

PHARMACOKINETICS AND IMMUNOLOGIC CONSEQUENCES OF REPEATED ADMINISTRATIONS OF PURIFIED BUTYRYLCHOLINESTERASES IN MICE

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ABSTRACT

Human serum butyrylcholinesterase (Hu BChE) was demonstrated to be an effective prophylaxis that can protect animals from organophosphate nerve agents. However, if the enzyme is to be used in humans, then knowledge of both the pharmacokinetics of the enzyme and its immunological consequences *in vivo*, is necessary. The present study sought to assess the pharmacokinetics and circulating anti-BChE antibodies produced following repeated administrations of purified Hu BChE and mouse BChE (Mo BChE) in mice. An i.m. injection of 100 U of Hu BChE in Balb/c or CD-1 mice displayed a mean retention time (MRT) of ~50 h, and area under the curve (AUC) of ~1250. The same dose of Mo BChE (purified from sera of CD-1 mice) injected in Balb/c mice exhibited a higher MRT of 78 h and AUC of 1815; the AUC increased to 2504 in CD-1 mice. As expected, a second injection of Hu BChE in mice exhibited a marked reduction in circulatory stability. Although, the second injection of Mo BChE displayed reduced circulatory stability in Balb/c mice, its stability was almost identical to the first injection in CD-1 mice. Consistent with these observations, circulating anti-BChE IgGs were observed in mice injected with Hu BChE and Balb/c mice injected with Mo BChE. No antibody response was detected in CD-1 mice following either injection of homologous Mo BChE. These results bode well for the potential use of human BChE as a detoxifying drug in humans.

INTRODUCTION

The exogenous administration of plasma-derived cholinesterases (ChEs) in both rodent and non-human primate models has been successfully used as a safe and efficacious prophylactic treatment to prevent poisoning by organophosphorus compounds (OP, Doctor *et al.*, 2001; Raveh *et al.*, 1997; Allon *et al.*, 1998). The protection displayed was not only against mortality but also against the adverse physiological and behavioral effects of nerve agent exposure. In these studies, the enzyme was administered by a single injection, however, multiple doses may be needed to achieve a long-lasting protective level of circulating enzyme to counteract the toxicity of multiple exposures to OPs. The prolonged circulatory residence time of the enzyme is influenced by the size of protein, the microheterogeneity of carbohydrate structures, and the induction (if any) of anti-enzyme antibodies following repeated injections of the enzyme. For example, a much shorter mean residence time of 34 h

Report Documentation Page				Form Approved OMB No. 0704-0188	
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1. REPORT DATE 16 NOV 2004		2. REPORT TYPE N/A		3. DATES COVERED -	
4. TITLE AND SUBTITLE Pharmacokinetics And Immunologic Consequences Of Repeated Administrations Of Purified Butyrylcholinesterases In Mice				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Division of Biochemistry, Walter Reed Army Institute of Research, Silver Spring, MD 20910-7500				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release, distribution unlimited					
13. SUPPLEMENTARY NOTES See also ADM001849, 2004 Scientific Conference on Chemical and Biological Defense Research. Held in Hunt Valley, Maryland on 15-17 November 2004.					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 5	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			

was observed for Hu BChE administered into macaques compared to 225 h for macaque BChE injected into macaques (Raveh *et al.*, 1997; Rosenberg *et al.*, 2002). There was no circulating anti-macaque IgG detected in macaques following repeated injections of homologous enzyme. In this study, we examined the consequences of repeated injections of heterologous (Hu BChE) and homologous (Mo BChE) BChE in mice following two i.m. injections of ~100 U (0.15 mg) on day 0 and on day 27, respectively. This dose is similar to that envisaged for use in humans (Ashani *et al.*, 2004). The effects of two heterologous and homologous injections were monitored by following blood BChE and anti-BChE IgG levels.

MATERIALS AND METHODS

Purification of Hu BChE and Mo BChE

Hu BChE was isolated from Cohn fraction IV (Luo *et al.*, 2002) and stored in lyophilized form at -20°C. Mo BChE was isolated from the sera of CD-1 mice and stored in solution at 4°C until use. The specific activities of the enzymes were 700-750 U/mg as measured in 50 mM sodium phosphate buffer at pH 8.0, at 25°C, using 1 mM butyrylthiocholine as the substrate (Ellman *et al.*, 1961).

