

20 May 2010

This supplement has been prepared to present scientific and technical news items that may be of more interest to technical personnel at RDT&E activities and the labs, or the medics rather than the broader readership of the basic CB Daily. Due to the nature of the material, the articles, if available online, are usually only available through subscription services thus making specific links generally unavailable. Thus, usually only the bibliographic citation is available for use by an activity's technical library.

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Chem-Bio News – S&T Edition

1. DEVELOPMENT AND VALIDATION OF A SENSITIVE GAS CHROMATOGRAPHY-AMMONIA CHEMICAL IONIZATION MASS SPECTROMETRY METHOD FOR THE DETERMINATION OF TABUN ENANTIOMERS IN HEMOLYSED BLOOD AND PLASMA OF DIFFERENT SPECIES:

"The limit of detection was 1 pg/ml for each enantiomer (approximately 500 fg on column) and the limit of quantification 5 pg/ml."

2. SURPRISING INFECTION-INDUCING MECHANISM FOUND IN BACTERIA:

"Research appearing in Nature, with the participation of doctors Susana Campoy and Jordi Barbé from the Department of Genetics and Microbiology at UAB, demonstrates that bacteria have a surprising mechanism to transfer virulent genes, causing infections. The research describes an unprecedented evolutionary adaptation and could contribute to finding new ways of treating and preventing bacterial infections."

3. STABLE ADDUCTS OF NERVE AGENTS SARIN, SOMAN AND CYCLOSARIN WITH TRIS, TES AND RELATED BUFFER COMPOUNDS--CHARACTERIZATION BY LC-ESI-MS/MS AND NMR AND IMPLICATIONS FOR ANALYTICAL CHEMISTRY:

"A reaction mechanism is proposed in which the amino function of the buffer serves as an intramolecular proton acceptor rendering the buffer hydroxyl groups nucleophilic enough for attack on the phosphorus atom of the agents. Results show that similar buffer adducts are formed with a range of hydroxyl and amino function containing buffers including TES, BES, TRIS, BIS-TRIS, BIS-TRIS propane, Tricine, Bicine, HEPES and triethanol amine."

4. MASS SPECTRAL CHARACTERIZATION OF ORGANOPHOSPHATE-LABELED, TYROSINE-CONTAINING PEPTIDES: CHARACTERISTIC MASS FRAGMENTS AND A NEW BINDING MOTIF FOR ORGANOPHOSPHATES:

"We have identified organophosphorus agent (OP)-tyrosine adducts on 12 different proteins labeled with six different OP. Labeling was achieved by treating pure proteins with up to 40-fold molar excess of OP at pH 8-8.6"

5. IDENTIFICATION OF CIPROFLOXACIN RESISTANCE BY SIMPLEPROBE, HIGH RESOLUTION MELT AND PYROSEQUENCING NUCLEIC ACID ANALYSIS IN BIOTREAT AGENTS: BACILLUS ANTHRACIS, YERSINIA PESTIS AND FRANCISELLA

TULARENSIS: *"While SimpleProbe and Pyrosequencing successfully identified all known mutants, the HRM assay identified all but those resulting from G<-->C or A<-->T substitutions."*

6. MICROENCAPSULATION OF ISLETS WITH LIVING CELLS USING POLYDNA-PEG-LIPID CONJUGATE: *"In this study, we propose an original method for microencapsulation of islets with living cells using an amphiphilic poly(ethylene glycol)-conjugated phospholipid derivative (PEG-lipid) and DNA hybridization."*

7. NEW RESEARCH POINTS TO PREVALENCE OF ACINETOBACTER IN THE COMMUNITY: *"New research by Sengstock, et al. in the latest issue of Clinical Infectious Diseases, a journal of the Infectious Diseases Society of America, points to increased prevalence and resistance of Acinetobacter species in the community; they point to patients with resistant isolates being discharged to nursing homes and longterm acute care facilities, thus introducing resistance to these facilities."*

CB Daily Report

Chem-Bio News

DEVELOPMENT AND VALIDATION OF A SENSITIVE GAS CHROMATOGRAPHY-AMMONIA CHEMICAL IONIZATION MASS SPECTROMETRY METHOD FOR THE DETERMINATION OF TABUN ENANTIOMERS IN HEMOLYSED BLOOD AND PLASMA OF DIFFERENT SPECIES

