17th Medical Chemical Defense Conference

Chemical Warfare Agents - old problems and new challenges

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Conference Talks (Abstracts)

Bundeswehr Institute of Pharmacology and Toxicology

T01 • History of nerve agent releases

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Tabun was discovered in Germany in 1936; sarin followed in 1939 and soman in 1944. In the early 1950s, a new insecticide (Amiton™; VG) discovered by ICI, UK was found to be too toxic for routine use and modification led to the development of VX. Independently, Russia produced a similar agent (Russian VX; R-33; VR) and a new generation of nerve agents, called Novichok (newcomer). Nerve agents, including tabun (often together with mustard), were employed by Iraq in the Iran-Iraq war in 1984-1988 and against that country's own Kurdish population in 1988. Sarin was released in Ghouta, Syria in August 2013 when 1,400 people were killed. In April 2017, sarin was used again in an attack in northern Syria, that killed more than 80 civilians. Terrorists from Aum Shinrikyo released nerve agents in Japan on at least 11 occasions in 1994–5. On 27 June 1994 six Aum members drove to the city of Matsumoto to poison three district court judges overseeing a land dispute with the group. Some 600 people were potentially exposed to sarin and seven died, but the three judges survived. Aum Shinrikyo used VX on several occasions in 1994-5 in attempts to murder its perceived enemies; one victim died and two others required lengthy hospital stays. On 20 March 1995 20 kg of crude sarin was released by Aum members puncturing plastic bags on five Tokyo subway trains. Over 5000 "casualties" sought medical attention, of whom 984 were moderately poisoned and 54 were severely poisoned; 12 died.1 VX was used as a weapon of assassination on 13 February 2017 when Kim Jong-nam, the estranged half-brother of the North Korean ruler, Kim Jong-un, was killed at Kuala Lumpur International airport. In Salisbury, England, on 4 March 2018, Sergei Skripal, his daughter Yulia, and a policeman investigating the incident were severely poisoned following exposure to a Novichok agent.2 With treatment, Mr Skripal, a former Russian double agent, his daughter, and Detective Sergeant Bailey recovered. Subsequently, on 30 June 2018, two more individuals, Dawn Sturgess and Charlie Rowley, who had found the original container used to release nerve agent, were also severely poisoned with Novichok; Sturgess died on 8 July 2018.

References:

1. Vale JA. What lessons can we learn from the Japanese sarin attacks? Przegl Lek 2005; 62:528-32.
2. Vale JA, Marrs TC, Maynard RL. Novichok: a murderous nerve agent attack in the UK. Clin Toxicol 2018; 56:1093-97.

T02 • The OPCW Technical Assistance Visits to the United Kingdom following the Salisbury and Amesbury incidents in 2018

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On 4 March 2018 Sergej and Yulia Skripal as well as police officer Nickolas Bailey were severely poisoned following exposure to a nerve agent in the city of Salisbury. The United Kingdom requested technical assistance from the OPCW Technical Secretariat under Article VIII subparagraph 38(e) of the Chemical Weapons Convention to independently verify analytical results.

The OPCW deployed a Technical Assistance Visit (TAV) in the week of 19-23 March taking both environmental and biomedical samples. These samples were analysed by OPCW designated laboratories and the results confirmed the findings of the UK regarding the identity of the chemical.

On 30 June Charles Rowlez and Dawn Sturgess got exposed to the same nerve agent in the town of Amesbury (close to Salisbury).This exposure led to the hospitalization of both and the subsequent death of Dawn Sturgess. Another request for technical assistance was made by the United Kingdom and the OPCW Technical Secretariat dispatched a second TAV deploying from 15 to 18 July and additionally on 13 August to obtain an additional sample. The TAV team collected environmental and biomedical samples and attended and observed the post-mortem of Dawn Sturgess. Sample analysis by OPCW designated laboratories again confirmed the findings of the UK regarding the identity of the chemical.

The presentation will explain the activities conducted by the TAVs on-site and the OPCW procedures followed during sampling, sample handling, dispatch and sample analysis by designated laboratories.

T03 • The management of organophosphates poisoning in Taiwan

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The poison center data base of Taiwan showed that, during the period of 1985 – 1993, with an total exposure rate of 29.3 %, pesticides was responsible for the top poisonous exposure, and organophosphates (OPs) poisoning was second to the paraquat in term of leading causes of poison-related fatalities. Another retrospective analysis using the same poison data bank (1985-2006) showed that the mortality rate of 4799 OPs poisoning exposure was 12.71 %. Organophosphates poisoning exposure has been always an important subject in toxicology emergency. How to effectively treat OPs poisonings to minimize the death rates as well as the days of hospital admission has been concerned by most of the medical doctors of both emergency medicine and critic care medicine in developing and under developed countries. Taiwan, in some way, has been considered as one of the well developed countries, however, even today, the treatment of OPs poisonings is still unwell defined. In Taiwan, chronologically, the pharmacological treatment of OPs poisoning could be divided into three stages: iv bolus use of atropine; dose titration use of atropine; the combination use of atropine titration and PAM. Atropine, a pharmacological antidote, has been well accepted to be used for treating OPs poisoning for many years, however, its toxicity or complications may be very significant if overdose does occur. In early years of 80, there had been toxicities and complications reported to be related to atropine overdose, until 1985 or so, when the titration method of atropine infusion was adapted. It was not until 1994, however, the PAM was considered seriously to be used in OPs poisonings with the experience shared by the European medical colleagues. However, what shall be an appropriate dose of PAM use, and whether continuous infusion or repeated bolus injection of PAM use will benefit the patients more is waiting for to be confirmed. More than that, facing the threat of terrorism attack, a cholinesterase activator with a character of both rapid action and long acting shall be considered as well.

T04 • Novel oximes in counteracting organophosphates exposure

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Organophosphates (OP) known as nerve agents (soman, sarin, tabun, VX) present a threat in terrorist attacks and conflicts, as was the case recently in Syria, Malesia and UK. The main targets of OP compounds are acetylcholinesterase (AChE), the essential enzyme in neurotransmission, and butyrylcholinesterase, its back-up enzyme. However, the antidotes currently in use, which act as reactivators of inhibited AChE, were empirically synthesized before the two enzyme's crystal structures were resolved. Due to structural requirements, their binding affinity and reactivation rate have not been well-balanced. Our computational and experimental studies of both cholinesterase interactions with a wide range of ligands defined favourable characteristic for potential new antidotes, and directed the subsequent synthesis of selected leads. Thorough *in vitro* and *in vivo* experimental evaluation demonstrated a feasible approach to the development of an oxime-assisted catalytic bioscavengers of soman, tabun and VX based on an efficient reactivation of cholinesterases. Ultimately, the oxime-assisted catalytic scavenging of the nerve agents in mice improved therapeutic outcomes preventing lethality and resulted in a delayed onset of toxicity symptoms.

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T05 • Non-oxime reactivators of organophosphate inhibited acetylcholinesterase

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Organophosphate (OP) compounds inhibit the enzyme acetylcholinesterase (AChE) resulting in severe symptoms and ultimately death. OP intoxications are currently treated by administration of atropine and certain oxime compounds (Obidoxime, HI-6 or 2-PAM). The latter compounds contain nucleophilic oximes that reactivate OP-inhibited AChE by liberating the phosphylated serine. However, these oximes have several drawbacks such as their intrinsic toxicity, their permanent charge which thwarts penetration of brain tissues and their inability to effectively reactivate all types of nerve agent inhibited AChEs. Therefore, the search for new (non-ionic) antidotes of nerve agent poisoning is of great importance. Recently, several papers reported on the discovery of non-oxime compounds as a result of the *in vitro* or in silico screening of libraries of bioactive compounds and approved drugs. For instance, Katz et al reported1 a novel class of compounds in which the 4-amino-2-(diethylamino)phenol (ADOC) appeared to be a key motif responsible for reactivation of OP-inhibited AChE.2 In addition, several structural derivatives of ADOC were synthesized and evaluated for OP-AChE reactivation by Cadieux et al.3 That study provided valuable information on key structural features of ADOC with respect to reactivation potency and enzyme inhibition, but unfortunately, none of the reported derivatives performed equal or better than the ADOC parent. We report4,5 the design and synthesis of ADOC derivatives of which a close structural derivative demonstrated promising *in vitro* reactivation characteristics.

