

28 August 2008

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Chem-Bio News – S&T Supplement

1. CLINICAL AND IMMUNE RESPONSE TO UNDILUTED AND DILUTED SMALLPOX

VACCINE: *"The existing smallpox vaccine stockpile might be expanded by administering three- or sixfold diluted vaccine doses combined with a careful pre-vaccination screening and extensive instructions to vaccinees."*

2. BUNDESWEHR INSTITUTE OF PHARMACOLOGY AND TOXICOLOGY [GE] - INHIBITION OF POLY(ADP-RIBOSE) POLYMERASE (PARP) INFLUENCES THE MODE OF SULFUR MUSTARD (SM)-INDUCED CELL DEATH IN HaCaT CELLS:

"Thus, the observed early proapoptotic effect of 3AB at lower SM concentrations may point to the influence of ATP-independent cell-death regulating mechanisms."

3. NEW EVIDENCE ON ADDICTION TO MEDICINES - DIAZEPAM HAS EFFECT ON NERVE CELLS IN THE BRAIN REWARD SYSTEM:

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4. CENTURY-OLD RULE OF CHEMISTRY OVERTURNED -- MAJOR IMPLICATIONS FOR DRUG DELIVERY:

"The new observations of the Warwick researchers suggest that the real transport rates could be up to a hundred times slower than predicted by the century old "Overton's Rule"

5. SUBSTRATE CLEAVAGE ANALYSIS OF FURIN AND RELATED PROPROTEIN CONVERTASES. A COMPARATIVE STUDY:

"Our results also suggest that pathogens, including anthrax PA83 and the avian influenza A H5N1 (bird flu) hemagglutinin precursor, evolved to be as sensitive to PC proteolysis as the most sensitive normal human proteins."

6. INDUCTION OF INNATE IMMUNITY BY LIPID A MIMETICS INCREASES SURVIVAL FROM PNEUMONIC PLAGUE:

"Combined, these results indicated that AGPs [aminoalkyl glucosaminide 4-phosphate] may be useful in protection of immunologically naive individuals against plague and potentially other infectious agents, and that AGP therapy may be used

synergistically with other therapies."

7. HIGH-THROUGHPUT PROTEIN MICROARRAYS ON THE WAY: *"Offering a fast, efficient and user-friendly route to immobilised functional proteins, it avoids the need for laborious protein purification or chemical tagging."*

8. PHOTONIC CRYSTAL DRUG DETECTIVE: *"A new high-throughput screening system based on photonic crystals could quickly and cheaply detect molecules that disrupt binding between proteins and DNA, offering a new way to look for novel classes of drugs, say scientists in the US."*

CB Daily Report

Chem-Bio News

CLINICAL AND IMMUNE RESPONSE TO UNDILUTED AND DILUTED SMALLPOX VACCINE

Drug Week
August 29, 2008

"To assess clinical reactions, immune responses and adverse events to undiluted, three- and sixfold diluted Lister strain vaccine stockpiled in Switzerland, a prospective, triple-blinded, randomised, parallel group clinical trial was performed."

"From 2001 to 2007 104 persons with an indication for vaccinia vaccination were recruited. They had a median age of 33 years (range 18-65), 56 (53.8%) were re-vaccinees and 48 (46.2%) primary vaccinees. There was no statistically significant variation in the proportion of re-vaccinees between diluted and undiluted vaccine groups (75% vs 51%, $p = 0.118$). With an overall clinical take rate (major reaction) of 97.1% the majority of the vaccinia-naive participants exhibited an at least fourfold increase of neutralising antibody titres (32/38, 84.2%) post-vaccination. Interestingly this proportion was lower among re-vaccinees (29/46, 63.0%, $p = 0.048$). No significant difference was observed in the take rate or at least fourfold seroconversion rate between the threefold and sixfold diluted vaccine doses. Adverse events were reported by 98 (94.2%) participants, not accounting for itching at the vaccination site. Subjects requiring immunisation were successfully (re-) vaccinated with undiluted as well as with three- or sixfold diluted vaccinia vaccine. Our findings complement previous studies with respect to the clinical take rate and immune response. The rate of adverse events was substantial but not unexpected and no severe adverse events occurred."