Hematology Week
May 17, 2010

"The aim of this study was to develop and validate a fast, sensitive and easily applicable GC-MS assay for the chiral quantification of the highly toxic organophosphorus compound tabun (O-ethyl-N,N-dimethylphosphoramidocyanidate, GA) in hemolysed swine blood for further use in toxicokinetic and toxicodynamic studies. These requirements were fulfilled best by a GC-MS assay with positive chemical ionization with ammonia (GC-PCI-MS)."

"Separation was carried out on a beta-cyclodextrin capillary column (Supelco BetaDex 225) after reversed phase (C18) solid-phase extraction. The limit of detection was 1 pg/ml for each enantiomer (approximately 500 fg on column) and the limit of quantification 5 pg/ml."

The full article can be found at: (O. Tenberken, et. al., "Development and validation of a sensitive gas chromatography-ammonia chemical ionization mass spectrometry method for the determination of tabun enantiomers in hemolysed blood and plasma of different species". Journal of Chromatography B, Analytical Technologies In the Biomedical and Life Sciences, 2010;878(17-18):1290-6). Link not available.

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SURPRISING INFECTION-INDUCING MECHANISM FOUND IN BACTERIA

Infection Control Today Magazine

May 18, 2010

“Research appearing in *Nature*, with the participation of doctors Susana Campoy and Jordi Barbé from the Department of Genetics and Microbiology at UAB, demonstrates that bacteria have a surprising mechanism to transfer virulent genes, causing infections. The research describes an unprecedented evolutionary adaptation and could contribute to finding new ways of treating and preventing bacterial infections.”

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“A team of researchers from Universitat Autònoma de Barcelona, together with members of the CSIC Institute for Agrobiotechnology, Public University of Navarre, Virginia Commonwealth University, and New York University Medical Center, coordinated by the Valencian Institute for Agronomic Research (IVIA) and CEU-Cardenal Herrera University, have studied the mechanisms producing virulence in staphylococcus bacteria and causing the toxic shock syndrome, a rare but potentially fatal illness in 50 percent of the cases.

Researchers observed how pathogenicity islands underwent an unprecedented evolutionary adaptation to be able to transfer pathogens to other innocuous bacteria and thus transform them into virulent bacteria.

Under normal conditions, pathogenicity islands produce the protein StI, which binds to the DNA segment containing virulent genes and represses the transfer of the island. However, sometimes bacteria become infected with a virus which packages and transfers these virulent genes to other bacteria.

Scientists have discovered that these islands can detect the presences of a virus, eliminate the repression produced by StI, and thus commence a replication and packaging cycle. The island is then capable of transference and of making other harmless bacteria turn virulent.”

The full article can be found at: <http://www.infectioncontroltoday.com/hotnews/infection-inducing-mechanism.html>

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STABLE ADDUCTS OF NERVE AGENTS SARIN, SOMAN AND CYCLOSARIN WITH TRIS, TES AND RELATED BUFFER COMPOUNDS--CHARACTERIZATION BY LC-ESI-MS/MS AND NMR AND IMPLICATIONS FOR ANALYTICAL CHEMISTRY

Health & Medicine Week

May 17, 2010

“Buffering compounds like TRIS are frequently used in chemical, biochemical and biomedical applications to control pH in solution. One of the prerequisites of a buffer compound, in addition to sufficient buffering capacity and pH stability over time, is its non-reactivity with

other constituents of the solution."

"This is especially important in the field of analytical chemistry where analytes are to be determined quantitatively. Investigating the enzymatic hydrolysis of G-type nerve agents sarin, soman and cyclosarin in buffered solution we have identified stable buffer adducts of TRIS, TES and other buffer compounds with the nerve agents. We identified the molecular structure of these adducts as phosphonic diesters using 1D (1)H-(31)P HSQC NMR and LC-ESI-MS/MS techniques. Reaction rates with TRIS and TES are fast enough to compete with spontaneous hydrolysis in aqueous solution and to yield substantial amounts (up to 20-40%) of buffer adduct over the course of several hours. A reaction mechanism is proposed in which the amino function of the buffer serves as an intramolecular proton acceptor rendering the buffer hydroxyl groups nucleophilic enough for attack on the phosphorus atom of the agents. Results show that similar buffer adducts are formed with a range of hydroxyl and amino function containing buffers including TES, BES, TRIS, BIS-TRIS, BIS-TRIS propane, Tricine, Bicine, HEPES and triethanol amine."