References:

1. Katz, F. S. et al. Discovery of New Classes of Compounds that Reactivate Acetylcholinesterase Inhibited by Organophosphates. Chembiochem 16, 2205–15 (2015).
2. Bierwisch, A., Wille, T., Thiermann, H. & Worek, F. Kinetic analysis of interactions of amodiaquine with human cholinesterases and organophosphorus compounds. Toxicol. Lett. 246, 49–56 (2016).
3. Cadieux, C. L. et al. Probing the activity of a non-oxime reactivator for acetylcholinesterase inhibited by organophosphorus nerve agents. Chem. Biol. Interact. 259, 133–141 (2016).
4. de Koning, M. C., Horn, G., Worek, F. & van Grol, M. Discovery of a potent non-oxime reactivator of nerve agent inhibited human acetylcholinesterase. Eur. J. Med. Chem. 157, 151–160 (2018).
5. Horn, G., de Koning, M. C., van Grol, M., Thiermann, H. & Worek, F. Interactions between acetylcholinesterase, toxic organophosphorus compounds and a short series of structurally related non-oxime reactivators: Analysis of reactivation and inhibition kinetics in vitro. Toxicol. Lett. 299, 218–225 (2018).

T06 • Research and development of bioscavengers for the pretreatment and emergency treatment of nerve agents intoxicatio

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Organophosphorus nerve agents (NA) irreversibly inhibit acetylcholinesterase (AChE) causing a major cholinergic syndrome. The medical counter-measures of NA poisoning have not evolved for the last 30 years with carbamates for pre-treatment, pyridinium oximes-based AChE reactivators, antimuscarinic drugs and neuroprotective benzodiazepines for emergency treatment. These drugs ensure protection of the peripheral nervous system and mitigate acute effects of NA lethal doses. They are life-saving countermeasures and despite treatment, intoxicated personnel will be incapacitated for days if not weeks for all but the lowest of exposures. The currently fielded drugs have significant limitations. Pyridostigmine and oximes do not protect/reactivate central AChE. Each licensed oxime is effective against a limited number of NA. Thus, there is large room for improvement of the prophylaxis and treatment of NA poisoning. The best pre-treatment approach involves neutralization of NA molecules before they reach their molecular targets by the means of administered bioscavengers. Recent data have shown that bioscavengers have also a therapeutic effect for persistent agents if administered at the first sign of poisoning. Human butyrylcholinesterase is the most advanced enzyme bioscavenger. GMP production at current costs limits its use to small populations like special forces, or to first responders and the few victims in case of a limited chemical event. Larger production at low cost must be actively developed. Among catalytic scavengers, able to process larger doses of NA, and therefore reducing the amount of enzyme that must be administered, phosphotriesterases are the most promising. The enzyme activity against NA has been optimised and effort on their development must focus on ways to produce a human-compatible injectable galenic form. Small molecules scavengers based on cyclodextrins or other caged-compounds are also of great interest due to their predicted low cost of production and use. These advanced bioscavengers are still at least a decade away from fielding but the benefits they will provide is enormous.

T07 • Salisbury Novichok Incident

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The generic clinical picture of nerve agent poisoning and current therapeutic management will be presented in the context of the Salisbury incident. Specific details of the patient presentation, pre-hospital medical interventions and subsequent supportive and directed medical management of the patients will be discussed. The important role of good routine clinical and nursing care, at all stages of the patients' treatment, and the contribution of specific medical countermeasures will also be revisited.

The utility of near-patient biomarker monitoring and bioanalytical toxicological analysis was demonstrated, as was recognition of the value of specialist advice from a CBRN clinical expert group who had daily clinical teleconferences with the hospital staff. Together this working collaboration informed the overall clinical management provided.

Speciality-relevant pitfalls and learning outcomes will be identified to enable future optimised therapeutic approaches, should this challenge be faced again in the medical community.

T08 • Phospholipid chlorohydrins as potential biomarkers for chlorine gas exposure

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Chlorine is a widely available threat agent that has been repeatedly used in alleged attacks in armed conflict globally. The Organization for the Prohibition of Chemical Weapons (OPCW) has on numerous occasions found "compelling confirmation" that chlorine gas have been used against civilians in northern Syria. However, there are no analytical methods available for unambiguous verification of exposure to chlorine gas. In this study, we describe the identification of biomarkers present in bronchoalveolar lavage fluid of chlorine gas exposed mice. The markers are detectable for >72 hours after exposure and are absent in non-exposed control animals. The markers are chlorohydrins of unsaturated pulmonary surfactant phospholipids, phosphatidylglycerols and phosphatidylcholine. The relevance of these markers for human exposure is also verified.

T09 • Outlook for upcoming therapeutics for chlorine induced acute lung injury

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Chlorine is a globally produced toxic chemical used in a variety of industrial processes and in water purification and as such its use cannot be restricted. Exposure may result in mass casualties from damage to the lungs that can result in respiratory failure, acute respiratory distress and death. While the mechanisms of action and medical management of chlorine have long been investigated, the exact molecular interactions that lead to the pathophysiology remain poorly understood. As a result, treatment of lung injury following exposure remains an intractable problem with a lack of clinically proven countermeasures. Supportive measures are based on the signs and symptoms following exposure and are generally anecdotal. On inhalation, chlorine initiates a complex set of reactions in the lung. It reacts with the moist lining of the airways forming hypochlorous acid with subsequent formation of highly reactive free radicals at the cellular level. Direct oxidative injury to the epithelium may also occur with migration and activation of inflammatory cells (e.g. neutrophils) and subsequent release of oxidants and proteolytic enzymes. Induction of nitric oxide synthase can lead to formation of nitric oxide and, secondarily, peroxynitrite which can contribute to further airways injury, oedema, inflammation, immediate airway constriction and persistent airways reactivity. More recently the role of selective TRP ion channel activation in chlorine induced lung injury has been proposed. In order to find new and more effective countermeasures it is essential that the molecular mechanisms of action of chlorine in the lung are understood. Emerging and recently developed technologies such as genomics and proteomics give us the ability to detect and identify many different molecular interactions and pathways following chemical exposure. These approaches enable us to develop a better understanding of the behaviour of cells at the molecular level and may therefore enable the identification of novel pathways for therapeutic intervention. Studies assessing therapeutic strategies in small animals are based on understanding key targets and pathways affected following exposure. The key challenge will be in transitioning successful candidate therapies from research to the clinic using appropriate model systems.

