20th World Congress of the International Society on Toxinology

"Toxinology in the 21st century: Public health impact from basic, translational and clinical sciences"

CONCURRENT SESSION ABSTRACTS

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Concurrent Session I

1A. Public Health and Toxinology.

Chairs: José M. Gutiérrez/Fan Hui Wen

1. Abdulrazaq Habib (Bayero University, Nigeria): Burden of Snakebite and Antivenom Supply Challenges in Africa. abdulrazaq_habib@yahoo.co.uk
2. Mohammad Afzal Mahmood: A framework for shifting the paradigm and developing coalitions to address neglected public health problems: Lessons from the Myanmar Snakebite Project. afzal.mahmood@adelaide.edu.au
3. Fan Hui Wen: (Instituto Butantan, Brazil): Public health policies to better manage the burden of scorpion sting envenoming. fan.hui@butantan.gov.br
4. Ymkje Stienstra (University of Groningen, the Netherlands): The neglected tropical disease Buruli ulcer; time to team up. y.stienstra@umcg.nl

1. Burden of Snakebite and Antivenom Supply Challenges in Africa

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Snakebite envenoming (SBE) is an important cause of mortality and morbidity in agricultural communities in Sub-Saharan Africa (SSA). Every year millions of people in SSA face death, disability and disadvantage from SBE without access to appropriate treatment. SBE is associated with economic privations and impoverishment associated with low GDP, Human Development Index and healthcare expenditure. To the individual patient debilitating financial toll is further exerted from both direct costs of treatment and indirect costs from lost income further perpetuating deprivation. Despite an annual burden estimated at over 1 million DALYs, substantially higher than for many Neglected Tropical Diseases (NTDs), resource allocation has been grossly inadequate. Antivenom remains the main therapy and has been shown to be highly cost-effective in SSA. A multitude of financial and commercial factors helped to cause, and now perpetuate, shortages of high quality, affordable and region-appropriate antivenom in areas where they are most needed. Components in the security of supply pose peculiar challenges to access ranging from affordability, capacity to adapt to market changes, diversity of supply, equity, expenditure, infrastructure, production, stability of prices, stability of exporting and importing countries to vulnerability to disruption. The recently launched roadmap for SBE strategy for prevention and control by WHO seeks to halve the burden by 2030. It utilizes multifaceted approaches involving phased-in antivenom stockpiling, hoping to make available 3 million antivenom treatments annually at full roll-out by 2030. Additionally, for long-term sustainability countries in SSA should be galvanized to support investment in local antivenom manufacturing as well as health systems and capacity strengthening.

2. A framework for shifting the paradigm and developing coalitions to address neglected public health problems: Lessons from the Myanmar Snakebite Project

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Neglected tropical diseases affect more than a billion poverty stricken people, causing a huge burden of disease, disability and death, and further entrenching destitution. Snakebite is among the most neglected and burdensome. This paper develops a theoretical foundation and practical approaches to address fundamental public health concerns, drawing on research and development processes employed in the Myanmar Snakebite Project (MSP). Informed coalitions are required, as neglect occurs when everyone thinks someone else may look after the victim. Neglect oppresses people further, from which they could be liberated through the process of 'getting together'. 'Getting together' for collective action may involve outside help but must be led by those who are affected, i.e. victims of snakebite, local healthcare workers and local health departments that, within the context of resource scarcity and a global neglect, find little support for their efforts. To initiate collective action commanded by locals, the MSP was founded on ideas suggested by local political leaders, the local public-sector antivenom producer, and representatives of the local public sector healthcare system. This principle was further emphasised through community's needs assessment and participatory approaches. To prevent silo-ism and fragmented approach, the initiative involved stakeholders, from ministers responsible for policy to community members, from antivenom producers to primary care staff, and from herpetologists, toxinologists and nephrologists to traditional healers. To incorporate local perspectives on policy and action, community women and men, snakebite victims, and health care providers across the villages were engaged as partners. To promote equity, community-based research and consultative processes were utilised, in addition to advocacy for sufficient antivenom production and distribution, and policy for village-based staff to be able to use antivenom. MSP provides a framework which could be beneficial for other neglected tropical diseases and for public health in general.

3. Public Health policies to better manage the burden of scorpion sting envenoming

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The public health relevance of scorpion sting envenoming in Brazil can be ascertained by the high frequency of cases, which increased from 37,367 to 123,964, from 2007 to 2017, with the incidence rates, which varied from 19 to 60 cases/100,000 pop. Such increase is more evident in the Northeastern region, where *Tityus stigmurus* is the predominant species, especially in the peripheral metropolitan areas, and associated with poor sanitary conditions and low economic and social indicators. More recently, São Paulo, the most developed State in Brazil, has also presented high figures, jumping from 738 cases (19% of all cases of envenoming reported in 1988) to 21,711 (61%) in 2017. *T. serrulatus* became the most predominant species in the Southeastern region and has been spreading to other parts of the national territory, representing 96% of all *Tityus* spp identified at Instituto Butantan, while *T. bahiensis* corresponded to only 3%, or 34 *T. serrulatus* for each *T. bahiensis*. Although case-fatality rate remained very low, the number of deaths doubled from 61 to 127 in the last decade-period, with a case-fatality of 0.3% in children under 14 yrs-old, 4 times higher than for other age-group patients, mostly associated with *T. serrulatus*. The greatest impact of the scorpion sting envenoming for the health systems lies in the predominance of fatal cases in children, usually in the first 3 hours after sting, and the failure in early antivenom and supportive treatment. Multidisciplinary studies analyzing biological aspects of the causative agent, climate factors, and social economic indicators are needed to fully understand the determinants for the increase of scorpion population in urban zones and the impacts of scorpion sting envenoming for the health systems.

4. The neglected tropical disease Buruli ulcer; time to team up

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Buruli ulcer is a neglected tropical disease caused by a toxin produced by *Mycobacterium ulcerans*. The infection leads to ulcers, especially on the limbs. Mortality in Buruli ulcer is low, but morbidity is high. Patients with Buruli ulcer need antimicrobial treatment and wound care. The long term burden mainly consists of permanent disabilities caused by contractures, stigma and subsequent socioeconomic problems. In this presentation I will talk about the Buruli ulcer research agenda and its challenges. I will relate these challenges to the priorities in snakebite envenoming research.

Concurrent Session I

1B. New Developments in Basic Toxinology I.

Chairs: Juan J. Calvete/ Sulan Luo

1. Vincent Viala: Long reads DNA sequencing in genomics and venom gland transcriptomics. vincent.viala@butantan.gov.br
2. Aida Verdes: Venom without glands: Novel methods to investigate toxin diversity, function and evolution in ribbon worms (Nemertea) aida.verdes@gmail.com
3. María Ikonomopoulou: The antiproliferative profile of a linear octopus-derived peptide in melanoma of BRAF-mutation. maria.ikonomopoulou@imdea.org
4. Denis Servent: Pinnatoxins, an emergent class of marine toxins interacting with nAChRs. Pharmacological characterization, biodistribution and musculo-skeletal effect of these neurotoxic agents. denis.servent@cea.fr
5. Sulan Luo: Preclinical Research of Analgesic αO-Conotoxin GeXIVA Without Addiction Side Effect. Sulan2017@qq.com
6. Alexander A. Vassilevski: P2X3 receptor antagonists from spider venom. avas@ibch.ru

1. Long reads DNA sequencing in genomics and venom gland transcriptomics

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Knowledge about the genomic organization of toxin genes is fundamental to better understand venom variation and evolution. State-of-the-art sequencing technologies, such as Illumina, generate high throughput short-reads data that allows to produce *de novo* draft genomes, as for the *Bothrops jararaca* (Serpentes:Viperidae) genome. However, it failed to generate long contiguous sequences at some multigenic toxin family *loci*. The high similarity between paralogue genes and the repetitive content of some genomic regions might be puzzling the genomic assemblers resulting in fragmented data independently from the sequencing depth. Long-reads sequencing technologies, also known as the 3rd generation of DNA sequencing, have shown the ability to overcome the issue by generating reads (> 30 kb) that span long repetitive regions. The same concept applies to transcriptomic analysis: data from short-reads can generate false-positive chimeric transcripts (i.e. mixture of sequences from distinct real mRNAs) which is the case for several multigenic toxin families. Long-reads can overcome this issue by sequencing full-length transcripts and excluding the step of assembly. In this presentation, I discuss the benefits and limitations of DNA long-reads sequencing technologies applied to the field of Toxinology. The discussion is supported with experimental data generated from the MinION sequencer of Bacterial Artificial Clones (BAC) containing ~200 kb regions of the *Bothrops jararaca* genome encoding for Snake Venom Metalloproteases (SVMP). The MinION technology generated ultra-long reads that allowed the assembly of 11 full BACs and the identification of 28 full SVMP genes. The data evidence that SVMP genes are organized in tandem clusters. 3rd generation sequencing technologies have proven to generate reads long enough (> 30 kb) to increase assembly statistics and to obtain full-length transcripts. Long-reads technologies have just emerged and some technical limitations will be quickly overcome turning it into a basic toolbox for molecular biology research.

2. Venom without glands: Novel methods to investigate toxin diversity, function and evolution in ribbon worms (Nemertea)

Aida Verdes

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Nemerteans, also known as ribbon worms, are active predators characterized by an eversible proboscis used to administer venom and subdue prey. There are approximately 1,300 species, mostly marine, that belong to three lineages (Palaeonemertea, Pilidiophora and Hoplonemertea) with important differences in the proboscis morphology. Hoplonemerteans have a calcareous stylet used to stab prey and possibly inject toxins, whereas palaeonemerteans and pilidiophorans contain rod-shaped structures that might puncture prey facilitating envenomation. Investigating nemertean venom is specially challenging because they don't have distinct multicellular glands, instead, toxins are produced by secretory cells lining the body wall and the proboscis epithelium, hypothesized to have defensive and predatory roles respectively. As a consequence, there is no empirical evidence supporting these hypotheses and our knowledge on nemertean venom is very limited. To overcome these obstacles, we implemented an RNA-Seq differential gene expression (DGE) analysis to identify putative toxin genes differentially expressed in the proboscis and the body wall, and performed the first proteotranscroptomic profiling of an hoplonemertean, the species *Antarctonemertes valida*. We identified numerous putative toxins, some of them differentially expressed in the body wall, and recovered parborlysin homologs, previously only known from two heteronemertean species. We also tested the toxic mucus of the heteronemertean *Lineus longissimus* for anticancer activity. Preliminary analyses show the mucus effectively reduced the viability of two melanoma cell lines whereas it had no deleterious effects on healthy fibroblasts. Our studies indicate there is a hidden diversity of nemertean toxins and suggest that ribbon worm venoms are an untapped source of novel bioactive compounds with biomedical potential. We discuss future steps to elucidate venom delivery mechanisms and sources of toxin production and storage, illustrating the value of nemerteans as model organisms to investigate poorly-known, challenging venom systems, such as those without distinct venom glands or where venom cannot be milked.

3. The antiproliferative profile of a linear octopus-derived peptide in melanoma of BRAF-mutation

Manuel Alejandro Fernandez-Rojo, Andreas Brust, Jeremy Potriquet, Joshua Daley, Lotten Ragnarsson, Asa Andersson, Pamela Mukhopadhyay, Patrick Wilhelm, Yanni Chin, Taylor Smallwood, Richard Clark, Glenn King, Grant Ramm, Nic Waddell, Richard Lewis, Glen Boyle, Paul Alewood, Jason Mulvenna, John Miles, Maria Ikonomopoulou

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Over 50% of melanoma patients bear BRAF mutation, prompting us to identify novel compounds with therapeutic applications against it. Here, we describe the antiproliferative profile of an octopus-derived peptide (MI1) in melanoma BRAF-mutated cells and in melanoma xenograft tumours. MI1 reduced the viability of melanoma BRAF-mutated cells of approximately 40% and had minimum effect on the neonatal foreskin fibroblasts. This was followed by an increase in reactive oxygen species while no change was observed on the mitochondrial membrane potential or cell cycle phases in melanoma cells. Interestingly, MI1 did not lead to apoptosis after 48 h. Due to the high homology of MI1 with tachykinin peptides, we examined its relationship with neurokinin receptors, but we found no obvious link with its antiproliferative capacity. Hence, to enlighten further on the mode of action of MI1 in melanoma cells, we performed in parallel proteomics and RNAseq analysis during a time-course. PI3K/AKT/mTOR pathway, a major driver of metabolism and cell proliferation, was one of the main affected signalling cascades, which was validated further by RNAseq and western-blotting. RNAseq also revealed alterations in other cancer, metabolic and immune-related pathways. *In vivo*, MI1 reduced the progression of melanoma-xenograft mouse tumours in comparison to the vehicle-treated mice group. In conclusion, our preliminary results suggest that the antiproliferative profile of MI1 in melanoma of BRAF-mutation could be regulated by metabolic changes.

4. Pinnatoxins, an emergent class of marine toxins interacting with nAChRs. Pharmacological characterization, biodistribution and musculo-skeletal effect of these neurotoxic agents

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Cyclic imines produced by marine dinoflagellate microorganisms, constitute a widely distributed group of phycotoxins with increasing prevalence in oceanic environment due to recent climate change. During active dinoflagellate blooms, phycotoxins may accumulate in shellfish tissues and can be transferred into fish, marine mammals and ultimately to humans. Among these phycotoxins, pinnatoxins (PnTx-A to H), produced by the dinoflagellate *Vulcanodinium rugosum*, were recently identified and shown to exhibit the highest oral acute mouse toxicity among cyclic imine toxins. We will describe binding and electrophysiological experiments highlighting the exceptional ability of these toxins to interact with various subtypes of nicotinic receptors (nAChRs), especially the muscle and neuronal (α7) subtypes. Biodistribution analyses by digital radioimaging in rats, after oral or i.v administration of tritiated PnTx-G, revealed the presence of the toxin in various peripheral organs, as well as in the central nervous system, highlighting its property to cross both the intestinal and the blood-brain barriers. Moreover, using the chick embryo as a first model, we show that during embryonic development, PnTx-A exposure reduces embryo motility by decreasing the embryo spontaneous movements which affected the maturation of the musculoskeletal system.

5. Preclinical Research of Analgesic αO-Conotoxin GeXIVA Without Addiction Side Effect

Dongting Zhangsun, Sulan Luo

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Neuropathic pain (Chronic pain) is a major public health problem and costly, which is affecting about 20% of the world general population. Chemotherapy-induced peripheral neuropathy (CIPN) is an adverse effect of many chemotherapeutic agents, which is a main cause of ongoing pain in cancer patients. Antagonists of the α9α10 nicotinic acetylcholine receptor (nAChR) subtype are of interest as pharmacological tools and potential therapeutics for neuropathic pain, as well as breast and lung cancers etc. αO-conotoxin GeXIVA is a potent inhibitor of the α9α10 nAChR and has been shown to be analgesic in the rat chronic constriction injury (CCI) animal models of neuropathic pain. GeXIVA is a 28-amino acid peptide. It has four cysteines which are able to form three isomers, which showed similar low IC50 values against both rat and human α9α10 nAChRs. Preclinical research of αO-conotoxin GeXIVA[1,2] (bead isomer) pilot product in large scale has being undergone currently. Stabilities of GeXIVA[1,2] in various conditions of serum, enzyme, thiol and forced degradation environment were evaluated systematically. Oxaliplatin-induced neuropathic pain (OINP) model in rats was constructed. GeXIVA[1,2] was administered through single or repeated intramuscular (IM) injections in rat OINP model. GeXIVA[1,2] can relieve and reverse oxaliplatin-induced mechanical and cold allodynia in OINP rats, which produced a cumulative analgesic effect without tolerance and promoted recovery from neuropathic pain. The long lasting analgesic effect of GeXIVA[1,2] on mechanical allodynia was shown, which continued until day 10 after the termination of the 16-day repeated treatment procedure. These results suggest that GeXIVA[1,2] would be a new promising drug for neuropathic pain medication.

6. P2X3 receptor antagonists from spider venom

Alexander Vassilevski

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P2X3 receptor is a validated molecular target in a number of syndromes, such as pain in arthritis or cystitis, trigeminal neuralgia or bone cancer. A few selective blockers of P2X3 receptors are known today, and most of them suffer from significant limitations in terms of drug development. I will describe two families of peptides from spider venom that affect specifically P2X3 receptors. One family contains purotoxins 1 and 6 (PT1 and PT6) from *Alopecosa marikovskyi* and *Thomisus onustus*. They are comparatively small peptides (35 and 31 amino acid residues respectively) conforming to the inhibitor cystine knot (ICK) motif. In contrast, PT2 from *A. marikovskyi* is larger (64 residues) and in addition to an N-terminal ICK domain contains a C-terminal linear domain with membrane-active properties. Purotoxins inhibit P2X3 receptors (IC_{50}~10 nM) by trapping them in the desensitized state and show no activity on P2X2 or P2X2/3 channels. PT6 properties were studied in greater detail. This peptide is resistant to proteases and heating and stable in human blood plasma. Subcutaneous administration of comparatively low doses of PT6 (0.1 mg/kg) into mice produces pronounced analgesia in CFA-induced inflammatory pain, acetic acid-induced writhing and formalin tests, surpassing that of conventional analgesics such as diclofenac. Moreover, PT6 restores grip strength in mice with inflamed joints modeling arthritis pain. ^{125}I-labeled PT6 was produced and used in ADME studies in mice. The peptide is cleared from the circulation via renal filtration and no accumulation in organs after multiple dosing is noted. Importantly, due to the P2X3 selectivity, PT6 shows no dysgeusia or ageusia effects in mice. PT6 is an attractive hit with potent analgesic effect

Concurrent Session II

2A. New Developments in Antivenoms.

Chairs: A. Alagón/A. de Roodt

1. Phillippe Billiald: Loxoscelism: Advances and challenges in the design of antibody fragments with therapeutic potential. philippe.billiald@u-psud.fr
2. Clara Guerra Duarte: Multi-epitope based immunogens applied to antivenom production. claragd@gmail.com
3. Fernando Goldbaum: Development by protein engineering of NEAST (neutralizing Equine Anti-Shiga Toxin): A new product to prevent the onset of Hemolytic Uremic Syndrome in STEC infected patients. fagoldbaum@gmail.com
4. Baltazar Becerril : Generation of a recombinant antivenom against scorpion stings in Mexico. baltazar@ibt.unam.mx
5. Jordan Benjamin: Bedside Perspective to Antivenom Policy: Practical Considerations for Selecting Antivenoms based on Regional Challenges. jordan@snakebitefoundation.org

1. Loxoscelism: Advances and challenges in the design of antibody fragments with therapeutic potential

Phillippe Billiald

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Envenoming due to *Loxosceles* spider bites still remain a neglected disease with a major impact on public health in temperate and tropical regions of North, Central and South Americas. In Brazil, *L. intermedia*, *L. gaucho* and *L. laeta* are of particular medical concern with more than 25,000 cases in 2015, of which 30 were fatal. It is commonly accepted that proteins of 30-35 kDa with sphingomyelinase D enzymatic activity play a key role in the process causing two main clinical manifestations: cutaneous (erythema, dermonecrosis) and cutaneous-visceral symptoms (hemolysis, renal failure). In this way, we can anticipate that recombinant therapeutic monoclonal antibodies could provide a reliable alternative to the only effective specific treatment which is associated to side effects and drawbacks including low specific activity.