"The existing smallpox vaccine stockpile might be expanded by administering three- or sixfold diluted vaccine doses combined with a careful pre-vaccination screening and extensive instructions to vaccinees."

The full article can be found at: (R. Gassmann, et. al., "Clinical and immune response to undiluted and diluted smallpox vaccine". Swiss Medical Weekly, 2008; 138(27-28):392-397). Link not available.

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BUNDESWEHR INSTITUTE OF PHARMACOLOGY AND TOXICOLOGY [GE] - INHIBITION OF POLY(ADP-RIBOSE) POLYMERASE (PARP) INFLUENCES THE MODE OF SULFUR MUSTARD (SM)-INDUCED CELL DEATH IN HaCaT CELLS

Proteomics Weekly

August 25, 2008

"These vesicant properties of SM have been linked to cell death of proliferating keratinocytes in the basal layer of the skin. Catalytic activation of the nuclear enzyme poly(ADP-ribose) polymerase (PARP-1) has been demonstrated to be a major event in response to high levels of DNA damage, and PARP-1 activation may be part of apoptotic signaling. In other contexts, overstimulation of PARP-1 triggers necrotic cell death because of rapid consumption of its substrate, beta-nicotinamide adenine dinucleotide (NAD⁺) and the consequent depletion of ATP. These findings prompted us to evaluate whether SM induces apoptosis in keratinocytes like HaCaT cells and to determine whether blocking of PARP enzyme activity with 3-aminobenzamide (3AB) can influence the mode of cell death. HaCaT cells were exposed to SM (10-1,000 µM; 30 min) and then cultivated in SM-free medium with or without 3AB for up to 48 h. This treatment resulted in a time and SM dose-dependent increase of apoptotic cell death characterized by PARP-1 cleavage and DNA fragmentation during the experimental period. After just 45 min of exposure to 1 mM SM, we observed a significant increase in PARP-1 activity in HaCaT cells. About 6 h after exposure, intracellular ATP levels were diminished by 22%, which seemed to be completely prevented by the addition of 3AB directly after exposure. However, 18 h later, this 3AB effect on the SM concentration-dependent loss of ATP was no longer detectable. Interestingly, the effect of SM on total cell viability was not changed by 3AB. However, the mode of cell death was influenced by 3AB exhibiting an increase of apoptotic cells and a concomitant decrease of necrotic HaCaT cells during the first 24 h after SM exposure. Our results indicate that SM concentrations of 1 mM or higher induce a prominent PARP activation leading to ATP depletion and necrosis. In contrast, lower concentrations of SM cause minor PARP activation and, especially, PARP-1 cleavage by caspase 3 without ATP depletion. Because ATP is required for apoptosis, we suggest that ATP acts as an early molecular switch from apoptotic to necrotic modes of SM-induced cell death, at least at high concentrations (>or=1 mM)."

"Thus, the observed early proapoptotic effect of 3AB at lower SM concentrations may point to the influence of ATP-independent cell-death regulating mechanisms."

The full article can be found at: (K. Kehe, et. al., "Inhibition of poly(ADP-ribose) polymerase (PARP) influences the mode of sulfur mustard (SM)-induced cell death in HaCaT cells". Archives of Toxicology, 2008;82(7):461-70). Link not available.

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NEW EVIDENCE ON ADDICTION TO MEDICINES - DIAZEPAM HAS EFFECT ON NERVE

CELLS IN THE BRAIN REWARD SYSTEM

Medical News Today

August 26, 2008

"Addictions to medicines and drugs are thought to develop over a relatively long period of time. The process involves both structural and functional changes in brain nerve cells that are still poorly understood. However, a single drug or alcohol dose is sufficient to generate an initial stage of addiction. Recent research conducted under the umbrella of the Academy of Finland Research Programme on Neuroscience (NEURO) has discovered the same phenomenon in the dosage of benzodiazepine diazepam."

