

6 November 2008

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CHLORPYRIFOS OXON, DIISOPROPYL FLUOROPHOSPHATE, AND FP-BIOTIN TO TYROSINES ON TUBULIN: A POTENTIAL MECHANISM OF LONG TERM TOXICITY BY ORGANOPHOSPHORUS AGENTS: *"It is concluded that OP bind covalently to tubulin, and that this binding could explain cognitive impairment associated with OP exposure."*

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treatment of acute tabun poisonings while the oxime HI-6 is still the most promising oxime for the treatment of acute soman and cyclosarin poisonings."

7. CHROMATOGRAPHIC RESOLUTION, CHARACTERISATION AND QUANTIFICATION OF VX ENANTIOMERS IN HEMOLYSED SWINE BLOOD SAMPLES: *"After an intravenous and percutaneous administration of a supralethal dose of VX in anaesthetised swine (+)-VX and (-)-VX could be quantified up to 720 min."*

8. A COLLABORATIVE ENDEAVOR TO DESIGN CHOLINESTERASE-BASED CATALYTIC SCAVENGERS AGAINST TOXIC ORGANOPHOSPHORUS ESTERS: *"It is anticipated that these new mutants will have OP hydrolase activity."*

9. INTERACTIONS OF BUTANE, BUT-2-ENE OR XYLENE-LIKE LINKED BISPYRIDINIUM PARA-ALDOXIMES WITH NATIVE AND TABUN-INHIBITED HUMAN CHOLINESTERASES: *"However, lower BChE affinities for K074 and K075 compared to AChE suggest that the fast tabun-inhibited AChE reactivation by these compounds would not be obstructed by their interactions with BChE in vivo."*

CB Daily Report

Chem-Bio News

POTENCY OF SEVERAL OXIMES TO REACTIVATE HUMAN ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE INHIBITED BY PARAOXON IN VITRO

Drug Week

November 14, 2008

"For the recovery of inhibited AChE, derivatives from the group of pyridinium or bispyridinium aldoximes (called oximes) are used. Their efficacy depends on their chemical structure and also type of organophosphorus inhibitor. In this study, we have tested potency of selected cholinesterase reactivators (pralidoxime, obidoxime, trimedoxime, methoxime and H-oxime HI-6) to reactivate human erythrocyte AChE and human plasma BuChE inhibited by pesticide paraoxon. For this purpose, modified Ellman's method was used and two different concentrations of oximes (10 and 100 microM), attainable in the plasma within antidotal treatment of pesticide intoxication were tested. Results demonstrated that obidoxime (96.8%) and trimedoxime (86%) only reached sufficient reactivation efficacy in case of paraoxon-inhibited AChE. Other oximes evaluated did not surpassed more than 25% of reactivation. In the case of BuChE reactivation, none of tested oximes surpassed 12.5% of reactivation. The highest reactivation efficacy was achieved for trimedoxime (12.4%) at the concentration 100 microM. From the data obtained, it is clear that only two from currently available oximes (obidoxime and trimedoxime) are good reactivators of paraoxon-inhibited AChE."

"In the case of BuChE, none of these reactivators could be used for its reactivation."

The full article can be found at: (D. Jun, et. al., "Potency of several oximes to reactivate human acetylcholinesterase and butyrylcholinesterase inhibited by paraoxon in vitro".

Chemico-Biological Interactions, 2008;175(1-3):421-4). Link not available.

ANALYST NOTE: Readers attention is drawn to the common affiliation of the authors of this article and the next.

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AN ATTEMPT TO ASSESS FUNCTIONALLY MINIMAL ACETYLCHOLINESTERASE ACTIVITY NECESSARY FOR SURVIVAL OF RATS INTOXICATED WITH NERVE AGENTS

Cardiovascular Week

November 10, 2008

"The death following acute nerve agent poisoning is due to central or peripheral respiratory/ cardiac failure. Therefore, the changes in AChE activity following nerve agents acting predominantly on the central (sarin, soman) or peripheral (VX) level were studied. It is known that AChE activity in different structures exists in relative excess. Female Wistar rats intoxicated with sarin, soman, and VX in different doses (0.5-2.0 x LD(50)) were divided into groups of survived and died animals. AChE activities in diaphragm, brain parts (pontomedullar area, frontal cortex, basal ganglia, in some cases other parts of the brain) were determined and the rest of activity (in %) was correlated with survival/death of animals."

"More precise elucidation of action of nerve agents and the assessment of minimal AChE activity in different organs compatible with the survival of organism poisoned with nerve agents were the aims of this study."

The full article can be found at: (J. Bajgar, et. al., "An attempt to assess functionally minimal acetylcholinesterase activity necessary for survival of rats intoxicated with nerve agents". Chemico-Biological Interactions, 2008;175(1-3):281-5). Link not available.