Here, starting from a mouse monoclonal antibody that targets rliD1, cross-reacts with some lower-MW components of the venom and neutralizes its dermonecrotic activity in animal models, we designed humanized antibody V-domains which were produced and purified as recombinant antibody fragments of various sizes and valences (scFv, diabody, minibody). These molecules were characterized in terms of hummanness, structural stability, antigen-binding activity and capacity of venom-neutralization. During the process, we clearly identified some blocking points that can impact the antigen-binding activity and neutralizing capacity. *In silico* modelling of the antigen/antibody interaction also allowed a better understanding of the neutralization mechanism and led us to reformat the humanized antibody fragment which, in the end, recovered the functional characteristics required for efficient *in vitro* neutralization of the venom.

This talk will also focus on pitfalls and points to anticipate prior to the pharmaceutical development of recombinant antibody fragments with therapeutic potential.

2. Multi-epitope based immunogens applied to antivenom production

Clara Guerra Duarte

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Venom availability is a bottleneck in antivenom production, especially for arachnid's and coral snake's venoms. Various approaches to substitute these venoms in antivenom production have been attempted, including the use of synthetic or recombinant epitopes. The combination of multiple epitopes from the most important toxins within a given venom showed to be a promising strategy to improve previous results in inducing neutralizing antibodies. As proof-of-concept, this approach has been employed for coral snakes and brown spider experimental antivenom development. For coral antivenom production, nine epitopes derived from five toxins from *Micrurus corallinus* venom were mapped, synthetized as soluble peptides and used to immunize rabbits. Anti-peptides antibodies neutralized phospholipase A2 and lethal activities of *M. corallinus* venom. When combined with priming doses of *Micrurus frontalis* venom, these peptides were even more efficient in eliciting neutralizing antibodies, that also cross-reacted with other *Micrurus* venoms. For *Loxosceles intermedia* venom, various epitopes mapped from the dermonecrotic toxin LiD1 combined in a single multi-epitopic recombinant protein were able to induce dermonecrotic and hemorrhagic protection against *L. intermedia* venom. When used together with crude venoms from three *Loxosceles* species for horse immunization, the multi-epitopic protein achieved neutralization parameters comparable to that obtained with venom exclusively, reducing by 67% the need of using crude venom for antivenom production. Further addition of epitopes from other relevant *Loxosceles* toxins to the multiepitopic recombinant protein elicited antibodies that can efficiently neutralize sphingomyelinase, hyaluronidase, and metalloproteinase activities of *Loxosceles* venoms. The use of multiepitopic immunogens seems to be an efficient strategy to achieve venom neutralization, with the extra benefit of being a non-toxic, safer immunogen concerning animal welfare in antivenom production. In addition, it has flexibility to be produced in large scale and can be modified to incorporate new insights in venom toxicity and epitope refining.

3. Development by protein engineering of NEAST (neutralizing Equine Anti-Shiga Toxin): A new product to prevent the onset of Hemolytic Uremic Syndrome in STEC infected patients

Fernando Goldbaum

Inmunova SA

Shiga toxin-producing Escherichia coli (STEC) are major food-borne pathogens worldwide. STEC causes 2,800,000 acute illnesses annually and leads to 53,000 cases of bloody diarrhea, where between 10-15% will develop the life-threatening hemolytic-uremic syndrome (HUS), mostly in children. HUS is an Orphan Disease with death rates of 3-5%. Around 40% of HUS patients will develop long-term sequelae. There are currently no specific therapeutic options on the market, thus leaving an unmet need for disease modifying therapies. HUS is more a toxemia than an infectious disease. One of the biggest challenges is to develop an effective and safe treatment with anti Shiga toxin (Stx) neutralizing capacity in order to block the onset of HUS in patients infected with STEC. Inmunova's core technology is a proprietary platform, called IMC® (Immuno Multi Carrier), which allows the design of state-of-the-art recombinant immunomodulators. Applying IMC® advantages, we engineered a new immunogen by inserting StxB to the IMC® platform. The resulting chimera (IMC®-Stx) demonstrated a strong capacity to induce long-lasting humoral immune responses with high neutralizing capacity for different Stx's variants. We took advantage of the strong immunogenic capacity of IMC®-Stx chimera to develop horse hyperimmune globulins (F(ab')2 fraction) for the treatment of STEC. The product called NEAST (Neutralizing Equine Anti-Shiga toxin), has successfully passed preclinical and phase I studies showing that this strategy works and the product has an excellent safety profile. Phase II/III clinical trials are ongoing in Argentina in around ten hospitals distributed across the country. We are recruiting up to 400 children with bloody diarrhea and presence of Stx2 in feces. The main objective of the trial is to prove the efficacy of NEAST to prevent the onset of HUS in STEC-infected patients. Orphan Drug Designation for NEAST was recently granted by EMA. Fernando Goldbaum: Development by protein engineering of NEAST (neutralizing Equine Anti-Shiga Toxin): A new product to prevent the onset of Hemolytic Uremic Syndrome in STEC infected patients

4. Generation of a recombinant antivenom against scorpion stings in Mexico

Baltazar Becerril, Lidia Riaño, Lourival Domingos Possani

Academic Leader. Department of Molecular Medicine and Bio-processes. Institute of Biotechnology. National Autonomous University of Mexico
Professor from the National Research Council (CONACYT) collaborating in my group

The recombinant antibody fragments generated against toxic components of scorpion venoms are considered a promising alternative for obtaining new antivenoms for therapy. In the World, Mexican population is one of the most affected by scorpion stings with an average of 300,000 accidents per year. Using phage display, directed evolution and site-directed mutagenesis, we have generated several human single-chain antibody fragments (scFv) with restricted or broad cross-reactivity against scorpion toxins. Depending on the complexity of the venoms, (number of medically important components), one or more of these scFvs are capable of neutralizing the whole venom. We have optimized two scFvs which are able to neutralize the vemoms of 9 out of the 21 species considered medically important in Mexico. In addition, we have confirmed the venom toxicity of 14 of the 21 different species known to exist in the country. Presently, we are optimizing a third scFv which will help to increase the number of neutralized venoms. Finally, we are generating another set of broadly neutralizing scFvs with the ideal purpose of formulating an antivenom with two independent antibodies against each medically important toxin.

These endeavors represent a very important advance for the modernization of current antivenoms. Due to the lower molecular size of scFv format and their high affinity towards toxins, it is expected the need of smaller amounts of protein to be required for achieving a total neutralization of the venoms. On the other hand, this new antivenom would have a minimum immunogenic character. Additionally, it would show a faster distribution in the body and a complete renal elimination of the toxins bound to the scFvs. Equally important is the fact that this scFv anti-venom would be fully characterized at a molecular and functional levels. Finally, these strategies would eliminate the use of animals for the production of anti-venoms.

5. Bedside Perspective to Antivenom Policy: Practical Considerations for Selecting Antivenoms based on Regional Challenges

Jordan Max Benjamin, Nicklaus Brandehoff, Benjamin Norman Abo, Kate Jackson, Jean-Philippe Chippaux

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Introduction: Snakebite envenomation is a neglected tropical disease that kills or maims over 500,000 people worldwide every year. The recent World Health Organization (WHO) global strategy listed antivenom as the first choice for treatment of systemic envenomation, but quality antivenoms may be unavailable in rural areas with the greatest need. This checklist provides relevant stakeholders with key criteria to consider in selecting the most appropriate antivenoms for the unique challenges of snakebite treatment in a given region.

Methods: A standard checklist should ensure that efficacy, safety, quality, affordability, and accessibility are considered in the context of the region and the bedside perspective. First, is the product registered for use in the country and is it designed to treat envenomations from local venomous species? An antivenom should directly neutralize venoms from the species of greatest medical and epidemiological importance in the region as listed in the WHO guidelines. If the correct species are covered, is there clinical evidence demonstrating that the product is safe and effective under realistically challenging field conditions? The safety profile is directly linked to the manufacturing process; the amount of proteins, type of immunoglobulin (whole IgG, Fab or F(ab')2 fragments), purification level and use of preservatives should be considered. Affordability should be evaluated not only as cost per vial but also the number of vials recommended for initial dosing and total cost of a treatment course. Lastly, antivenom formulation is often a key determinant especially in tropical regions where highly-purified, lyophilized antivenoms do not need cold chain distribution or storage.

Conclusion: This practical checklist is intended to help health care providers and policy makers choose the most appropriate antivenoms for their regions. Once an antivenom has been selected and deployed, clinical practice and periodic reassessment should complete the evaluation and confirm that it performs as expected.

Concurrent Session II

2B. New Developments in Basic Toxinology II.

Chairs: Juan J. Calvete/Sulan Luo

1. Eivind A. B. Undheim: New mass spectrometry-based tools for unravelling toxin function and evolution. e.undheim@uq.edu.au
2. Jeroen Kool: Picofractionation & MS imaging: Analytics for pathology profiling of venoms. j.kool@vu.nl
3. Daniel Petras: Large scale top-down venomics – A bird’s-eye view of genus wide venom composition. dpetras@ucsd.edu
4. Bruno Lomonte: Lys49 myotoxins: emerging insights into their modes of action. bruno.lomonte@ucr.ac.cr
5. Elda Sanchez: A Myotoxin From The Venom Of Crotalus oreganus helleri: Its Role In Snake Envenoming. Elda.Sanchez@tamuk.edu
6. Tim Luddecke: Recent advances in Salamandra skin poison research. tim.lueddecke@outlook.com
7. David Salazar-Valenzuela, An evolutionary framework for venom variation patterns in terciopelo pitvipers (Bothrops asper), a model organism in toxinology davidsalazarv@gmail.com

1. New mass spectrometry-based tools for unravelling toxin function and evolution

Eivind A. B. Undheim

Centre for Biodiversity Dynamics, Department of Biology, Norwegian University of Science and Technology, Norway
Centre for Ecology and Evolutionary Synthesis, Department of Biosciences, University of Oslo, Norway
Centre for Advanced Imaging, The University of Queensland, Australia

Despite decades of research, we still have a poor understanding of the functional biology of most toxins in animal venoms. Part of this poor understanding stems from a lack of knowledge about the way venoms are produced and stored in the venom gland and associated structures, which is in large part due to the limited molecular information that is gained by targeted imaging approaches. In contrast to these methods, mass spectrometry imaging (MSI) allows non-targeted simultaneous detection of numerous compounds directly off tissue sections. It is thus an ideal technique for investigating the spatial distributions of toxins and other venom components across venom producing tissues, which are in turn excellent model systems for development of new MSI-based methods with novel biomedical applications. In combination with “traditional” venomics techniques, these new mass spectrometry-based tools have shed new light on the functional biology of venoms and their toxins. This has revealed a surprising spatial heterogeneity of toxin production in a range of venomous animals, and helped us put the evolution of venoms and toxins into both a functional and morphological context.

2. Picofractionation & MS imaging: Analytics for pathology profiling of venoms

Jeroen Kool

AIMMS Division of BioMolecular Analysis, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands

Venoms need to be researched in order to better understand envenoming for development of next-generation snakebite treatments. Next to this, the number of protein-based pharmaceuticals is rapidly increasing and venoms are interesting sources for finding new pharmaceutical lead molecules. Venoms are often only available in low amounts, are very complex, and the bioactives often are present in a low concentration. Unraveling this complexity required advanced analytics and bioassays. Capillary chromatography separations are ideal techniques for venom separation prior to bioassaying. To hyphenate capillary separations with miniaturized bioassays however comprises a major technical difficulty.

We recently developed so called picofractionation analytics in which nanoLC is post-column connected to a piëzo-controlled nanoliter droplet spotter. After nanoLC, eluent is spotted onto microarray glass slides followed by printing a microarray bioassay of choice on top of the eluent droplets. The spotted microarray bioassay is then placed in an in-house developed fluorescence microscope array reader, which acquires data on all bioassay droplets. Finally, reconstructed bioactivity chromatograms are plotted and then compared with parallel obtained nano-ESI-MS data for direct identification of bioactives.

This presentation will end with introducing our latest research effort: “Tissue physiology and venom toxin distribution in the envenomed mouse”, which deals with *in vivo* venom distribution after envenoming and organ specific localization and (cyto)toxicity profiling of individual venom components from crude venoms, measured by MALDI-MS-Imaging and parallel H/E-organ-cellular-tissue stains.

3. Large scale top-down venomics - A bird's-eye view of genus wide venom composition

Daniel Petras

University of California - San Diego, Skaggs School of Pharmacy & Pharmaceutical Sciences, La Jolla, USA

To understand, fight and utilize the molecular mechanism of venoms, a detailed knowledge of their composition is needed. Besides next generation sequencing, which provides the fundamental architecture of venom peptides and proteins, several bottom-up proteomics strategies are available that enable the measurement of actual toxin expression and post-translational modifications. However, most of these methods can suffer from toxin co-elution during pre-fractionation, limited quantification capabilities as well as long analysis times that often limit the analysis of statistical relevant numbers of individuals. Additionally, the proteolytic digestion causes an insuperable obstacle to differentiate closely-related toxin proteoforms. In the last decade, high-resolution mass spectrometry as well as snake genome and transcriptome databases have become increasingly available, paving the way for top-down protein analysis. To complement potential limits of current bottom-up venomics approaches, we adapted a top-down venomic workflow and showed its feasibility in a case study on King Cobra venom. Since then, we used this method for the routine analysis of a multitude of elapid and viper venom proteomes. In addition to a scalable data acquisition routine, we implemented different software tool for improved protein spectrum matching as well as multivariate statistical approaches, in order to make full use of high-throughput top-down venomics. In this presentation I will highlight the basic methodology as well as recent developments and applications of our pipeline for the genus scale analysis of mamba and cobra venoms, showcasing the tremendous potential of top-down approaches for the large scale analysis of venom proteomes.

4. Lys49 myotoxins: emerging insights into their modes of action

Bruno Lomonte

Instituto Clodomiro Picado, Facultad de Microbiología, Universidad de Costa Rica, San José, Costa Rica

Since the discovery of the first Lys49 myotoxin, from the venom of Agkistrodon p. piscivorus, more than 80 proteins of this kind have been reported from many viperids. The Lys49 myotoxins possess a conserved group IIA phospholipase A2 (PLA2) structural scaffold, but are devoid of phospholipolytic activity. Hence, they have been referred to as "PLA2 homologues" or "PLA2-like" proteins. Thus far, all share the property of inducing skeletal muscle necrosis locally, albeit not systemically. In addition to myotoxicity, a wide variety of bioactivities have been observed for these proteins in diverse experimental setups.

The ability of Lys49 myotoxins to induce rapid necrosis of skeletal muscle fibers in vivo, as well as cytolysis of diverse cell types in vitro, has been mapped to their C-terminal region, encompassing cationic and hydrophobic amino acids. Recent advances on the crystallographic structures of Lys49 myotoxins support the proposal of a multi-step model for their action, which involves allosteric activation and the participation of a "membrane-docking" and a "membrane-disrupting" site. However, several fundamental aspects of the overall mechanism of myotoxicity remain poorly understood. Identification of functional cellular targets for Lys49 myotoxins has represented a major challenge, but recently cell surface nucleolin has been demonstrated to play a role in the toxic mechanism. Internalization of Lys49 myotoxins, and the possibility of exerting actions on organelles, is another important aspect only recently being addressed. Likewise, the concerted action of Lys49 myotoxins together with other co-existing venom components has now disclosed phenomena that are both intriguing and revealing, in the light of the tissue pathology induced by whole venoms rather than by isolated toxins. Experimental studies addressing some of these aspects will be discussed. A more complete understanding of mechanisms underlying the toxicity of Lys49 proteins may have useful implications for developing inhibitors or therapeutic strategies against snakebite.

5. A Myotoxin From The Venom Of Crotalus oreganus helleri: Its Role In Snake Envenoming

Elda Eliza Sanchez, Emelyn Salazar Castillo, Walter Cromer, Montamas Suntravat, Jacob Galan, Alexis Rodriguez-Acosta, Hector Finol

Texas A&M University-Kingsville
Texas A&M Health Science Center
Universidad Central de Venezuela

Crotamine and crotamine-like peptides are non-enzymatic polypeptides, belonging to the family of myotoxins, which are found in high concentration in the venom of the genus Crotalus. Helleramine was isolated and purified from the venom of the Southern Pacific rattlesnake, *Crotalus oreganus helleri*. This peptide had a similar, but unique, homology to crotamine and crotamine-like proteins isolated from other rattlesnakes species. The variability of crotamine-like protein amino acid sequences may allow for different biological targets or optimization to the same target of different prey. Helleramine was capable of increasing intracellular Ca2+ in Chinese Hamster Ovary (CHO) cell line. It inhibited cell proliferation as well as cell viability (IC50 = 11.60 μM) of C2C12, immortalized skeletal myoblasts in a concentration-dependent manner, and promoted early apoptosis and cell death under our experimental conditions. Skeletal muscle harvested from mice 24 h after helleramine injection showed contracted myofibrils and profound vacuolization under the subsarcolemmal that enlarged the subsarcolemmal space; a loss of plasma and basal membrane integrity was observed. Hellaramine also targets lymphatic tissue, providing further insights and evidence of crotamine-like peptides' important role in the pathophysiology of snake envenomation.

6. Recent advances in *Salamandra* skin poison research

Tim Lüddecke

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True salamanders of the genus *Salamandra* comprise some of the most charismatic amphibians on earth, such as the common fire salamander (*Salamandra salamandra*) or the alpine salamander (*Salamandra atra*). That members of this genus are poisonous is known since antiquity. Salamanders were as a consequence not only connected to witchcraft and folklore but were further among the first species to be studied when natural compound chemistry started to discover nature 200 years ago. Although the research on *Salamandra* poison was a fruitful subject, the field was mostly on hold since the 1980s. Nevertheless, it was recently re-evoked by the emergence of the lethal salamander plague. Here we report on a variety of novel findings around the biology of *Salamandra* poison that we acquired recently from a plethora of studies using the common fire salamander as a model system. We describe how new instrumental technological approaches for bioprospecting were developed and present two newly discovered alkaloid toxins from fire salamander secretions. We studied the metamorphosis of the species and were able to determine the developmental origin of toxicity. In a series of experimental approaches we were able to test the signal honesty of fire salamander aposematism and found no quantitative correlation between toxicity and coloration, neither in terms of hue or brightness nor with proportion of yellow. However our data suggests that the fire salamander generally is conspicuous to its predators and thus should be considered qualitatively aposematic. Interestingly, the coloration instead with toxicity rather correlates with sex, suggesting that intraspecific mechanisms such as sexual selection are the driving forces behind the aposematic coloration of fire salamanders.

7. An evolutionary framework for venom variation patterns in terciopelo pitvipers (*Bothrops asper*), a model organism in toxinology

David Salazar-Valenzuela, Diana Mora-Obando, Davinia Pla, Juan J Calvete, H Lisle Gibbs

Centro de Investigación de la Biodiversidad y Cambio Climático, Universidad Tecnológica Indoamérica
Department of Evolution, Ecology and Organismal Biology, The Ohio State University
Unidad de Venómica Evolutiva y Traslacional, Instituto de Biomedicina de Valencia

The Neotropics is the most species-rich region in the world. The current diversity and distribution of lineages present in this region is in part the result of complex ecological and evolutionary trends determined by environmental variables that have operated at diverse spatial and temporal scales. Also, recent studies have demonstrated the importance of demographic processes influencing the structure of present-day phylogeographic patterns. We used a combination of genomic, morphological, and venom-protein data to explore diversification patterns and evolutionary mechanisms implicated in the divergence of the *Bothrops asper* species complex, a medically important group of snakes and a model organism in toxinological research. We identified extensive phylogeographic structure, suggesting the influence of geographic barriers and/or differences in ecological niches in the recent diversification of the group. A deep divergence between two major clades is evident, but more recently diverged groups in South America show complicated patterns suggestive of recent divergence and/or gene flow among lineages. Next, we used this information to perform model-based analyses to investigate the demographic processes involved in the origin of Andean montane lineages. This approach allowed us to resolve some of the discrepancies of evolutionary relationships identified with tree-methods, but also to find evidence for the recent isolation of montane lineages in dry inter-Andean valleys. Finally, we identified three protein families (snake venom metalloproteinases, C-type lectins, and phospholipases A2) in venoms of Andean lineages whose abundances show remarkable differences when compared to Costa Rican and neighboring *B. asper* populations. These results highlight the importance of Andean montane habitats as drivers for the diversification of terciopelo pitvipers, and provide a much-needed historical framework that could promote a better understanding of appropriate mixtures of their venoms for immunization and clinical manifestations of terciopelo snakebite accidents in humans.

