JOURNAL OF APPLIED BIOANALYSIS, Jan. 2015, p. 35-37.
http://dx.doi.org/10.17145/jab.15.006
Vol. 1, No. 1

OPEN ACCESS

SHORT COMMUNICATION

Method Development for Extraction of Butyrylcholinesterase using Protein-G Agarose Spin Columns

Amruta S. Indapurkar, Padmanabhan Eangoor, Jennifer S. Knaack*

Mercer University, Department of Pharmaceutical Sciences, 3001 Mercer University Dr. Atlanta, GA 30341, USA

(Received: 30 November 2014, Revised 12 January 2015, Accepted 14 January 2015).

Butyrylcholinesterase (BuChE) is a biomarker of organophosphate (OP) poisoning and can be used as a diagnostic marker to measure exposure to OP compounds. The purpose of this study was to develop a method to extract BuChE from human plasma. BuChE was extracted from plasma using the NAb protein-G Agarose Spin Kit. Factors affecting extraction like incubation time, plasma volume and cross-linking of antibodies to agarose beads were evaluated. All samples were analyzed for BuChE activity using the Ellman's assay. The incubation times of plasma and anti-BuChE antibodies marginally affected the extraction efficiency of BuChE whereas a decrease in plasma volume increased the extraction efficiency. Cross-linking of anti-BuChE antibodies on agarose increased the extraction efficiency. The NAb protein-G Spin Kit can be used successfully to extract BuChE from human plasma. This extraction technique may be coupled to downstream analytical analyses for diagnosing exposure to OP compounds.

Keywords: butyrylcholinesterase, organophosphorus compounds, organophosphate poisoning, Ellman's assay.

Introduction

Organophosphorus (OP) compounds are used as warfare agents, insecticides, pesticides and anti-wear agents [1–4]. OP compounds covalently bind to serine esterase enzymes like acetylcholinesterase (AChE), butyrylcholinesterase (BuChE) and carboxylesterase and inhibit them irreversibly.[1] BuChE is used as a preferred biomarker for the detection of OP poisoning because of its ability to adduct to broad range of OP compounds and long half-life of 16 days [2–7]. Here, we have evaluated a new, more rapid and universally accessible method to extract BuChE from human plasma using protein-G agarose spin columns. Incorporation of this extraction technique into a diagnostic protocol of OP compound exposure will reduce costs, enable universal access to the method, and reduce the time needed to analyze blood specimens.

***Correspondence:**
Mercer University, Department of Pharmaceutical Sciences, 3001 Mercer University Dr., Atlanta, GA 30341 Phone. +1-678-547-6737.
E-mail: Knaack_JS@Mercer.edu

Materials and methods

BuChE IgG antibodies (clone 3E8) and NAb protein-G Spin Kit were obtained from Pierce Biotechnology (Thermo Scientific, Waltham, MA). Butyrylthiocholine iodide and Ellman's reagent (5,5'-dithiobis-(2-nitrobenzoic acid) or DTNB) were obtained from Alfa Aesar (Ward Hill, MA). Monobasic potassium phosphate and dibasic potassium phosphate were obtained from VWR International (Radnor, PA). LC-MS grade acetonitrile and water were obtained from MedSupply Partners (Atlanta, GA). Pooled human plasma was purchased from Lee Biosolutions (St. Louis, MO). Triethanolamine buffer solution 0.2 M with magnesium ions, EDTA, and <0.1% sodium azide as a preservative, phosphate buffered saline (PBS), 10X concentrate diluted to 1X with LC-MS grade water for a final concentration of 10 mM sodium phosphate (0.9% NaCl), dimethyl pimelimidate dihydrochloride (DMP), 0.2M tris buffered saline (TBS), 10X solution, diluted to 1X with LC-MS grade water for a final concentration of 20 mM Tris with 0.9% NaCl were obtained from Sigma Aldrich (St. Louis, MO).

Results and discussion

Refinement of this new method involves evaluation of three factors: incubation time, plasma volume and crosslinking of anti-BuChE antibodies.

Effect of incubation time of the antibodies and the plasma on extraction was determined by incubating antibodies and plasma with agarose beads at room temperature with end-over-end mixing for 30, 45 and 60 min. 500 µL of the antibody solution (20 µg of anti-BuChE antibody to 480 µL PBS) was incubated followed by incubation of 500 µL of the plasma.

