Plant-derived human acetylcholinesterase-R provides protection from lethal organophosphate poisoning and its chronic aftermath

Running Title:
Plant-derived human acetylcholinesterase

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OP exposure induces two seemingly inverse effects: First, it decreases ChE activities by blockade, and second, it induces long-lasting feedback overproduction of AChE-R (1, 2). In another set of experiments, we examined the ability of AChE-R_{ER} to protect from these delayed effects of OP poisoning. To this end, mice were pre-treated by i.v. administration of PBS +/- AChE-R_{ER} (400 U) followed by a sublethal (0.8 LD_{50}) or lethal (1.13 LD_{50}) paraoxon challenge. Unprotected, sub-lethally-challenged mice, or AChE-protected, lethally-challenged mice all exhibited severe cholinergic symptoms followed by recovery (survival rates were 100% and 70% for sub-lethally- and lethally-challenged mice, respectively). Ten days post-treatment, mice were euthanized and plasma and skeletal muscle samples were harvested. At this time, all mice predictably experienced a small increase in their plasma AChE levels induced by the OP challenge (Fig. 4B, Fig. S1A). Plasma AChE activity at this delayed time point was substantially lower in enzyme-treated animals as compared to unprotected sub-lethally challenged mice, suggesting an effective yet incomplete offset of the long-lasting AChE-R over-production by administration of plant-derived AChE-R_{ER}. Administration of plant-derived AChE-R_{ER} alone significantly limited the levels of plasma AChE-R activity 10 days post injection (Fig. 4B, Fig. S1A), supporting the notion that this offset was a feedback response to the high levels of AChE molecules in the circulatory system. Further more, the enzyme-pretreatment modulation of the OP-induced increase in plasma AChE activity is mirrored by corresponding changes in AChE monomers, probably murine AChE-R, observed by non-denaturing gels of samples obtained 10 days post-challenge (Fig. S1A).

The enzyme-pretreatment modulation of the OP-induced increase in plasma AChE activity is mirrored by corresponding changes in AChE monomers, probably murine AChE-R, observed by non-denaturing gels of samples obtained 10 days post-challenge (Fig. S1A). These monomers migrate somewhat slower than the plant-derived pure

AChE-R_{ER}, which were completely cleared by the mice 2 hours after their administration (Fig. 3). The slower migration species can either reflect protein-protein associations or distinct glycosylation patterns of post-challenge murine AChE-R.

To detect long-term changes in the total amount of AChE (active and inactive), we resolved plasma proteins by SDS-PAGE followed by detection of the AChE protein using specific Abs directed against the AChE common domain or against the unique carboxyl-terminal domain of murine AChE-R (Fig. S1B). Individual mice in each treatment group presented considerable variability. Nevertheless, paraoxon-induced, dose-dependent elevation of murine AChE in the blood appeared to be countered by the enzyme pretreatment. Thus, plasma accumulation of murine AChE-R paralleled the trend of changes in total AChE (Fig. S1B) and matched the trends seen with AChE activity and active AChE monomers (Fig. 4B, S1A). We therefore conclude that the post-poisoning increase in the plasma levels of endogenous murine AChE, specifically murine AChE-R, can be at least partially mitigated by exogenously applied plant-derived enzyme.

Enzyme pretreatment exerted considerable attenuation effects on the post-exposure accumulation of the murine AChE-R in skeletal (leg) muscles following both lethal and sublethal OP insults and prevented its inhibition (Fig. S1C).

In muscles, the vast majority of AChE molecules, even following insult, is of the synaptic variant, AChE-S(3). AChE-S can be further organized into several molecular forms, including soluble and membrane bound ones, which can be separated by sequential extraction of tissue homogenates(4). Homogenization in low-salt buffer releases soluble (monomeric) AChE-S and AChE-R (together representing ~15% of total AChE activity, Fig. 5E). Subsequent extractions, first with buffer containing 1% Triton X-100 (Low Salt-Detergent, ~65%), then with 1 M NaCl (High Salt, ~20%) release the AChE-S species, mostly as tetramers that are associated with membranes (data not

shown)(4). Administration of plant-derived AChE-R_{ER} had no significant effect on the total muscle AChE activity, but a lethal dose of paraoxon caused a ~50% decrease in membrane-bound detergent-soluble forms of AChE (Fig. 5E), most probably tetrameric AChE-S. In unprotected mice, paraoxon also caused a significant decrease in the low-salt soluble AChE activity (presumably AChE-R) (-16.4%, Fig 5E insert), suggesting that host muscle AChE-R is irreversibly inhibited by the OP, yielding “aged” enzyme. AChE-R_{ER} treatment reversed this trend, so that AChE activities were apparently similar to those of PBS controls (Fig. 5E insert) and compatible with the hypothesis that the administered AChE-R_{ER} served as a “decoy” protecting muscle AChE-R from inhibition. In conclusion, administration of plant-derived AChE-R_{ER} reciprocally modulated the OP-induced changes in AChE gene expression, preventing its inhibition in muscles while decreasing OP-induced AChE-R production in the mouse circulation.

Figure Legend:

Figure S1. Plant-derived AChE-R_{ER} and paraoxon can reciprocally modulate accumulation of murine AChE-R *in vivo*. Samples of plasma (a-b) and quadriceps muscles (c-e) were harvested 10 days post treatments and were analyzed for their content of AChE protein and enzymatic activity. (a) Plasma proteins were resolved by non-denaturing PAGE followed by staining of active AChE. Plant-derived AChE-R_{ER} served for comparison. (b) Plasma proteins were resolved by SDS-PAGE followed by immunoblotting. Total AChE protein was detected with Abs directed to the common domain of all mouse AChE variants and murine AChE-R by Abs specific to its unique C-terminal domain (mARP). Representative blots are shown and above them densitormetric quantitation of band intensities (Analysis was conducted 5 times per sample, with 3-6 animals per group). (c) Muscle proteins were sequentially fractionated based on their solubility in Low Salt buffer (containing 144 mM NaCl, 50 mM $MgCl_2$ and 10 mM sodium phosphate, pH 7.4), Low Salt + Detergent (1% Triton X-100), and finally High Salt (1 M NaCl and 10 mM sodium phosphate) as previously described (4). Proteins in the Low Salt + Detergent fraction were resolved by SDS-PAGE followed by immunoblotting and detection with the AChE-R specific Ab. Synthetic mARP peptide served as a positive control. (d) AChE activity was assayed in all three fractions (mean±SEM) (e) Percent change (relative to PBS control) of AChE activity in the Low Salt fraction. Asterisk denotes statistical significance (P<0.04, Student's t test).

Supplemental References

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