Concurrent Session III

3A. Emerging Technologies in Toxinology.

Chairs: M. Kini/R. Valente

1. Somasekar Seshagiri: Genomic analysis of venomous animals and its application for antivenom development. sekar@migenome.com
2. Kushal Suryamohan: Bioinformatics driven high-quality genome assembly and annotation of venomous animals for effective antivenom development. suryamohan.kushal@gene.com
3. Mrinalini: Venomous snake biology in the age of genomics. dbsmrin@nus.edu.sg
4. Ana Gisele C. Neves-Ferreira: Integrative structural biology in Toxinology: focus on natural inhibitors of snake venom toxins. anagextra@gmail.com
5. Richard H. Valente: Inferring venom peptidomic biological activities with connectivity mapping. richardhemmi@gmail.com
6. Manjunatha Kini: Subtleties of sequences in protein folding and function dbskinim@nus.edu.sg

1. Genomic analysis of venomous animals and its application for anti-venom development

Sekar Seshagiri

SciGenom Research Foundation, Bangalore, India
ModMab Therapeutics, USA

Traditionally proteomic approaches have been more widely used to study venom and venom components. Recently, though genomics has been applied to study a number of venomous animals the number of high quality genomes is limiting. We have collected both genomic and transcriptomic data types and performed an integrated analysis to generate high quality genomes for multiple venomous organisms. The predicted protein coding genes encoded by the genome was combined with tissue specific expression data to identify venom specific genes and gene products. Using the venom relevant gene information we have initiated the production of synthetic recombinant venom and anti-venom. The talk will cover genomics of venomous animals and its application in development of anti-venom.

2. Bioinformatics driven high-quality genome assembly and annotation of venomous animals for effective anti-venom development

Kushal Suryamohan

Genentech, South San Francisco, CA 94080, USA

With over 200,000 extant venomous animals, the number of high-quality genomes of venomous species are exceedingly sparse and limited to only a handful of taxa. To reveal the evolutionary history of toxins as well as to elucidate the venom repertoire, it is imperative to produce genome assemblies and annotations for venomous species across different lineages.

The recent development of long-read sequencing, linked-read sequencing, optical mapping and chromatin interaction mapping technologies now enable cost-effective approaches to near-chromosomal-level assemblies, thus overcoming many of the shortcomings of short read sequencing technology. We have developed a bioinformatics frame work that enables generating high quality contiguous assembly and annotation of genomes of venomous animals. We have assembled and annotated the Indian cobra genome and identified venom specific toxin genes. Our pipeline resulted in a genome assembly with a scaffold N50 of 223 Mbp, making it the most contiguous reptilian genome assembled to-date. Transcriptomic data from 14 different tissues was used to comprehensively identify genes expressed in the venom gland including a minimal set of 19 venom gland-specific toxin genes that likely form the core toxic components of the venom and can be used towards the development of recombinant antivenom.

3. Venomous snake biology in the age of genomics

Mrinalini, R. Manjunatha Kini

Department of Biological Sciences, National University of Singapore, Singapore

Genomics is an emerging area in venom and toxinological research. 'Omics technologies benefit both fundamental and applied venom research by facilitating a better understanding of venom evolution and venom variation. Snake genomes in particular, are rich resources that provide exciting new avenues for multi-disciplinary scientific discoveries and can contribute to betterment of antivenom design and snakebite management. Several snake genomes have been sequenced so far, however genomic resources and the use of genomic approaches in venom research continue to be rather limited. We sequenced the genome of an Old-World venomous snake, the Temple Pitviper (*Tropidolaemus wagleri*). Next and Third Generation Sequencing were used to sequence the genome to a depth of 140X using Illumina and 75X using PacBio long read technology. A de novo assembly resulted in a genome of 1.47 Gbp with a final coverage of over 100X, and the genome was contained in 5380 scaffolds with an N50 of 500 Kbp (0.5 Mbp). Comparative evolutionary genomics analyses, in combination with transcriptomics and proteomics, provided a better understanding of evolution of snakes, and we present these results here.

4. Integrative Structural Biology in Toxinology: Focus on natural inhibitors of snake venom toxins

Ana Gisele C. Neves-Ferreira

Laboratory of Toxinology, Oswaldo Cruz Institute, Fiocruz, Brazil

By 2030, the World Health Organization aims to halve the global rates of death and disability caused by snake envenomation. To meet this challenge, a comprehensive strategy has been recently devised, which includes the need to “accelerate research into new therapies”. In this context, the use of effective inhibitors of tissue-damaging toxins [phospholipases A2 (PLA2) and metalloendopeptidases (SVMP)] constitutes an attractive (pre-hospital) adjunctive therapy. The repurposing of drugs, such as varespladib, an inhibitor of human non-pancreatic secretory PLA2, is currently under evaluation and shows promising results. We argue that the biochemical defense observed in venom-resistant animals could represent a rich source of structurally diverse inhibitor scaffolds, which have been fine-tuned by co-evolution. Although several natural protein inhibitors of snake venom PLA2 and SVMP have been characterized, their structure-function relationship remains largely overlooked. This talk will highlight the potential of Integrative Structural Biology to foster our understanding of the high-affinity noncovalent antitoxin-toxin complexes. The main analytical technique used was high-resolution mass spectrometry (MS), combined with chemical cross-linking and/or hydrogen-deuterium exchange. Complementary methodologies, such as analytical ultracentrifugation and/or small-angle X-ray scattering, were also employed. For the structural characterization, distance constraints generated by MS-based techniques were integrated into molecular modeling/docking pipelines (I-TASSER and Rosetta software suites). Results on the following inhibitors will be discussed: a) antimyotoxin DM64 (5 immunoglobulin-like domains) isolated from Didelphis aurita´s serum and its complex with K49-myotoxin II from *Bothrops asper* venom; b) antihemorrhagin BJ46a (2 cystatin-like domains) isolated from *B. jararaca´*s serum and its complex with the SVMP jararhagin. Using this hybrid low-resolution strategy, crucial structural information on the possible regions of interaction between the inhibitors and their target toxins was generated. The acquired knowledge will be used for the rational design of innovative therapeutic peptides aiming to neutralize venom-induced local pathological effects. Financial support: FIOCRUZ (Inova VPPCB-007-FIO-18-2-9).

5. Inferring venom peptidomics biological activities with connectivity mapping

Richard Hemmi Valente

Laboratory of Toxinology, Oswaldo Cruz Foundation (FIOCRUZ), Brazil

Historically, most drugs have been developed based on small molecules (mass <500 Da). However, because of their low selectivity and potency, their adverse effects/toxicity limit the number of new drugs approved for use in humans. On the other hand, after millions of years of evolutionary process, nature offers a vast library of natural products with a wide range of biological activities, i.e, natural peptides, the new bet on biopharmaceuticals. Research on venom components (including peptides) from different sources (e.g. snakes) led to the development of diagnostic kits as well as therapeutic drugs. Our group has recently undertaken a deep proteopeptidome analysis of the venom of the South American pit viper *Bothrops jararaca* demonstrating that this venom's peptidome is much richer than previously thought, which led us to hypothesize that such diversity could enclose cryptides, whose potential bioactivities remained yet undetermined. The challenge was to screen for new activities without a priori knowledge of what to look for. For that effect, we have submitted *B. jararaca* venom peptide fraction to connectivity map analysis, an approach well suited for biosimilar drug discovery. Data indicated antihypertensive, antimalarial, antibacterial, and antitumor potential activities, among others. We have assayed (in vitro) the peptide pool against *Trypanosoma cruzi* and Plasmodium falciparum and demonstrated that it is effective against both parasites without, most importantly, being cytotoxic to a mammalian cell lineage (murine macrophage). Even though there is a long road ahead, we propose that this natural (bioactive) peptide library might represent a good source for designing alternative antiparasitic and antitumoural drugs, analgesics, anesthetics, antibiotics, and drugs to treat neurological disorders. Financial support: CNPq (Universal 28/2018) and FIOCRUZ (Inova VPPCB-007-FIO-18-2-9).

6. Subtleties of sequences in protein folding and function

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Venoms are complex cocktails of both enzymatic and nonenzymatic protein toxins. These toxins are grouped in to various superfamilies of toxins. Toxins within each superfamily share significant primary, secondary and tertiary (at times, quaternary) structural similarities. We have ample data to show that toxins within each superfamily exhibit distinct pharmacological activities. With closer evaluation of structure-function relationships, it is evident that subtle changes indeed contribute to significant differences in toxin folding and function. In this talk, I will highlight several subtleties in various superfamilies on toxins, along with the specific impact on their folding and pharmacology. These observations make it imperative to pay attention to subtle sequence changes with diligence.

Concurrent Session III

3B. Organ Systems and Toxins I.

Chair: A.M. da Silva/J. Eble

1. Jay W. Fox: Biomolecular investigation into systemic vascular leakage and kidney dysfunction induced by Russell's viper: A unique type of vascular toxicity and pathophysiology in viperid envenomings. jwf8x@virginia.edu
2. Sarah Natalie Cirilo Gimenes: Local damage in human envenomings by *Bothrops atrox* in Brazilian Amazon. sarah.gimenes@butantan.gov.br
3. Dilza Trevisan Silva: Systemic response of mice kidneys to the injection of HF3, a hemorrhagic SVMP from *B. jararaca* snake venom. dilzatrevisan@gmail.com
4. Johannes Eble: Neuropilin-1, a novel target of snake venom toxins on endothelial cells, influences inflammatory processes and tumor vessel leakage johannes.eble@uni-muenster.de
5. Jan Tytgat: Beyond hemostasis: a potassium channel blocker snake venom serine protease with potential antitumor activity. jan.tytgat@kuleuven.be

1. Biomolecular investigation into systemic vascular leakage and kidney dysfunction induced by Russell's viper: A unique type of vascular toxicity and pathophysiology in viperid envenomings.

Jay Fox, Alexandra Rucavado, Teresa Escalante, Erika Camacho, Jose Maria Gutiérrez

Instituto Clodomiro Picado, Facultad de Microbiología, Universidad de Costa Rica, San José, Costa Rica; University of Virginia School of Medicine, Charlottesville, VA.

Envenoming by *Daboia russelii* (Russell's viper) is a significant public health concern in Asian and India and is characterized by kidney dysfunction and acute kidney injury (AKI) in addition to the more common effects associated with coagulopathies. Generally, treatment with appropriate antivenom successfully ameliorates both coagulopathy and often AKI. Delayed or lack of treatment often leads to significant pathophysiology requiring hemodialysis putting more burden on patients and health care systems. Our laboratory is investigating the molecular mechanisms of systemic vascular leakage and whether these are connected to kidney dysfunction. Both, phospholipase A2s and snake venom VEGFs have been demonstrated capable to produce vascular leakage. To assess whether this pathology is related to kidney dysfunction we utilized the PLA2 inhibitor varespladip and sv-VEGF anti-body to determine whether these could attenuate vascular permeability and subsequently kidney dysfunction. In the case of varespladip, complete enzymatic inhibition of venom PLA2s did not block vascular leakage or kidney dysfunction. Similarly, anti-svVEGF or anti-human VEGF was unable to completely block kidney dysfunction. Although we cannot fully discount the role of svVEGF in kidney dysfunction given the nature of these experiments the data suggests consideration of other factors that occur during envenoming that may contribute to kidney dysfunction. These will be discussed and are currently under investigation.

2. Local damage in human envenomings by *Bothrops atrox* in Brazilian Amazon

Sarah Natalie Cirilo Gimenes, Mônica Colombini, Hiochelson Najibe dos Santos Ibiapina, Allyson Guimarães Costa, Jacqueline Sachett, Wuelton Marcelo Monteiro, Fan Hui Wen, Jay William Fox, Ana Maria Moura da Silva

Butantan Institute
Tropical Medicine Hospital, Manaus
University of Virginia

Brazil's northern region has registered highest prevalence of snakebites, and *Bothrops atrox* is responsible for majority of accidents with reports of severe local damage. Its venom presents a rich composition of metalloproteases P-III and P-I class, which hydrolyze and bind to extracellular matrix proteins (ECM). According to the lower capacity of antivenom act on local damage, we seek to understand the local complications triggered by envenoming, analyzing the presence of venom/antivenom and investigate the protein profile on the blister content. Five patients who had suffered *B.atrox* snakebite and were attended at Tropical Medicine Hospital, Manaus, Brazil were included in this study. The venom/antivenom presence in the blisters was quantified by ELISA/Western Blotting, the proteomics technique was used to analyze the blister content. The blisters were collected after 48hrs, and it was possible to identify the presence of venom in the blister. Interestingly, at it this same time we also identified the presence of the antivenom, which could recognize by Western Blotting the region of SVMPs. The antivenom concentration was higher than venom on the blister content, and the venom/antivenom levels had not correlation with the severity of envenoming. Even the antivenom being present in the blister, patients bitten by *B.atrox* suffer severe local damage. These data suggest that other factors are exacerbate in the local damage. Then, we analyzed the blister proteomic profile, and approximately 647 proteins were identified, these include proteases, ECM fragments, which could amplify the proinflammatory effect, such as DAMPs; and immunodulators, such as protein S-100 and C-reactive protein, which are described exacerbated in ulcers due to tissue damage. Thus, our studies contribute to understanding the blister environment of *B. atrox* envenoming and may add to the development of new strategies to improve the local treatment.

3. Renal effects induced by HF3, a metalloproteinase isolated from *Bothrops jararaca* venom, in a murine model.

Dilza Trevisan-Silva; Débora Andrade-Silva; Milton Y. Nishiyama-Jr; Ursula C. Oliveira; Milene C. Menezes; Inácio L. M. Junqueira-de-Azevedo; Oliver Schilling; Solange M. T. Serrano.

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Institute of Surgical Pathology, Medical center, University of Freiburg, Germany.

Metalloproteinases are abundant in viperid venoms and are implicated in several envenomation effects. *Bothrops jararaca* venom is reported to induce kidney injury in rats or in isolated kidney tissue. HF3 is an extremely hemorrhagic metalloproteinase from *B. jararaca* venom and participates in local events of envenomation. We are applying omics approaches to evaluate the effects of HF3 on mice kidneys after 2 h and 6 h of HF3 injection (5 g) on the thigh muscle (CEUAIB 8174030915). For transcriptomic analysis, next generation sequencing was performed on Illumina HiSeq™ 1500 and differentially abundant mRNAs were estimated applying DESeq2 methods. For proteomic analysis, kidney proteins were extracted and submitted to trypsin digestion and peptides were TMT-labeled for relative quantitative proteomic analysis. For N-terminomic analysis, a modified. Terminal Amine Isotopic Labeling of Substrates (TAILS) protocol was applied, using TMT-tags for free-amine blocking and relative quantification. Raw files obtained from proteomic approaches were submitted to MaxQuant search and statistical analyses were performed using the Limma package in R. Transcriptomic analysis showed differential expression of 31 and 137 genes related to kidney injury, after, respectively, 2 h and 6 h. The proteomic analysis showed that among 2,921 quantified proteins, those that were differentially abundant are related to inflammation and kidney injury pathways. N-terminomic analysis resulted in 2,937 quantified peptides and the Proteomic Identification of protease Cleavage Sites (PICS) analysis showed that leucine was not the preferred amino acid at the P1' position, indicating the activation of host proteinases upon HF3 injection. Taken together, these findings indicate that an isolated hemorrhagic metalloproteinase promotes various host reactions in response to its local and systemic effects, altering the abundance of many proteins and affecting relevant signaling pathways in the kidneys.

4. Neuropilin-1, a novel target of snake venom toxins on endotelial cells, influences inflammatory processes and tumor vessel leakage.

Johannes A. Eble

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Neuropilin-1 has primarily been known as a coreceptor for the vascular endothelial growth factor (VEGF)-receptor 2 (VEGFR2) on endothelial cells, which contributes to VEGF signaling, angiogenic sprouting and increased vessel permeability. On the other hand, rhodocetin (RC) from the venom of Malayan pit viper (*Calloselasma rhodostoma*) was identified as the prototypic member of C-type lectin-related protein (CLRPs) subfamily, which selectively inhibits the collagen-binding α2β1 integrin, one out of 24 members of this cell adhesion molecule family. RC consists of four chains, which pairwise heterodimerize into the two subunits, αβ and γδ, which associate into a cross-shaped tetramer [1]. RC targets several molecules of the envenomed victim. Whereas the γδ-subunit firmly binds to integrin α2β1, its αβ-subunit (RCαβ) recognizes neuropilin-1 (NRP1) on the surface of endothelial cells (ECs)[2]. After having bound to NRP1, RCαβ induces the association of NRP1 with cMET, but not with VEGFR2. Via phosphorylation of paxillin, it thus causes the rearrangement of the actin cytoskeleton and the restructuring of adhesomes from focal adhesions into focal contacts, resulting in a higher motility of the ECs. Moreover, transcriptome analysis revealed that RCαβ induces the transcription of inflammatory response genes and the activation of confluent ECs within a monolayer in an in vitro system [4]. These events, together with other endothelium-destructing venom components, may contribute to the sepsis-like symptoms of snake-bite envenomation. In an in vivo mouse tumor model, RCαβ almost exclusively affects blood vessels within the tumor tissue [3]. ECs in normal blood vessels expose both NRP1 and cMET on their abluminal face and are inaccessible to blood-borne RCαβ. In contrast, tumor cells integrated into the endothelium or replacing ECs in tumor mosaic and vasculogenic mimicry vessels, respectively, are the prime target for this snake venom component. Therefore, the tumor cells becomes motile and open the way for RCαβ to the abluminal face of ECs. There, the CLRP forms a trimeric complex with NRP1 and cMET and causes disintegration of the tumor blood vessels. This eventually leads to a tumor-specific hemorrhage and might be pharmacologically harnessed to bring anti-cancer agents to the tumor mass.

5. Beyond hemostasis: a potassium channel blocker snake venom serine protease with potential antitumor activity

Johara Boldrini-França, Ernesto Lopes Pinheiro-Junior, Steve Peigneur, Tássia Rafaella Costa, Rafael Junqueira Borges, Manuela Berto Pucca, Felipe Augusto Cerni, Sante Emmanuel Carone, Marcos Roberto de Mattos Fontes, Suely Vilela Sampaio, Eliane Candiani Arantes, Jan Tytgat

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Snake venom serine proteases (SVSPs) are complex and multifunctional enzymes that act primarily on hemostasis. In this work, we report the hitherto unknown inhibitory effect of a SVSP isolated from the venom of *Crotalus durissus collilineatus*, named collinein-1, on the voltage-gated and cancer-relevant hEAG1 potassium channel (Kv10.1). Of all potassium channel types tested, we found that collinein-1 selectively inhibits hEAG1 and that the inhibition occurs independently from its enzymatic activity. Corroboratively, it was found that collinein-1 reduces the cell viability of human breast cancer MCF7 (high expression of hEAG1), but neither affects cells from liver carcinoma HepG2 (low expression of hEAG1) nor from non-tumorigenic epithelial breast cell line (also low expression of hEAG1). In order to obtain both functional and structural confirmation of this unexpected finding, where an unusually large ligand acts as an inhibitor of an ion channel, a recombinant and catalytically inactive mutant of collinein-1 (His43Arg) was made and found to preserve its capability of inhibiting hEAG1. A molecular docking model is also proposed.