T10 • Fast-acting irritant gases / chlorine gas – clinical effect, symptoms and treatment

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Irritant gas exposure is a common cause in household or industrial accidents but may also occur in chemical warfare. It may cause an acute inhalation injury, ocular or dermal symptoms. Acidic and alkaline radicals are released and react with the respiratory tract mucosa. Depending on their water solubility they react immediately or with a certain latency. Highly soluble agents like ammonia result in quick upper respiratory tract symptoms. Chlorine gas has a medium solubility, also causing delayed symptoms of the lower airways. The severity and duration of the symptoms depend on the gas concentration and duration of exposure. The onset is within seconds and lasts a few hours. Eyes may show a conjunctival injection, abrasions and corrosions. A burning sensation of lips and mouth is common. Corrosions of the nasal and oral mucosa down to the trachea can occur. Patients usually present with a dry cough and possible retrosternal or thoracic pain. Dyspnea along with tachypnea, inspiratory stridor and bronchospasm occur frequently. Glottis edema or laryngospasm are emergency symptoms. In chlorine gas exposure pneumonia and lung edema may occur delayed. Inspection of eyes and mouth and thorough pulmonary auscultation are required in all exposed persons. Oxygen saturation should be monitored and arterial blood gases obtained in symptomatic patients. A routine thoracic x-ray is not recommended – except if pulmonary edema is suspected, which may manifest even up to 48 hours. In case of chest pain, an EKG and cardiac enzymes should be obtained. A laryngoscopy should be performed if glottis edema is suspected. Treatment is mainly symptomatic as no specific antidote is available. Exposed eyes should be irrigated immediately. Oxygen and - in case of bronchospasm - inhalatory β-sympathomimetics or theophylline could be administered. A benefit of sodium bicarbonate inhalation after chlorine gas inhalation is discussed controversially. In case of massive laryngospasm mechanical ventilation with CPAP or intubation/tracheotomy with a sufficiently high positive-end-expiratory-pressure (PEEP) may be necessary. Neither inhalatory glucocorticoids nor prophylactic antibiotics are recommended. Intravenous glucocorticoids for the prophylaxis of a toxic pulmonary edema should – if ever – applied highly restrictive. If symptoms are mild, patients can be safely discharged after brief observation. After chlorine gas exposure patients should be monitored for up to 24 hours.

T11 • Opioids fundamentals

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Chemicals related structurally to the plant substances opiates are made synthetically for medicinal use and illegally as drugs of abuse; the synthetic derivatives are known as opioids. Among these are fentanyl and analogues, which are solids employed in human and veterinary treatments to cause anaesthesia (loss of sensation accompanied by unconsciousness) and analgesia (a reduction in pain sensitivity). The margin between the therapeutic and lethal doses is generally quite narrow, and overdosing often results in death, predominantly through respiratory depression. Some fentanyls have lethality comparable to organophosphorus nerve agents if misused outside the clinic in the absence of medical support [1]. Only a few fentanyls are produced worldwide for medicinal purposes and are made in multi-kilogram batches. The last few years have witnessed a surge in the illicit production of fentanyls and their emergence as street drugs, resulting in many accidental deaths. Despite the past use of an aerosolised mixture of fentanyl compounds to end a siege [2], fentanyls are not riot control agents (RCAs) as they do not meet the definition of a RCA under the Chemical Weapons Convention [3]. In view of the increasing availability of fentanyl and analogues, and the potential for their misdirection to cause harm, fundamental aspects of opioid science are described to set the scene for the presentations by others that will follow on opioid toxicology and medical countermeasures.

References:

1. C.D. Lindsay, J.R. Riches, N. Roughley, C.M. Timperley. Chemical defence against fentanyls, in: Chemical Warfare Toxicology, Volume 2: Management of Poisoning; F. Worek, J. Jenner, H. Thiermann (Eds.), Issues in Toxicology No. 27, The Royal Society of Chemistry, Cambridge, UK, 2016, Chapter 8, 259-313.
2. J.R. Riches, R.W. Read, R.M. Black, N.J. Cooper, C.M. Timperley. Analysis of clothing and urine from Moscow theatre siege casualties reveals carfentanil and remifentanil use. J. Anal. Toxicol. 2012, 36, 647-656.
3. C.M. Timperley, J.E. Forman, et al. Advice from the Scientific Advisory Board of the Organisation for the Prohibition of Chemical Weapons on riot control agents in connection to the Chemical Weapons Convention. RSC Adv. 2018, 8, 41731.

T12 • Identification of Fentanyl Exposures through Analysis of Clinical Samples using Mass Spectrometry

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Human exposures to fentanyls, which significantly contribute to the ongoing U.S. opioid overdose epidemic, can be confirmed through the analysis of clinical samples. Our laboratory has developed multiple diagnostic methods, using simple sample preparation of dried blood spots, plasma, or urine, followed by LC-MS/MS for the targeted identification of known fentanyl analogs and select metabolites. We have also evaluated various LC-HRMS screening approaches to address novel fentanyl analogs. Plasma and urine samples fortified with many fentanyl analogs were assessed to confirm the capabilities of these LC-HRMS methods. The application of both targeted and screening methods enables the real-time detection of known and novel fentanyls present in clinical samples.

T13 • Derivation of human estimate of toxicity for ultra-potent opioid carfentanil

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Carfentanil is an ultra-potent opioid of public health and chemical weapons defense concern. Exposure to carfentanil has been seen in both illicit drug use and in the resolution of a hostage situation in Moscow, Russia in 2002. While opioid pharmacology and toxicology are well researched topics, carfentanil remains unstudied relative to its clinically used counterparts like fentanyl. Similarly to fentanyl, carfentanil elicits is toxicity from central nervous system depression, largely in regions of the brain involved in spontaneous respiratory rhythmogenesis. By depressing the respiratory centers of the brain, respiratory failure can occur, and can lead to death. Carfentanil is of concern because it has demonstrated 100 times higher potency than its prototype, fentanyl, in analgesic assays in rodents. Carfentanil has very little human relevant data to indicate its toxicity in man for use in public health or chemical defense risk assessments. The present study was designed to test the hypothesis that carfentanil physiologically based pharmacokinetic (PBPK) modeling could accurately reflect observed PK *in vivo* in a surrogate animal model, and be translated to a human equivalent predication of toxicity. Studies were carried out to assess opioid receptor subtype specificity, potency, and efficacy. Carfentanil metabolism was studied in both rabbit and human liver microsomes to assess its intrinsic clearance. A metabolite identification study was undertaken to identify metabolites that could contribute to prolonged exposure or toxicity, and to generate a library of metabolites to be used in a forensic setting to identify carfentanil as a culprit agent in overdose or mass casualty exposures. Additionally, two key physicochemical properties of carfentanil were quantified in both rabbit and human blood: plasma protein binding and red blood cell – plasma partitioning. These properties have important roles in PBPK modeling, and can be used in a forensic setting to indicate where carfentanil can be found. Finally, the rabbit *in vitro* derived properties were incorporated into the PBPK model to accurately model the *in vivo* PK seen in the very limited rabbit exposures. This model was then translated to a human PBPK model and an equivalent toxic dose was optimized. This predicted human toxic dose (0.34 μg/kg) was calculated to be very close to a post hoc human LD50 value published in a study by researchers at the U.S. Drug Enforcement Agency (0.29 μg/kg).

T14 • Environmental hazards of potent opioids and implications for national defense

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Potent opioids such as fentanyl and fentanyl derivatives are increasingly prevalent as adulterants or substitutes for opioid drugs of abuse in Europe and in North America. Trafficking and distribution of these chemicals is inadequately characterized. Rescue and emergency personnel are increasingly concerned about the possibility of unintentional environmental exposures that might occur in the course of their operational duties. There is evidence that opioid agonists have been broadcast offensively as incapacitating agents. Defending against toxicity from such agents requires a thoughtful plan for protection, decontamination, and treatment.