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ADVANTAGES OF THE WRAIR [WALTER REED ARMY INSTITUTE OF RESEARCH] WHOLE BLOOD CHOLINESTERASE ASSAY: COMPARATIVE ANALYSIS TO THE MICRO-ELLMAN, TEST-MATE CHE, AND MICHEL (DELTAPH) ASSAYS

Biotech Week

November 12, 2008

"Red blood cell AChE (RBC-AChE) and plasma BChE can be used as sensitive biomarkers to detect exposure to OP nerve agents, pesticides, and cholinergic drugs. In a comparative study, RBC-AChE and serum BChE activities in whole blood was obtained from forty seven healthy male and female human volunteers, and then exposed separately ex vivo to three OP nerve agents (soman (GD), sarin (GB) and VX) to generate a wide range of inhibition of AChE and BChE activity (up to 90% of control)."

"These samples were measured using four different ChE assays: (i) colorimetric microEllman (using DTNB at 412 nm), (ii) Test-mate ChE field kit (also based on the Ellman assay), (iii) Michel (delta pH), and (iv) the Walter Reed Army Institute of Research Whole Blood (WRAIR WB) cholinesterase assay. The WRAIR assay is a modified Ellman method using DTP at 324 nm (which minimizes hemoglobin interference and improves sensitivity), and determines AChE and BChE in a small whole blood sample simultaneously. Scatter plots of RBC-AChE activities were determined using the WRAIR ChE assay versus the micro-Ellman, Test-mate and Michel after exposure to varying concentrations of soman, sarin and VX. Regression analyses yielded mostly linear relationships with high correlations ($r^2=0.83-0.93$) for RBC-AChE values in the WRAIR assay compared to the alternate methods. For the plasma BChE measurements, individual human values were significantly more variable (as expected), resulting in lower correlations using WRAIR ChE versus the alternate assays (r^2 values 0.5 - 0.6). To circumvent the limitations of simple correlation analysis, Bland and Altman analysis for comparing two independent measurement techniques was performed. For example, a Bland and Altman plot of the ratio of the WRAIR whole blood AChE and Michel AChE (plotted on the y-axis) vs. the average of the two methods (x-axis) shows that the majority of the individual AChE values are within ± 1.96 S.D. of the mean difference, indicating that the two methods may be used interchangeably with a high degree of confidence."

"The WRAIR ChE assay can be thus be used as a reliable inter-conversion assay when comparing results from laboratory-based (Michel) and field-based (Test-mate ChE kit), which use different methodology and report in different units of AChE activity."

The full article can be found at: (J.R. Haigh, et. al., "Advantages of the WRAIR whole blood cholinesterase assay: comparative analysis to the micro-Ellman, Test-mate ChE, and Michel (DeltapH) assays". *Chemico-Biological Interactions*, 2008; 175(1-3):417-20). Link not available.

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SOFTWARE CUTS INFECTIONS BY ALMOST HALF

Infection Control Today Magazine

November 5, 2008

"A new software program developed by Tel Aviv University (TAU) researchers to fight hospital-acquired infections (HAIs) is catching on faster than the flu. Professor Yehuda Carmeli from the Sackler Faculty of Medicine at TAU has developed a security system for preventing hospital epidemics. Integrating basic sanitary procedures, his system uses the tools of e-mail alerts and other online communication to alert hospital staff of potential threats.

Two years ago Carmeli's team adopted this system in its own institutions, and the work paid off. "We stopped 45 percent of the primary hospital-borne organisms that attack patients from spreading," says Carmeli. His most recent paper on the topic appeared in *Antimicrobial Agents and Chemotherapy* this year."

The full article can be found at: <http://www.infectioncontrolday.com/hotnews/software-cuts-infections.html>

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MASS SPECTROMETRY IDENTIFIES COVALENT BINDING OF SOMAN, SARIN, CHLORPYRIFOS OXON, DIISOPROPYL FLUOROPHOSPHATE, AND FP-BIOTIN TO TYROSINES ON TUBULIN: A POTENTIAL MECHANISM OF LONG TERM TOXICITY BY ORGANOPHOSPHORUS AGENTS

Drug Week

November 14, 2008

"Chronic low dose exposure to organophosphorus poisons (OP) results in cognitive impairment. Studies in rats have shown that OP interfere with microtubule polymerization."