Concurrent Session IV

4A. Non-antibody and Adjuvant – Based Therapeutics.

Chair: A. Yanagihara

1. Greg Neely: CRISPR screening used to identify an effective antidote for box jellyfish venom. greg.neely@sydney.edu.au
2. Angel Yanagihara: Cubozoan Envenomation: Mechanisms, Models and Management ayanagih@hawaii.edu
3. Yoon Hwang; Improving envenomation outcomes by inhibiting venom spreading factors (e.g. hyaluronidases, gelatinases, phospholipase A2s) yoon.y.hwang.civ@mail.mil
4. Richard Lewis (University of Queensland): Re-evaluating the nirvana cabal deployed by piscivorous cone snails r.lewis@imb.uq.edu.au
5. Nilgun Tumer: Inhibition of the activity of ricin by targeting its interaction with the ribosome. tumer@sebs.rutgers.edu

1. “CRISPR screening used to identify an effective antidote for box jellyfish venom“

Greg Neely

The Charles Perkins Centre, School of Life & Environmental Sciences, The University of Sydney

The box jellyfish *Chironex fleckeri* is extremely venomous, and envenoming causes tissue necrosis, extreme pain and death within minutes after severe exposure. Despite rapid and potent venom action, basic mechanistic insight is lacking. Here we perform molecular dissection of a jellyfish venom-induced cell death pathway by screening for host components required for venom exposure-induced cell death using genome-scale lenti-CRISPR mutagenesis. We identify the peripheral membrane protein ATP2B1, a calcium transporting ATPase, as one host factor required for venom cytotoxicity. Targeting ATP2B1 prevents venom action and confers long lasting protection. Informatics analysis of host genes required for venom cytotoxicity reveal pathways not previously implicated in cell death. We also discover a venom antidote that functions up to 15 minutes after exposure and suppresses tissue necrosis and pain in mice. These results highlight the power of whole genome CRISPR screening to investigate venom mechanisms of action and to rapidly identify new medicines.

2. Cubozoan Envenomation: Mechansims, Models and Management

Angel Anne Yanagihara, Noel Saguil, Christie Wilcox, Armin Coronado, Noel Stephen F Saguil, Raechel Kadler

Bekesy Lab, School of Ocean and Earth Sciences and Technology, AND Dept of Tropical Medicine, John A. Burns School of Medicine, University of Hawaii
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Leyte Normal University, Tacloban, Philippines
University of San Carlos, Cebu, Philippines
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Polytechnic University of the Philippines
Department of Tropical Medicine, University of Hawaii
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Cubozoan envenomations are the leading cause of severe and lethal human sting injuries from marine life. The total amount venom discharged into sting-site tissues, “venom load”, correlates with tentacle contact length and sequelae severity. Optimal first aid measures prevent additional venom discharge into skin and reduce the activity of venom already discharged. Since 1% of tentacle cnidae discharge upon initial contact, rapid inactivation and effective removal of adherent tentacles is critical. We evaluated whether common rinse solutions or scraping increased venom load as measured in a direct functional assay of venom activity (hemolysis). Scraping significantly increased hemolysis. For *Alatina alata*, increases did not occur if the tentacles were first doused with vinegar or if heat (45oC for 45 min) was immediately applied. However, in *Chironex fleckeri* and other life threatening chirodropid species, vinegar dousing and heat treatment were somewhat less effective; the best outcomes occurred with the use of copper gluconate containing spray and cream formulations. Pretreatment with a newly identified venom inhibitor, 2-hydroxypropyl-β-cyclodextrin(HPβCD), of blood agar plates before live tentacle application or of red blood cell solutions in hemolytic assays before addition of venom, markedly reduced venom-induced hemolysis, whereas post treatment with HPβCD showed no effect in solution based hemolytic assays and worsened outcomes in live tentacle blood agar assays. Surprisingly, seawater rinsing, considered a “no-harm” alternative to vinegar, significantly increased venom load. The application of ice severely exacerbated *A. alata* stings, but had a less pronounced effect on *C. fleckeri* stings, while heat application markedly reduced hemolysis for both species. Our results do not support scraping or seawater rinsing to remove adherent tentacles but support the use of vinegar dousing followed by application of skin safe heat as well as the utility of novel chemical inhibitors.

3. Improving envenomation outcomes by inhibiting venom spreading factors (e.g. hyaluronidases, gelatinases, phospholipase A2s)

Angela R. Jockheck-Clark, Thomas E Bird, Yoon Y Hwang

Naval Medical Research Unit San Antonio

Background: Bites from medically-relevant snake species cause more than 400,000 amputations and/or permanent disabilities every year. The sometimes brutal local pathophysiological effects of envenomation are caused by enzymatic venom components known as "spreading factors." Spreading factors start to distort the extracellular matrix within minutes of envenomation and can cause severe local effects such as edema, blistering, hemorrhage, tissue necrosis, and damage to nerve terminals. While some envenomation victims can be treated with antibody-derived antivenoms to prevent fatality, these therapies cannot diffuse into the tissue at the envenomation site. Therefore, to reduce the local symptoms of envenomation, reduce the burden of survival, and potentially delay the systemic spread of life-threatening venom components, we have developed two therapies that target multiple spreading factor classes.

Methods: Candidate spreading factor inhibitors (SFIs) were screened in vitro for the ability to inhibit hyaluronidase, gelatinase, and phospholipase A2 activities found in the crude venom extracts of *Naja naja kaouthia* (monocled cobra), *Vipera russelli* (Russel's viper), *Agkistrodon piscivorus piscivorus* (cottonmouth), *Bungarus caeruleus* (common krait) and *Bungarus candidus* (Malayan krait). Spreading factor activities were assessed using PLA2 and gelatinase assay kits (ThermoFisher), or a hyaluronidase assay protocol (Sigma-Aldrich 3.2.1.35).

Results: Two SFI combinations were highly effective at neutralizing the spreading factor activities found in all five of the aforementioned venoms. Inhibition occurred in less than 5 minutes, functioned in a dose-dependent manner, and inhibited spreading factor activities for several hours.

Conclusions: Because snake venom compositions differ significantly from one species to the next, these data strongly suggest that the SFI combinations will be effective against spreading factors found in a variety of other venom sources. Importantly, the individual SFIs are FDA-approved as injectable treatments for various disorders, and will likely be compatible with commercial self-injection systems.

4. Re-evaluating the nirvana cabal deployed by piscivorous cone snails.

Mriga Dutt, Jean Giacomotto, Lotten Ragnarsson, Asa Andersson, Andreas Brust, Zoltan Deakan, Paul F Alewood, Richard J Lewis

The University of Queensland

Cone snails use separately evolved venoms for prey capture and defence. While most use a harpoon for prey capture, the Gastridium clade that includes the well-studied *Conus geographus* and *C. tulipa*, have developed a net hunting strategy to catch fish. This unique feeding behaviour requires secretion of "nirvana cabal" peptide(s) to dampen the escape response of targeted fish allowing for their capture directly by mouth. However, the active components of the nirvana cabal remain poorly defined. Surprisingly, the conantokins (NMDAR antagonists) and/or conopressins (vasopressin receptor agonists and antagonists) found in *C. geographus* and *C. tulipa* venom failed to produce a nirvana cabal-like effect in zebrafish. In contrast, low concentrations of the non-competitive α1-adrenoceptor antagonist rho-TIA found in *C. tulipa* venom dramatically reduced the escape response of zebrafish larvae when added directly to water. rho-TIA inhibited the zebrafish α1-adrenoceptor, confirming rho-TIA has the potential to reverse the known stimulating effects of norepinephrine on fish behaviour. r-TIA can act alone and not as part of a cabal, since it did not synergise with conopressins and/or conantokins. This study highlights the importance of applying ecologically relevant animal behaviour models to decipher the complex neurobiology underlying the prey capture and defensive strategies of cone snails.

6. Inhibition of the activity of ricin by targeting its interaction with the ribosome

Xiao-Ping Li, Nilgun E Tumer, Jennifer Nielsen Kahn

The plant toxin ricin is a type II ribosome inactivating protein (RIP), which consists of an active A chain (RTA) covalently linked to a cell binding B chain (RTB). Ricin is one of the most potent toxins known. Currently, there is no FDA approved treatment for ricin intoxication. Ricin has been a uniquely challenging drug target. Small molecule inhibitors of enzymatic activity with high potency have not been identified. Interaction of the A subunit with ribosomes has not been examined as a potential drug target. We identified the host target of ricin as the conserved C-termini of the ribosomal P stalk proteins and showed that ricin binds the ribosome by a two-step mechanism. In the first step slow and non-stalk specific electrostatic interactions concentrate RTA on the ribosome and in the second step fast electrostatic interactions occur with the P stalk. We identified Arg235 as the most critical arginine for the electrostatic interactions of RTA with the P stalk. The X-ray structure analysis indicated that only the last six residues of P proteins bind to a hydrophobic pocket on RTA. To understand the relative importance of the hydrophobic interactions we mutated residues in the hydrophobic pocket and showed that toxicity of RTA can be eliminated in mammalian cells by combining mutations in critical electrostatic and hydrophobic residues. We identified peptides that interact with the ribosome binding site and inhibit the depurination activity of RTA by disrupting its interaction with the ribosome. RTA activity was inhibited by targeting the ribosome binding site without targeting the active site. These studies established toxin-ribosome interactions as a new target for inhibitor discovery. We propose a new model for molecular recognition of the P stalk by ricin, which may be applicable to other RIPs and translation factors that interact with the P stalk.

Concurrent Session IV

4B. Organ Systems and Toxins II.

Chairs: Ayvazyan/Krizaj

1. Maria Elena de Lima: How a potent neurotoxin can become a promising drug. lima.mariaelena@gmail.com
2. Igor Križaj: Understanding the molecular mechanism underlying the presynaptic toxicity of sPLA2s is a window into pathophysiology of their mammalian orthologues. igor.krizaj@ijs.si
3. Yuri N. Utkin Three finger neurotoxins: new discoveries and arising questions. utkin@ibch.ru
4. Igor E. Kasheverov: Channel blockers from scorpion venoms inhibit nicotinic acetylcholine receptors. shak_ever@yahoo.com
5. Jordi Molgó: Gambierol, a marine dinoflagellate toxin, potently increases evoked quantal transmitter release and reverses pre- and post-synaptic neuromuscular block at vertebrate junctions jmolgo@yahoo.com
6. Naira Ayvazyan: The specificity of Middle East vipers´ venom action on the nervous tissue. taipan@ysu.am
7. Choo Hock Tan: Insights into the evolutionary and medical significance of unique alphaneurotoxin and phospholipase A2 compositions in Naja spp. (cobra) venoms. tanch@um.edu.my

1. How A Potent Neurotoxin Can Become A Promising Drug.

Maria Elena De Lima, Carolina Nunes da Silva, Kênia Pedrosa Nunes, Perla Villani Borges, Paulo G.S Lacativa

Grupo Santa Casa de Belo Horizonte: Instituto de Ensino e Pesquisa, Belo Horizonte, MG, Brazil.
Universidade Federal de Minas Gerais, Belo Horizonte, MG. Brazil
Department of Biomedical and Chemical Engineering and Sciences, Florida Institute of Technology,Melbourne, FL, USA
Biozeus Biopharmaceutical S.A, Rio de Janeiro, RJ

PnTx2-6 (or δ-CNTX-Pn1c) is a potent neurotoxin (LD50=0.7 mg/Kg, 48 aminoacid residues, six disulfide bridges), from the venom of the spider *Phoneutria nigriventer*, targeting different sodium channels. It causes priapism. Based on the sequence of PnTx2-6, a smaller peptide, called PnPP-19 (or BZ371), was designed and synthesized. BZ371 was able to potentiate erectile function, in vivo and ex vivo, in normotensive rats and mice, besides to restore erectile function in hypertensive and diabetic animals. In addition, the peptide is active by topical application. BZ371 did not target Navs. It increased the cGMP levels, the expression/activity of iNOS and nNOS and did not show apparent toxicity. Considering that erectile dysfunction (ED) is a growing world health problem, especially in patients affected by vascular diseases, i.e. diabetes and hypertension, BZ371 was investigated as a potential drug to treat ED. In a conceptual study in humans, BZ371 was tested in 12 healthy subjects, 6 men and 6 women (mean age=33), by topical application in the genital area. Physical examination, blood hemogram and standard biochemistry, blood pressure measurements and electrocardiogram (ECG) were used to evaluate safety. A doppler scan was used to detect blood flow changes in the genital area of both genders. Topical administration of BZ371 was well tolerated and no adverse effects were observed, with no local irritation, edema, headache, hypotension, ECG changes, blood hemogram/biochemistry changes, prolonged erection, priapism or penile pain. Doppler Scan demonstrated an increase in penile arterial inflow (around 113), in subjects who received 2 mg/ml of treatment compared to placebo at 30 min, following the application. In conclusion, topical administration of BZ371 was considered safe on both genders. Increased genital blood flow was compatible with preclinical data. BZ371 has the potential to be a safe and efficacy option for erectile dysfunction patients.

2. Understanding the molecular mechanism underlying the presynaptic toxicity of sPLA2s is a window into pathophysiology of their mammalian orthologues

Igor Križaj, Adrijan Ivanušec, Jernej Šribar

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University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia

The neuro-muscular junction (NMJ) is an area between the motor neuron and the muscle cell fibre, where these two cell types communicate. At the NMJ, electrical signals arriving along the axon are converted into chemical signals in the form of neurotransmitter released into the synaptic cleft. Neurotransmitter diffuses a narrow gap between the nerve and the muscle cell, where it binds to a specific receptor, resulting in membrane depolarisation and consequent muscle contraction. Given its fundamental importance for survival, it is not a surprise that the NMJ is attacked by plethora of different neurotoxins produced by different bacteria, plants and animals, as an important part of their molecular arsenal to hunt or defend. Most neurotoxins disturb the NM communication by acting in different ways on the nerve cell (presynaptic toxins), some of them also on the muscle cell (postsynaptic toxins). Neurotoxins mainly acting presynaptically are also secreted phospholipases A2 (sPLA2s). They are particularly abundant in snake venoms. Many molecular details of their toxic action at the NMJ have already been revealed [1], but the picture is still not completely clear. In this lecture, the most recent insights into the mechanism of sPLA2 neurotoxicity will be presented [2, 3]. Importantly, knowledge gained by these toxins is advancing our understanding of functioning of their mammalian orthologues in the nervous system, in particular of the group IIA sPLA2. The latter has been namely indicated to regulate neuro-transmission by fine-tuning of acetylcholine release and conductance of the nicotinic acetylcholine receptor, neurite sprouting and fitness of the neuronal mitochondria. On the other hand, dysregulation of this enzyme seems to be an important factor of commencement of Alzheimer´s disease.

[1] Šribar et al. (2014) Toxicon 89, 9–16; [2] Oberčkal et al. (2015) PLoS One 10, e0120692; [3] Šribar et al. (2019) Sci. Rep. 9, 293.

3. Three finger neurotoxins: recent discoveries and arising questions

Yuri Utkin, Ulrich Kuch, Alexey Osipov, Igor Kasheverov, Denis Kudryavtsev, Vladislav Starkov, Rustam Ziganshin, Dietrich Mebs, Victor Tsetlin

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Three-finger neurotoxins (TFN) are the most toxic components of Elapid venoms. The first TFN α-bungarotoxin (α-Bgt) has been discovered almost fifty years ago and is still widely used as a specific marker of neuronal α7, α8 and α9α10 as well as muscle type nicotinic acetylcholine receptors (nAChR).

In the venom of krait Bungarus candidus, we found new TFNs called αδ-bungarotoxins (αδ-Bgt). Similar to α-Bgt, the toxins consist of a chain of 73 amino acid residues crosslinked with 5 disulfide bridges and exhibit a high affinity for nAChR of α7 and muscle type. However, in contrast to α-Bgt, αδ-Bgts distinguish two binding sites in muscle-type receptors, demonstrating a higher affinity for the α/δ site; the binding of αδ-Bgt is easily reversible.

We showed that TFN α-cobratoxin *Naja kaouthia* completely blocked GABA-induced currents in the GABA-A receptor expressed in Xenopus oocytes. The receptor was also inhibited by several other TFNs in a mixed competitive and noncompetitive way.

Two toxins, TFT-AF and TFT-VN from the vipers *Azemiops feae* and *Vipera nikolskii*, respectively, were prepared by heterologous expression in E. coli. The study of their biological activity showed that the viper TFNs are antagonists of nAChRs of neuronal as well as muscle type.

As a result of a proteomic analysis of the *Naja kaouthia* cobra venom, a number of post-translational modifications (PTM) in the structure of TFNs was detected. The most prevalent PTMs were found to be acetylation, phosphorylation, and formylation. A large number of peptides with acetylated and formylated N-terminal amino acid residues was observed indicating the presence of processed and modified TFNs in the venom.

Several questions arising from these findings will be discussed.

4. Channel blockers from scorpion venoms inhibit nicotinic acetylcholine receptors

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Sechenov First Moscow State Medical University, Institute of Molecular Medicine, Moscow, Russia

Nicotinic acetylcholine receptors (nAChRs) are the targets of a wide range of compounds of very different chemical nature ranging from quaternary amines to proteins. The most well-known and widely used for nAChR studies are α-neurotoxins and α-conotoxins from the venoms of Elapidae snakes and Conus mollusks, respectively. Interestingly, in our studies of the biological activity of several scorpion venoms we revealed anticholinergic activity. The search and isolation of individual components responsible for this activity led to already known peptide toxins, namely, OSK-1 from *Orthochirus scrobiculosus*, spinoxin from *Heterometrus spinifer* and HelaTx1 from *Heterometrus laoticus*, all of them being blockers of voltage-gated potassium channels. Based on these data, a series of other well-known potassium channel blockers from scorpion venoms was investigated, revealing their micromolar and sub-micromolar affinities towards muscle-type nAChR from *Torpedo californica* ray electric organ. The most active compounds (OSK-1 and spinoxin) in competition with radiolabeled α-bungarotoxin showed IC50 of about 0.5 μM. Similar blocking efficacy was revealed in the functional test on mouse muscle nAChR expressed in Xenopus oocytes. The affinity of all tested scorpion toxins to the human neuronal α7 receptor was significantly lower. Our results indicate that scorpion neurotoxins present a target promiscuity.

This work was supported by RFBR grant no. 18-04-01366 and the Molecular and Cell Biology program of the Russian Academy of Sciences.