"It is recommended to use alternative buffers like MOPS, MES and CHES when working with G-type nerve agents especially at higher concentrations and over prolonged times."

The full article can be found at: (J. Gab, et. al., "Stable adducts of nerve agents sarin, soman and cyclosarin with TRIS, TES and related buffer compounds--characterization by LC-ESI-MS/MS and NMR and implications for analytical chemistry". Journal of Chromatography B, Analytical Technologies In the Biomedical and Life Sciences, 2010;878(17-18): 1382-90). Link not available.

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MASS SPECTRAL CHARACTERIZATION OF ORGANOPHOSPHATE-LABELED, TYROSINE-CONTAINING PEPTIDES: CHARACTERISTIC MASS FRAGMENTS AND A NEW BINDING MOTIF FOR ORGANOPHOSPHATES

Life Science Weekly

May 18, 2010

"We have identified organophosphorus agent (OP)-tyrosine adducts on 12 different proteins labeled with six different OP. Labeling was achieved by treating pure proteins with up to 40-fold molar excess of OP at pH 8-8.6."

"OP-treated proteins were digested with trypsin, and peptides were separated by HPLC. Fragmentation patterns for 100 OP-peptides labeled on tyrosine were determined in the mass spectrometer. The goals of the present work were (1) to determine the common features of the OP-reactive tyrosines, and (2) to describe non-sequence MSMS fragments characteristic of OP-tyrosine peptides. Characteristic ions at 272 and 244 amu for tyrosine-OP immonium ions were nearly always present in the MSMS spectrum of peptides labeled on tyrosine by chlorpyrifos-oxon. Characteristic fragments also appeared from the parent ions that had been labeled with diisopropylfluorophosphate (216 amu), sarin (214 amu), soman (214 amu) or FP-biotin (227, 312, 329, 691 and 708 amu). In contrast to OP-reactive serines, which lie in the consensus sequence GX SXG, the OP-reactive tyrosines have no

consensus sequence. Their common feature is the presence of nearby positively charged residues that activate the phenolic hydroxyl group. The significance of these findings is the recognition of a new binding motif for OP to proteins that have no active site serine. Modified peptides are difficult to find when the OP bears no radiolabel and no tag."

"The characteristic MSMS fragment ions are valuable because they are identifiers for OP-tyrosine, independent of the peptide."

The full article can be found at: (L.M. Schopfer, et. al., "Mass spectral characterization of organophosphate-labeled, tyrosine-containing peptides: characteristic mass fragments and a new binding motif for organophosphates". Journal of Chromatography B, Analytical Technologies In the Biomedical and Life Sciences, 2010;878(17-18): 1297-311). Link not available.

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IDENTIFICATION OF CIPROFLOXACIN RESISTANCE BY SIMPLEPROBE, HIGH RESOLUTION MELT AND PYROSEQUENCING NUCLEIC ACID ANALYSIS IN BIOTHRREAT AGENTS: BACILLUS ANTHRACIS, YERSINIA PESTIS AND FRANCISELLA TULARENSIS

Medical Letter on the CDC & FDA
May 23, 2010

"The potential for genetic modification of biological warfare agents makes rapid identification of antibiotic resistant strains critical for the implementation of suitable infection control measures. The fluorinated quinolone, ciprofloxacin, is an antibiotic effective for treating bacterial infections by inhibiting the enzyme activity of the DNA type II topoisomerases DNA gyrase and topoisomerase IV."

