13 November 2008

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Chem-Bio News– S&T Supplement

1. NEW BISPYRIDINIUM OXIMES: IN VITRO AND IN VIVO EVALUATION OF THEIR BIOLOGICAL EFFICIENCY IN SOMAN AND TABUN POISONING: *"The combination of HI-6 and atropine is the therapy of choice for soman poisoning."*

2. ACQUISITION OF CLASSICAL CTX PROPHAGE FROM VIBRIO CHOLERAE O141 BY EL TOR STRAINS AIDED BY LYTIC PHAGES AND CHITIN-INDUCED COMPETENCE: *"Furthermore, the O141 strains apparently represent an alternative reservoir of the CTXclass phage genome, because the classical V. cholerae O1 strains are possibly extinct."*

3. 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."*

4. OXIMES: REACTIVATORS OF PHOSPHORYLATED ACETYLCHOLINESTERASE AND ANTIDOTES IN THERAPY AGAINST TABUN POISONING: *"Since the reactivating efficacy of the oximes in vitro corresponded to their therapeutic efficacy in vivo, it seems that pharmacological effect of these oximes is indeed primarily related to the reactivation of tabun-phosphorylated AChE."*

5. SIALYLOLIGOSACCHARIDES INHIBIT CHOLERA TOXIN [Ctx] BINDING TO THE GM1 RECEPTOR: *"It is proposed that SOS [sialyloligosaccharides] could be used as a conventional food additive, such as in emulsifiers, stabilisers or sweeteners, and are classified as nondigestible oligosaccharides that pass into the small intestine, which is the site of Ctx pathogenesis."*

6. EIGHT NEW BISPYRIDINIUM OXIMES IN COMPARISON WITH THE CONVENTIONAL OXIMES PRALIDOXIME AND OBIDOXIME: IN VIVO EFFICACY TO PROTECT FROM DIISOPROPYLFLUOROPHOSPHATE TOXICITY: *"Dosage calculations based on in vitro IC50 measurements may therefore in future replace in vivo LD50 determinations, thereby reducing the number of animals required."*

7. POLYVALENCY: A PROMISING STRATEGY FOR DRUG DESIGN: *"We also highlight promising future avenues for research in polyvalent drug design."*

8. LIPID A MIMETICS ARE POTENT ADJUVANTS FOR AN INTRANASAL PNEUMONIC PLAGUE VACCINE: *"We conclude that lipid A mimetics are highly effective adjuvants for an i.n. plague vaccine."*

9. DETECTION OF VIABLE YERSINIA PESTIS BY FLUORESCENCE IN SITU HYBRIDIZATION USING PEPTIDE NUCLEIC ACID PROBES: *"The PNA-FISH assays described in this study allow for the sensitive and specific detection of viable Y. pestis bacteria in a timely manner."*

10. ISOLATION OF PEPTIDE TRANSPORT SYSTEM-6 FROM BRAIN ENDOTHELIAL CELLS: THERAPEUTIC EFFECTS WITH ANTISENSE INHIBITION IN ALZHEIMER AND STROKE MODELS: *"This represents the first isolation from BBB tissue of a peptide transporter and shows that inhibition of peptide efflux pumps is a potential strategy for drug delivery to brain."*

CB Daily Report

Chem-Bio News

NEW BISPYRIDINIUM OXIMES: IN VITRO AND IN VIVO EVALUATION OF THEIR BIOLOGICAL EFFICIENCY IN SOMAN AND TABUN POISONING
Biotech Week
November 12, 2008

"This study investigated the interactions of four bispyridinium oximes with human erythrocyte acetylcholinesterase (AChE) and their effects on soman-and tabun-poisoned mice."

"Oximes HI-6 and TMB-4 were used for comparison. These oximes inhibited AchE with inhibitory potency (IC(50)) ranging from 0.02 to 1.0 mM. The best reactivating potency (% R) was obtained with K074, when AChE was inhibited by tabun. The protective potency (P (50)) of all oximes in human erythrocyte AChE inhibited by soman and tabun could not be determined. In tabun-poisoned mice very good antidotal efficacy was obtained with K027, K048, and K074, which makes them interesting for future investigation."

"The combination of HI-6 and atropine is the therapy of choice for soman poisoning."

The full article can be found at: (S. Berend, et. al., "New bispyridinium oximes: in vitro and in vivo evaluation of their biological efficiency in soman and tabun poisoning". Chemico-Biological Interactions, 2008;175(1-3):413-6). Link not available.