5. Gambierol, a marine dinoflagellate toxin, potently increases evoked quantal transmitter release and reverses pre- and post-synaptic neuromuscular block at vertebrate junctions

Jordi Molgó, Sébastien Schlumberger, Haruhiko Fuwa, Evelyne Benoit, Denis Servent

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Gambierol is a marine polycyclic ether toxin that was first isolated together with ciguatoxins from cultured *Gambierdiscus toxicus* dinoflagellates collected in French Polynesia. The chemical synthesis of gambierol permitted the analyses of its mode of action, which includes the selective inhibition of voltage-gated K+ (KV) channels in various cells and tissues expressing such channels. In the present study, we investigated the action of synthetic gambierol at vertebrate skeletal neuromuscular junctions using conventional techniques. Nanomolar concentrations of gambierol inhibited the fast K+ current and prolonged the duration of the presynaptic action potential in motor nerve terminals, as revealed by presynaptic focal current recordings, and increased stimulus-evoked quantal transmitter release in neuromuscular junctions blocked either by a high Mg2+-low Ca2+ medium, or by botulinum neurotoxin type-A. Also, gambierol reversed the postsynaptic block produced by d-tubocurarine. In motor nerve terminals loaded with fluo-3/AM, gambierol increased the transient Ca2+-signals in response to nerve-stimulation at 1-10 Hz. The results suggest that gambierol, which on equimolar basis is more potent than 3,4-diaminopyridine, can have potential application in pathologies in which it is necessary to antagonize pre- or post-synaptic neuromuscular block, or both.

6. The specificity of Middle East vipers' venom action on the nervous tissue

N.Ayvazyan, A. Kishmiryan, D. Terzyan, A. Voskanyan

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Macrovipera lebetina obtusa (MLO) and *Montivipera raddei* (MR) represent the most medically important poisonous snakes in Armenia, Iran, and neighbor countries. Most of the studies concern the function of phospholipase A2 (PLA2) in these venoms and its action on the lipids of cell membranes. But it is known that purified PLA2 has only 1% of the toxic activity in these venoms. The most important role in the intoxication is ascribed to low molecular weight components of the venom (such as obtustatin, lebein, and other disintegrins) and metalloproteases, but the scientific literature on this question is contradictory. It is also not clear whether the venom of these vipers is pure hemorrhagic or could have some neurotoxic effects as well: there are some cases of clinical manifestations quite characteristic for the neurotoxic mode of intoxication. Many opinions by the problem may show that we know neither the underlying mechanism nor the respective determining factors exactly.

Synaptic transmission is a fundamental neurobiological asset enabling the exchange of signals between neurons and between neurons and their non-neuronal effectors. At the core of neurotransmission lies regulated release of active substances from the pre-synaptic terminal onto the receptive surface of the downstream target. Characterization of the toxic effect of the MLO and MR venoms on the neuromuscular junction (NMJ) was done both through the in vitro and in vivo experimental approaches. Also, the microglia activity of rat brain following exposure of the mentioned venoms was investigated.

The obtained data is compared with the similar results concerning other Middle East Viperinae snake venoms, which are very scarce in spite of the richness of the herpetofauna's biodiversity of this region.

7. Insights into the evolutionary and medical significance of unique alpha-neurotoxin and phospholipase A2 compositions in Naja spp. (cobra) venoms

Choo Hock Tan, Kae Yi Tan, Nget Hong Tan, Kin Ying Wong

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Department of Molecular Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia.

Cobras (Naja spp.) are typically Category 1 medically important venomous snakes in Asia and Africa. Three-finger toxins and phospholipases A2 (PLA2) are two commonly reported toxin families in cobra venoms. Our recent proteomic and transcriptomic findings, nonetheless, indicate that there are considerable inter- and intra-specific variations across cobra lineages. We found a strong correlation between PLA2 enzymatic activities with PLA2 protein abundances in the cobra venoms. High PLA2 activities were shown in the venoms of Asiatic spitting cobras (*Naja sputatrix*, *Naja sumatrana*), followed by moderate activities in Asiatic non-spitters (*Naja naja, Naja atra, Naja kaouthia*), African spitters (subgenus Afronaja) and forest cobra (subgenus Boulengerina). African non-spitting cobras of subgenus Uraeus (*Naja haje, Naja annulifera, Naja nivea, Naja senegalensis*) showed exceptionally low venom PLA2 activities, consistent with the negligible PLA2 abundance in venom proteomes. The lack of PLA2 in Uraues cobra venoms implies that PLA2 is not ubiquitous in snake venoms. Meanwhile, the abundance of short- and long alpha-neurotoxins correlates significantly with the lethal potency of cobra venoms from various species and locales in Asia. Cobra venoms containing alpha-neurotoxins >25% of total venom proteins (*Naja philippinensis,* Pakistani *Naja naja, Thai Naja kaouthia*) are most lethal (LD50 0.22 μg/g), consistent with the severe neuromuscular paralysis observed in clinical envenomation. With alpha-neurotoxins 15% of total venom proteins, the venoms of Indonesian *N. sputatrix*, Malaysian *N. sumatrana*, Chinese *N. atra*, and *N. kaouthia* from China, Vietnam and Malaysia show higher LD50 (0.5–1.0 μg/g) and are less neurotoxic. *N. philippinensis* venom is intriguing as its alpha-neurotoxins are composed solely of short neurotoxins, whereas the Thai *N. kaouthia* venom is dominated by long neurotoxins. The findings support that alpha-neurotoxins, regardless of short- or long-chain subtype, are principal lethal components in cobra venoms. Antivenom production should be tailored toward targeted neutralization of these toxins.

Concurrent Session V

5A. North American Society on Toxinology.

Chairs: C. Vogel/J. Fox

1. Micaiah Ward (Florida State University, Tallahassee, Florida, USA): Experimental evolution of venom resistance. mward@bio.fsu.edu

2. Marcelo Strauch (Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil): Apilic antivenom, a new treatment for bee attacks, is effective in preclinical studies. strauchmarcelo@yahoo.com.br

3. Marcos Monteiro-Machado (Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil: Effects of fucosylated chondroitin sulfate (fucCS) and N-acylhydrazone derivative LASSBio-785 on *Apis mellifera* venom activities (15 min) marcosmmachado@gmail.com

4. Pamella Nogueira-Souza (Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil): Neutralization of bee venom activities by wedelolactone. pamdourila@gmail.com

1. Experimental Evolution of Venom Resistance

Micaiah Ward, Alex Oliver, Lauren Maquet, Darin Rokyta

Florida State University

The therapeutic properties offered by venom-derived toxins have led to the development of many life-saving medications. As this medicinal library continues to grow, so does the need to fully understand these toxins and their potential targets, many of which remain unknown. We have taken an experimental-evolution approach to venom target identification by evolving venom resistance in fruit flies, Drosophila melanogaster, using venom from the Florida blue centipede, Scolopendra viridis. The advantages of Drosophila genetics enables the identification of genetic (allele frequency) changes resulting from evolved resistance in comparison to a control population, narrowing down potential targets to a set of candidate genes that have responded to the strong selective pressure of venom. Because the ability to evolve the trait of venom resistance relies on genetic variation for which selection can act, variation in venom resistance was first established by assessing variation in response to venom injection in 97 inbred fly lines using a calculated median lethal dose. After establishing variation, a genetically mixed population of Drosophila was separated into experimental and control populations. The experimental populations were subjected to selection for venom resistance by injecting 500 flies with a median lethal dose of S. viridis venom approximately every other generation. Control groups were subjected to the same selection process using sterile PBS, and additional control groups were non-injected. After 18 months and approximately 35 generations, whole-genome sequencing was performed on the starting population and each of the experimental and control populations to identify allele frequency changes. Measurements of life history traits, such as offspring and average body size, were also taken throughout the experiment to assess the potential costs of evolved venom resistance. Although sequencing results are forthcoming, the phenotypic results indicate that all experimental populations were significantly more resistant to S. viridis venom in comparison to control groups.

2. Apilic antivenom, a new treatment for bee attacks, is effective in preclinical studies

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Massive bee venom envenomation induces intense local pain, tissue damage, and serious systemic alterations. In the last years, bee attacks and death has increased in Brazil and doesn't exist a specific treatment able to direct antagonize bee venom toxicity. Recently, it was developed by CEVAP (UNESP) and Instituto Vital Brazil (IVB) an apilic antivenom (AAV) after horse's immunization with fractionated bee venom. Thus, we evaluated if the AAV is able to antagonize bee venom and melittin toxicity. Mice (20-25 g) were used to perform in vivo experiments. In all protocols the AAV post treatment was performed by intravenous injection. Hemathocrit and lethality experiments were performed using A. mellifera crude venom (10 mg/kg, i.p.). Intraplantar edema induced by bee venom (0.1 µg/paw) and was evaluated using a digital caliper rule. Vascular permeability was assessed by Evans Blue (2.5%) transudation after intradermic bee venom injection (1 mg/kg). Miotoxicity and myeloperosidase activity were evaluated in vivo after bee venom perimuscular injection (1 mg/kg). Phospholipase and hyaluronidase activities were evaluated by turbidimetric methods. Citotoxicity by bee venom (10 µg/mL) and melittin (10µg/mL) were assessed in tubular renal cells (LLC-PK1) by measuring lactate dehydrogenase. It was observed that AAV antagonizes in a great extent (higher than 70%) the lethality, hemoconcentration, myotoxicity and edema, but not the myeloperoxidase activity. The AAV abolishes the increase of vascular permeability and the phospholipase and hyaluronidase activities. The AAV was able to antagonizes cytotoxicity induced by bee venom and melittin. The AAV was able to antagonize important in vivo and in vitro toxicity induced by bee venom and melittin. These results can be used to improve and validate the quality of the AAV and can be used in future to treat massive bee venom envenomation.

3. Effects of fucosylated chondroitin sulfate (fucCS) and N-acylhydrazone derivative LASSBio-785 on Apis mellifera venom activities

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Introduction: The proliferation of Africanized bees in Brazil has led to an increase in the incidence of accidents which is a public health problem. In 2017, an estimated 17,000 cases of accidents involving bee stings have resulted in more than 50 deaths. In this work, we investigated the effect of LASSBio 785 and fucosylated chondroitin sulfate (fucCS) on the different activities (cytotoxic and inflammatory) of Apis mellifera venom. Methods and Results: Although it was not able to inhibit the cardiotoxic and phospholipase actions of A. mellifera venom, LASSBio 785 (10 and 15 mg/kg) significantly reduced the venom edematogenic activity 15 and 30 minutes after injection, and abolished this activity with a dose of 30 mg/kg. It was observed that LASSBio 785 (30 mg/kg) was able to significantly reduce the increase in capillary permeability, marked by cutaneous extravasation of Evans Blue, as well as was able to inhibit tissue MPO activity. In relation to the actions of fucCs, it was able to significantly reduce edema in the three doses evaluated (0.3, 1.5 and 3 µg/paw), besides inhibiting the increase in vascular permeability, hemoconcentration and lethality induced by A. mellifera crude venom. It is noteworthy that fucCs presented promising activity on the venom cardiotoxicity, characterized by the reduction, in vivo, of plasma CKMB activity, and prevention of the fall of heart tension and heart rate, in vitro. Thus, fucCS appears as promising substance for the development of new treatment for bee accidents.

4. Neutralization of bee venom activities by wedelolactone

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Africanized Apis mellifera attacks in humans lead to a clinical condition that include rhabdomyolysis, cardiac, respiratory and renal failure. Bee venom is a complex mixture of proteins, peptides, amines and cytotoxic substances. It is important the development substances against bee sting. With this aim our research group developed a study using the natural substance found in the crude extract of the plant Eclipta prostrata: a coumestan called wedelolactone. We investigated the antagonism of wedelolactone in different experimental models in vitro, such as phospholipase A2 (PLA2) activity, hyaluronidase activity and myotoxicity. In the study of PLA2 activity the bee venom (1 µg/mL) was preincubated with wedelolactone (3-100 µM) for 30 min at 37 °C. The wedelolactone inhibited the PLA2 activity of the venom in a concentration-dependent manner. The wedelolactone (10-150 µM) also inhibited the hyaluronidase activity of the venom (10 µg/ml) in a concentration-dependent way. Extensor digitorum longus muscles (EDL) isolated from Swiss mice (25-30 g, protocol CEUA UFRJ n° DFBCICB072-04/16) were used to evaluate myotoxic activity of the venom alone (10-25 µg/mL) and preincubated with wedelolactone (1-10 µM) through the releasing rate of the sarcoplasmic enzyme, creatinocinase (CK), in U.g-1.h-1. The muscles were perfused for 90 minutes with a PSS, renewed every 30 minutes. The crude venom (25 µg/mL) increased the basal CK release rate of 0.78 ± 0.1 U.g-1.h-1 for 9.82 ± 1.5 U.g-1.h-1 at 60 min, about 12.5 times the baseline. In addition, 10 µM of wedelolactone reduces the venom induced CK release rate by about 97%. Our data suggests that wedelolactone has the ability to neutralize some of the bee venoms enzyme effects and it's myotoxicity, and that further studies should be performed to better understand that ability.

Concurrent Session VI

6A. North American Society on Toxinology.

Chairs: C. Vogel/J. Fox

1. Elda Sanchez (Texas A&M University, Kingsville, Texas, USA): The role of snake venom CRiSP toxins on blood and lymphatic endothelial cell permeability and pro-inflammatory responses: New insights into the pathophysiology of snake bites. kuees002@tamuk.edu

2. Jacob Galan (Texas A&M University, Kingsville, Texas, USA): Proteomic identification and quantification of snake venom biomarkers in plasma extracellular vesicle. jacob.galan@tamuk.edu

3. Paulo A. Melo (Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil): A synthetic metalloproteinase inhibitor derivatives from lapachol. melo.pa@gmail.com

4. Emelyn Salazar: (Texas A&M University, Kingsville, Texas, USA): Biochemical characterization and comparative analysis of two phospholipases A2 from venoms of North American snakes. emelynsalazar.87@gmail.com

5. Carl-Wilhelm Vogel (University of Hawaii, Honolulu, Hawaii, USA): Identification of Functionally Important Amino Acid Residues for C3 Convertase Activity Using Chimeric Proteins of Human C3 and Cobra Venom Factor. cvogel@cc.hawaii.edu

1. The role of snake venom CRiSP toxins on blood and lymphatic endothelial cell permeability and pro-inflammatory responses: new insights into the pathophysiology of snakebite

Elda Eliza Sanchez, Montamas Suntravat, Emelyn Salazar Castillo, Jessenia Marquez, Oscar Sanchez

Texas A&M University-Kingsville

Snakebite is a substantial global health problem, causing severe injury to 2.7 million men, women and children and claiming an estimated 125,000 deaths annually. In spite of its massive toll on human health, very little is known of the pathophysiology of snakebite. Although several studies have reported the lymphatic system plays a critical role in venom absorption and distribution following a snakebite, the role of the lymphatic system in envenomation remains unclear. Several snake venom Cysteine-Rich Secretory Proteins (svCRiSPs) have been shown to possess ion channel-blocking activities and affect the activity of vascular endothelial cells. This work aimed to investigate the role of svCRiSPs from three of the most medically significant species of North American Rattlesnakes (Crotalus atrox, C. adamanteus, C. scutulatus scutulatus) in snakebite, focusing specifically on the effects of these toxins on the function of the blood and lymphatic vessels and pro-inflammatory responses. By using both in vitro assays on Human Dermal Lymphatic Epithelial Cells (HDLECs) and Human Dermal Blood Endothelial Cells (HDBECs) cell permeability and in in vivo assays of vascular permeability, we have been able to provide clear evidence for the direct effects of svCRiSPs (Catrox-CRiSP, Css-CRiSP, and Cada-CRiSP) on endothelial cell barrier function and pro-inflammatory responses. Knowledge gained from these studies will provide insights into the molecular mechanisms that underlie the effects of svCRiSPs on vascular function and pro-inflammatory responses and will contribute to a new level of understanding of the pathophysiology of snakebite and the development of novel therapeutic strategies for the treatment of snakebite and possibly other vascular and lymphatic diseases.

2 Proteomic identification and quantification of snake venom biomarkers in plasma extracellular vesicles

Jacob A Galan, Elda E Sánchez, Emelyn Salazar, Montamas Suntravat

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Department of Chemistry, Texas A&M University-Kingsville

Exploration of the pathophysiology of snakebites requires the use of quantitative omics approaches to characterize snake venom and as it enters into the systemic circulation. Transcriptomics and proteomics of venom, called venomics has led to recent advancements in the understanding of snake venom composition between different species and also intra-species variation. These omic approaches give insights into the venom proteome, but further method development is warranted to analyze the response to snake venom and to explore the venom-reactome for the identification of snake venom biomarkers. The recent discovery of extracellular vesicles (EVs), including microvesicles/exosomes, and their critical cellular functions has presented them as intriguing sources for biomarker discovery and disease diagnosis. Indeed, analysis of snake venom toxins in EVs offers an unprecedented potential for understanding the pathophysiology of snake envenomation. Herein, we analyzed purified extracellular vesicles using EVTRAP technology and quantitative mass spectrometry from mice injected subcutaneously with a sub-lethal dose of crude Crotalus atrox venom. Proteomics analysis revealed that 1200 proteins could be identified and quantified. Over 300 proteins were up and down-regulated, and many of these regulated responses were involved in cytochrome P450, acute phase inflammation, lipid metabolism, NADH, and mitochondrial electron transport. Interestingly, we also found in mouse extracellular vesicles, venom peptides consisting of Cysteine-Rich Secretory Proteins (CRiSPs) and Cobra Venom Factors. These findings show a possible evolutionary conserved mechanism of CRiSPs and Cobra Venom factors using extracellular vesicles in snake envenomation. These data demonstrate the ability to detect active toxins and responses to snakebites, providing more direct real-time evidence for venom detection, progression, and the envenomated organism's physiological condition.

3 A synthetic metalloproteinase inhibitor derivatives from lapachol

Paulo Assis Melo

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Local tissue injuries incurred by snakebites can cause extensive, irreversible, severe tissue destruction. Such injuries are partially neutralized by the available antivenins, which in general are focused on halting systemic effects. We have previously used Bothrops snake venoms activities to identify, and develop new planed synthetic agents. In this issue are selecting Bothrops asper venom to design and synthesize a compound a quinone derivatives of lapachol that could inhibit it's hemorrhagic and proteolytic activities. We tested the ability of some new potential active analogues based on the 2-hydroxi-naphthoquinone scaffold to antagonize important activities of Bothrops atrox venom, under different experimental protocols and bioassays either in vitro or in vivo. We investigated the venom-induced hemorrhage, edematogenic, and myotoxic effects in mice in vivo as well as procoagulant, phospholipase A, collagenase and proteolytic activities in vitro. Proteolytic and collagenase activities of Bothrops atrox venom were shown to be inhibited by lapachol analogues named 3a, 3b, 3c, 3e. The inhibition of these enzymatic activities might help to explain the effects of the analogue 3a in vivo, which decreased skin hemorrhage induced by the venom. The analogues 3a and 3b did not inhibit the myotoxic activity and partially inhibited, less than 20% the phospholipase A induced by Bothrops atrox venom. The lack of protective effect of these compounds against the myotoxicity can be partially explained by their lack of ability to effectively inhibit phospholipase venom activity. The edema induced by Bothrops atrox venom was significantly reduced by the compound analogue 3a which is especially a metalloproteinase inhibitor acting decrecreasing collagenase and proteolytic activities in vitro.