T15 • *In vitro* and *ex vivo* models in medical chemical defence research

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The recent use of nerve agents (OPNA) in the Syrian Civil War and for assassinations in Malaysia and the UK highlights the threat by these compounds and the need for optimized medical countermeasures (MedCM). As animal experiments are expensive, time-consuming and increasingly under public scrutiny, well-conceived *in vitro* and *ex vivo* models are important to pre-select candidate MedCM. The main target of OPNA is inhibition of the pivotal acetylcholinesterase (AChE). AChE is also expressed on erythrocytes and purified membranes are a valuable tool for initial MedCM research. Dynamic flow exposure of erythrocyte AChE to OPNA and MedCM simulates toxico- and pharmacokinetic processes combined with real time analysis of AChE activity. In this model, a combination of HI-6 and obidoxime showed no additive effect but the reactivation spectrum was extended, which could bridge efficiency gaps until a broad-spectrum reactivator is available. A retrogradely perfused Langendorff heart and hiPS-derived cardiomyocytes in a multi electrode array (MEA) were established to address dysrhythmic effects induced by OPNA and potentially by MedCM themselves. The OPNA cyclosarin resulted in a dose-dependent increase in field potential duration as marker for dysrhythmia (600 nM; 9.4±0.5%), which was antagonized by atropine but not by HI-6. Obidoxime, pralidoxime and the candidate reactivators HI-6 and MMB-4 did not induce dysrhythmia in the MEA and the Langendorff heart. Inhibited AChE results in smooth muscle contraction. Consequently, a small bowel and lung tissue/airway model were established. Small bowel tissue from patients undergoing routine surgery was exposed to sarin and treated with scopolamine as most potent drug with an EC50 of 50 nM. Interestingly, HI-6 was more potent than obidoxime (EC50 3.8 µM vs. 197.8 µM) in this model. As lung model, precision cut lung slices allow to assess the efficacy of supportive asthma and COPD therapeutics in OPNA-poisoning . Beta-2-agonists had a negligible effect whereas glycopyrrolate (EC50 15.8 nM) and ipratropium (EC50 2.3 nM) efficiently reversed airway contractions. Significant effects were also achieved by combining beta-2-agonists with atropine, advocating a clinical trial in patients poisoned by OP pesticides. Complex *in vitro* and *ex vivo* models are successfully established and can serve as important tools to accelerate development of MedCM and allow optimized planning of focused *in vivo* experiments.

T16 • Developing medical countermeasures against aerosolized opioids: animal models and inhalation exposures

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Carfentanil is a powerful synthetic opioid that is approximately 100 times more potent than fentanyl and 10,000 times more potent than morphine. Originally intended to be a sedative for big game animals in a veterinary setting, carfentanil has increasingly been recognized for its potential battlefield applications as demonstrated in 2002, when the Russian military used the air handling system in the Moscow Dubrovka Theater Center to introduce an aerosolized mixture of carfentanil and remifentanil to subdue Chechen rebels who had taken 912 hostages. Although the attackers were neutralized, 130 hostages died from the effects of the chemicals. The hostage deaths were in part attributed to delayed or inaccurate treatment to opioid exposure because the composition of the aerosol mixture was not immediately released to first responders. We have developed animal models that utilize realistic routes of exposure and relevant carfentanil exposure doses to investigate medical countermeasures against inhaled opioids. In our models we characterized exposure by timelines and symptoms matching those in humans. This experimental design provided a wealth of biochemical and physiological information relevant to advanced drug development and medical intervention strategies. Our exposure systems are custom-designed for individual, whole-body exposures to an aerosolized solution of CRF and integrate real-time physiological monitoring capabilities. Various clinical observations and physiological parameters were recorded during exposure and up to 24 hr post-exposure in both mice and ferrets exposed to aerosolized carfentanil. Common to both models were profound respiratory depression and cardiac function abnormalities in exposed animals. Across the range of carfentanil exposure doses studied, mortality was observed in ferrets but not in mice. In both models, post-exposure administration of naloxone intramuscularly at human equivalent doses ranging from 4 to 40 mg significantly reduced the time that animals were incapacitated following exposure and also reversed respiratory depression, and cardiac dysfunction. Our results suggest that naloxone is a viable treatment against the effects of potentially lethal dose of inhaled carfentanil.

The experimental protocol was approved by the Animal Care and Use Committee of the US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals (National Research Council), and the Animal Welfare Act of 1966 (P.L. 89-544), as amended. This research was supported by DTRA RD-CB.

T17 • The evaluation of continuing medical management strategies in the VX-poisoned guinea-pig

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Post exposure treatment for nerve agent exposure includes the administration of an oxime, which acts to restore the function of the inhibited enzyme acetylcholinesterase (AChE), alongside atropine and diazepam. For immediate treatment, these are usually delivered by an autoinjector however, it is likely that personnel exposed to nerve agent will require further medical treatment, particularly if exposure is by the percutaneous route. An intravenous infusion of HI-6 DMS at a dose rate of 0.24 or 0.48 mg/kg/min, in combination with atropine, at 0.07 mg/kg/min, was efficacious against a percutaneous VX challenge (2.5 x 24 h LD_{50}) in guinea-pigs [Whitmore et al, 2018, Tox Lett, 293, 207]. Survival was improved compared to atropine alone, with all animals receiving HI-6 surviving until the end of the study at 48 h. A PKPD relationship between erythrocyte AChE activity, plasma levels of HI-6 DMS and survival was determined. Increasing the dose rate of HI-6 DMS in the infusion from 0.24 to 0.48 mg/kg/min increased the both plasma levels of HI-6 and AChE activity. There was no difference in the survival, or the condition of the animals between the two HI-6 dose rate groups when observed post-poisoning, suggesting that both doses of HI-6 were maximally effective against this challenge dose of agent. This presentation will discuss the rationale for the HI-6 dose selection and experimental design, present more details of the assessment of signs of poisoning and explore the suitability of the guinea-pig for experimentally assessing medical management regimens.

T18 • Aiding the warfighter from chemical warfare agents using innovative medical diagnostics

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The Defense Threat Reduction Agency (DTRA) within the U.S. Department of Defense is developing innovative diagnostic platforms to aid in diagnosing exposure to traditional and non-traditional chemical warfare agents (CWAs). Emphasis is being placed on platforms capable of providing rapid test results to aid the warfighter on the battlefield. To meet this need, DTRA is focusing research and development investments in three platforms: (1) an electrochemical-based platform for detecting red blood cells-acetylcholinesterase (AChE) and plasma butyrylcholinesterase (BuChE) activity from whole blood, (2) a platform for detecting AChE-inhibition, and (3) a "lab on a disk" immunoassay platform. The first platform will be capable of providing real-time warning of exposure to organophosphate (OP) nerve agents by measuring blood levels of AChE and BChE. The lightweight, low powered, palm sized platform resembling a glucometer requires a single drop of blood (finger prick capillary blood) and can provide results in less than 2 minutes. The second platform is designed to only measure AChE-inhibition by measuring free thiols in the thiocholine product generated by AChE. It employs antibodies with no detectable cross-reactivity to BuChE from a single drop of blood and can provide results in less than 4 minutes. The third platform, "lab on a disk" SpinDx immunoassay platform, is able to detect protein biomarkers from blood indicative of exposure to OP nerve agents. The light-weight, field-portable device does not require sample preparation and is multiplexable up to 20 targets per assay. This platform requires whole blood and can provide results in less than 15 minutes. DTRA will conduct a number of evaluations and technology demonstrations to down select to the platform that meets warfighter requirements and needs for use in the battlefield. The development and use of these novel platforms will greatly benefit warfighters in battlefield environments to quickly and correctly aid in the diagnosis of exposure to CWAs.