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ACQUISITION OF CLASSICAL CTX PROPHAGE FROM VIBRIO CHOLERAE O141 BY EL TOR STRAINS AIDED BY LYTIC PHAGES AND CHITIN-INDUCED COMPETENCE

Gene Therapy Weekly
November 13, 2008

"The El Tor biotype of Vibrio cholerae O1, causing the current seventh pandemic of cholera, has replaced the classical biotype, which caused the sixth pandemic. The CTX prophages encoding cholera toxin in the two biotypes have distinct repressor (rstR) genes."

"Recently, new variants of El Tor strains that carry the classical type (CTXclass) prophage have emerged. These ''hybrid'' strains apparently originate through lateral gene transfer and recombination events. To explore possible donors of the CTXclass prophage and its mode of transfer, we tested environmental V. cholerae isolates for the presence of CTXclass prophage and mobility of the phage genome. Of the 272 environmental V. cholerae isolates tested, 6 were found to carry the CTXclass prophage; all of these belonged to the O141 serogroup. These O141 strains were unable to produce infectious CTXclass phage or to transmit the prophage to recipient strains in the mouse model of infection; however, the CTXclass prophage was acquired by El Tor strains when cultured with the O141 strains in microcosms composed of filtered environmental water, a chitin substrate, and a V. cholerae O141-specific bacteriophage. The CTXclass, prophage either coexisted with or replaced the resident CTXET prophage, resulting in El Tor strains with CTX genotypes similar to those of the naturally occurring hybrid strains. Our results support a model involving phages and natural chitin substrate in the emergence of new variants of pathogenic V. cholera."

"Furthermore, the O141 strains apparently represent an alternative reservoir of the CTXclass phage genome, because the classical V. cholerae O1 strains are possibly extinct."

The full article can be found at: (S.M.N. Udden, et. al., “Acquisition of classical CTX prophage from Vibrio cholerae O141 by El Tor strains aided by lytic phages and chitin-induced competence”. Proceedings of the National Academy of Sciences of the United States of America, 2008;105(33):11951-11956). 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 organophosphosphorus (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 phosphylated 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 phosphonylation 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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OXIMES: REACTIVATORS OF PHOSPHORYLATED ACETYLCHOLINESTERASE AND ANTIDOTES IN THERAPY AGAINST TABUN POISONING
Drug Week
November 14, 2008

"We tested oximes varying in the type of ring (pyridinium and/or imidazolium), the length and type of the linker between rings, and in the position of the oxime group on the ring to find more effective oximes to reactivate tabun-inhibited human erythrocyte AChE. Three of our tested pyridinium oximes K027, K048, K074, along with TMB-4, were the most promising for AChE reactivation. Promising oximes were further tested in vivo on tabun poisoned mice not only as antidotes in combination with atropine but also as pretreatment drug. Herein, we showed that a promising treatment in tabun poisoning by selected oximes and atropine could be improved if oximes are also used in pretreatment."

"Since the reactivating efficacy of the oximes in vitro corresponded to their therapeutic efficacy in vivo, it seems that pharmacological effect of these oximes is indeed primarily related to the reactivation of tabun-phosphorylated AChE."

The full article can be found at: (Z. Kovarik, et. al., “Oximes: Reactivators of phosphorylated acetylcholinesterase and antidotes in therapy against tabun poisoning”.

Chemico-Biological Interactions, 2008;175(1-3):173-9). Link not available.

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SIALYLOLIGOSACCHARIDES INHIBIT CHOLERA TOXIN [Ctx] BINDING TO THE GM1 RECEPTOR
Biotech Week
November 12, 2008

“Ctx vaccines are prohibitively expensive and only give short-term protection."

"Consequently, there is scope for the development of alternative control strategies or prophylactics. This may include the use of oligosaccharides as functional mimics for the cell-surface toxin receptor (GM1). Furthermore, the sialic acid component of epithelial receptors has already been shown to contribute significantly to the adhesion and pathogenesis of Ctx. Here, we demonstrate the total inhibition of Ctx using GM1-competitive ELISA with 25mgmL (-1) of a commercial preparation of sialyloligosaccharides (SOS). The IC(50) value was calculated as 5.21mgmL(-1). One-hundred percent inhibition was also observed at all concentrations of Ctx-HRP tested with 500ngmL(-1) GM1-OS. Whilst SOS has much lower affinity for Ctx than GM1-OS, the commercial preparation is impure containing only 33.6% carbohydrate; however, the biantennary nature of SOS appears to give a significant increase in potency over constituent monosaccahride residues."