4 Biochemical Characterization and Comparative Analysis of two Phospholipases A2 from venoms of North American snakes

Emelyn Salazar, Carla Zavala, Oscar Sanchez, Montamas Suntravat, Jacob Galan, Elda E Sánchez

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Chemistry Department, Texas A&M University- Kingsville

Snake venoms are comprised of enzymatic and non-enzymatic components that attribute for a broad spectrum of pharmacological activities. Although these components are found in every species, their proportions and their effects vary as well. Phospholipases A2 (PLA2s) are main toxins in viperid venoms that, besides their enzymatic activity, present multiple functional sites that induce various effects including myotoxicity, hemolytic activity, and inflammation. Using reverse phase HPLC fractionation, acidic and basic PLA2s were purified from Crotalus adamanteus (Cada) and Agkistrodon piscivorus piscivorus (App) crude venoms, respectively. Both N-terminal amino acid sequences revealed Asp at position 49 (D-49), which were homologous with catalytically active PLA2s from Viperidae venoms. The PLA2s were determined to have indirect hemolytic activity through a turbidimetric method with red blood cells and egg yolk as phospholipid substrates. In addition, we evaluated the myotoxic, hemolytic, and inflammatory effects to characterize their biological functions, thus providing a comparative analysis of the enzymes. These findings can be used for in vivo and in vitro experiments to further characterize their activities and roles in snake envenomation, as well as for the production of specialized antivenoms that target PLA2s and their biological activities.

5 Identification of Functionally Important Amino Acid Residues for C3 Convertase Activity Using Chimeric Proteins of Human C3 and Cobra Venom Factor

Carl-Wilhelm Vogel, Brian E. Hew, David C. Fritzinger

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Cobra venom factor (CVF) is the complement-activating protein in cobra venom. CVF is a structural and functional analog of complement component C3. CVF, like C3b, forms a convertase with factor B. This bimolecular complex CVF,Bb is an enzyme that cleaves C3. The CVF,Bb convertase is physico-chemically very stable, and completely resistant to an activation by Factors H and I. These two properties are in stark contrast to the C3b,Bb convertase. Our previous work using recombinant chimeric proteins of CVF and human C3 as well as comparative crystallographic analysis demonstrated that the C-terminal C345C domain harbors the important structures for the CVF-specific functions. Here we report the functional significance of individual amino acids for convertase activity. In several places, CVF and stable chimeric proteins form ionic or hydrogen bonds with Factor B. Replacing the CVF residues with the ones found in C3b resulted in the loss of the bonds and significantly lower convertase stability. Examples include T1539P, T1657V, and several residues between 1571 and 1578. We also exchanged two neutral amino acids with the charged amino acids found in human C3 (T1499D, L1501K). The resulting chimeric protein was stable and exhibited good functional activity; but its formation was significantly slower. In conclusion, our work demonstrates that hybrid proteins of human C3 and CVF are valuable tools to fine map functionally important amino acid residues in CVF and C3.

Concurrent Session VII

7A. Clinical I

Chairs: J. White/A. de Roodt

1. Julian White: Latrodectism; evidence of “failure” or a failure of evidence? toxinoz@gmail.com

2. Abdulrazaq G Habib: Clinico-Epidemiologic Determinants of Limb-Loss following Snakebite in Nigeria. Garba Iliyasu ilyasug@yahoo.com

3. Jordan Benjamin: Bringing Snakebite Treatment to the Point of Injury: The Asclepius Snakebite Foundation Model for Field Treatment. jordan@snakebitefoundation.org

4. Fouad Chafiq: Assessment of Use of Inoserp®MENA in the management of snake envenomation in Morocco. chafiqfouad@yahoo.fr

5. Adolfo de Roodt: Relationship between separation between fangs and fang mark at the bite site and size of coral snakes in Argentina and their usefulness for early diagnose of snakebites. aderoodt@gmail.com

6. Caitlyn Rogers: Green snake” bites; characteristics and significance of this subset of snakebites in the Mandalay region of Myanmar. caitlyn.rogers@adelaide.edu.au

7. Caitlyn Rogers: The effect of snake length on the extent of envenoming in Russell’s Viper (*Daboia siamensis*) snake bite cases in Myanmar. caitlyn.rogers@adelaide.edu.au

8. José María Gutiérrez: Ability of the phospholipase A2 inhibitor Varespladib to abrogate or delay lethality induced by neurotoxic snake venoms (15 min) jose.gutierrez@ucr.ac.cr

9. Julian White: Envenoming by monitor lizards; a modern mythology? toxinoz@gmail.com

10. Adolfo de Roodt: Change in the distribution of *Tityus* species of sanitary importance in Argentina. aderoodt@gmail.com

1 Latrodectism; evidence of “failure” or a failure of evidence?

Julian White, Scott Weinstein, David Warrell

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Latrodectism, an envenoming syndrome caused by widow spider bites, genus Latrodectus (Theriidae), is arguably the most important form of spider envenoming globally. The syndrome is characterised by neurorexcitation, presumably by α-latrotoxins, leading to local, regional and systemic pain, increased sweating, hypertension, painful muscle spasms and a wide variety of other clinical effects. These may take hours to become significant and days or longer to resolve; very rarely deaths have been associated with latrodectism but are poorly documented. Without effective treatment, latrodectism may result in prolonged major and distressing discomfort for patients, often requiring a period of hospitalisation and time off work, thereby generating a significant social and economic cost. Multiple studies have indicated that antivenom can neutralise venom of major Latrodectus species. Clinical experience and a number of clinical studies, reported over many decades, indicate that antivenom is the most effective treatment. Despite this a single study has provided an “evidence based” recommendation that antivenom is ineffective and should not be used and this is radically altering patient care pathways in at least one health system. We argue that this is a failure of interpretation and application of evidence based medicine that will have detrimental effects on patient care and outcomes. For envenoming syndromes with significant subjective symptomatology and logistic difficulty in enrolling sufficient numbers of patients, the results of randomised clinical trials should be considered together with clinical experience in developing treatment pathways, rather than an RCT being used to dismiss past clinical experience and studies, leading to potentially suboptimal clinical outcomes. Replicability should be emphasised in all areas of toxinology research to ensure independent confirmation of any single or initial study. We provide published evidence in support of this opinion.

2 Clinico-Epidemiologic Determinants of Limb-Loss following Snakebite in Nigeria

Ibrahim Nashabaru, Mohammed Sulaiman, Saidu Balla Abubakar, Magaji Mahmud, Sadiq Halilu, Adefolarin Opawoye, Emmanuel Ekeria, Garba Iliyasu, Muhammad Hamza, Robert A Harrison, Abdulrazaq G Habib

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Introduction: Snakebite envenoming (SBE) is a major cause of mortality and morbidity. Limb loss (LL) has been recognized as a significant morbidity but exact estimates and determinants are unknown. Here, we determine the characteristics and predictors of carpet viper bite related LL. Methods: The study was conducted at Kaltungo General Hospital, Nigeria. It has a 12 month prospective arm and a retrospective arm. Patients with SBE with LL are designated as cases and compared to control patients without LL. Data was analyzed using student's t-test, chi square, measures of effect and binary logistic regression (LR). Results: A total of 140 cases with equal control were studied (32 prospective and 108 retrospective). There were 98 (70%) males with median age of 16 years. Among cases 15 had spontaneous amputation, 32 surgical amputation and 112 severe gangrene clinically judged to be non-viable limb. The 20WBCT was incoagulable at presentation in 266 (97.8%). Antivenom was administered to 97.1% of the 280 cases and controls. There were no differences in age, gender, bitten limb, use of first-aid, traditional medicines, clinical presentations, frequency of compartment syndrome and severity of SBE. Delay from bite to antivenom administration was longer among cases compared to controls: 6.17 ± 8.13 vs 3.57 ± 2.55 days. In univariate analysis the following were related to LL [Odds Ratio, OR (95% Confidence Interval)]: tourniquet use 1.66 (1.00-2.75; p=0.0378), blister 8.26 (4.20-16.95; p0.0001) and compartment syndrome 2.09 (1.15-3.81; p=0.0092). In the LR only presence of blister/bulla was significantly associated with LL with adjusted OR (95%CI) of 5.95 (2.43-14.58). The mean hospital stay among cases compared to controls was 187.7± 113.4 vs 147.4 ± 66.0 hours (p=0.0016). Conclusions: SBE was complicated by LL in 4% of patients. Overall, only presence of blister/bulla is an independent predictor of LL prolonging hospital stay following SBE.

3 Bringing Snakebite Treatment to the Point of Injury: The Asclepius Snakebite Foundation Model for Field Treatment

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Rocky Mountain Poison and Drug Center, Denver, Colorado, 80204

The overwhelming majority of snakebites worldwide occur in remote areas far from well-equipped medical facilities. Most clinicians are unprepared for the challenges of managing snakebite patients in field conditions and are unfamiliar with the concept of administering antivenom in the prehospital environment. Treatment delay is strongly associated with increased morbidity and mortality in snakebite patients, and earlier administration of antivenom therapy should always be the goal when managing a serious snake envenomation.

Physicians and paramedics specializing in wilderness emergency medicine from the Asclepius Snakebite Foundation have developed a model to train and equip advanced life support (ALS) providers with the essential medications and equipment to deliver a high level of care, including antivenom treatment, to snakebite patients in the field. This case-based lecture discusses the essential medications and supplies to pack in snakebite kit for ALS providers and presents our model for the assessment, diagnosis, stabilization, and treatment of envenomation patients in austere conditions in sub-Saharan African and beyond.

4 Assessment of Use of Inoserp®MENA in the management of snake envenomation in Morocco

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Introduction: The hospital management of venomous snakebites consists mainly of antivenom. In Morocco,currently there is only one antivenom for the treatment of viper and elapidae envenomation: Inoserp®MENA. The purpose of this study was to assess the efficacy and safety of Inoserp®MENA administered to patients victims of snakebites as a part of the treatment.

Methods and Results: We conducted a retrospective study on cases of snakebites, who received Inoserp®MENA, reported to the Moroccan poison center over three years (2016-2018). Data collected included , age, ,sex, time to arrival in hospital, snake species whenever information is available , clinical features, time of administration of antivenom. Efficacy was assessed by improvement of thrombocytopenia. Outcomes were also assessed.

Over the three years, 290 cases, who were reviewed, met inclusion criteria. The mean age of the study population was 33 ans with a sex ratio of 1.8. The species which were identified were Daboia mauritanica (63, 3%). %). Time of administration of antivenom was of an average of 13,9 hours. Viper syndrome was of a percentage of 99,3% (n=288), the average dose administered per patient was 1,73 vials. Only 86 patients had thrombocytopenia, among these latters, the rate of platelets was improved for 63. Cobraic syndrome was of a percentage of 0.6% (n=2), the average dose administered per patient was 3,5 vials. Over a total of 7 adverse reactions, 3 were linked to an anaphylactic shock. Outcomes of the envenomation were favorable for 278 patients, though 2 patients had sequelae. Death occurred in 10 cases.

Conclusion : This study demonstrated the efficacy and safety of Inoserp®MENA in improving hemostasis. However, further prospective studies are needed to confirm this.

5 Relationship between separation between fangs and fang mark at the bite site and size of coral snakes in Argentina and their usefulness for early diagnose of snakebites.

Adolfo de Roodt, Laura Cecilia Lanari, Rodrigo Daniel Laskowicz, Vanessa Costa de Oliveira, Adolfo Rafael de Roodt, Juan Carlos Stazonelli-Sadir, Gustavo Scrocchi, Jorge Williams, Sergio Rossett, Fernando Morón Goñi, Marcela Alejandra Desio, Jantine Henriette van Grootheest, Emiliano Lértora, Daniel Dozoretz, Carlos Fabián Damin

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Crotalus durissus terrificus (Cdt) and *Micrurus* (M.) snakes are the only species responsible for neurotoxic envenomation in Argentina. Although envenomation can be diagnosed clinically by anamnesis of some evident signs and symptoms, in absence of clear data early diagnose can be difficult and impair early treatment with specific antivenom. Due to morphological differences in size and dentition between both groups of snakes, the mark of fangs at the bite site could facilitate early diagnose in absence of reliable anamnestic data (for example for accidents at night or in pediatric cases). We measured corporal length and the separation between fangs (SBF) of 309 coral snakes from different collections and serpentariums (194 M. pyrrhocryptus, 63 M. altirostris, 13 M. balyocoriphus, 10 M. frontalis and 29 M. corallinus) and of 79 live Cdt of different sizes. Additionally, the relationship between the fang mark (FM) at the bite site and SBF was determined. Coral snakes showed a length of Md= 65.5 cm (min. 22-max. 133 cm; n=297), SBF= 5.17±1.53 mm (1.4-9.3 mm; n=275) and FM= 5.6±1.1 mm (Md=5.5; 3.3-8.0 mm; n=42). The relationship between SBF and length resulted in r2 0.808 (p0.0001). No differences in FM and SBF were found between samples from museums or live animals (p>0.2). The Cdt (n=79 live specimens) studied showed a length of Md= 61 cm (37-156 cm) and SBF 14 mm (8.5-29.6 mm; p0.0001). Smaller Cdt, inclusive newborns, showed larger SBF and FM than coral snakes (SBF= 10 mm; p0.0001) with venom yields around 2.5 mg (0.2-6.4 mg). A neurotoxic syndrome caused by a snake bite, with a fang mark under 10 mm, is therefore very possibly due to Micrurus snakes since Cdt specimens of the size compatible with this bite mark or separation between fangs can´t inject enough venom to produce a neurotoxic envenomation in humans.

6 “Green snake” bites; characteristics and significance of this subset of snakebites in the Mandalay region of Myanmar

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Snakebite is a significant problem in Myanmar. Bites by Daboia siamensis and Naja kaouthia are considered medically important, but “green snake” bites are not. However, “green snakes” may include Green Pit Vipers (Trimeresurus spp.) for which there is no Burmese antivenom although these snakes cause significant local and systemic effects in other countries. As part of the Myanmar Snakebite Project, prospective case data was collected over 3 years from a number of hospitals including 3,803 snakebite cases reported from the Mandalay region. 355 of these were listed as bites from a green-coloured snake, either identified by the patient, or by the doctor. In only 22 cases was the culprit snake retained and preserved, then identified by an expert; 21 were identified as venomous Green Pit Vipers (Trimeresurus albolabris), and 1 was identified as a non-venomousAhaetulla prasina. Amongst patients bitten by Trimeresurus albolabris, 15/21 developed swelling of the bitten limb, and 3/21 developed coagulopathy. No patient developed necrosis, blistering, thrombocytopenia or AKI in these 21 cases. Of the remaining 333 patients, without expert snake identification, 241 (72 %) developed swelling of the bitten limb, and 62 (19 %) developed coagulopathy. The criteria for AKI were met in 22/333 patients, but only 1 required dialysis. In 11/22 the snake was likely to be a green pit viper as the snake was seen and identified by the doctor. This provides an indication that T. albolabris may possibly cause AKI and has important implications for snakebite management in Myanmar, as previously a snakebite presenting with local envenoming, coagulopathy and AKI was considered to be a Russell’s viper bite. Further collection of confirmed green pit viper bites is required in Myanmar, to better define the syndrome of envenoming and determine if a specific antivenom should be developed.

7 The effect of snake length on the extent of envenoming in Russell's Viper (Daboia siamensis) snake bite cases in Myanmar

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Snakebite is a major problem in Myanmar, particularly bites by Russell's Viper (Daboia siamensis). Some clinicians consider bites by small snakes cause less severe envenoming, requiring less antivenom. This study aimed to determine if there is a difference in the severity of envenoming based on the length of Russell's Vipers, and whether basing treatment on the size of snake is appropriate. Data was analysed from a prospective clinical audit of snakebites in the Mandalay region (part of the larger 'Myanmar Snakebite Project'), involving patients bitten by Russell's Viper and monitored for the development of significant local and systemic effects, measured against antivenom use and final outcome. Where snakes were brought in by patients, these were collected, identified and measured. 132 snakes were confirmed as Daboia siamensis, ranging from 18 cm to 105 cm in length. The clinical manifestations in patients bitten by smaller snakes (≤30 cm in length, n=52) were compared to those in patients bitten by larger snakes (≥65 cm in length, n=38). The rates of local necrosis, regional lymphadenopathy, thrombocytopenia, coagulopathy, renal damage (AKI), shock and capillary leak, were higher in the group bitten by the larger snakes. There were 2 deaths in the group of 132 patients, both bitten by snakes larger than 65 cm. However, some patients bitten by snakes ≤30 cm still developed significant systemic effects: 29 % showed features of coagulopathy and 3.8 % showed features of AKI. There was a positive correlation between the length of snake that caused the bite and the number of days the patient was in health care (r=0.4; p0.001). This study indicates that bites by larger Daboia siamensis are more likely to result in severe envenoming, but bites by smaller snakes can also result in severe envenoming, therefore initial antivenom dosage should not be based on snake size/length.

8 Ability of the phospholipase A2 inhibitor Varespladib to abrogate or delay lethality induced by neurotoxic snake venoms

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The phopholipase A2 (PLA2) inhibitor Varespladib (LY315920) and its orally bioavailable prodrug, methyl-Varespladib (LY333013) inhibit PLA2 activity of a wide variety of snake venoms, and abrogate toxicity in some of them. These drugs have the potential to be used in field conditions in snakebite envenomings, and as adjunt therapy to antivenoms in the hospital setting. In this study, the ability of these two forms of Varespladib to halt or delay lethality of potent neurotoxic snake venoms was tested in a mouse model. The venoms of Oxyuranus scutellatus, Notechis scutatus, Bungarus multicinctus and Crotalus durissus terrificus, all of which have potent neurotoxic PLA2s, were used. A dose of each venom, corresponding to 3 Minimum Lethal Doses (3 LD50s), was injected subcutaneously in mice, followed by the intravenous (LY315920) or oral (LY333013) administration of the inhibitors, immediately and at various time intervals after envenoming. Control mice receiving venom alone died within 3 hr of envenoming. Observations in mice receiving Varespladib were carried out at 6 and 24 hr. Mice injected with O. scutellatus venom and treated with the inhibitors survived the 24 hr observation period, whereas those receiving B. multicinctus and C. d. terrificus venoms survived at 6 hr but not at 24 hr. In contrast, mice receiving N. scutatus venom and then the inhibitors died within 3 hr, similarly to control animals injected with venom alone. Venoms were analyzed for the relative amounts of presynaptic PLA2 neurotoxins and postsynaptic α-neurotoxins. In the case of N. scutatus, the relatively high concentration of α-neurotoxins explains the inefficacy of Varespladib. Results suggest that the two forms of Varespladib are effective in abrogating, or delaying, neurotoxic manifestations of envenomings whose neurotoxicity is mainly dependent on presynaptically-acting PLA2s, but not in venoms in which α-neurotoxins play a significant role in the overall toxicity.

9 Envenoming by monitor lizards; a modern mythology?

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Monitor lizards, Family Varanidae, comprise an anatomically homogeneous assemblage of hunting lizards in a single genus, Varanus, found from Africa, through Asia, to Australia, where the peak species diversity is found. They vary in size from small cryptic species to the largest lizards known. All have numerous sharp teeth and large specimens can inflict severe injuries to large animals, including humans, but humans are not normal prey, even for the largest species, Varanus komodoensis. The Toxicofera hypothesis postulates that venomousness in reptiles has arisen only once and that varanid lizards are included within the venomous reptile clade. Several papers have stated that varanids are venomous and there are now 3 papers reporting severe or fatal outcomes from alleged varanid bites, two claiming envenoming had occurred and in one case, fatal envenoming. This contrasts with a large global experience with these lizards, particularly the larger species, without any other reports of clinical effects consistent with envenoming, as described in the above 3 cases. Bites by V. komodoensis are the subject of speculation about effects of its bite on large prey including an unproven claim they are venomous. We consider the 3 clinical reports of alleged varanid bites speculatively causing envenoming, examining their variable clinical presentation, with similarities including significant local tissue injury at the bite site, and variables including acute kidney injury and rhabdomyolysis. Necrotizing soft-tissue infection (NSTI) is a well described disease, with 2 subtypes, the polymicrobial Type I and monomicrobial Type II. Both are associated with major local effects and secondary systemic effects, similar to those in the 3 reported alleged varanid bites, but Type II can also be associated with significant myonecrosis. We argue that these 3 cases far more likely represent NSTI, not envenoming by a varanid and do not support speculation about venomousness in varanids.

