



Letter

The impact on the activity of acetylcholinesterase of a polylysine-ApoE peptide carrier targeting the blood brain barrier

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ABSTRACT — The K16ApoE peptide has been demonstrated to deliver a supraphysiological level of protein therapeutics to the brain and further increase the life-span of mice with a lysosomal storage disorder (LSD). If successfully developed, K16ApoE would provide new treatments for LSD and many other neurological diseases. However, while the K16ApoE can cross the blood-brain barrier, data indicates a toxic response associated with it. The mechanism of toxicity must be resolved for further clinical translation. The toxic response towards the peptide was hypothesized to be induced by inhibition of acetylcholinesterase (AChE) activity at neuro-muscular junction. Here, the dose-response analysis between AChE and K16ApoE was conducted in both female and male mice. Results demonstrated that AChE activity was significantly reduced with increasing dose of K16ApoE except for the mid-dose where a dramatic increase in AChE activity was observed. Also, obvious difference in response to K16ApoE was shown when considering the influence from sex and body weight. Though the statistical analysis of the dose response and survival ratio suggested that AChE is not the primary mechanism of action for the acute toxicity of K16ApoE, the complex inhibition/stimulation response of AChE indicated that this enzyme must play a role in the toxicity of the peptide.

Key words: Blood-brain barrier, Peptide carrier, K16ApoE, Toxicity, Acetylcholinesterase, Inhibition

INTRODUCTION

The blood-brain barrier (BBB) presents a big challenge to deliver drugs into the brain limiting the effective drug development for many neurological disorders. Systemic delivery of therapeutics to the central nervous system (CNS) is the most ideal way in terms of safety and even distribution in the brain. However, various approaches have demonstrated marginal level of the drug achievable in the brain (Cho *et al.*, 2015; Meng *et al.*, 2012; Sorrentino *et al.*, 2013; Wang *et al.*, 2013).

A peptide carrier containing 16 polylysine residues and 20 amino acids of apolipoprotein E sequences (K16ApoE) has been demonstrated to deliver a variety of large pro-

tein therapeutics and small molecule drugs (Sarkar *et al.*, 2011, 2014). In a mouse model of Late-infantile neuronal ceroid lipofuscinosis (LINCL) with deficiency in the lysosomal protease tripeptidyl peptidase I (TPPI) (Sleat *et al.*, 1997, 2004), we have achieved supraphysiological levels (~800% of wild-type) of intravenously-administered recombinant TPP1 throughout the brain upon co-injection with K16ApoE. Furthermore, the mice demonstrated a significant improvement in neurological function and increased lifespan in either acute or chronic treatment with the mixture of TPP1 and K16ApoE (Meng *et al.*, 2014, 2017).

However, K16ApoE exhibits the dose-dependent toxicity administered alone or in conjunction with TPP1

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(Meng *et al.*, 2014). When injected with moderate to high doses of the K16ApoE, mice exhibited significant difficulty in breathing and muscle spasms ultimately leading to death at high doses. Therefore, K16ApoE has a very narrow therapeutic index. Characterization of peptide variants suggested that the toxicity and efficacy both are associated with the positive charge of K16ApoE. Eliminating the toxicity by removing the positive charges from K16ApoE resulted in diminishing the uptake of TPP1 to the brain (Meng *et al.*, 2017).

Based on the observations of symptoms similar to neurotransmitter inhibition, it was hypothesized that the K16ApoE was inhibiting acetylcholinesterase (AChE) at the neuro-muscular junction and it was this inhibition that resulted in the observed toxicity of the peptide. To test this hypothesis, a series of experiments were conducted to determine the role that AChE inhibition plays in the observed toxicity of the peptide.

MATERIALS AND METHODS

K16ApoE (KKKKKKKKKKKKKKKKKLRVR-LASHLRKLRKRLRDA) was synthesized in GenScript (Nanjing, China) with the purity above 98%. ICR inbred mice, between 12 and 14 weeks of age were purchased from Wenzhou Medical University (Wenzhou, China) and maintained under a 12/12-hr light/dark schedule. Forty-eight mice were anesthetized and subsequently dosed by tail vein injection with K16ApoE in phosphate

buffered saline. Anesthetized animals were sacrificed. Tissues were collected and snap frozen in liquid nitrogen for enzyme activity analysis. AChE analysis was conducted as previously described (Ellman *et al.*, 1961) All statistical analysis was conducted using Stata, version 11 statistical software package (StataCorp., 2009). All animals were maintained by protocols approved by the Wenzhou-Kean University and Wenzhou medical University Institutional Animal Care and Use Committee.