"It is proposed that SOS could be used as a conventional food additive, such as in emulsifiers, stabilisers or sweeteners, and are classified as nondigestible oligosaccharides that pass into the small intestine, which is the site of Ctx pathogenesis."

The full article can be found at: (H.R. Sinclair, et. al., “Sialyloligosaccharides inhibit cholera toxin binding to the GM1 receptor”. Carbohydrate Research, 2008;343(15):2589-94). Link not available.

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EIGHT NEW BISPYRIDINIUM OXIMES IN COMPARISON WITH THE CONVENTIONAL OXIMES PRALIDOXIME AND OBIDOXIME: IN VIVO EFFICACY TO PROTECT FROM DIISOPROPYLFLUOROPHOSPHATE TOXICITY
Drug Week
November 14, 2008

"To assess K-oxime efficacy in vivo, the extent of protection from mortality induced by diisopropylfluorophosphate (DFP) was quantified by Cox survival analysis and compared with that of the clinically available oximes. Oximes were administered in an equitoxic dosage, i.e. half the LD01. Best protection was conferred by K-27, reducing the relative risk of death (RR) to 16% of control RR (p <or=0.05), which was statistically significantly better (p <or=0.05) than all other tested oximes, except obidoxime, K-53 and K-75. The efficacy of

obidoxime (RR=0.19), K-48 (RR=0.28), K-53 (RR=0.22), K-74 (RR=0.38) and K-75 (RR=0.29) was significantly (p <or=0.05) better than that of 2-PAM (RR=0.62) and K-113 (RR=0.73). No significant protective effect was observed for K-107 and K-108. Our LD50 data show that K-107, K-108 and K-113 (which strongly inhibit AChE in vitro) are in vivo markedly more toxic than all other oximes tested and can therefore only be safely administered at a low dosage which is insufficient to protect from DFP-induced mortality."

"Dosage calculations based on in vitro IC50 measurements may therefore in future replace in vivo LD50 determinations, thereby reducing the number of animals required."

The full article can be found at: (D.E. Lorke, et. al., “Eight new bispyridinium oximes in comparison with the conventional oximes pralidoxime and obidoxime: in vivo efficacy to protect from diisopropylfluorophosphate toxicity”. Journal of Applied Toxicology, 2008;28 (7):920-8). Link not available.

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POLYVALENCY: A PROMISING STRATEGY FOR DRUG DESIGN
Drug Week
November 14, 2008

This concept of ''polyvalency'' can be used to design molecules that are potent inhibitors of toxins and pathogens. We describe the design of potent polyvalent inhibitors that neutralize anthrax toxin in vivo as well as our attempts to elucidate the relationship between inhibitor structure and activity."

"We also highlight promising future avenues for research in polyvalent drug design."

The full article can be found at: (D. Vance, et. al., “Polyvalency: A promising strategy for drug design”. Biotechnology and Bioengineering, 2008;101(3):429-434). Link not available.

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LIPID A MIMETICS ARE POTENT ADJUVANTS FOR AN INTRANASAL PNEUMONIC PLAGUE VACCINE
Biotech Week
November 12, 2008

“The formulation employed two synthetic lipid A mimetics as adjuvant combined with Yersinia pestis-derived V-and F1-protective antigens."

"The two nontoxic lipid A mimetics, classed as amino-alkyl glucosaminide 4-phosphates (AGPs) are potent ligands for the Toll-like receptor (TLR) 4. Using a murine (BALB/c) pneumonic plague model, we showed a single i.n. application of the vaccine provided 63% protection within 21 days against a Y. pestis CO92 100 LD50 challenge. Protection reached

100% by 150 days. Using a homologous i.n. 1 degrees /2 degrees dose regimen, with the boost administered at varying times, 63% protection was achieved within 7 days and 100% protection was achieved by 21 days after the first immunization. Little or no protection was observed in animals that received antigens alone, and no protection was observed when the vaccine was administered to BALB/c TLR4 mutant mice. Vaccine-induced serum IgG titers to F1 and V-antigen were reflected in high titers for IgG1 and IgG2a, the latter reflecting a bias for a cell-mediated (TH1) immune response. This intranasal vaccine showed 90% protection in Sprague-Dawley rats challenged with 1000 LD50."

