In vitro Characterization of Recombinant Human BuChE (Protexia) as a Potential Bioscavenger

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Objectives

- Demonstrate improved medical protection against nerve agents
- Develop a prophylactic that detoxifies nerve agents at a rate sufficient to protect against 5LD\(_{50}\) exposure
- Prophylactic should:
  - Be non-toxic
  - Produce no adverse side effects
  - Have no adverse effect on performance
  - Be easy to administer
  - Have a long biological half-life

3-D model of BuChE allows for rational drug design and molecular biology approaches to development of biological scavengers.
Concept

- Provide a pretreatment capable of protecting against up to $5 \times \text{LD}_{50}$ of nerve agent with no side effects and no need for additional protective clothing or therapy.
Potential Bioscavengers

Efforts to date have focused on BuChE of human origin

• Production of Hu BChE From Cohn Fraction IV-4
  • single band on SDS-PAGE
  • ~ 20 g = 14 million units of purified BChE
  • >98% pure; specific activity ~700 U/mg

• Material (Protexia™) from milk of transgenic goats is now available
  • Hu-BuChE gene fused to a milk promoter
  • Expression is directed to the milk of transgenic goats
  • Purified material has Specific activity ~595 U/mg

Need to characterize Protexia™ versus plasma derived material
Protexia™ Binds H³-Soman

![Graph showing Protexia™ binds H³-Soman](image)

- Pretreated: CBDP, (-), Cold GD
- No Protein

DPM

- Protexia™: 100, 200, 300, 400
- No Protein: 100, 200, 300, 400
Protexia™ Binds H³-DFP

![Graph showing the binding of Protexia™ and No Protein with different pretreatments.](image-url)
Protexia™ BuChE is Inhibited by OPs

Molar Ratio of BuChE to Nerve Agent
8:1  4:1  1:1  1:4

% of uninhibited activity

Agent Concentration (µM)

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Titration of h-BuChE and Protexia™ by Soman and VX

- **Graph 1**: Titration of h-BuChE and Protexia™ by VX (ng/mL) vs. BChE (U/ml)
  - **Y-axis**: BChE (U/ml)
  - **X-axis**: VX (ng/mL)
  - Data points show a linear relationship between VX concentration and BChE activity for both Hu BChE and Protexia™.

- **Graph 2**: Titration of h-BuChE and Protexia™ by GD (ng/mL) vs. BChE (U/ml)
  - **Y-axis**: BChE (U/ml)
  - **X-axis**: GD (ng/mL)
  - Similar to Graph 1, shows a linear relationship with data points for Hu BChE and Protexia™.

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Structures of Inhibitors

Pyridostigmine bromide

Pyridostigmine bromide
Structures of Inhibitors

Phenerine

Tolserine

Phenylethylcynserine tartrate

Ethyl carbamoyl

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Tolserine with h-BuChE

IC50 graph: tolserine inhibitor with Hu-BuChE

% inhibition

inhibitor concentration (M)
Tolserine with h-BuChE

![Graph showing the relation between tolserine concentration and 1/V.](image)
Tolserine with RHu-BuChE (Protexia™)

IC50 graph: tolserine inhibitor with RHu-BuChe

% inhibition

inhibitor concentration (M)

1.00E-10 1.00E-09 1.00E-08 1.00E-07 1.00E-06 1.00E-05 1.00E-04

0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0

1.00E-04 1.00E-05 1.00E-06 1.00E-07 1.00E-08 1.00E-09 1.00E-10
Tolserine with Protexia™

Ki plot: Tolserine inhibitor with RHuBuChe

1/N

-1.00E-07 -5.00E-08 0.00E+00 5.00E-08 1.00E-07

concentration (M)

-5 0 5 10 15 20 25

10^-3 5.00E-04 2.50E-04 1.67E-04
Phenserine with h-BuChE

![Graph showing the relationship between Inhibitor Concentration (M) and 1/V for Phenserine with h-BuChE. The graph includes data points for different concentrations: 10^-3, 5.00E-04, 2.50E-04, and 1.67E-04. The x-axis represents Inhibitor Concentration (M), ranging from -2.00E-06 to 1.00E-06. The y-axis represents 1/V, ranging from 0 to 5.]}
Phenserine with Protexia™

Kᵢ plot: Phenserine inhibitor with RHuBuChe

Inhibitor concentration (M) vs. 1/V
### Kᵢ and IC50 data for Set of Inhibitors

<table>
<thead>
<tr>
<th>Inhibitor compound</th>
<th>Protexia™</th>
<th>Hu-BuChE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC 50</td>
<td>Kᵢ (M)</td>
</tr>
<tr>
<td>Phenserine</td>
<td>5 x 10⁻⁷</td>
<td>1.3 x 10⁻⁶</td>
</tr>
<tr>
<td>Tolserine</td>
<td>8 x 10⁻⁸</td>
<td>1.4 x 10⁻⁷</td>
</tr>
<tr>
<td>Phenethyl cynserine</td>
<td>5 x 10⁻¹¹</td>
<td>5.1 x 10⁻¹⁰</td>
</tr>
<tr>
<td>Ethyl carbamoyl</td>
<td>5 x 10⁻¹¹</td>
<td>6.2 x 10⁻¹⁰</td>
</tr>
<tr>
<td>Pyridostigmine bromide</td>
<td>5 x 10⁻⁷</td>
<td>4.6 x 10⁻⁷</td>
</tr>
</tbody>
</table>
Comparison of Hu-BuChE and ProtexiaTM

- In vitro comparison of both forms of human BuChE with a variety of inhibitors

- Properties of recombinant HuBuChE from milk of transgenic goats (Protexia™)
  - Binds the nerve agents GA, GB, GD, VX
  - In vitro properties similar to human plasma BuChE

- Results to date support similarity in the two sources.
Summary

Plasma BuChE is current standard.
• Provides protection
• PK known in three species

Protexia™ binds all nerve agents
• Binding is active site specific
• Reaction with a variety of inhibitors is the same for both types of BuChE

While further characterization of Protexia™ is needed to include PK and efficacy data, in vitro tests suggest both forms of the enzyme behave in a similar manner.