T19 • Vesicant-induced lung damage: The DNA damage response in lung epithelial cells

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Nitrogen mustard and sulfur mustard are potent vesicants that modify and disrupt cellular macromolecules including DNA resulting in cytotoxicity and tissue injury. This can occur directly or via oxidative stress. As a consequence of vesicant-induced nucleic acid damage, DNA damage signaling pathways including ataxia telangiectasia mutated (ATM), ataxia telangiectasia and Rad3-related (ATR), as well as DNA-dependent protein kinase (DNA-PK) are upregulated. Expression of these proteins is cell cycle related, for example, in A549 lung epithelial cells, nitrogen mustard (HN2) causes a concentration-dependent arrest of cells in the S and G2/M phases of the cell cycle. This is associated with time- and concentration-dependent increases in expression of phosphorylated ATM (S1981), Chk2 (T68), H2AX (Ser139), and p53 (ser15). Activation of DNA damage signaling was most pronounced in S-phase cells followed by G2/M-phase cells and suppressed by the ATM and DNA-PK inhibitors, KU55933 and NU7441, respectively, and to a lesser extent by VE-821, an ATR inhibitor. This was correlated with abrogation of DNA damage checkpoints signaling. In the case of p53, HN2-induced increases in protein expression were not dependent on a mutant phenotype. Thus, HN2 caused a concentration- and time-dependent induction of p53 in HaCaT keratinocytes which express mutant p53 (H179Y, R282W), A431 epidermoid cells which also express mutant p53 (R273H), and p53 wild type HEK293 kidney epithelial cells and A549 lung epithelial cells. These data indicate that the actions of HN2 on induction of p53 are status independent. HN2 was found to cross-link p53 forming several high molecular weight complexes. Mechanisms mediating protein cross-linking were studied using recombinant wild type human p53 cross-linked with HN2. LC-MS MS analysis revealed that HN2 selectively alkylated Cys135, Cys141, Cys229, Cys238, Cys242 on the protein, forming both monoadducts and peptide loop links. Additionally, HN2 cross-linked Cys124 on one molecule of p53 and Cys229 on a second molecule of p53, forming protein dimers. Taken together, our data demonstrate that DNA damage signaling is likely to be important in downstream responses to vesicants. This may contribute to vesicant-induced cytotoxicity and tissue injury.

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T20 • Natural history of inhaled sulfur mustard poisoning in rats: mitigation of tissue injury by anti-TNFα antibody

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Inhalation of the chemical warfare agent sulfur mustard (SM, 2 (bis-chloroethyl) sulfide) causes acute debilitating lung injury which progresses to fibrosis. In these studies, we characterized the acute and long-term manifestations of SM-induced pulmonary injury in rats. Spontaneously breathing male Wistar rats were anesthetized, intratracheally intubated, and exposed to 0.4 mg/kg SM by vapor inhalation. Animals were euthanized 3, 7, 16 and 28 d post-exposure and bronchoalveolar lavage fluid (BAL) and lung tissue collected. At 3 d post SM, pulmonary edema, inflammatory cell accumulation, thickened alveolar septal walls and ulceration of bronchial epithelium were observed in histologic sections. Expression of heme oxygenase (HO)-1 and inducible nitric oxide synthase (iNOS), markers of proinflammatory macrophages, were also markedly upregulated in the lung, along with mucin, fibrinogen and cyclooxygenase (COX)-2, consistent with SM-induced oxidative and nitrosative stress, lung injury and inflammation. This was associated with increases in BAL cell and protein content and levels of high mobility group box (HMGB)-1, receptor for advanced glycation end products (RAGE), matrix metalloproteinase (MMP)-9, and surfactant protein (SP)-D, demonstrating alveolar epithelial injury and derangements in lung lipids. Similar structural and inflammatory changes were also noted in the lung at 7 d post SM, but at reduced levels. At 16 d post SM, lung HO-1 and mucin expression were increased, relative to 7 d. In addition, bronchial epithelial- and mesothelial-hypertrophy/hyperplasia, interstitial fibrosis and dysregulated epithelial repair were evident. At 28 d post SM, a secondary increase in BAL cells and levels of MMP-9, RAGE, SP-D and fibrinogen, as well as HO-1 and COX-2 expression were observed which was comparable to or greater than increases observed 3 d post SM. This was correlated with the appearance of proteinaceous bronchiolar and alveolar exudate entrapping inflammatory cells, diffuse squamous metaplasia, aberrant bronchial epithelial repair and multifocal alveolar interstitial and peribronchial fibrosis. Enlarged anti-inflammatory/profibrotic macrophages were also noted in the tissue at this time. Treatment of rats with anti-TNFα antibody (15 mg/kg, i.v., 1x/week) beginning 15-30 min after SM mitigated the effects of the vesicant on lung pathology, inflammation and fibrosis. These data demonstrate a similar pathologic sequala of events occurs in rats and humans following SM exposure. Moreover, blocking TNFα represents an important approach to mitigating acute lung injury induced by vesicants.

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T21 • A mass spectrometric platform for the quantitation of sulfur mustard-induced nucleic acid adducts as mechanistically-relevant biomarkers of exposure

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Despite its worldwide ban, the warfare agent sulfur mustard (SM) still represents a realistic threat, due to potential release in terroristic attacks and asymmetric conflicts. Therefore, the rigorous and quantitative detection of SM exposure is crucial for diagnosis, health risk assessment, and surveillance of international law. Alkylation adducts of nucleic acids can serve as valuable toxicologically-relevant 'biomarkers of SM exposure'. Here, we developed a robust and versatile bioanalytical platform based on isotope dilution UPLC-MS/MS to quantify major SM-induced DNA and RNA adducts, as well as adducts induced by the monofunctional mustard 2-chloroethyl-ethyl-sulfide (CEES). We synthesized 15N/13C-labeled standards, which allowed absolute quantitation with full chemical specificity and sub-femtomol sensitivities. DNA and RNA mono-alkylation adducts and crosslinks were carefully analyzed in a dose and time dependent manner in various matrices, including human cancer and primary cells, derived of the main SM-target tissues. Nucleic acid adducts were detected up to 6 d post exposure, indicating long persistence, which highlights their toxicological relevance and proves their suitability as forensic and medical biomarkers. Finally, we investigated ex-vivo-treated rat skin biopsies and human blood samples, which sets the basis for the implementation into the method portfolio of OPCW-designated laboratories to analyze authentic samples from SM-exposed victims.

References:

1. Zubel, T. et al. A mass spectrometric platform for the quantitation of sulfur mustard-induced nucleic acid adducts as mechanistically relevant biomarkers of exposure. Archives of Toxicology, doi:10.1007/s00204-018-2324-7 (2018).
2. Zubel, T., Bürkle, A. & Mangerich, A. Mass spectrometric analysis of sulfur mustard-induced biomolecular adducts: Are DNA adducts suitable biomarkers of exposure? Toxicology Letters 293, 21-30, doi:10.1016/j.toxlet.2017.12.014 (2018).

T22 • Interaction of sulfur mustard with proteins – Are there potential cellular effects in addition to DNA-alkylation?

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The chemical warfare agent sulfur mustard (SM) is known to alkylate DNA but also proteins. Especially DNA alkylation, resulting in mono-functional adducts but also bi-functional crosslinks, is thought to be the main reason for cytotoxicity. Interactions of SM with other biomacromolecules have already been described. Biological effects resulting thereof were paid only little attention. However, a plethora of chemicals exhibit characteristic odors, suggesting an immediate and more specific interaction with the human body. During the last few years, G-protein-coupled receptors and especially chemosensory ion channels of the transient receptor potential family (TRP channels) were identified as defined targets for several chemicals and were suggested to be fundamentally involved in the molecular (patho)physiology. In line with this concept, own studies revealed activation of TRPA1 channels, which are both expressed in lung epithelial cells, by alkylating compounds. Heat shock protein A6 (HSPA6) was identified as SM-induced and TRPA1-mediated protein. However, initial *ex vivo* experiments using isolated and perfused lungs from TRPA1-/- mice revealed that lung edema formation in TRPA1-/- lungs was significantly enhanced compared to wt-lungs. Peptide hormones were recently identified as new targets for SM. Steroid hormones exhibit also reactive residues which may also be covalently modified by SM. Using MALDI-TOF analysis, we were able to proof covalent modifications of steroid hormones. This was accompanied by impaired hormone function which was assessed by *in vitro* reporter assays and *ex vivo* leukocyte stimulation experiments. Taken together, our findings document interactions of SM with proteins that have impact on cellular functions.