"Since microtubules are required for transport of nutrients from the nerve cell body to the nerve synapse, it has been suggested that disruption of microtubule function could explain the learning and memory deficits associated with OP exposure. Tubulin is a major constituent of microtubules. We tested the hypothesis that OP bind to tubulin by treating purified bovine tubulin with sarin, soman, chlorpyrifos oxon, diisopropylfluorophosphate, and 10-fluoroethoxyphosphinyl-N-biotinamidopentyldecanamide (FP-biotin). Tryptic peptides were isolated and analyzed by mass spectrometry. It was found that OP bound to tyrosine 83 of alpha tubulin in peptide TGTyr, tyrosine 59 in beta tubulin peptide YVPR, tyrosine 281 in beta tubulin peptide GSQQYR, and tyrosine 159 in beta tubulin peptide EEYPDR. The OP reactive tyrosines are located either near the GTP binding site or within loops that interact laterally with protofilaments."

"It is concluded that OP bind covalently to tubulin, and that this binding could explain cognitive impairment associated with OP exposure."

The full article can be found at: (H. Grigoryan, et. al., "Mass spectrometry identifies covalent binding of soman, sarin, chlorpyrifos oxon, diisopropyl fluorophosphate, and FP-biotin to tyrosines on tubulin: a potential mechanism of long term toxicity by organophosphorus agents". *Chemico-Biological Interactions*, 2008;175(1-3):180-6). Link not available.

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A COMPARISON OF REACTIVATING EFFICACY OF NEWLY DEVELOPED OXIMES (K074, K075) AND CURRENTLY AVAILABLE OXIMES (OBIDOXIME, HI-6) IN SOMAN, CYCLOSARIN AND TABUN-POISONED RATS

Proteomics Weekly

November 10, 2008

"The potency of newly developed oximes (K074, K075) and commonly used oximes

(obidoxime, HI-6) to reactivate nerve agent-inhibited acetylcholinesterase was evaluated in rats poisoned with soman, tabun or cyclosarin at a lethal dose corresponding to their LD(50) value. In vivo determined percentage of reactivation of soman-inhibited blood and brain acetylcholinesterase in poisoned rats showed that only the oxime HI-6 was able to reactivate soman-inhibited acetylcholinesterase in the peripheral (blood) as well as central (brain) compartment."

"In vivo determined percentage of reactivation of tabun-inhibited blood and brain acetylcholinesterase in poisoned rats showed that obidoxime is the most efficacious reactivator of tabun-inhibited acetylcholinesterase among studied oximes in the peripheral compartment (blood) while K074 seems to be the most efficacious reactivator of tabun-inhibited acetylcholinesterase among studied oximes in the central compartment (brain). In vivo determined percentage of reactivation of cyclosarin-inhibited blood and brain acetylcholinesterase in poisoned rats showed that HI-6 is the most efficacious reactivator of cyclosarin-inhibited acetylcholinesterase among studied oximes."

"Due to their reactivating effects, both newly developed K oximes can be considered to be promising oximes for the antidotal treatment of acute tabun poisonings while the oxime HI-6 is still the most promising oxime for the treatment of acute soman and cyclosarin poisonings."

The full article can be found at: (J. Kassa, et. al., "A comparison of reactivating efficacy of newly developed oximes (K074, K075) and currently available oximes (obidoxime, HI-6) in soman, cyclosarin and tabun-poisoned rats". *Chemico-Biological Interactions*, 2008; 175(1-3):425-7). Link not available.

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CHROMATOGRAPHIC RESOLUTION, CHARACTERISATION AND QUANTIFICATION OF VX ENANTIOMERS IN HEMOLYSED SWINE BLOOD SAMPLES

Medical Devices & Surgical Technology Week

November 16, 2008

"The present study was initiated to develop a sensitive and highly selective method for the analysis of the enantiomers of the nerve agent VX (O-ethyl S-[2(diisopropylamino)ethyl] methylphosphonothioate) in blood samples for toxicokinetic and therapeutic research. To achieve this goal, analytical and semi-preparative enantioseparation of VX were carried out with gas and liquid chromatography."

"The GC chiral stationary phase was HYDRODEX-beta-TBDAC (beta cyclodextrin), on which VX was baseline-resolved. On the chiral HPLC phase CHIRALCEL OD-H the enantiomers of VX were isolated with enantiomeric excess >99.99%. They were characterised by specific optical rotation (± 25.8 deg ml dm⁽⁻¹⁾g⁽⁻¹⁾ at 20 degrees C and 589 nm) and by determination of cholinesterase inhibition rate constants. For the quantitative chiral detection of VX the enantioresolution was realized on the HPLC chiral phase CHIRAL AGP. A specific procedure was developed to isolate VX from swine blood samples thereby stabilising its enantiomers. The limit of detection was 200 fg per enantiomer on column. The absolute

recovery of the overall sample preparation procedure was 75%."

"After an intravenous and percutaneous administration of a supralethal dose of VX in anesthetised swine (+)-VX and (-)-VX could be quantified up to 720 min."

The full article can be found at: (G. Reiter, et. al., "Chromatographic resolution, characterisation and quantification of VX enantiomers in hemolysed swine blood samples". Journal of Chromatography B, 2008;873(1):86-94). Link not available.