Animals

Mice (Balb/c & CD-1) were purchased from Charles River Laboratory at 7-8 weeks of age and housed in a controlled specific-pathogen-free environment (12 h light-12 h dark photoperiod; $22 \pm 1^\circ\text{C}$; $60\% \pm 10\%$ relative humidity) in the animal facility at Walter Reed Army Institute of Research (WRAIR). Animals were housed in groups of 3 per cage with free access to food and water. Animal studies were approved by the WRAIR Animal Care and Use Committee, and all animal care procedures conformed to *the Guide for the Care and Use of Laboratory Animals*.

Pharmacokinetics and Immunologic Consequences of Repeated Exposures of Mice to BChEs

Purified Hu BChE or Mo BChE, 100 U each, was administered into mice (n=6, 3 male/3 female) by a single bolus i.m. injection, followed by a 2nd injection of 100 U of the same enzyme four weeks later. Five μl of blood was drawn from the tail vein at multiple time points following the two injections for determination of blood BChE activity (Ellman *et al.*, 1961) and anti-BChE IgG levels.

Anti-BChE Antibody Assay

For antibody detection in mouse plasma, 0.2 U of Hu BChE or Mo BChE per well was used as the plate-coating antigen. Mouse antibody binding to Hu BChE or Mo BChE was detected with peroxidase-labeled goat anti-mouse IgG using 2,2'-azino-bis-(3-benzthiazoline-6-sulfonic acid) (ABTS) substrate. Standard curves using purified mouse IgG were run with each assay to allow quantification of antibody response.

Determination of Pharmacokinetic Parameters

Pharmacokinetic parameters were calculated using a Windows-based computer software for noncompartmental analysis of pharmacokinetic data (Laub and Gallo, 1996). The area under the blood BChE concentration vs time curve (AUC_∞) was calculated by the trapezoidal method. Half-life ($T_{1/2}$) was calculated as $T_{1/2} = \ln(2)/\text{kel}$. C_{max} was defined as the concentration of blood BChE activity at peak. Mean retention time (MRT) = AUMC/AUC , where AUMC is area under the moment curve.

Statistical Analysis

Group means and standard deviations were calculated for all numerical data. Statistical evaluations were performed on pharmacokinetic parameters using the student T-test. *P* value of less than 0.05 was considered significant.

RESULTS AND DISCUSSION

1. Both Hu BChE and Mo BChE displayed extended circulatory stabilities *in vivo* following the first injection in both Balb/c and CD-1 mice (Top panels of Figure 1 & Figure 2, and Table 1). Mo BChE, however, displayed a significantly larger AUC and MRT compared to Hu BChE in mice, indicating that homologous BChE has much better bioavailability compared to heterologous BChE.
2. As expected, the 2nd injection of Hu BChE exhibited a marked reduction in all pharmacokinetic parameters (Table 1), which is consistent with a striking elevation of blood anti-BChE IgG levels (Bottom panels of Figure 1 & Figure 2).
3. The 2nd injection of Mo BChE into Balb/c mice also caused an apparent reduction in all pharmacokinetic parameters (Table 1). This observation was validated by the existence of anti-BChE IgG in Balb/c mice following the 2nd injection. These results suggest that the production of anti-BChE antibodies upon injection of Mo BChE, purified from the sera of CD-1 mice, into Balb/c mice may be due to differences in BChEs from the two strains of mice.
4. This suggestion was validated by the follow up study in which the two injections of Mo BChE purified from the sera of CD-1 mice into CD-1 mice produced an almost identical pharmacokinetic profile. Consistent with this observation, there was no detectable anti-BChE IgG in the blood of CD-1 mice following the two homologous injections of Mo BChE.

