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Research paper

A novel expression cassette delivers efficient production of exclusively tetrameric human butyrylcholinesterase with improved pharmacokinetics for protection against organophosphate poisoning

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ABSTRACT

Butyrylcholinesterase is a stoichiometric bioscavenger against poisoning by organophosphorus pesticides and nerve agents. The low level of expression and extremely rapid clearance of monomeric recombinant human butyrylcholinesterase (rhBChE) from bloodstream ($t_{½} \approx 2$ min) limits its pharmaceutical application. Recently (Ilyushin at al., PNAS, 2013) we described a long-acting polysialylated recombinant butyrylcholinesterase (rhBChE-CAO), stable in the bloodstream, that protects mice against 4.2 LD_{50} of VR. Here we report a set of modifications of the initial rhBChE expression vector to improve stability of the enzyme in the bloodstream and increase its production in CHO cells by introducing in the expression cassette: (i) the sequence of the natural human PRAD-peptide in frame with rhBChE gene via "self-processing" viral F2A peptide under control of an hEF/HTLV promoter, and (ii) previously predicted *in silico* MAR 1-68 and MAR X-29 sequences. This provides fully tetrameric rhBChE (4rhBChE) at 70 mg/l, that displays improved pharmacokinetics ($t_{½} = 32 \pm 1.2$ h, MRT = 43 ± 2 h). 3D Fluorescent visualization and distribution of ^{125}I-labeled enzyme reveals similar low level 4rhBChE and rhBChE-CAO accumulation in muscle, fat, and brain. Administered 4rhBChE was mainly catabolized in the liver and breakdown products were excreted in kidney. Injection of 1.2 LD_{50} and 1.1 LD_{50} of paraoxon to BALB/c and knockout BChE−/− mice pre-treated with 4rhBChE (50 mg/kg) resulted in 100% and 78% survival, respectively, without perturbation of long-term behavior. In contrast, 100% mortality of non-pre-treated mice was observed. The high expression level of 4rhBChE in CHO cells permits consideration of this new expression system for manufacturing BChE as a biopharmaceutical.

1. Introduction

Chemical and nuclear weapons gave many dramatic war episodes in the 20th century. The Geneva Protocol, signed in 1925,

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