T23 • Is sulfur mustard (SM) exposure induced chronic senescence the reason for the chronic wound healing disorder?

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After SM exposure, the most prominent clinical symptom is the development of large, poorly healing skin wounds. This chronic wound healing disorder persists for weeks to months. Mesenchymal stem cells (MSC) are known to play an important role in wound healing. In addition, it is also known that patients with chronic wound healing disorder have MSC with impaired stem cell functionality. Based on these observations and the known relationship between wound healing disorder and MSC function, we investigated the effects of SM on human MSC. A reason for this chronic wound healing disorder could be that SM exposed MSC undergo chronic senescence. In this state MSC may persist over long time periods and secrete proinflammatory cytokines known as the senescence-associated secretory phenotype. In this study, we analyzed whether SM is able to induce senescence in MSC and if they develop the senescence-associated secretory phenotype (SASP). Human MSC were isolated from the bone marrow of donor femoral heads and their quality was determined using specific cell surface markers by flow cytometry. Cells were exposed to single doses of SM (1 – 40 µM) or H_2O_2 (200 µM) and the senescence-associated β-galactosidase (SA-β-gal) was stained up to 31 days afterwards. Besides the observation of chronic senescent MSC after one-time SM exposure a SASP was observable. In addition to the increased secretion of typical SASP factors such as MMP-1, MMP-2 and IL-6, we also could reveal the secretion of SM induced pro-inflammatory factors such as IL-12, CXCL13 and CXCL16. It could be suggested that this dysregulation of pro- and anti-inflammatory factors could further lead to chronic inflammatory processes as they occur after SM exposure. SM single dose exposure is sufficient to induce chronic senescence in human MSC. These SM-induced senescent MSC may be unable to fulfill their regenerative role and contribute to the wound healing disorder. Further research is necessary to identify other mechanisms to selectively remove senescent cells which would be an innovative treatment strategy after SM exposure.

T24 • Novel substituted phenoxyalkyl pyridinium oximes show promise for central neuroprotection against organophosphate anticholinesterase poisoning

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Few acetylcholinesterase (AChE) reactivators, most of which are pyridinium oximes, have the ability to cross the blood-brain barrier and restore AChE activity in the brain following inhibition by organophosphates. Our laboratory has invented and patented a platform of substituted phenoxyalkyl pyridinium oximes which incorporate a large lipophilic moiety into the structure (US patent 9,277,937; European patents pending; licensed by Defender Pharmaceuticals). These novel oximes have shown evidence of reducing the brain AChE inhibition in rats challenged with high sublethal doses of a highly relevant sarin surrogate (nitrophenyl isopropyl methylphosphonate, NIMP) and paraoxon (the active metabolite of the insecticide parathion), whereas 2-PAM does not. These oximes have also shown evidence of enhancing 24-hr survival of lethal dosages of these two organophosphates, and with some of the oximes, more effectively than 2-PAM at the same therapeutic molar equivalent. These oximes have decreased the time until cessation of seizure-like behavior, while 2-PAM does not, in rats treated with two organophosphates. These oximes also have provided neuroprotection as evidenced by histology in select brain regions comparable to vehicle controls in glial cells using glial fibrillary acidic protein (GFAP) as a marker and in neurons using neuN as a marker in rats treated with high dosages of organophosphates. In addition, these oximes also have shown a reduction in the severity of signs of poisoning in guinea pigs treated with sarin. Mechanistic studies have indicated that our more effective oximes are poor substrates for the blood-brain barrier exporter P-glycoprotein, suggesting that these oximes will be retained within the brain. Therefore there is experimental evidence that these novel oximes can penetrate the blood-brain barrier, providing central protection from neurotoxicity induced by organophosphates of both nerve agent and insecticidal chemistries.

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T25 • Low molecular weight scavengers for V-type nerve agents derived from cation-binding synthetic receptors

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A promising approach for the treatment of poisonings with neurotoxic organophosphates relies on scavengers that detoxify the nerve agents before they can reach their target. Considerable progress has been made in recent years in the development of protein-based scavengers, of which some exhibit high activity even against the persistent V-type nerve agents. The therapeutic use of proteins can be problematic, however, which is why low molecular weight synthetic scavengers represent an interesting alternative. In a proof of concept study, we could show that appropriately functionalized cyclodextrin derivatives can indeed protect from cyclosarin poisonings *in vivo*. Cyclodextrins proved to be unsuitable for the development of scavengers that detoxify VX and other V-type nerve agents, however. We therefore turned our attention to the use of functionalized sulfonatocalix[4]arenes because we expected these compounds to bind cationic organophosphates more strongly than cyclodextrins, a property that should in turn have a favorable effect on the detoxification activity. Indeed, one of the compounds synthesized in our lab was able to substantially accelerate the deoxification of various V-type nerve agents. Unfortunately, the rates of detoxification that could be achieved were still too low to allow an *in vivo* use. Two approaches were then pursued to obtain even more efficient scavengers. One involved improving the reactivity of the functional group that is responsible in the scavenger for the reaction with the nerve agent and its concomitant detoxification. Alternatively, we also considered the use of structural units in our scavengers that induce a higher organophosphate affinity than sulfonatocalix[4]arenes. In this context, we tested whether the strongly cation-binding acyclic cucurbiturils could serve as a scavenger basis. In this talk, the concept underlying our approach, our synthetic strategies, and the properties of the cucurbituril-based scavengers obtained so far will be summarized.

T26 • Catalytic bioscavenger with improved substrate profile, stability and plasma half-life to treat acute poisoning with neurotoxic organophosphorous compounds (OPs)

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Organophosphorous nerve agents pose a severe toxicological threat, both at disposal, during use as pesticides, in military conflicts and if considering potential acts of terrorism. Compared with the volatile and rapidly autohydrolyzing G-agents, the sulfur-organophosphates of the V-type (VX, VR, CVX) are highly problematic due to their persistence, low vapour pressure and the slow biodistribution following contact via skin or lung while satisfying treatments for detoxification are unavailable. Hydrolytic enzymes, which may be administered into the blood stream by injection and decompose the circulating nerve agent into less toxic compounds *in vivo* could provide a solution. Indeed, nature offers some enzymes that can hydrolyze OPs; however, their catalytic activities are low, in particular with regard to the synthetic V-agents. Thus, improvement of such natural biocatalysts via enzyme engineering is mandatory. For the phospho¬triesterase found in the bacterium Brevundimonas diminuta (BdPTE) mutants with improved catalytic efficiencies have been described; yet, their biochemical properties are insufficient for therapeutic application. Hence, the goal of our research project is the development of novel variants of BdPTE using the methodology of rational and combinatorial protein design. These will be produced as recombinant proteins in the laboratory bacterium Escherichia coli, purified to homogeneity and their structural, biochemical and enzymological properties will be assessed in order to obtain a biopharmaceutical drug candidate for the catalytic detoxification of various OPs. Thereby, the identification of an engineered PTE with broad substrate spectrum, including pesticides as well as V-type nerve agents, constitutes an important task. The optimization of a highly stable enzyme with prolonged pharmacokinetics – to meet the slow biodistribution of many OPs – and potentially reduced immunogenicity is another relevant aspect of our project.