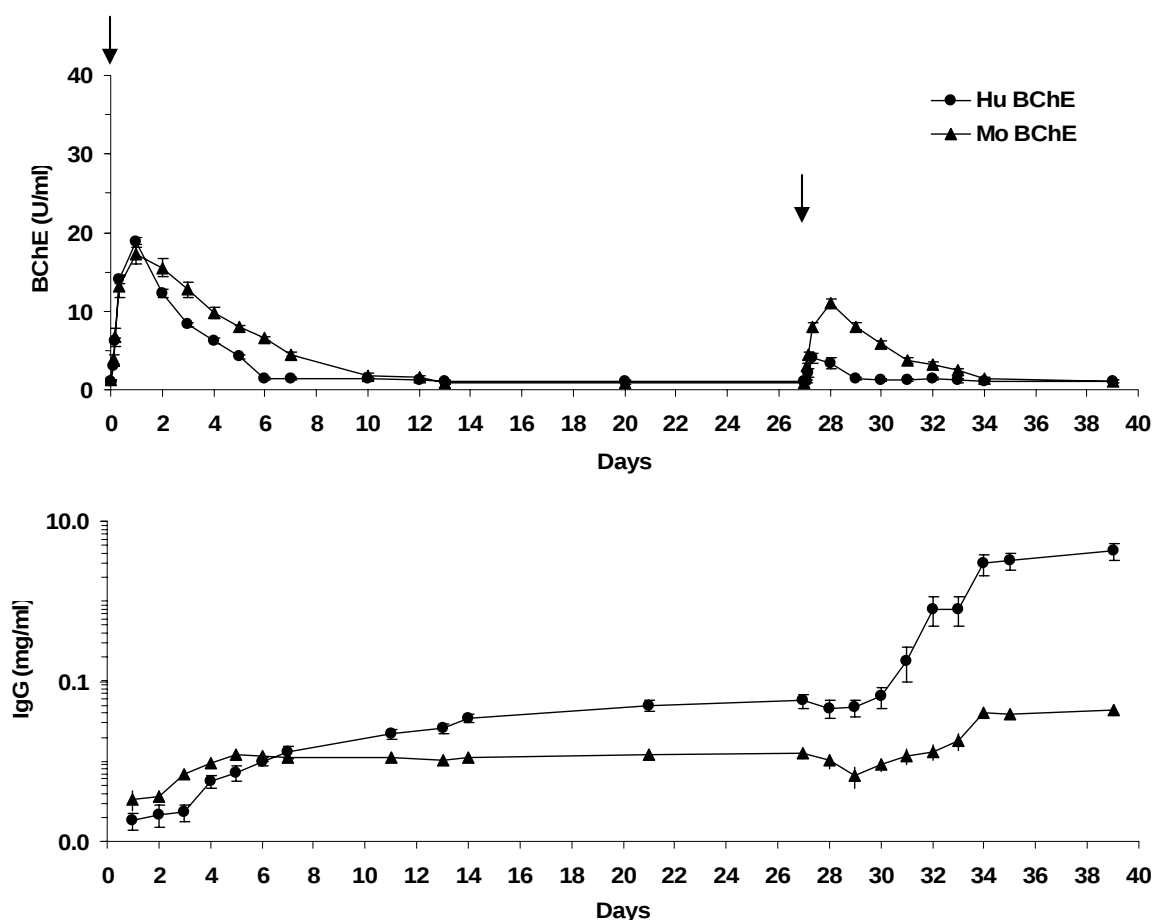


FIGURE 1. Top panel: Time courses of Hu BChE and Mo BChE in the blood of Balb/c mice following two injections of purified Hu BChE and Mo BChE, respectively; Bottom panel: Antibody levels in the sera of Balb/c mice following two injections of purified Hu BChE and Mo BChE. The arrows indicate the times of the first and second injections.

