Evaluation of atypical butyrylcholinesterase from Oryzias latipes as a scavenger of nerve agents

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Introduction

Organophosphorus (OP) nerve agents, when introduced to the human body, bind covalently to the active site serine of acetylcholinesterase (AChE) and inhibit the enzyme. This inhibition prevents the hydrolysis of the neurotransmitter acetylcholine (ACh) by AChE, which results in multi-organ failure and ultimately death. Current post-OP exposure therapy includes administration of an ACh receptor antagonist, an anticonvulsant, and an oxime. Oximes are nucleophiles that can remove the nerve agent molecule from the active site of the enzyme. Human butyrylcholinesterase (BuChE) has widely gained success as a stoichiometric bioscavenger due to its ability to mimic the irreversible binding of nerve agent to AChE. Bioscavengers bind and remove OP compounds from the bloodstream before they inhibit AChE (Mumford et al., 2011). Atypical BuChE (aBuChE) from Medaka Oryzias latipes has intermediate properties of both AChE and BuChE (Pezzementi, Nachon, & Chatonnet, 2011). It was hypothesized that if aBuChE binds to nerve agents, then the enzyme may have the potential to act as a bioscavenger. The purpose of this study was to assess the ability of aBuChE to bind to nerve agents and reactivate in the presence or absence of oximes.

Materials and Methods

Expression of aBuChE

The cDNA encoding aBuChE as well as an empty vector, pcDNA3.1 (control), were transfected into Human Embryonic Kidney 293T cells using Lipofectamine™ 2000. Cells were incubated at 37 °C in a CO₂ incubator for 48 hours. Media was harvested, filtered, and stored at 4 °C.

Reactivation of aBuChE

Fifty μL enzyme was incubated with 100 μL substrate (2.6 mM – 5.28 mM acetylthiocholine (AtCh); 2.6 μM – 1.32 mM butyrylthiocholine (BtCh) or propionylthiocholine (PtCh) and reporter (2 mM 5,5′-dithiobis-(2-nitrobenzoic acid)) in 100 mM KPO₄, pH 7.4 for 5 min at room temperature. Extracts were centrifuged at 20,000g for 20 min, and the supernatants were kept at -20 °C.

Results

The cDNA encoding aBuChE was transfected into Human Embryonic Kidney 293T cells and the supernatants were kept at -20 °C. Extracts were centrifuged at 20,000 g, and the supernatants were kept at -20 °C. After incubation, samples were passed over a gel-filtration column to remove unbound nerve agent. Samples were diluted in 100 mM KPO₄, pH 7.4 for 5 min at room temperature. Extracts were centrifuged at 20,000 g, and the supernatants were kept at -20 °C. After incubation, samples were passed over a gel-filtration column to remove unbound nerve agent. Samples were diluted in 100 mM KPO₄, pH 7.4 and at specific time intervals were tested for hydrolysis of PtCh in the presence or absence of 1 mM oxime.

Results (cont.)

In this evaluation of aBuChE from O. latipes as a scavenger of nerve agents, comparison of cell lysates and conditioned media from cells transfected with aBuChE (Graph 1) confirmed that the protein remained in the cell, but was also secreted into the media. Fetal bovine serum in the media contributed to background activity, which necessitated usage of cell media. Similarly, the presence of oximes had intermediate properties of both AChE and BuChE (Pezzementi, Nachon, & Chatonnet, 2011). It was hypothesized that if aBuChE binds to nerve agents, then the enzyme may have the potential to act as a bioscavenger. This hypothesis was supported by the results presented in Figure 1, which shows that aBuChE binds to nerve agents and reactivates in the presence of oximes.

Conclusion

In this evaluation of aBuChE from O. latipes as a scavenger of nerve agents, comparison of cell lysates and conditioned media from cells transfected with aBuChE (Graph 1) confirmed that the protein remained in the cell, but was also secreted into the media. Fetal bovine serum in the media contributed to background activity, which necessitated usage of cell media. Similarly, the presence of oximes had intermediate properties of both AChE and BuChE (Pezzementi, Nachon, & Chatonnet, 2011). It was hypothesized that if aBuChE binds to nerve agents, then the enzyme may have the potential to act as a bioscavenger. This hypothesis was supported by the results presented in Figure 1, which shows that aBuChE binds to nerve agents and reactivates in the presence of oximes.

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References