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A COLLABORATIVE ENDEAVOR TO DESIGN CHOLINESTERASE-BASED CATALYTIC SCAVENGERS AGAINST TOXIC ORGANOPHOSPHORUS ESTERS

Drug Week

November 14, 2008

"Wild-type human butyrylcholinesterase (BuChE) has proven to be an efficient bioscavenger for protection against nerve agent toxicity. Human acetylcholinesterase (AChE) has a similar potential."

"A limitation to their usefulness is that both cholinesterases (ChEs) react stoichiometrically with organophosphorus (OP) esters. Because OPs can be regarded as pseudo-substrates for which the dephosphylation rate constant is almost zero, several strategies have been attempted to promote the dephosphylation reaction. Oxime-mediated reactivation of phosphorylated ChEs generates a turnover, but it is too slow to make pseudo-catalytic scavengers of pharmacological interest. Alternatively, it was hypothesized that ChEs could be converted into OP hydrolases by using rational site-directed mutagenesis based upon the crystal structure of ChEs. The idea was to introduce a nucleophile into the oxyanion hole, at an appropriate position to promote hydrolysis of the phospho-serine bond via a base catalysis mechanism. Such mutants, if they showed the desired catalytic and pharmacokinetic properties, could be used as catalytic scavengers. The first mutant of human BuChE that was capable of hydrolyzing OPs was G117H. It had a slow rate. Crystallographic study of the G117H mutant showed that hydrolysis likely occurs by activation of a water molecule rather than direct nucleophilic attack by H117. Numerous BuChE mutants were made later, but none of them was better than the G117H mutant at hydrolyzing OPs, with the exception of soman. Soman aged too rapidly to be hydrolyzed by G117H. Hydrolysis was however accomplished with the double mutant G117H/E197Q, which did not age after phosphorylation with soman. Multiple mutations in the active center of human and Bungarus AChE led to enzymes displaying low catalytic activity towards OPs and unwanted kinetic complexities. A new generation of human AChE mutants has been designed with the assistance of molecular modelling and computational methods. According to the putative water-activation mechanism of G117H BChE, a new histidine/aspartate dyad was introduced into the active center of human AChE at the optimum location for hydrolysis of the OP adduct. Additional mutations were made for optimizing activity of the new dyad."

"It is anticipated that these new mutants will have OP hydrolase activity."

The full article can be found at: (P. Masson, et. al., "A collaborative endeavor to design cholinesterase-based catalytic scavengers against toxic organophosphorus esters". *Chemico-Biological Interactions*, 2008;175(1-3):273-80). Link not available.

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INTERACTIONS OF BUTANE, BUT-2-ENE OR XYLENE-LIKE LINKED BISPYRIDINIUM PARA-ALDOXIMES WITH NATIVE AND TABUN-INHIBITED HUMAN CHOLINESTERASES

Proteomics Weekly
November 10, 2008

"Kinetic parameters were evaluated for inhibition of native and reactivation of tabun-inhibited human erythrocyte acetylcholinesterase (AChE, EC 3.1.1.7) and human plasma butyrylcholinesterase (BChE, EC 3.1.1.8) by three bispyridinium para-aldoximes with butane (K074), but-2-ene (K075) or xylene-like linker (K114). Tested aldoximes reversibly inhibited both cholinesterases with the preference for binding to the native AChE."

"Both cholinesterases showed the highest affinity for K114 ($K(i)$ was 0.01 mM for AChE and 0.06 mM for BChE). The reactivation of tabun-inhibited AChE was efficient by K074 and K075. Their overall reactivation rate constants were around $2000 \text{ min}^{-1}\text{M}^{-1}$, which is seven times higher than for the classical bispyridinium para-aldoxime TMB-4. The reactivation of tabun-inhibited AChE assisted by K114 was slow and reached 90% after 20 h. Since the aldoxime binding affinity of tabun-inhibited AChE was similar for all tested aldoximes (and corresponded to their $K(i)$), the rate of the nucleophilic displacement of the phosphoryl-moiety from the active site serine was the limiting factor for AChE reactivation. On the other hand, none of the aldoximes displayed a significant reactivation of tabun-inhibited BChE. Even after 20 h, the reactivation maximum was 60% for 1 mM K074 and K075, and only 20% for 1 mM K114."

"However, lower BChE affinities for K074 and K075 compared to AChE suggest that the fast tabun-inhibited AChE reactivation by these compounds would not be obstructed by their interactions with BChE in vivo."

The full article can be found at: (M. Calic, et. al, "Interactions of butane, but-2-ene or xylene-like linked bispyridinium para-aldoximes with native and tabun-inhibited human cholinesterases". *Chemico-Biological Interactions*, 2008;175(1-3):305-8). Link not found.

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