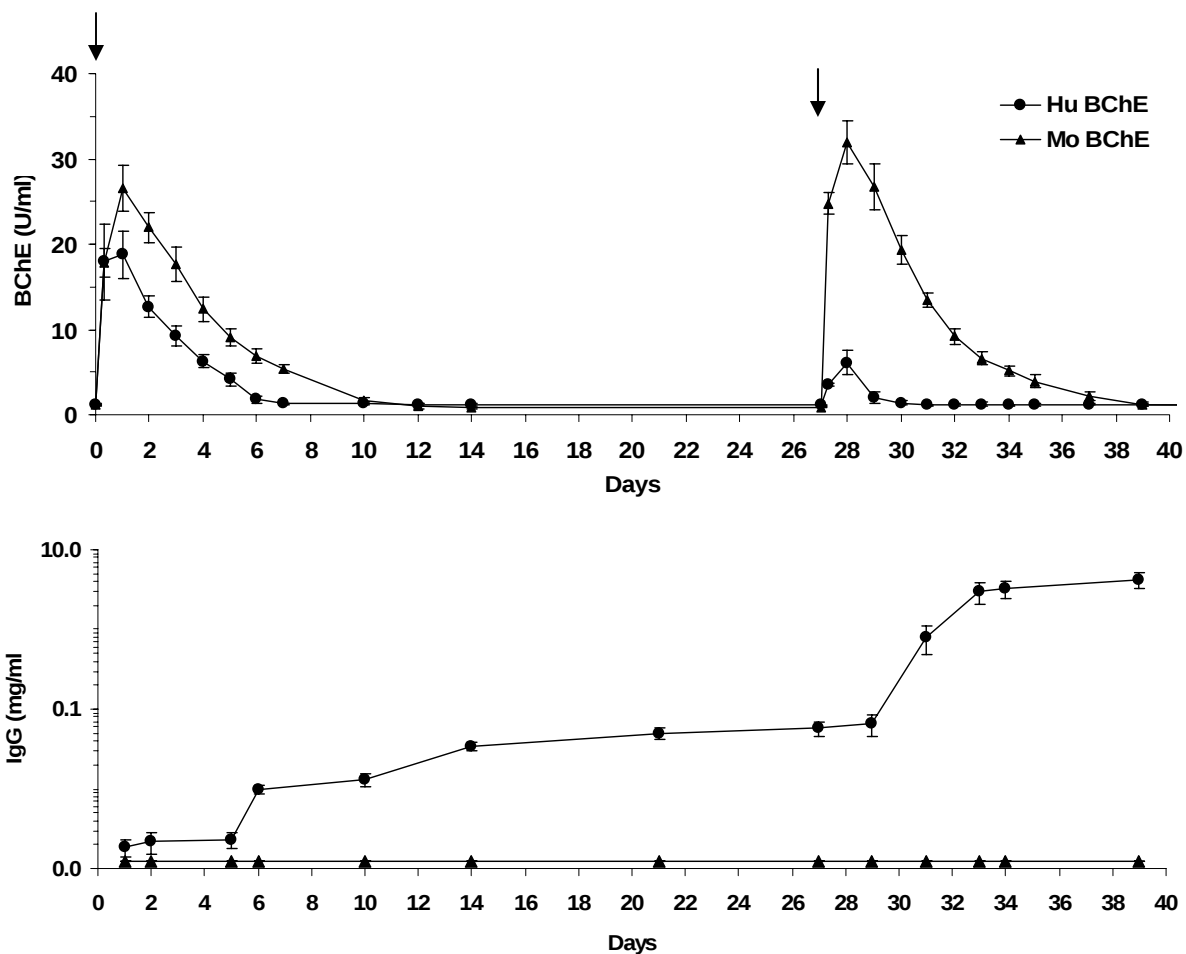


FIGURE 2. Top panel: Time courses of Hu BChE and Mo BChE in the blood of CD-1 mice following two injections of purified Hu BChE and Mo BChE, respectively; Bottom panel: Antibody levels in the sera of CD-1 mice following two injections of purified Hu BChE and Mo BChE. The arrows indicate the times of the first and second injections.

TABLE 1. Pharmacokinetic parameters of purified Hu BChE and Mo BChE in Balb/c and CD-1 mice.

Parameters	<u>Balb/c mice</u>				<u>CD-1 mice</u>			
	1 st Injection		2 nd Injection		1 st Injection		2 nd Injection	
	Hu BChE	Mo BChE	Hu BChE	Mo BChE	Hu BChE	Mo BChE	Hu BChE	Mo BChE
MRT	51±1	78±1*	34±10	60±2 [#]	48±2	73±3*	26±1	79±6 [#]
T _{1/2}	24±2	33±2*	27±8	24±3	18±4	22±2	10±2	32±2 [#]
C _{max}	18±1	16±1	3±1	10±0 [#]	19±3	25±3	5±1	31±2 [#]
AUC	1,217±35	1,815±116*	110±21	791±44 [#]	1,301±217	2,504±239*	165±43	3,071±194 [#]

* p<0.05 vs Hu BChE group following 1st injection.

[#] p<0.05 vs Hu BChE group following 2nd injection.

CONCLUSIONS

In this study, we examined the consequences of repeated injections of heterologous (Hu BChE) and homologous (Mo BChE) BChE in mice following two i.m. injections of ~100 U (0.15 mg) on day 0 and on day 27, respectively. This dose is similar to that envisaged for use in humans (Ashani *et al.*, 2004). The effects of two heterologous and homologous injections were monitored by following blood BChE and anti-BChE IgG levels. The rate of clearance of heterologous Hu BChE following the first injection was faster compared to that of homologous Mo BChE in mice. As expected, the second injection of Hu BChE cleared much faster compared to the first injection. On the other hand, the pharmacokinetic profiles of the two homologous Mo BChE injections were very similar, suggesting a lack of humoral response to the injected enzyme. Consistent with this observation, no circulating anti-Mo BChE IgG was detected following either of the two homologous Mo BChE injections. The results of this study in conjunction with macaque study suggest that Hu BChE is a safe and effective bioscavenger that should be developed as a product that can protect humans against all OP nerve agents.

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