Effect of plasma volume on extraction was determined by incubating different volumes of human plasma (50, 75, 100 and 500 µL) with antibody-conjugated columns for 45 minutes. Effects of crosslinking of antibodies on extraction were determined by crosslinking the antibodies on agarose beads using dimethylpimelimidate in triethanolamine buffer (5.4 g/L) at room temperature for 30 minutes followed by incubation with 100 µL of plasma at room temperature with end-over-end mixing for 45 min. For all the experiments, antibody-BuChE conjugates were eluted three times using elution buffer. The columns were regenerated four times using elution and binding buffer. All samples were collected by centrifuging the column at 4500 rpm (1358 *x g*). Samples collected were analyzed using the Ellman's assay. The absorbance was measured at 405 nm and readings were taken every minute for 15 minutes using a Cytation 3 Imaging Reader, Biotek Instruments Inc. (Winooski, Vermont) [8]. All statistical analyses were performed using Microsoft Excel (Redmond, Washington).

The effect of incubation times on the percent activity of BuChE extracted from human plasma is shown in **Figure 1**.

The percent activity of BuChE in plasma after extraction at 30, 45 and 60 minutes was 46.5 ± 2.32, 52.9 ± 1.69, and 37.4 ± 1.5%, respectively (*P-value < 0.05). The percent activity of BuChE in elutions at 30, 45 and 60 minutes was found to be statistically similar. The results indicate that incubation time marginally affects extraction. Extraction efficiency was less than 64% for all the time points. One possible explanation for low extraction efficiency is overloading of the column with 500 µL of plasma. To increase the overall extraction efficiency, either the amount of anti-BuChE antibodies should be increased or volume of plasma should be decreased. To reduce the cost-per-sample, the volume of plasma was decreased. The effect of plasma volume on percent activities of BuChE extracted from human plasma is shown in **Figure 2**. The percent activity of BuChE in 50, 75, 100 and 500 µL plasma after extraction of

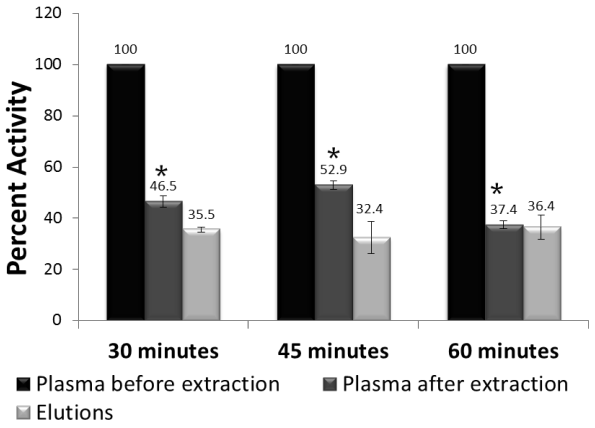

Figure 1. Comparison of BuChE activity in plasma before and after extraction and elutions when incubated for different time periods. Incubation time marginally affects extraction efficiency (n=3) (* P-value < 0.05).

BuChE was 1.8 ± 0.15, 4.1 ± 0.57, 8.29 ± 0.39 and 52.9 ± 1.69%, respectively (*P-value < 0.05) giving percent extractions of 98.2 ± 0.15, 95.9 ± 0.57, 91.7 ± 0.39 and 47.1 ± 1.69%, respectively. The percent activity of BuChE in 50, 75, 100 and 500 µL elutions was 62.4 ± 9.42, 58.4 ± 6.7, 24.9 ± 3.9 and 32.4 ± 6.18, respectively. Statistical differences were observed for extracted BuChE activity between 50 and 100 µL of plasma, 50 and 500 µL of plasma, 75 and 100 µL of plasma, and 75 and 500 µL of plasma (*P-value < 0.05). The results indicate that the percent extraction increases with decreasing plasma volumes.

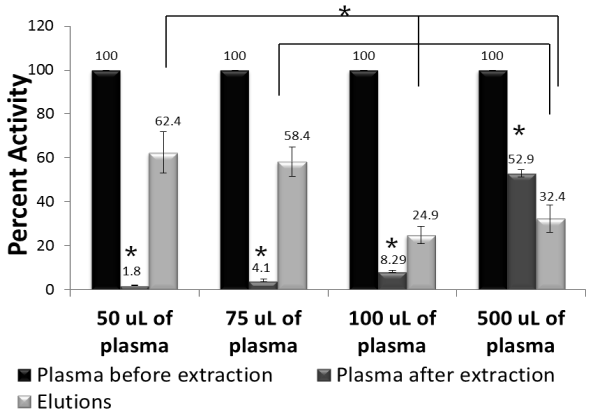

Figure 2. Comparison of BuChE activity in plasma before extraction, plasma after extraction, and in column elutions from varying plasma sample volumes. Decreasing plasma volumes results in an increase in extraction efficiency (*n=3*) (* P-value < 0.05).