10 Change in the distribution of *Tityus* species of sanitary importance in Argentina.

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Scorpions of Tityus genus are responsible for envenomation and deaths in Argentina and these scorpions were mostly distributed from the Center to the North of the country. In the last years, several species have been described in regions where their presence had not been previously described. These are: 1- Tityus bahiensis in the provinces of Entre Ríos and Buenos Aires (in the localities of Lanús and San Clemente del Tuyú), and 2- Tityus confluens in the city of Buenos Aires and in the province of Buenos Aires in the localities of Pilar, La Plata, Mar del Plata and Bahía Blanca. These findings modify the distribution map of scorpions of sanitary importance in Argentina, reason for which this new distribution must be considered when facing a scorpion sting. This is especially important in the city of Buenos Aires and the province of Buenos Aires, where most of the accidents by scorpions are caused by Tityus trivittatus, and where at least in some of their regions, T. confluens or T. bahiensis can be found at present. The possible reasons of this new distribution, as well as the possible causes for the occurrence of severe envenomations in regions where these were not observed historically, are not clear. Based on the severe envenomations historically observed and on this new distribution map, emphasis must be placed on the need to capacitate health personnel in general in diagnostic and treatment and intensivists or critical care physicians in particular to adequately treat scorpion accidents. The surveillance of the distribution of the scorpions is necessary due the modifications observed and the sanitary importance of specimens of the genus Tityus.

Concurrent Session VIII

8A. New Biology and Evolution of Venomous Organisms I.

Chairs: Casewell/Richardson.

1. Ronald Jenner (NHM, London): Parallel evolution of complex centipede venoms. r.jenner@nhm.ac.uk

2. Ashlee Rowe (University of Oklahoma): Molecular mechanisms of resistance to lethal scorpion neurotoxins in a scorpion predator. ahrowe@ou.edu

3. Ray Norton (Monash University): Correlations among sequence, physicochemical properties and function in peptide toxins. ray.norton@monash.edu

4. Luciana Freitas-de-Sousa (Instituto Butantan) Individual variability and ontogenetic variation in Bothrops jararacussu snake venom. luciana.sousa@butantan.gov.br

5. Nick Casewell (Liverpool School of Tropical Medicine): Solenodon genome reveals convergent evolution of venom in eulipotyphlan mammals. nicholas.casewell@lstmed.ac.uk

1 Parallel evolution of complex centipede venoms

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Centipedes are an ancient group of venomous terrestrial animals, with a fossil record going back about 420 million years. Living species belong to five monophyletic lineages that are classified as orders: Scutigeromorpha (house centipedes), Lithobiomorpha (stone centipedes), Geophilomorpha (soil centipedes), Scolopendromorpha (the familiar big-bodied centipedes), and Craterostigmomorpha (two species restricted to Tasmania and New Zealand). However, our understanding of centipede venoms rests almost entirely on data from scolopendromorphs. Combined proteomic and transcriptomic (proteotranscriptomic) venom profiles are available for only six scolopendromorph species and a single scutigeromorph. In this study we present the first proteotranscriptomic venom profiles for representatives of the remaining three orders, as well as a second species of scutigeromorph. We use these data to perform the first comparative analysis of centipede venoms that includes all currently available proteotranscriptomic venom profiles. Our analyses reveal that centipede venoms contain more than 90 phylogenetically distinct protein and peptide families. Strikingly, not a single one of these is found in all five orders, with 67 families being found only in single orders. Phylogenetic analyses of proteome-annotated gene trees show that the composition of centipede venoms is highly dynamic across macroevolutionary time scales, with numerous gene duplications as well as functional recruitments and losses. Our analyses further suggest that the ancestral centipede venom was a simple cocktail comprising just four protein families, with complex venoms only evolving after the five orders had diverged from each other. Complex venoms then evolved in parallel in each of the orders, with scolopendromorphs evolving particularly complex arsenals. Our data highlight the striking evolutionary plasticity of centipede venom composition throughout their evolutionary history. More compositional evolution happened along the lineage of *Scolopendra subspinipes*after it diverged from its congener *S. morsitans*than happened along the stem lineages of the five orders combined.

2 Molecular mechanisms of resistance to lethal scorpion neurotoxins in a scorpion predator

Ashlee Rowe

University of Oklahoma

Voltage-gated sodium ion channels (Nav) encode environmental stimuli and regulate motor responses to that information by producing the action potentials underlying neuronal signaling and muscle contraction. Consequently, Navchannels are targets of diverse neurotoxins produced by multiple taxa across the animal kingdom. Arizona bark scorpions (Centruroides sculpturatus) produce a cocktail of neurotoxins to subdue their prey and defend against predators. Their venom includes alpha and beta toxins that disrupt Nav channel function, causing pain, muscle paralysis and respiratory failure in animals. Toxins that cause pain and death may impose strong selection on the receivers, driving the evolution of adaptions that mediate interactions between bark scorpions and their enemies. Predatory rodents (grasshopper mice, Onychomys) hunt bark scorpions. In response to selection by scorpion venom, grasshopper mice have evolved physiological resistance to toxins that cause pain and death. Although previous work identified amino acid changes in one grasshopper mouse Navchannel (Nav1.8) that provide resistance to venom pain, mechanisms underlying resistance to muscle paralysis and death were unknown. In skeletal muscle, Nav1.4 regulates contraction, making it a potential target of bark scorpion toxins. Thus, we investigated the structural and functional properties of grasshopper mouse Nav1.4 channels, and their potential role in resistance to the lethal components in bark scorpion venom. While bark scorpion venom produced both alpha and beta toxin effects on rat Nav1.4, grasshopper mouse Nav1.4 was less sensitive to the alpha and beta toxin effects of the venom. We identified amino acid changes in domains I and III, and a C-terminus insert in the alpha subunit of the grasshopper mouse Nav1.4 protein that are crucial for resistance to bark scorpion alpha and beta toxins.

3 Correlations among sequence, physicochemical properties and function in peptide toxins

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Sea anemones are a rich source of peptides that are potent and often selective probes of the structure and function of ion channels and receptors. For example, several peptides from sea anemones and analogues thereof are potent blockers of the voltage-gated potassium channel Kv1.3, which plays a major role in the activation of effector memory T cells. As these cells have a key role in autoimmune diseases such as multiple sclerosis, psoriasis, type 1 diabetes and rheumatoid arthritis, peptide blockers of Kv1.3 that selectively inhibit the activation of T_{EM} cells show considerable potential as therapeutics for autoimmune diseases.

These and other studies of sea anemones highlight how widespread the ShK fold is in nature, not only in the phylum Cnidaria, but also in parasitic worms mammals and even plants. A comparison of available ShK sequences based on various physicochemical properties reveals that they cluster into discrete sub-sets; the relationships among these clusters, their functional activity and potential as therapeutics are currently being explored, but it is clear that this fold can support several other activities beyond potassium channel blockade.

4 Individual variability and ontogenetic variation in Bothrops jararacussu snake venom

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Bothrops jararacussu snakes present a Type II venom with predominance of phospholipases A_2 (PLA_2) in its composition. In other *Bothrops* snakes, Type II venoms are apparently more subject to ontogenetic variability than Type B venoms. However, very little is known about *B. jararacussu* venom variability. Here, we analyzed the individual transcriptome and proteome from 3 juvenile and 3 adult *B. jararacussu* specimens and evaluated the variability on venom composition and on the major toxin functions. Comparing the individual venom composition, a pronounced variability in the abundance of PLA_2 and metalloproteinases (SVMPs) protein families was observed either among adult or juvenile specimens. As expected, PLA_2s were the most abundant components in adult venoms while venoms from juvenile snakes presented metalloproteinases as the major component, a pattern comparable to venoms from most other *Bothrops* species. The PLA_2 isoforms predominant in adults were the basic myotoxic Bothropstoxins I and II, while in the venom from juveniles these PLA_2 isoforms were the least abundant. The PLA_2 enzymatic activity was similar for both, adults and juveniles, which suggests that the highly expressed isoform in adults are related to the K-49 analogues. Moreover, juveniles showed higher SVMP catalytic activity (*p*0.001) and the hemorrhagic activity *in vivo* was inversely correlated to the ontogenetic stage. The juveniles (50 cm) induced the most pronounced hemorrhagic activity, similarly to the smaller adult (83 cm), and a reduced activity in adults larger than 100 cm. Serine proteinases and C-type Lectin were the other components most abundant in the venom from juveniles and adults. The serine proteinases activity was similar between juvenile and adult venoms. Thus, we show for the first time the individual variability and the ontogenetic variation in *B. jararacussu* venom, which mechanisms of generation are under further studies of our group.

5 Solenodon genome reveals convergent evolution of venom in eulipotyphlan mammals (15 min) nicholas.casewell@lstmed.ac.uk

Nick Casewell

Liverpool School of Tropical Medicine

Venom systems are important adaptations that have evolved on many independent occasions, and typically function to facilitate predation and/or defence. Despite venoms being model systems for studying a variety of evolutionary and physiological processes, many taxonomic groups remain understudied. For example, multiple representatives of eulipotyphlan mammals are venomous, but little is known about the evolutionary history and composition of their oral venom systems. Here, we investigated the origin and evolution of venom in eulipotyphlans by characterising the venom system of the endangered Hispaniolan solenodon (*Solenodon paradoxus*). We constructed a genome to underpin proteomic identifications of solenodon venom toxins, before undertaking evolutionary analyses of those constituents, and functional assessments of the secreted venom. Our results demonstrate that molecular components of solenodon and shrew venoms have evolved convergently, with hypotensive kallikrein-1 proteins co-opted as toxins in both lineages following their divergence over 70 million years ago. Our findings represent a striking example of convergent molecular evolution, and highlight that certain mammalian venom systems may be subjected to evolutionary constraints.

Concurrent Session VIII

8B. Clinical II. Emerging Clinical Topics: Safety and Effectiveness of Current Antivenoms; Clinical Presentations of Intoxication and Management; Epidemiology.

Chairs: Wuelton Monteiro / Fan Hui Wen

1. Charles Gerardo (Duke University): Need for better evidences and methodological aspects of clinical trials in snakebites. charles.gerardo@duke.edu

2. Ceila Málaque (Butantan Institute): Severe snakebite envenomations and management. ceila.malaque@butantan.gov.br

3. Joao Ricardo Vissoci (Duke University): Bottlenecks for access to treatment of snakebites and scorpion stings, with special attention to clinical consequences joaovissoci@gmail.com

4. Jacqueline Sachett (Universidade do Estado do Amazonas): Barriers to access to antivenom serum in the Amazon. jacenfermagem@hotmail.com

5. Fernando Val (Fundação de Medicina Tropical Dr. Heitor Vieira Dourado): Disabilities from snakebites in the Amazonia region. ffaval@gmail.com

1 Need for better evidences and methodological aspects of clinical trials in snakebites

Charles Gerardo

Duke University

Background: Modern snake antivenoms and novel agents in development require clinical studies of efficacy and safety prior to implementation in patient populations. There are substantial challenges to conducting these studies. The challenges include research infrastructure to conduct these trials in certain locales, challenges in enrollment and follow-up of patients, the lack of patient centered outcomes measures, agreement on acceptable study designs, and generalizability of study results to other snake envenoming populations.

Methods/Results: We will discuss important considerations for evaluating efficacy and safety in future studies. The need for development of patient-centered outcome measures, alternative study designs, and alternative analyses, and the feasibility of clinical research networks to prospectively assess these therapies will be discussed.

Conclusion: Only through by a detailed evaluation of prior approaches to snake envenoming clinical trials and a commitment to refining these techniques will yield high value results that can be applied for maximum benefit in this neglected tropical disease.

2 Severe snakebite envenomations and management

Ceila Málaque

Butantan Institute

Bothrops snake occurs from the Mexico to the Argentina and the Bothrops envenomation is the most frequent snakebite in that region. In Brazil the case fatality rate of Bothrops envenomation is 0.40%. Bothrops venom causes tissues damage at the site of venom injection, hemostatic alteration and systemic haemorrhage. Therefore, in human envenomation the patients may evolve with spontaneous bleeding that can be severe and lead to death, such as in the bleeding occur in central nervous system, and complications such as necrosis, infection and acute kidney injury. The complications and death are associated with delayed application of appropriate antivenom. In management of snakebite envenomation, therefore, the cornerstone is early administration of appropriate antivenom. For this reason it is important that antivenom be allocated where the majority of snakebites occur, as well as patients have the facilities to access the health system. In addition, the door-to-antivenom time should be short. Furthermore, measure to avoid or reduce complications such as adequate hydration, avoid nephrotoxic drugs, treat infection with appropriate antimicrobials, avoid unnecessary invasive procedures, are very important. Therefore, it is essential continuous education for health personnel to improve knowledge about the physiopathology of envenomation by snakebites, and improve management of the envenomed patients.

3 Bottlenecks for access to treatment of snakebites and scorpion stings, with special attention to clinical consequences

Joao Ricardo Vissoci

Duke University

According to the World Health Organization, 4.5–5.4 million people are bitten by snakes each year with the number of deaths ranging from 81,000 to 138,000. Most of these snakebites occur in tropical and sub-tropical, low- and middle-income countries (LMIC). Between 2001 and 2012 in Brazil, 28% of injuries and 54% of deaths from venomous land animals were attributed to snakes. Mitigating the morbidity and mortality of these bites depends on timely administration of antivenom. Using data from 2010 to 2015 in Brazil's national healthcare databases, we identified areas of higher snake and scorpions envenomation prevalence, mortality and time to reach care through GIS. Using a generic optimization method based on geospatial simulations, we identified the optimal scenario for antivenom dispensation that would reduce the time to reach antivenom care, using the primary care facility network across the country. This talk will approach the methodological potential impacts on time to reach care and outcomes of snake and scorpion envenomation by using the primary care facilities network and the family health strategy as surrogates for antivenom care.

4 Barriers to access to antivenom serum in the Amazon

Jacqueline Sachett

Universidade do Estado do Amazonas

Snakebite accidents are a major public health problem. They are accidents caused by venomous snakes, especially in tropical countries, due to their frequency and associated morbidity and mortality. In South America, Brazil has the highest annual average of snakebite accidents, with approximately 29,000 cases and a case fatality rate of about 0.4%. The North region occupies the second place in this incidence of the country (24 / 100,000 inhabitants), second only to the Midwest region (33 / 100,000 inhabitants). It is known that the Amazon has a particular and atypical geography of the rest of the country, because the main transport route between its inland municipalities is fluvial. This mode of transport is not as fast when emergency medical attention is needed. The average time between snakebite accident and medical treatment is longer than 6 hours regardless of severity and mortality, since most of the population victims of poisonous snake accidents are from rural and indigenous people, who live on agriculture and extractivism and the risk in these areas is about six times higher. The only treatment advocated by the Brazilian Ministry of Health in cases of snake poisoning is the application of specific anti-acid serum. However, due to the difficulty of access to the specialized health system, as in regions of the Amazon, populations are forced to seek therapeutic alternatives usually through plant species, popular medicine, in an attempt to block the biological activities induced by the poisons of snakes. Then, it is important to discuss the efficiency of health care for people who are victims of snakebite accidents, identifying the main difficulties of access to specialized medical services as well as the quality of prehospital care, and the differences in care for cases of different localities, environmental variables and local economic activities, species involved and alternative therapies.

5 Disability secondary to snakebites in rural Amazon: what are the impacts?

Fernando Val, João Arthur Alcântara, Guilherme Kemeron Maciel Salazar, Altair Seabra Farias, Wuelton Marcelo Monteiro, Jacqueline Gonçalves de Almeida Sachett

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In rural communities of the Amazon, extractivism and pastoralism are the main sources of subsistence and income. Victims of snakebite envenoming are usually involved in such activities and face great physical and psychological disability, hampering socioeconomic sustainability and stability. Due to the lack of proper access to health services, it becomes difficult to assess the impact after such accidents. Aiming to evaluate health and disability across rural communities in the Solimões and Juruá rivers, we applied the WHODAS 2.0 (World Health Organization's Disability Assessment Schedule) in victims of snakebite envenoming. We evaluated health status and their ability to perform daily activities necessary to fulfill their role at home, work, school or within the local community. A total of 58 victims of snakebite were interviewed between January and April, 2019. The accident had greater impact in the cognition and social engagement domains (51.6% and 51.5%, respectively). Mobility was affected in 48.3% and daily life activities, 42.1%. The less impacted domains were interpersonal relations (36.4%) and self-care (35.4%). Furthermore, the tool showed that 56 (96.5%) victims needed to reduce their work hours or intensity, impacting family and community income. Deficiency, secondary to snakebite envenoming, is multidimensional and is the product of an interaction between attributes of individual characteristics and the social environment. Snakebite may lead to chronic disability. As a result, quality of life and economic activities in the community may become compromised. Rehabilitation should be available to better improve functional outcome. Fast and effective treatment, rehabilitation availability, and proper access to education and prevention strategies regarding ways to approach activities in rural areas without being exposed to such risks are essential to tackle this worldwide problem.

Concurrent Session IX

9A. New Biology and Evolution of Venomous Organisms II.

Chairs: Casewell/Richardson

1. Fernanda Cardoso (University of Queensland): Harnessing multifunctional spider-venom peptides to modulate pain pathways. f.caldascardoso@uq.edu.au
2. Jens Puschhof (Hubrecht Institute): Slithering stem cells – understanding snake venom production in vitro using organoids. j.puschhof@hubrecht.eu
3. José Antonio Portes-Junior (Instituto Butantan): The venom variability of the Bothrops jararaca complex and its correlation with the speciation processes in continental islands. portes.junior@butantan.gov.br
4. Tim Lüddecke (Fraunhofer IME) Translational tarantula phylogenomics: Evolution of theraphosid spiders and their defensive arsenal with implications for venom bioprospecting. tim.lueddecke@outlook.com
5. Juan Calvete (CSIC, Valencia): Comparative venomics of Brazilian coral snakes: Micrurus frontalis, Micrurus spixii spixii, and Micrurus surinamensis. jcalvete@ibv.csic.es
6. Mike Richardson (Leiden University) Evo-devo and genomics of snakes m.k.richardson@biology.leidenuniv.nl

1 Harnessing multifunctional spider-venom peptides to modulate pain pathways

Fernanda Cardoso

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Animal venoms have evolved into potent neurotoxic peptides for prey capture and defence. Many of these peptides modulate the function of ion channels involved in the generation and transmission of electrical signals in neurons and muscles, thereby inducing paralysis, pain or death in a diverse range of organisms, including humans. Spider venom-derived cysteine knot peptides, in particular, are a mega-diverse class of molecules that exhibit unique pharmacological properties to modulate key ion channels. Voltage-gated sodium and calcium channels are often targeted by these molecules that allosterically promote opening or closing of these channels by binding to structural domains outside the channel pore. Although such effects are often deleterious, naturally occurring multifunctional and selective spider peptides are showing potential to treat a range of neurological disorders. In order to investigate these spider peptides, we are using phylogenetic analysis to disclose patterns of evolutionary pressure and clues about their structure-activity relationships and action mechanisms. In addition, we have applied high-throughput fluorescent screens to identify spider-venom peptides that preferably target ion channels involved in pain pathways. From the venom of the Venezuelan tarantula *Theraphosa apophysis,* novel ion channels inhibitors named Tap1a and Tap2a selectively inhibited pain-related sodium and calcium channels subtypes. Potent modulation of pain pathways in afferent nerve fibers in the colon and in the bladder by Tap1a have highlighted multifunctionality as key factor for this modulation. Finally, structure-activity relationships have been investigated using peptide rational design and molecular docking to disclose key regions and residues involved on the inhibitory properties of these spider peptides. This work contributes to the understanding of the multifunctionality of naturally occurring bio-active peptides modulating sodium and calcium channels and the research into new therapeutics to treat chronic pain.