"Previously, addiction to benzodiazepines has been explained by reference to negative rather than positive reinforcement. In other words, the thinking has been that the reason people continue to use the medicine is that it helps to alleviate their distressing withdrawal symptoms and general discomfort, rather than because it provides a sense of reward," says Professor Esa Korpi, who has been in charge of the research project at the University of Helsinki.

However, according to the latest research it seems that diazepam causes a similar change in the brain's reward-inducing dopamine cells as a dose of alcohol, morphine, amphetamine or cocaine. Furthermore, neural message transmission in the dopamine cells is reinforced for up to 72 hours after ingestion of diazepam. "Our studies have shown that diazepam also affects the dopamine system, which adds a new positive reinforcement mechanism of reward learning to the theory of benzodiazepine addiction," Korpi explains.

Article published on the subject in Neuropsychopharmacology, 18 June 2008: Long-lasting modulation of glutamatergic transmission in VTA dopamine neurons after a single dose of benzodiazepine agonists."

The full article can be found at: <http://www.medicalnewstoday.com/articles/119284.php>

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CENTURY-OLD RULE OF CHEMISTRY OVERTURNED -- MAJOR IMPLICATIONS FOR DRUG DELIVERY

ScienceDaily

August 25, 2008

"The new observations of the Warwick researchers suggest that the real transport rates could be up to a hundred times slower than predicted by the century old "Overton's Rule". This could have major implications for the development and testing of many future drugs.

Overton's rule says that the easier it is for a chemical to dissolve in a lipid (fat) the easier and faster it will be transported into a cell. The Rule was first outlined in the 1890s by Ernst Overton of the University of Zürich. He declared that substances that dissolve in lipids pass more easily into a cell than those that dissolve in water. He then set forth an equation that predicted how fast that diffusion would happen. One of the key parameters in that equation

is K which defines the lipophilicity (oil-liking nature) of the chemical. The higher the value of K, the faster the predicted cell permeation rate. For over a century, medicinal chemists have used this relationship to shape their studies and clinical trials."

"The results stunned the researchers. While the acids did diffuse across a lipid membrane, they did so at rates that were diametrically opposite to the predictions of the Rule, i.e. the most lipophilic molecules were actually transported slowest. The researchers studied four acids (acetic, butanoic, valeric, and hexanoic) that had increasingly larger "acyl" (or carbon) chains. The longer the carbon chain, the easier the chemical dissolves in lipids and, therefore, according to Overton, the faster they should diffuse across a lipid membrane. In fact, the University of Warwick researchers observed that for these four acids the exact opposite is true: the easier it is for an acid to dissolve in a lipid, the slower it is transported across the membrane."

"The researchers are quick to concede that as it stands their demonstration is a far cry from a viable protein microarray. However, they stress that by combining their method with mechanical spotting techniques, arrays with large numbers of proteins could readily be manufactured. With this in mind, they add, a number of industrial groups have already expressed an interest in the technology."

The full article can be found at: <http://www.sciencedaily.com/releases/2008/08/080825174959.htm>

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SUBSTRATE CLEAVAGE ANALYSIS OF FURIN AND RELATED PROPROTEIN CONVERTASES. A COMPARATIVE STUDY

Virus Weekly

August 26, 2008

"We present the data and the technology, a combination of which allows us to determine the identity of proprotein convertases (PCs) related to the processing of specific protein targets including viral and bacterial pathogens. Our results, which support and extend the data of other laboratories, are required for the design of effective inhibitors of PCs because, in general, an inhibitor design starts with a specific substrate."

