice. *Biochem Pharmacol* **33**: 3807–3811, 1984.