"We conclude that lipid A mimetics are highly effective adjuvants for an i.n. plague vaccine."

The full article can be found at: (C.L. Airhart, et. al., “Lipid A mimetics are potent adjuvants for an intranasal pneumonic plague vaccine”. Vaccine, 2008;26(44):5554-61). Link not available.

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DETECTION OF VIABLE YERSINIA PESTIS BY FLUORESCENCE IN SITU HYBRIDIZATION USING PEPTIDE NUCLEIC ACID PROBES
Medical Letter on the CDC & FDA
November 16, 2008

“"A successful method has been developed for the detection of live Yersinia pestis, the plague bacillus, which incorporates nascent RNA synthesis. A fluorescent in situ hybridization (FISH) assay using peptide nucleic acid (PNA) probes was developed specifically to differentiate Y. pestis strains from closely related bacteria."

"PNA probes were chosen to target high copy mRNA of the Y. pestis caf1 gene, encoding the Fraction 1 (F1) antigen, and 16S ribosomal RNA. Among Yersinia strains tested, PNA probes Yp-16S-426 and Yp-F1-55 exhibited binding specificities of 100% and 98%, respectively. Y. pestis grown in the presence of competing bacteria, as might be encountered when recovering Y. pestis from environmental surfaces in a post-release bioterrorism event, was recognized by PNA probes and neither hybridization nor fluorescence was inhibited by competing bacterial strains which exhibited faster growth rates. Using fluorescence microscopy, individual Y. pestis bacteria were clearly differentiated from competing bacteria with an average detection sensitivity of 7.9x10(3) cells by fluorescence microscopy. In the current system, this would require an average of 2.56x10(5) viable Y. pestis organisms be recovered from a post-release environmental sample in order to achieve the minimum threshold for detection."

"The PNA-FISH assays described in this study allow for the sensitive and specific detection of viable Y. pestis bacteria in a timely manner."

The full article can be found at: (J.H. Kenny, et. al., “Detection of viable Yersinia pestis by fluorescence in situ hybridization using peptide nucleic acid probes”. Journal of Microbiological Methods, 2008;75(2):293-301). Link not available.

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ISOLATION OF PEPTIDE TRANSPORT SYSTEM-6 FROM BRAIN ENDOTHELIAL CELLS: THERAPEUTIC EFFECTS WITH ANTISENSE INHIBITION IN ALZHEIMER AND STROKE MODELS

By Dilek Dogrukol-Ak, Vijaya B Kumar, Jan S Ryerse, Susan A Farr, Sulekha Verma, Naoko Nonaka, Tomoya Nakamachi, Hirokazu Ohtaki, Michael L Niehoff, John C Edwards, Seiji Shioda, John E Morley and William A Banks
Journal of Cerebral Blood Flow & Metabolism
November 12, 2008

"By isolating for the first time ever a peptide transporter from the blood–brain barrier (BBB) and developing an antisense that selectively targets the brain-to-blood efflux component, we were able to deliver a therapeutic concentration of the neurotrophic peptide pituitary adenylate cyclase-activating polypeptide (PACAP) 27 to brain in animal models of Alzheimer's and stroke. Efflux pumps at the BBB are major causes of BBB impermeability to peptides. PACAP is neuroprotective in vitro in femtomole amounts, but brain uptake of PACAP27 is limited by an efflux component of peptide transport system-6 (PTS-6). Here, we characterized, isolated, and sequenced this component of PTS-6, identifying it as beta-F1 ATPase, and colocalized it with PACAP27 on BBB endothelial cells. Antisenses targeting the BBB inhibited PACAP27 efflux, thus increasing brain uptake of PACAP27. Treatment with antisense+PACAP27 improved cognition in a mouse model of Alzheimer's disease and reduced infarct size after cerebral ischemia. This represents the first isolation from BBB tissue of a peptide transporter and shows that inhibition of peptide efflux pumps is a potential strategy for drug delivery to brain.

The full article can be found at: http://www.nature.com/jcbfm/journal/vaop/ncurrent/abs/jcbfm2008131a.html

ANALYST NOTE: This approach may offer a model for treating some kinds of chemical agent casualties, such as those caused by nerve agents.

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