RESULTS

The results of the AChE analysis of brain tissues indicate a complex dose/response relationship. The analysis demonstrates a significant ($p < 0.05$) reduction in AChE activity in mice exposed to lower concentrations of the peptide compared to animals in the control groups. A statistically significant ($p < 0.05$) recovery of AChE at mid-range dose was also observed.

There was 100% mortality of both male and female mice at the 160 and 320 nmole peptide doses. There was 1 death in the female cohort at 80 nmole peptide. An analysis of the survivability data and AChE data indicates no significant difference ($p < 0.05$) in AChE activity between individuals that survived and those that did not.

The data indicate a sexual dimorphic response in AChE activity when the data is normalized for body weight. There is a significant difference ($p < 0.05$) in weight normalized AChE activity with female mice have

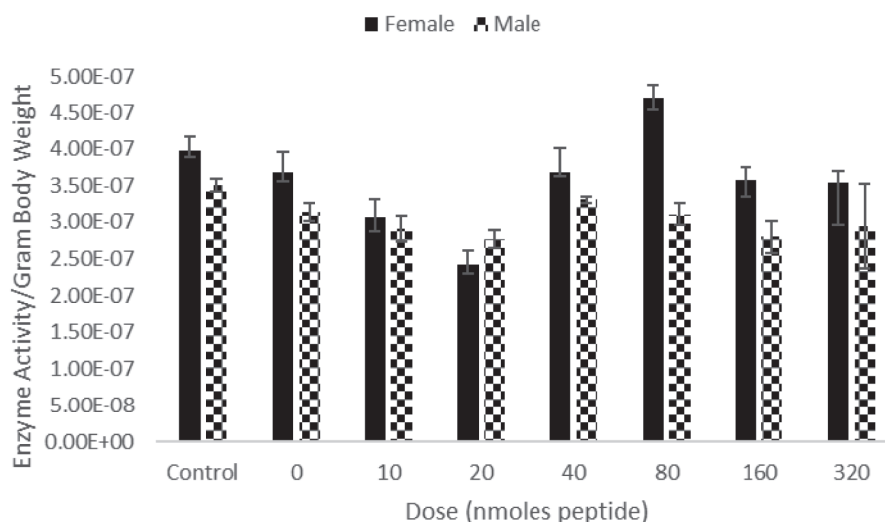


Fig. 1. Impact of Sex on Weight-Normalized AChE Activity in Response to K16ApoE. K16ApoE of different concentrations was injected in the mice via tail vein. There were three female and male mice for each dose group. AChE activity for either female or male mice was shown in dark and mosaic bars, respectively. ($n = 48$).

a higher activity level. There is also a significant interaction between sex and dose with respect to AChE activity (Fig. 1).

DISCUSSION

This investigation was designed to test the hypothesis that the K16ApoE peptide inhibited AChE ultimately leading to respiratory failure and death. The results indicate a significant reduction in AChE activity in organisms exposed to the K16ApoE peptide. There is also a clear difference between male and female mice in response to the peptide. However, an analysis of the dose-response curve and survivability data clearly demonstrates that AChE is not the primary mechanism of action for the acute toxicity of the peptide.

Although AChE is not the primary mechanism of action, the results indicate that it does play a role in the toxicity. The key portion of the dose/response curve is the increase in AChE activity in the mid-range of peptide concentration. This mid-dose increase in AChE activity was also observed in preliminary peptide-AChE experiments (data not included). This result was unexpected and may indicate some form of biochemical or epi-genetic response that drives an enhanced protein expression. This is consistent with peptide induced protein expression in the literature (Ikeno and Haruyama, 2013; Choi *et al.*, 1998).

The data also demonstrate a difference in AChE response between male and female mice. Sexual dimorphism in structure and physiological processes, including with AChE response, in mice is recognized throughout the scientific literature (Haizlip *et al.*, 2015; Steegenga *et al.*, 2014; Loy and Sheldon, 1987). The observed statistically significant ($p < 0.05$) interaction between sex and dose (Fig. 1) further demonstrates a dimorphic response to the peptide.

K16ApoE is a promising tool for treating neurological disorders. With a demonstrated ability to transport compounds across the blood-brain barrier, this peptide has great potential (Sarkar *et al.*, 2011, 2014; Meng *et al.*, 2014, 2017) but, the toxicity of the peptide must be resolved. Although AChE is not the primary toxicity mechanism of action, it clearly plays a role. The next steps to be accomplished are to identify alternative targets that may work in conjunction with AChE to solve the mechanism of toxicity and thereby allow further development of K16ApoE as an effective drug delivery carrier. Solving the mechanism of toxicity will allow further development of K16ApoE as an effective drug delivery carrier for neurological disorders.

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