A volume of 100 µL of plasma was chosen for further experiments to provide measurable BuChE activity and near-complete extraction of BuChE (91.7 ± 0.39%). The effect of crosslinking on percent activities of BuChE ex-

tracted from human plasma is shown in **Figure 3**.
The extraction efficiencies of BuChE in plasma after extraction for crosslinked and non-crosslinked agarose beads were 98.04 ± 0.20 and 91.7 ± 0.39%, respectively. The percent activity in elutions for crosslinked and non-crosslinked agarose beads was 31.8 ± 6.37 and 24.9 ± 3.9%, respectively (*P-value > 0.05).

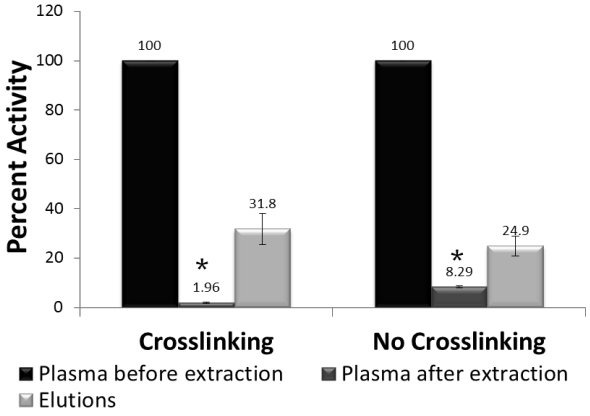

Figure 3. Comparison of BuChE activity in extraction fractions from cross-linked and non-crosslinked antibody-coated agarose columns. Cross-linking can increase the extraction efficiency by approximately 7 % (n=3) (* P-value < 0.05).

These results indicate that crosslinking can increase the extraction activity by approximately 7%. However, crosslinking increases the time to perform an extraction as well.

Conclusions

Here, a new method for extracting BuChE from plasma using protein G agarose spin columns was demonstrated and refined. The use of agarose beads to extract BuChE for diagnostic purposes is feasible and can reduce the cost-per-sample for analysis while making extraction of BuChE feasible for laboratories. However, the extracted samples will require further digestion and analysis by an HPLC-MS/MS method which is currently being developed. The extraction and analytical methods will then be validated. Future studies will be directed at coupling this extraction with downstream analysis for the development of a complete diagnostic method.

References

1. Marsillach J, Costa LG, Furlong CE. Protein adducts as biomarkers of exposure to organophosphorus compounds. Toxicology 307, 46-54 (2013).
2. Aryal UK, Lin CT, Kim JS et al. Identification of phosphorylated butyrylcholinesterase in human plasma using immunoaffinity purification and mass spectrometry. Anal Chim Acta 723, 68-75 (2012).
3. Wang Y, Boeck AT, Duysen EG, Van Keuren M, Saunders TL, Lockridge O. Resistance to organophosphorus agent toxicity in transgenic mice expressing the G117H mutant of human butyrylcholinesterase. Toxicol Appl Pharmacol 196(3), 356-366 (2004).
4. Huang YJ, Huang Y, Baldassarre H et al. Recombinant human butyrylcholinesterase from milk of transgenic animals to protect against organophosphate poisoning. Proc Natl Acad Sci U S A 104(34), 13603-13608 (2007).
5. Carletti E, Aurbek N, Gillon E et al. Structure-activity analysis of aging and reactivation of human butyrylcholinesterase inhibited by analogues of tabun. Biochem J 421(1), 97-106 (2009).
6. Pantazides BG, Watson CM, Carter MD et al. An enhanced butyrylcholinesterase method to measure organophosphorus nerve agent exposure in humans. Anal Bioanal Chem 406(21), 5187-5194 (2014).
7. Parikh K, Duysen EG, Snow B et al. Gene-delivered butyrylcholinesterase is prophylactic against the toxicity of chemical warfare nerve agents and organophosphorus compounds. J Pharmacol Exp Ther 337(1), 92-101 (2011).
8. Ellman GL, Courtney KD, Andres V, Jr., Feather-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7, 88-95 (1961).

Citation:
Indapurkar AS, Eangoor P, Knaack JS. Method Development for Extraction of Butyrylcholinesterase using Protein-G Agarose Spin Columns. J Appl Bioanal 1(1), 35-37, (2015).

Funding/Manuscript writing assistance:
The authors have no financial support or funding to report and also declares that no writing assistance was utilized in the production of this article.

Competing interest:
The authors have declared that no competing interest exist.