"Seven proteinases of the human PC family cleave the multibasic motifs R-X-(R/K/X)-R downward arrow and, as a result, transform proproteins, including those from pathogens, into biologically active proteins and peptides. The precise cleavage preferences of PCs have not been known in sufficient detail; hence we were unable to determine the relative importance of the individual PCs in infectious diseases, thus making the design of specific inhibitors exceedingly difficult. To determine the cleavage preferences of PCs in more detail, we evaluated the relative efficiency of furin, PC2, PC4, PC5/6, PC7, and PACE4 in cleaving over 100 decapeptide sequences representing the R-X-(R/K/X)-R downward arrow motifs of human, bacterial, and viral proteins. Our computer analysis of the data and the follow-on cleavage analysis of the selected full-length proteins corroborated our initial results thus allowing us to determine the cleavage preferences of the PCs and to suggest which PCs are

promising drug targets in infectious diseases."

"Our results also suggest that pathogens, including anthrax PA83 and the avian influenza A H5N1 (bird flu) hemagglutinin precursor, evolved to be as sensitive to PC proteolysis as the most sensitive normal human proteins."

The full article can be found at: (A.G. Remacle, et. al., "Substrate cleavage analysis of furin and related proprotein convertases. A comparative study". Journal of Biological Chemistry, 2008; 283(30):20897-906). Link not available.

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INDUCTION OF INNATE IMMUNITY BY LIPID A MIMETICS INCREASES SURVIVAL FROM PNEUMONIC PLAGUE

Drug Week

August 29, 2008

"This study analysed the effect of priming the innate immune system using synthetic lipid A mimetics in a *Yersinia pestis* murine pulmonary infection model. Two aminoalkyl glucosaminide 4-phosphate (AGP) Toll-like receptor 4 (TLR4) ligands, delivered intranasally, extended time to death or protected against a lethal *Y. pestis* CO92 challenge."

"The level of protection was dependent upon the challenge dose of *Y. pestis* and the timing of AGP therapy. Protection correlated with cytokine induction and a decreased bacterial burden in lung tissue. AGP protection was TLR4-dependent and was not evidenced in transgenic TLR4-deficient mice. AGP therapy augmented with subtherapeutic doses of gentamicin produced dramatically enhanced survival."

"Combined, these results indicated that AGPs may be useful in protection of immunologically naive individuals against plague and potentially other infectious agents, and that AGP therapy may be used synergistically with other therapies."

The full article can be found at: (C.L. Airhart, et. al., "Induction of innate immunity by lipid A mimetics increases survival from pneumonic plague". Microbiology, 2008; 154(Pt 7):2131-8). Link not available.

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HIGH-THROUGHPUT PROTEIN MICROARRAYS ON THE WAY

By Fred Campbell

Royal Society of Chemistry [UK]

August 26, 2008

"A new method to rapidly generate protein microarrays has been developed by UK researchers at the University of Manchester. Offering a fast, efficient and user-friendly route

to immobilised functional proteins, it avoids the need for laborious protein purification or chemical tagging."

"Jason Micklefield and colleagues have now developed a new way to attach proteins to the plate, using direct enzyme catalysis - taking an existing, mild synthetic strategy from solution and applying it to a functional surface. In practice, three ingredients are essential: an active enzyme; a functionalised surface; and a supply of tagged protein of the scientist's choosing. With all three in place, the reaction to attach the tagged protein to the surface is complete in just a few hours."

The full article can be found at: <http://www.rsc.org/chemistryworld/News/2008/August/26080803.asp>

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PHOTONIC CRYSTAL DRUG DETECTIVE

By James Mitchell Crow

Royal Society of Chemistry [UK]

August 26, 2008

"A new high-throughput screening system based on photonic crystals could quickly and cheaply detect molecules that disrupt binding between proteins and DNA, offering a new way to look for novel classes of drugs, say scientists in the US.

Paul Hergenrother and Brian Cunningham at the University of Illinois at Urbana-Champaign say their device could screen 22,000 small molecules a day that might disrupt protein-DNA interactions - and the technique is also applicable to studying protein-protein or protein-RNA interactions. The authors presented their research on 1 August at an RSC conference, Transatlantic frontiers of chemistry, in Cheshire, UK."

The full article can be found at: <http://www.rsc.org/chemistryworld/News/2008/August/26080801.asp>

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