2 Slithering stem cells – understanding snake venom production in vitro using organoids

Jens Puschhof

Hubrecht Institute

Recent advances in organoid technology have proven this system to be a valuable tool in understanding human organ development and pathologies. These adult stem cell derived cultures closely recapitulate structural and functional properties of their organ of origin. Here, we expand the organoid technology toolbox by describing a protocol to culture non-mammalian organoids derived from a snake venom gland. The complexity of venom production, composition and function remains largely unknown for many species. Organoids derived from an Aspidelaps lubricus venom gland can be long-term expanded and histologically resemble the gland. Expression of typical venom-related transcripts (3FTx and Kunitz-type protease inhibitors) can be detected in proliferating organoids with RNA sequencing. Single cell RNA sequencing reveals distinct venom expressing cell types, as well as proliferating cells with features of mammalian stem cells. Using mass spectrometry, we identify peptides in the culture medium supernatant that match the composition of the crude venom of the same species. Venom gland organoids furthermore consist of specialized secretory cells visible by transmission electron microscopy. The system enables investigation of venom production and function on a cellular level in controlled conditions and without the need of experimental animals. This study describes the adaption of organoid technology to a non-mammalian species, providing a model to understand the complexity of the snake venom gland.

3 The venom variability of the Bothrops jararaca complex and its correlation with the speciation processes in continental islands.

José Antonio Portes-Junior, Pollyanna Fernandes Campos, Gesiele Almeida Barros-Carvalho, Ana Maria Moura-da-Silva, Lisle H. Gibbs, Inácio de Loiola Meirelles Junqueira-de-Azevedo, Felipe Gobbi Grazziotin

Instituto Butantan
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We still do not have a complete understanding of how complex evolutionary novelties are generated. Venomous snakes isolated on islands are considered a powerful model to study the evolutionary processes that create phenotypic diversity. Populations of B. jararaca underwent several episodes of recent speciation on the continental islands off the southeastern coast of Brazil. This repeated speciation from the same ancestral population makes this system an extreme case of recent adaptation to distinct niches. In this study we evaluate the evolutionary forces and mechanisms that shaped the variability of venom within the B. jararaca complex. We assess the venom variability through the combined analyses of venom gland transcriptomics and reversed-phase high-performance liquid chromatography. We studied forty venoms and 30 glands from mainland populations from the states of Santa Catarina (SC), São Paulo (SP), Espirito Santo (ES), Minas Gerais and Bahia; and the islands of Arvoredo (SC), Queimada Grande (SP), Moela (SP) and Franceses (ES). Considering the levels of expression of the main toxin classes, we identified high variability among individuals, and we were able to classify the transcriptomes in four major patterns of predominant toxin expression: (1) PLA2; (2) SVMP; (3) SVMP and CTL; (4) PLA2, CTL and SVSP. We confirmed these main patterns of expression by comparison with the venom chromatographic profiles, that were used to perform Principal Component Analysis (PCA) using the isometric logratio transformed proportion of expression of the main toxin classes. PCA results showed that the composition of venoms correlates with geographic distribution. The venom intrapopulational variability for the island specimens is relatively low, although the population from Arvoredo Island presents high venom variability. This island is by far the largest island included in this study suggesting that food supply or population size could be affecting the variability of the venom in the Bothrops jararaca complex.

4 Translational tarantula phylogenomics: Evolution of theraphosid spiders and their defensive arsenal with implications for venom bioprospecting

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The family Theraphosidae, commonly referred to as tarantulas, represents the most diverse group of mygalomorph spiders. Given their remarkably popular position compared to other spiders, it is rather surprising that theraphosid systematics still largely relies on morphological data, although recent studies demonstrated that theraphosids are affected by high degrees of morphological homoplasy. Their evolutionary history remains as well only poorly understood since reliable phylogenetic trees for its major radiations are lacking so far. Here we used phylogenetic and phylogenomic approaches to unravel the intra-familial relationships within Theraphosidae. For the first time we reconstructed a highly supported backbone phylogeny for the family and by that identified several sub-familial groups in need for taxonomic revision. We discovered a clade inside our phylogeny that is characterized by the presence of urticating setae as a defensive mechanism. Members within this clade are known to comprise less potent venoms than other Theraphosidae and they further contain the most speciose lineages within the family. Based on these observations we discuss that the evolution of urticating setae might have posed a selective pressure onto the theraphosid venom system, leading to the subsequent loss of defensive-toxicity. Therefore the importance of evolutionary costly venom as a means of defense might be reduced in these spiders and, finally, provided those taxa with a more economic alternative that contributed to their outstanding diversification. Lastly, we used the available data of previously identified venom components from publicly available databases. These were subsequently correlated with our phylogenetic tree to identify major genetic lineages within Theraphosidae that have so far been neglected in venom-based bioprospecting attempts. We highlight theraphosid priority groups for future venom surveys and flag phylogenetic studies in general as a useful tool that can be used supportively for the rapid identification of interesting target species for venom biodiscovery in the future.

5 Comparative venomics of Brazilian coral snakes: Micrurus frontalis, Micrurus spixii spixii, and Micrurus surinamensis

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A comparative venom proteomic analysis of the Brazilian southern coral snake, M. frontalis, the Amazon coral snake M. spixii spixii, and the aquatic coral snake M. surinamensis is reported. Venoms from M. frontalis and M. s. spixii were composed mainly (> 90% of the total venom proteome) by 3FTxs and PLA2s in different proportions, and minor proteins from 2 to 5 protein families. Conversely, the aquatic coral snake expressed a streamlined (95%) 3FTx venom with low abundance (4.2%) of PLA2 molecules. A compositional-lethal activity for natural prey correlation analysis suggests that M. surinamensis venom may has evolved under strong pressure to quickly immobilize aquatic prey. On the other hand, venoms from M. frontalis and M. s. spixii, whose diet consist mainly of amphisbaenians and colubrid snakes, may have been shaped through balancing selection. Our work provides strong evidence for the occurrence in M. frontalis venom, but not in those from M. s. spixi and M. surinamensis, of a KUN-PLA2 complex homologue to heterodimeric venom toxins from some long-tailed monadal coral snakes that target acid-sensing receptors ASIC1a/2 evoking pain. The M. frontalis protein would represent the first example of a KUN-PLA2 heterodimer in a South American short-tailed triadal coral snake venom.

6 Evo-devo and genomics of snakes

Mike Richardson

Leiden University

The application of new technologies will help solve many classic questions in snake biology. Genome sequencing in this and other labs is revealing how toxin genes have duplicated, clustered and diverged during snake evolution. Our transcriptome profiling of toxin genes in embryos is showing that there is commonality between the venom gland and salivary glands of the mandible. Finally, we are applying single-cell mRNA sequencing to address the most fundamental question of all in snake biology: how did the numerous snake adaptations (venom gland and fangs, elongated body, loss of limbs) evolve.

Concurrent Session IX

9B. SBTX: Innovation in Clinical and Basic Research in Toxinology.

Chairs: G. Picolo /M. E. de Lima.

1. Gisele Picolo, Special Laboratory of Pain and Signaling, (Butantan Institute, São Paulo, Brazil) Crotoxin induces analgesic and immunomodulatory effects on chronic pain models that is potentiated by nanostructured silica SBA-15 gisele.picolo@butantan.gov.br
2. Jose M. Gutiérrez, (Universidad de Costa Rica, San Jose, Costa Rica): Novel alternatives for improving the therapy of snakebite envenomings jose.gutierrez@ucr.ac.cr
3. Maria Elena de Lima, (IEP/SCBH, Belo Horizonte, MG, Brazil): In vitro and in vivo antimicrobial activity of peptides derived from the venom of the spider Lycosa erythrognatha. lima.mariaelena@gmail.com
4. Kenneth Shea: Synthetic antibodies. Polymer nanoparticles that sequester the medically relevant protein toxins in snake venom. kjshea@uci.edu
5. Laura-Oana Albulescu: Repurposing DMPS, a metal chelator, as a rapid field intervention for treating hemotoxic snakebite. Laura-Oana.Albulescu@lstmed.ac.uk
6. Yaroslav Andreev: Sea anemone peptide modulates TRPA1 activity, produces analgesia andenhances process of regeneration. shifter2007@gmail.com
7. Eliécer Jiménez Charris: Antitumor potential of Pllans–II, an acidic Asp49–PLA2 from Porthidium lansbergii lansbergii snake venom on human cervical carcinoma HeLa cells. eliecer.jimenez@correounivalle.edu.co

1. Crotoxin induces analgesic and immunomodulatory effects on chronic pain models that is potentiated by nanostructured silica SBA-15

Gisele Picolo

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Crotoxin (CTX) is the main toxic component of the *Crotalus durissus terrificus* snake venom, which, despite its neurotoxic effect, has been extensively studied due to its immunomodulatory, anti-inflammatory, antitumor and antinociceptive properties. However, its toxicity is a limiting factor for its use. The mesoporous silica (MSN) SBA-15 is a nanoparticle that when used as adjuvant or carrier, may reduce the toxicity and potentiate the immune response to various compounds. Since the treatment for chronic pain is still a challenge, the therapeutic potential of CTX:SBA-15 for chronic pain and for autoimmune disease was considered. CTX:SBA-15 induced an increase of 35% in the LD_{50} when compared to unconjugated CTX. Treatment with five consecutive doses of CTX:SBA-15 in the acute (from 0 to 4^{th} day) and chronic (from 14^{th} to 18^{th} day) phases of the partial sciatic nerve ligation model, induced a long-lasting reduction of the mechanical hypernociception. Furthermore the treatment with CTX:SBA-15 reduced IL-6 and increased IL-10 expression. Interestingly, when complexed with SBA-15, CTX showed antinociceptive effect when orally administered. When evaluated in the MOG_{35-55} induced-experimental autoimmune encephalomyelitis (EAE), an accepted animal model for multiple sclerosis, crotoxin induced antinociception and reduced EAE progression, delaying the onset of the disease, decreasing the clinical scores and decreasing the incidence of the disease among the animals. When conjugated to the SBA-15, CTX has its antinociceptive effect enhanced, maintaining its interference on the immune and inflammatory responses in EAE model. In contrast to treatment with CTX, a single dose of CTX:SBA-15 was effective in modulating both mechanical hypernociception and clinical signs in animals with EAE. These results confirms the therapeutic potential of CTX for chronic pain and for the modulation of EAE, and points out for the use of silica as a reducer agent for toxicity.

2. Novel alternatives for improving the therapy of snakebite envenomings

José María Gutiérrez

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The mainstay in the treatment of snakebite envenomings is the administration of animal-derived antivenoms. When designed using appropriate mixtures of venoms and produced following good manufacturing practices, antivenoms are highly safe and effective therapeutic tools. Nevertheless, antivenoms present a number of limitations. In particular, they need to be administered in health facilities by trained staff, and have specificity towards the venoms used in immunization and other related venoms. In settings where antivenom administration is delayed due to limitations in the provision of health services, there is a need for innovative therapies that could be applied in the field rapidly after the onset of envenoming. Advances in the development of novel therapies for snakebite envenoming will be discussed, including (a) recombinant human or chimeric antibodies directed against key venom toxins; (b) natural and synthetic inhibitors of enzymatic venom components that play key roles in toxicity, such as phospholipases A2 and metalloproteinases; (c) nanoparticles, designed to have the ability to bind different families of venom components; and (d) aptamers and other synthetic molecules which inactivate particular types of toxins. Innovations in this field demand interdisciplinary efforts to identify the key toxins in medically-relevant venoms through toxicovenomics, design novel inhibitory molecules, and develop high throughput systems for assessing their inhibitory potential against a variety of snake venoms at the preclinical level. Likewise, these innovations should be followed by the development of clinical trials, which represent a great challenge for moving this field forward, especially when assessing the ability of these therapies to reduce venom-induced local tissue damage.

3. In vitro and in vivo antimicrobial activity of peptides derived from the venom of the spider Lycosa erythrognatha

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Super-resistant bacteria are a public health problem, increasing the need to develop new drugs and strategies to combat the superbugs. Our group isolated and characterized a potent antimicrobial peptide, LyeTx1, from the venom of the spider Lycosa erythrognatha. LyeTx1 and a new peptide derivative, LyeTx1b, were synthetized and had their secondary structure solved by NRM technique. The minimum inhibitory concentration (MIC) for these peptides was determined in 12 different clinically isolated strains. In the strains *Acinetobacter baumannii*, *Echerichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, LyeTx1b was slightly more active than LyeTx1. However, on other tested strains, the activities for both peptides were similar, but they were not active on two strains of *Serratia sp*. In resistance evaluation experiments, by using two different methods, LyeTx1b does not favor the selection of resistant mutants in strains of *S. aureus* resistant to methicillin. In the *S. aureus* strain biofilm assay, LyeTx1b activity was more potent than the native peptide. LyeTx1b showed, *in vitro*, greater activity than the original molecule, in planktonic and biofilm of bacteria. Then, LyeTx1b was tested *in vivo*, by injection in the knee of a mouse model of septic arthritis, infected with *S. aureus* (ATCC 6538) and by topical application, in a rabbit keratitis model. The rabbit eyes were infected with *Staphylococcus aureus* resistant to penicillin, erythromycin and ampicillin, isolated from ocular samples of a 22 years old female patient, presenting keratitis. In the septic arthritis model LyeTx1b reduced the number of bacteria's load, the migration of immune cells, the level of IL-1b cytokine and CXCL1 chemokine, as well as prevented cartilage damage, being much more active than clindamicine. In summary, these results showed that LyeTx1b could potentially be indicated as an antibiotic against clinically resistant strains of bacteria and also as a template for the development of new drugs, apparently, without inducing resistance in strains of medical importance.

4. Repurposing DMPS, a metal chelator, as a rapid field intervention for treating hemotoxic snakebite

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Snakebite kills up to 138,000 people annually and ~400,000 are left with permanent disabilities. Envenomings by saw-scaled vipers (Viperidae: *Echis*) are responsible for high incidences of snakebite mortality and morbidity in Africa and Asia, and bites are characterized by systemic hemorrhage and coagulopathy. Antivenom, the only currently available specific treatment for snakebite, has poor specificity, low affordability for the affected impoverished populations, and must be administered in clinical settings due to its intravenous delivery and high rates of adverse reactions. Moreover, delays in accessing these healthcare facilities result in poorer outcomes for many snakebite victims. Here, we investigated the value of small molecule inhibitors, specifically metal chelators, as novel snakebite therapeutics. We show that these licensed, safe and affordable repurposed medicines effectively neutralize saw-scaled viper venoms *in vitro* and *in vivo*. Among the tested candidates, dimercaprol (British anti-Lewisite) and its derivative 2,3-dimercapto-1-propanesulfonic acid (DMPS), were found to effectively antagonize the procoagulant activity of Zn-dependent snake venom metalloproteinases from a variety of saw-scaled viper venoms. Both chelators prevented lethality in murine preclinical models of envenoming when the drug was delivered intravenously in conjunction with *Echis ocellatus* venom. Critically, DMPS significantly prolonged survival in envenomed mice when drug administration was delayed until after venom delivery. As DMPS is available as an oral therapeutic, we also investigated whether oral administration could delay or rescue lethality. Venom-induced lethality was partially prevented when the drug was given orally after venom administration, and was completely abolished when the clinical scenario of venom, immediate oral drug administration, and delayed antivenom treatment was tested. Thus, DMPS shows great promise as a novel therapeutic for treating snakebite envenomings caused by venoms rich in metalloproteinases. Its early oral administration may prove to be a valuable community-based intervention for the treatment of envenomings caused by many hemotoxic snake species.

5. Sea anemone peptide modulates TRPA1 activity, produces analgesia and enhances process of regeneration

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The TRPA1 channel is an important player in the perception of pain and the development of inflammation. This channel is a promising target for the development of new drugs for the treatment of a number of pathological conditions.

A peptide named Ms 9a-1 was isolated from the venom of the sea anemone Metridium senile. Ms 9a-1 produced a significant potentiating effect on the currents of TRPA1 induced by allyl isothiocyanate (AITC) - and diclofenac. Ms9a-1 (300 nM) significantly increased calcium intake in DRG neurons in response to AITC. This peptide acted as a positive TRPA1 modulator in vitro but did not cause pain or heat hyperalgesia when injected into the hind paw of mice. At the same time, intravenous administration of Ms 9a-1 (0.1-0.3 mg/kg) caused a significant reduction in the pain and inflammatory response to AITC (TRPA1 agonist) and reversed Freund's complete adjuvant-induced inflammation and thermal hyperalgesia. TRPA1 activation is necessary for the analgesic action of the peptide as shown by the selective TRPA1 antagonist (A-967079). We consider that Ms 9a-1 potentiates the TRPA1 response to endogenous agonists, followed by the permanent loss of functionality of TRPA1-expressing neurons. Potentiation of TRPA1 can be a useful therapeutic approach since the administration of peptide Ms 9a-1 has an analgesic and anti-inflammatory effect.

The regenerative processes are regulated by the nervous system through the mechanisms of neuroinflammation, and Ms 9a-1 was tested in the models of wound healing and arthritis. The peptide caused a significant increase in the rate of wound healing and significantly reduced the symptoms of experimental arthritis and improved joint recovery.

Thus, the regulation of inflammation by peptide Ms9a-1 can be a promising approach to improve regeneration after injuries and inflammatory diseases.

6. Antitumor potential of Pllans–II, an acidic Asp49–PLA2 from Porthidium lansbergii lansbergii snake venom on human cervical carcinoma HeLa cells

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This work shows for the first time the antitumoral and angiostatic potential effects of *Pllans–II*, an acidic monomeric Asp49–PLA_2 from *Porthidium lansbergii lansbergii* snake venom on HeLa cells. *Pllans–II* exhibited a predominant dose–dependent cytotoxicity on cervical carcinoma HeLa cells more than human breast cancer MCF7 and MDA–MB–231 cells. Interestingly, *Pllans–II* showed a negligible effect *in vitro* on normal cells such as MCF 10A, a non–tumorigenic breast epithelial cell and endothelial cells–HUVEC. Besides, *Pllans–II* induced a significative cell cycle arrest in the G1 phase of treated HeLa. Flow Cytometry analysis showed that *Pllans–II* induced both early and late apoptosis on HeLa cells, verified by the modulation of important genes mediators of apoptosis pathways such as CASP8, BCL2L1, BCL2, BAX, BAD, and BIRC5, showing a possible apoptotic cell death triggered through extrinsicpathways. *Pllans–II* was also able to inhibit cell migration on HeLa cells by interfering with α5 and β1–containing integrins. In addition, *Pllans–II* inhibited *in vitro* angiogenesis on endothelial HUVEC cells through VEGF–independent pathway. Our results display that *Pllans–II* is able to be an anticancer target for the development of a new antitumoral prototype.