T27 • Correlation between positive allosteric modulation at human α7 nicotinic acetylcholine receptors and functional recovery of soman-inhibited respiratory muscles

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The poisoning with organophosphorus compounds leads to the inhibition of acetylcholinesterase and to a dysfunction of the neuromuscular transmission, which ends in a fatal paralysis of respiratory muscles, if not treated adequately. Treatment with atropine and obidoxime are insufficient in the case of intoxication with soman and tabun. The 4-tert-butyl-substituted bispyridinium compound (BP) MB327 showed first promising therapeutic effects in vivo and in vitro. In the research project E / U2AD / CF514 / DF561, further BP compounds were synthesized and tested on the rat diaphragm by myograph measurements and on the α7 subtype of the human nicotinic acetylcholine receptor (hα7-nAChR) by automated whole-cell patch-clamp technique. After application of the agonist nicotine (100 µM), the cation influx of hα7-nAChR was induced. At higher nicotine concentrations (1 mM), the receptor current was inhibited due to receptor desensitization. The tert-butyl BP compounds MB327, PTM0001, PTM0002 and the methoxy BP compounds PTM0008, PTM0009 and PTM0010 were able to abolish this desensitization ("resensitizing", positive allosteric modulation type II, PAM type II). Muscle preparations were stimulated by indirect electric field stimulation (20, 50, 100 Hz). With application of 3 µM soman the muscle function was completely inhibited. With addition of the above-mentioned BP compounds, a partial restoration of muscle strength was achieved. These BP compounds, exhibiting PAM type II activity at hα7-nAChRs, also led to the recovery of soman-inhibited muscle strength in the rat diaphragm. Hence, it can be assumed that the desensitization of the muscle-type-nAChR could be reversed. Studies on muscle-type nAChR, prepared from the electric organ of Torpedo californica and using SSM-based electrophysiology, support this assumption. Although different target structures were investigated, comparable pharmacological profiles could be identified. The findings support the search for therapeutically effective substances.

References:

1. Niessen, K.V., Seeger, T., Rappenglück, S., Wein, T., Höfner, G., Wanner, K.T., Thiermann, H., Worek, F., 2018. In vitro pharmacological characterization of the bispyridinium non-oxime compound MB327 and its 2- and 3-regioisomers. Toxicol. Lett.;293:190-197.
2. Scheffel, C., Niessen, K.V., Rappenglück, S., Wanner, K.T., Thiermann, H., Worek, F., Seeger, T., 2018. Conteracting desensitization of human α7-nicotinic acetylcholine receptors with bispyridinium compounds as an approach against organophosphorus poisoning. Toxicol. Lett. 293, 149-156

T28 • Acute severe intoxication with cyclopropylfentanyl, a novel synthetic opioid

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Background: The use of novel psychoactive substances (NPS) is a global problem. Since 2016 an increase has been observed in the availability of new synthetic opioids in Europe. They are one of the fastest-growing groups of NPS monitored by the European Monitoring Center for Drugs and Drug Addiction (EMCDDA). Cyclopropylfentanyl (N-phenyl-N-[1-(2-phenylethyl)piperidin-4-yl]cyclopropanecarboxamide) is a very potent and selective μ-opioid agonist (Ki = 0.088 nM; [1]). It was reported as an NPS for the first time in August 2017 in Europe.

Methods: The case was included in a prospective observational study of patients treated in emergency departments (ED) after the intake of NPS. Clinical features and follow-up information were acquired using a structured questionnaire for physicians. Serum and/or urine samples of ED patients were analyzed using liquid chromatography-electron spray ionization-tandem mass spectrometry (LC-ESI-MS/MS) screening methods for NPS.

Case report: A 25-year-old male reported intranasal administration of a fentanyl containing spray. Within 10 minutes he suffered from nausea, profuse sweating and dyspnoe. Soon afterwards coma and respiratory insufficiency was noticed by his friends. They started cardiopulmonary resuscitation (CPR) and contacted emergency medical services. The emergency physician administered oxygen and 0.8 mg of the antidote naloxone. On admission in hospital the patient presented with coma, miosis, and a breathing rate of 14/min. The patient was monitored in the intensive care unit. He developed recurrent decreases of oxygen saturation during the next 12 hours. The minimum oxygen saturation was 23 % SpO2.The intake of cyclopropylfentanyl was analytically confirmed (7 ng/ml in the urine sample). Other compounds identified were naloxone, cocaine and THC-COOH.

Discussion and Conclusion: The here reported acute poisoning with analytical confirmation of cyclopropylfentanyl was recorded in July 2017 in the context of our study. Between June 2017 and December 2017, a total of 78 cyclopropylfentanyl-related deaths with analytical confirmation in post-mortem samples were reported by three European countries [2]. Highly potent synthetic opioids such as cyclopropylfentanyl pose a risk of life-threatening poisoning due to the pronounced respiratory depression caused by overdoses. The patient's life could only be saved because his friends immediately started cardiopulmonary resuscitation.

T29 • Characterizing carfentanil intoxication in a laboratory primate model: behavioral safety and efficacy of naloxone therapy

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Carfentanil is an opioid agonist 10,000 times more potent than morphine. This study characterized carfentanil toxicity and evaluated medical countermeasure efficacy in African green monkeys. Initial studies characterized the progression of carfentanil (SC, 0.50-1.0 μg/kg) intoxication, and all doses produced lethargy, hunched posture, and ataxia with higher doses producing loss of posture and bradypnea. A second experiment evaluated the efficacy of naloxone in reversing carfentanil (1.15 μg/kg) intoxication. Naloxone (IM, 0.039-1.35 mg/kg) was administered when bradypnea (≤ 10 breaths/min) or loss of posture occurred (average 27.6 min). Recovery following treatment was generally rapid; respiration increased and posture improved within 10 minutes. Animals receiving lower doses of naloxone (≤ 65 μg/kg) required supplemental naloxone within 3 hours to fully recover. The third experiment evaluated the behavioral safety and efficacy of naloxone using monkeys trained on an automated choice reaction-time test. Animals received carfentanil (1.15 μg/kg) or vehicle and were subsequently treated with naloxone (IM, 71-710 μg/kg) at one of two different delays. Carfentanil (0.575 μg/kg) was used to evaluate behavioral impairment without treatment. This dose nearly abolished responding (≤ 10 % of baseline responding). The lowest dose of naloxone (71 μg/kg) partially attenuated the effects of carfentanil (1.15 μg/kg), resulting in an approximately 40 % decrease in responding, whereas higher naloxone doses fully reversed carfentanil intoxication within 15 minutes. Naloxone alone had no significant adverse effects on behavior. Naloxone is effective in counteracting carfentanil intoxication, and our results suggest that higher doses should be administered to ensure rapid and complete reversal of overdose.

T30 • Recent sulfur mustard attacks in Middle East and experience of health professionals

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Sulfur mustard (SM) is a vesicant chemical warfare agent (CWA) which affects ocular, cutaneous, and respiratory system. Its toxicity and persistency made SM an ideal CWA. Although the production and use of CWA's is banned by CWC, non-state actors in Middle East had used both blister agents against civilian groups after 2013. It is believed that non-state actors in Middle East able to produce CWA's and they have capability of exporting them to developed countries. This action could be ended up with a horrible chemical attack which would terrorize and destabilize Western societies. In this study, we aimed to share our experiences on medical management of SM casualties that were recorded after two chemical attack. We noted typical symptom-free latency period of SM exposure and we concluded that the duration of this phase correlated inversely with the absorbed dose. Itching and erythema following reddiness of the skin, numerous small vesicles, and characteristic pendulous thin-walled blisters about 18 h after exposure at different body regions of cases were noted. Bone marrow suppression is the most serious but less frequent acute effect of SM and two cases died in two weeks because of secondary bacterial infections due to bone marrow suppression. After the evacuation from the hot zone, all chemical casualties should be transferred to the designated intensive care units. After detailed medical decontamination and stabilization, biological samples should be collected from all chemical casualties as soon as possible for the verification of the exposure. Medical history and existing symptoms reveal the clinical diagnosis of SM exposure. Cases with suspicious SM exposure with minor symptoms should be observed at least 24 h because of typical symptom-free latency period. Medical management of SM casualties should focus on "good nursing care" including prophylactic short-course systemic antibiotic therapy, treatment of pain and itching, fluid and electrolyte replacement therapy, respiration and nutrition support. Despite of improved clinical skills, treatment of SM lesions is still non-specific which aims to relieve symptoms and to prevent infections. Treatment modalities of SM are still investigated. Clinicans who deal with victims of SM should share their field and clinical experiences on medical management of SM in scientific platforms because we need all available clinical information in order to improve our diagnostic capabilities and treatment approaches.