"The genes that encode the subunits of DNA gyrase (gyrA and gyrB) and topo IV (par C and parE) contain hotspots within an area known as the quinolone resistance-determining region (QRDR). Base pair changes within this region give rise to mutations that cause resistance to the antibiotic by altering amino acids within the enzymes. Ciprofloxacin-resistant (cipro(r)) strains of Bacillus anthracis, Yersinia pestis, and Francisella tularensis with one or more known mutations within the QRDR of gyrA, gyrB, parC, and parE genes were tested with SimpleProbe and High Resolution Melt (HRM) dye chemistries and Pyrosequencing genetic analysis to evaluate the ability to rapidly detect ciprofloxacin-induced mutations."

"While SimpleProbe and Pyrosequencing successfully identified all known mutants, the HRM assay identified all but those resulting from G<-->C or A<-->T substitutions."

The full article can be found at: (B.M. Loveless, et. al., "Identification of ciprofloxacin resistance by SimpleProbe, High Resolution Melt and Pyrosequencing nucleic acid analysis in biotthreat agents: Bacillus anthracis, Yersinia pestis and Francisella tularensis". Molecular and Cellular Probes, 2010;24(3): 154-60). Link not available.

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MICROENCAPSULATION OF ISLETS WITH LIVING CELLS USING POLYDNA-PEG-LIPID CONJUGATE

Blood Weekly

May 13, 2010

"Microencapsulation of islets with a semipermeable membrane, i.e., bioartificial pancreas, is a promising way to transplant islets without the need for immunosuppressive therapy for insulin-dependent diabetes mellitus (type I diabetes). However, materials composing a bioartificial pancreas are not ideal and might activate defense reactions against foreign materials."

"In this study, we propose an original method for microencapsulation of islets with living cells using an amphiphilic poly(ethylene glycol)-conjugated phospholipid derivative (PEG-lipid) and DNA hybridization. PolyA and polyT were introduced onto the surfaces of the islets and HEK 293 cells, respectively, using amphiphilic PEG-lipid derivatives. PolyA20 modified HEK cells were immobilized onto the islet surface where polyT20-PEG-lipid was incorporated. The cells spread and proliferated on the islet surface, and the islet surface was completely encapsulated with a cell layer after culture."

"The encapsulated islets retained the ability to control insulin release in response to glucose concentration changes."

The full article can be found at: (Y. Teramura, et. al., "Microencapsulation of islets with living cells using PolyDNA-PEG-lipid conjugate". Bioconjugate Chemistry, 2010;21(4):792-6). Link not available.

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NEW RESEARCH POINTS TO PREVALENCE OF ACINETOBACTER IN THE COMMUNITY

Infection Control Today Magazine

May 19, 2010

"New research by Sengstock, et al. in the latest issue of Clinical Infectious Diseases, a journal of the Infectious Diseases Society of America, points to increased prevalence and resistance of Acinetobacter species in the community; they point to patients with resistant isolates being discharged to nursing homes and longterm acute care facilities, thus introducing resistance to these facilities.

In their latest work, Sengstock and colleagues describe the epidemiology, resistance patterns, and outcomes of older adults with Acinetobacter infection in community hospitals. The researchers investigated the microbiology databases at Oakwood Healthcare System in Michigan (four hospitals with 632, 259, 199 and 168 beds) for clinical Acinetobacter cultures obtained from 2003 to 2008. Patients aged 60 years who were admitted from home or

nursing homes were included. The researchers recorded the initial Acinetobacter isolate and susceptibility to eight antibiotics; cultures obtained 48 hours after hospitalization were categorized as hospital-acquired. Administrative databases provided patients' origins (home or nursing home) and discharge destinations (home, nursing home, longterm acute care facility, another hospital, or hospice care or death)."

The full article can be found at: <http://www.infectioncontrolday.com/hotnews/acinetobacter-in-the-community.html>

The original article can be found at: Sengstock DM, Thyagarajan R, Apalara J, Mira A, Chopra T and Kaye KS. Multidrug Resistant Acinetobacter baumannii: An Emerging Pathogen among Older Adults in Community Hospitals and Nursing Homes. Clinical Infectious Diseases 2010;50:1611-1616. Link not available.

ANALYST NOTE: While this article admittedly does not have a CB connection, it is pertinent in a force health protection context. Although the research was based on a geriatric population, the concentrated living conditions of the subjects may also make the research relevant to troop billeting situations such as unit barracks and troop training establishments.

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