Post inhibitory reactions of human acetylcholinesterase inhibited by mipafox and a sarin-analog by kinetics and mass spectrometry

Iris Mangas (PQ)[1,2,3,*], Palmer Taylor (PQ)[2], Eugenio Vilanova (PQ)[1], Jorge Estévez (PQ)[1], Zoran Radić (PQ)[2], Tanos C. C. Franca (PQ)[3,4]

*imangas@ime.eb.br

[1]*Institute of Bioengineering. Unit of Toxicology and Chemical Safety. University "Miguel Hernandez" of Elche. Alicante, Spain.*
[2]*Department of Pharmacology, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, La Jolla, CA 92093 United States.*
[3]*Laboratório de Modelagem Aplicada a Defesa Química e Biológica, Instituto Militar de Engenharia, Rio de Janeiro, Brazil.*
[4]*Center for Basic and Applied Research, Faculty of Informatics and Management, University of Hradec Kralove, Hradec Kralove, Czech Republic.*

Keywords: Organophosphorus compounds, mass spectrometry, acetylcholinesterase, reactivation, oximes, aging

Introduction

The hydroxyl oxygen of the catalytic triad serine in the active center of serine hydrolase acetylcholinesterase (AChE) attacks organophosphorus compounds (OPs) at the phosphorus atom to displace the primary leaving group and to form a covalent bond. Inhibited AChE can be reactivated by cleavage of the Ser-P bond either spontaneously or through a reaction with nucleophilic agents.[1,2] The inhibited AChE adduct can also lose part of the molecule by progressive dealkylation over time in a process called aging. Reactivation of the aged enzyme has not yet been demonstrated. Here our goal was to study oxime reactivation and aging reactions of human AChE inhibited by mipafox or a sarin analog (Flu-MPs, fluorescent methylphosphonate[4]) by *in vitro* kinetics and mass spectrometry using a method in MALDI-TOF and MALDI-TOF-TOF.

Results and Discussion

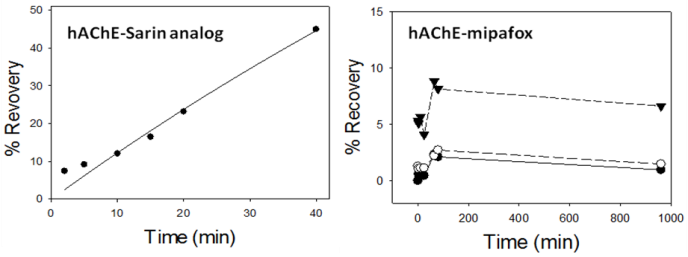

Figure 1. Progressive reactivation was observed after Flu-MPs inhibition using oxime 2-PAM. However, no reactivation was observed after mipafox inhibition with 2-PAM or the more potent oximes used.

A peptide fingerprinted mass spectrometry (MS) method, which clearly distinguished the peptide with the active serine (active center peptide, ACP) of the human AChE (hAChE) adducted with OPs, was developed by MALDI-TOF-TOF. The ACP was detected with a diethyl phosphorylated adduct after paraoxon inhibition, and with an isopropylmethyl phosphonylated and a methyl phosphonylated adduct after Flu-MPs inhibition and subsequent aging (Figure 2). Nevertheless, nonaged nonreactivated complexes were seen after mipafox inhibition and incubation with oximes, where MS data showed an ACP with an N,N diisopropyl phosphoryl adduct (Table 1).

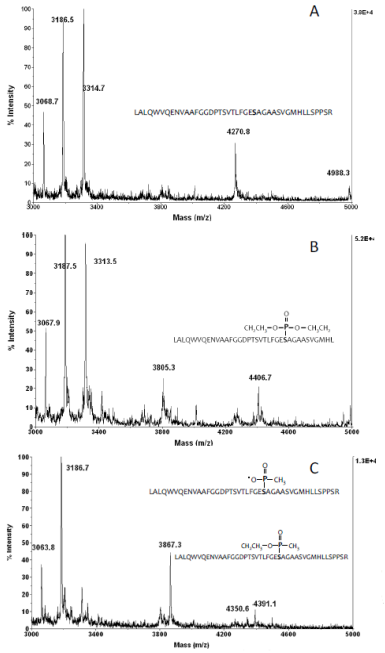

Figure 2. Representative MALDI-TOF MS of hAChE.
A. Control hAChE.
B. hAChE inhibited by paraoxon;
C. hAChE inhibited Flu MPs.
Peaks represent the ACP unmodificated and modificated according to Table 1.

Table 1. Masses of theoretical and observed of unmodified and modified active ACP of hAChE with paraoxon, mipafox and a sarin analog.

	MH+ (m/z)	
Active Center Peptide	***Theoretical***	***Observed***
unmodified	4270.8	4270.8-4270.9
inhibited by mipafox	4432.8	4432.8-4433.9
aged by mipafox that lost two isopropylamine groups	4349.8	not observed
aged by mipafox and lost an isopropylamine group	4396.8	not observed
inhibited by paraoxon	4406.8	4406.7
inhibited by analog of sarín	4391.8	4390.6-4391.8
inhibited and aged by analog of sarin	4350.8	4350.6-4350.9

Conclusions

We document here direct evidence for a phosphorylated hAChE by mipafox refractory to oxime reactivation, although we observed no aging by kinetics and MS.

Acknowledgements

CNPq/CAPES/IME/Ministério da Defesa/UCSD.

References

[1] *Sultatos LG.* ***1994.*** *J Toxicol Environ Health. 43(3):271;.*[2] *Taylor P, Radić Z.* ***1994****. 34:281-320.;*[3]*Taylor, P.* ***2001****.Hardman, J. G., and Limbird, L. E., Eds. pp 175-19;* [4] Amitai et al.,Toxicology 233. **2007**. 187–198.