T31 • Alkylation of Methionine329 in human serum albumin by sulfur mustard

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Sulfur mustard (SM) is a banned chemical warfare agent which belongs to the class of vesicants. Exposure of SM may lead to various symptoms including erythema and blistering with delayed wound healing as well as respiratory problems and temporary blindness. Despite its prohibition by the chemical weapons convention, that is controlled by the Organisation for the Prohibition of Chemical Weapons (OPCW), SM still represents a serious threat for civilians and military personnel. As confirmed by the OPCW, SM was used in Syria and Iraq in 2015 by members of the Islamic state. Therefore, development of new analytical methods as well as a better understanding of the molecular toxicity of SM is still of high concern. On the molecular level, toxicity of SM is assumed to be based on the alkylation of endogenous bio-macromolecules like DNA and proteins. Thereby, the characteristic hydroxyethylthioethyl-(HETE-) residue is attached to these molecules. Protein adducts have been shown to be very suitable biomarkers to verify exposure to SM. Herein we present a new target for SM in human serum albumin (HSA), Met329. After the reaction of SM with the thioether in the Met329-sidechain and subsequent hydrolysis, a metastable HETE-methionyl sulfonium ion is formed. Proteolysis of SM-incubated HSA or human serum using pepsin gained the alkylated tetrapeptide LeuGluMet329(-HETE)Phe, LGM(-HETE)F, which was detected by microbore liquid chromatography-electrospray ionization high-resolution tandem-mass spectrometry. This novel marker showed only a marginal higher limit of identification (32.3 nM SM in serum) than the established markers HETE-Cys34Pro obtained after pronase cleavage or HETE-Cys34ProPhe resulted from cleavage with Proteinase K (both 15.6 nM SM in serum). *In vitro* stability of alkylated Met329 was shown to be limited to 5 days, which makes this modification a beneficial short-time marker. In addition, the transfer of the HETE-moiety from the methionyl sulfonium ion to the sidechains of Cys34 and Glu in HSA as well as to the N-terminus of the LGMF tetrapeptide was observed *in vitro* accordingly providing new insights into the molecular toxicity of SM.

T32 • Using precision cut lung slices to evaluate treatment strategies for nerve agents

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Background: Precision cut lung slices (PCLS) in combination with electric field stimulation (EFS) offers an *ex vivo* model in order to study mechanisms of airway contractions following nerve agent exposure and efficacy of respiratory treatment. By using EFS, a physiological release of the cholinergic neurotransmitter acetylcholine (ACh) can be achieved, enabling appropriate studies of anticholinergic treatment. The method has been established in rats by others for other purposes and our aim was to evaluate EFS in our mouse model.
Methods: PCLS from C57Bl/6 mice were prepared and exposed to EFS at a frequency of 50 Hz, a width of 1 ms and an output of 200 mA for 2.5 s at each minute. After establishing airway contractions, VX was applied to the lung slices (to a final concentration of 10 μM) and airway contractions were measured during ten minutes. Atropine in different concentrations was added and the airways were observed until the end of the experiment.
Results: EFS of PCLS caused a nerve-dependent release of ACh in physiologically relevant concentrations which gave rise to a weaker airway contraction than if exogenous ACh would have been used. Applying EFS to PCLS caused the airways to repeatedly contract to 90 % of the initial area. Exposure to VX further increased the contractions to ~83 % of the initial area. Addition of atropine counteracted the contractions completely.
Conclusion: By using EFS in the *ex vivo* PCLS mouse model, a robust method to evaluate anti-cholinergic treatment of VX poisoning was obtained, although the model is still in need of optimization and further characterization.

T33 • Brain perfusion and oxygenation as markers of physiological response to soman exposure in a rat model: a 9.4T MRI and fiber-optic oxygen sensor study

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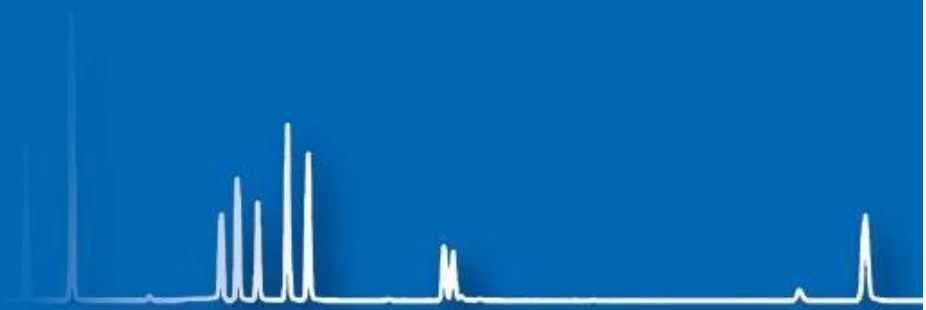
Introduction: Nerve agents (NAs) continue to be a threat to civilian and military personnel. Exposure to NAs can result in debilitating neurological damage through prolonged seizure activity. Excitotoxicity from nerve agent-induced seizures is believed to be the main contributor to the neuropathology. There is growing evidence within the areas of seizure and epilepsy research that suggest the involvement of hypoxia/ischemia-related neuropathology. The objective of this study was to use multimodal MRI perfusion and pO_2 quantification to determine if hypoxia/hypoperfusion are part of the pathophysiology following soman-induced seizures.

Methods: Male Sprague-Dawley rats (n = 6) were implanted with fiber-optic pO_2 probes at least one week prior to soman exposure. MRI was performed on isoflurane-anaesthetized rats in vivo using a 9.4T MRI Bruker Avance console and a 35 mm volume coil. Perfusion imaging was measured using continuous arterial spin labeling. Baseline perfusion images and pO_2 measurements were taken at a minimum of 24 hours prior to soman exposure. Rats were pretreated with HI-6 dimethanesulfonate (125 mg/kg) and atropine methyl nitrate (20 mg/kg), 20 minutes prior to soman exposure (90 μg/kg; subcutaneous). Additional doses of HI-6 and atropine were given at 20, 40 and 60 minutes post exposure. Perfusion and pO_2 measurements were acquired 60 minutes post soman exposure.

Results The perfusion map 1 hour after soman exposure shows restricted cerebral blood flow (CBF) throughout the brain. CBF decreased in the sensory cortex (70 %, $p < 0.001$), piriform cortex (64 %, $p < 0.001$), hippocampus (77 %, $p < 0.001$), and motor cortex (58 %, $p < 0.001$). There was no significant change in pO_2 1 hour after soman: baseline pO_2 was 52.4±10 mmHg and post-soman exposure was 52.1±15.1mmHg.

Conclusions In simultaneously acquiring cerebral blood flow and pO_2 after soman exposure, we have shown the potential uncoupling between cerebral metabolism and CBF. pO_2 is determined by the balance of oxygen delivery (CBF) and utilization (metabolism). In measuring pO_2 and CBF, metabolism was indirectly determined. The reduced CBF and constant pO_2 following soman exposure in isoflurane-anaesthetized rats likely indicates a decrease in metabolism. This is potentially the result of cellular damage following soman exposure resulting in the inability of cells to use available oxygen.



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