The status of taxonomy and venom in sea snakes
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Event Dates: 29th - 30th August 2017
Event Website: http://lpmhealthcare.com/venoms-2017
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PODIUM AGENDA (subject to change)

DAY 0: Monday 28th August | Mad Hatter, Iffley Road, Oxford

1830: Meet up at St Hilda’s Lodge
1900-2200: Social event at Mad Hatter, Iffley Road, Oxford

DAY 1: Tuesday 29th August | The Edward Boyle Auditorium, St Hilda’s College

0800: Registration and coffee
0845: Housekeeping
0850: Welcome

Session I: Antivenoms – Chair Professor David Warrell
0900: Dr Robert Harrison (Inaugural Keynote), Liverpool School of Tropical Medicine, UK
Visions of improved antidotes against animal venoms
0930: Dr Andreas Laustsen, Technical University of Denmark, Denmark
In vivo neutralization of elapid neurotoxins by human IgG antibodies
0950: Professor Iran Mendonça da Silva, Amazonas State University, Brazil
Efficacy and safety of freeze-dried trivalent (Bothrops-Lachesis-Crotalus) antivenom for snakebites in Brazilian Amazon: a randomized controlled trial
1010: Dr Thomas Lamb, University of Edinburgh, UK
A national prospective study of ViperaTAb for the management of moderate-severe Vipera berus envenoming
1030: Refreshments and posters
1100: Dr Marieke A Dijkman, University Medical Center Utrecht, The Netherlands
Is antivenom always necessary to treat post-synaptic neurotoxicity in high care hospitals?

Session II: Snake venoms – Chair Professor David Warrell
1120: Professor Arne R Rasmussen, The Royal Danish Academy of Fine Arts, Denmark
The status of taxonomy and venom in sea snakes
1140: Miss Bianca op den Brouw, The University of Queensland, Australia
Functional variation in the venom of desert vipers Pseudocerastes and Eristicophis (Viperinae: Viperidae)
1200: Miss Jordan Debono, The University of Queensland, Australia
Coagulating colubrids: Evolutionary, pathophysiological and biodiscovery implications of venom variations between Dispholidus typus and Thelotornis mossambicanus
1220: Professor Jeffrey Klein, University of California, Irvine, USA
Tumescent Contravenom: Pre-hospital Treatment for Snake Envenomation
1300: Presentation of Life-Time Achievement Award to Professor John B Harris
1345: Professor David Warrell (Keynote), University of Oxford, UK
Clinical effects of snake-bite envenoming on the nervous system: lessons and challenges
1415: Dr Ulrich Kuch (University of Frankfurt, Germany) and Professor John Harris (Newcastle University, UK)
Bungarus walli

Session III: Snakebites – Chair Professor Dietrich Mebs
1345: Professor David Warrell (Keynote), University of Oxford, UK
Clinical effects of snake-bite envenoming on the nervous system: lessons and challenges
1415: Dr Ulrich Kuch (University of Frankfurt, Germany) and Professor John Harris (Newcastle University, UK)
Bungarus walli

Session IV: Global Snakebite Initiative Symposium on “Snakebite as a Neglected Tropical Disease: Road map for action” – Chair Dr David Williams
1435: Dr Bernadette Abela-Ridder, Dept of the Control of Neglected Tropical Diseases, WHO, Switzerland
Snakebite Envenoming: Moving towards improved control
1445: Dr C Micha Nübling, Essential Medicines & Health Products (EMP), WHO, Switzerland
Recent WHO initiatives to ensure quality and efficacy of snake antivenoms
1455: Dr David Williams, Global Snakebite Initiative & University of Melbourne, Melbourne, Australia
Building functional capacity for snakebite projects in LMIC settings

1505: Professor Abdulrazzaq Habib, Global Snakebite Initiative & Bayero University, Kano, Nigeria
Reducing Snakebite Burden in the Developing World – Targets for the Next Decade!

1515: Dr Robert Harrison, Liverpool School of Tropical Medicine, Liverpool, United Kingdom
Research activity at the Liverpool School of Tropical Medicine

1525: Dr Isabela Ribeiro, Drugs for Neglected Diseases Initiative (DNDi), Geneva, Switzerland
DNDi exploratory assessment of R&D needs and opportunities in snakebites

1535: Refreshments and posters

1605: Dr Gabriel Alcoba, Geneva University Hospitals and Médecins Sans Frontières, Geneva, Switzerland
Snakebite survival priorities: case-management and mapping in Africa and Asia

1615: Dr Rafael Ruiz de Castañeda, Institute of Global Health, University of Geneva, Switzerland
Snakes and Snakebite in the Digital Era: Neglected Opportunities for Innovation

1625: Dr Benjamin Waldmann, Health Action International, Amsterdam, The Netherlands
Empowering Civil Society in Snakebite Prevention, Treatment and Advocacy in Sub-Saharan Africa

1635: Discussion & close of Day-1

1700: The European premiere of ‘Minutes to Die’

1830: Drinks reception

1915: Networking Dinner (by prior booking or invitation only)

DAY 2: Wednesday 30th August | The Edward Boyle Auditorium, St Hilda’s College

Session V: Venomics and transcriptomics – Chair Dr Nick Casewell

0900: Professor Juan Calvete (Keynote Speaker), Instituto de Biomedicina de Valencia, Spain
Absolute venomics

0930: Dr Nicholas Casewell, Liverpool School of Tropical Medicine, UK
Retention of a compositionally diverse and functionally active venom phenotype in a pitviper produced by automictic parthenogenesis

0950: Dr Timothy Jackson, University of Melbourne, Victoria, Australia
Exapted exochemicals – the role of co-option in the evolution of toxins

1010: Dr Stuart Ainsworth, Liverpool School of Tropical Medicine, UK
Genus-wide mamba venom gland transcriptomes and venom proteomics

1030: Refreshments and posters

Session VI: Diverse venomous taxa – Chair Professor Anna Nekaris

1100: Dr Ronald Jenner, The Natural History Museum (London), UK
Venomics of remipede crustaceans reveals novel peptide diversity and illuminates biological role of the venom

1120: Mr John Dunbar, National University of Ireland, Galway, Ireland
Steatoda nobilis (Thorell, 1875): investigating Great Britain’s “most venomous spider”

1140: Ms Tania Goncalves, CEA, France
Comparison of D peptide and huwentoxin-IV as potential antinociceptive agents targeting the NaV1.7 subtype of voltage-gated sodium channels

1200: Mr Krzysztof Kowalski, Adam Mickiewicz University in Poznań, Poland
Physiological activity of venom from the Eurasian water shrew Neomys fodiens

1220: Dr Ian Mellor, University of Nottingham, UK
The discovery of nicotinic acetylcholine receptor antagonists from ladybird defensive secretions: leads for new insecticidal compounds?

1240: Dr Rodrigo Ligabue-Braun, Universidade Federal do Rio Grande do Sul, Brazil
Are domestic cats poisonous animals? The evidence so far

1300: Mrs Akriti Rastogi, BITS Pilani K K Birla Goa Campus, India
Studies on Anticoagulant Potential of Barrel Jellyfish (Rhizostoma pulmo) Tentacle Extract

1320: Lunch and posters
Session VII: Poisoning and venom targets – Chair TBA

1400:  Professor Dietrich Mebs (Keynote), University of Frankfurt, Germany

Enjoying a toxic meal

1430:  Professor John B Harris, Newcastle University, UK

*Equine Grass Sickness is associated with enhanced exocytosis, synaptic vesicle depletion and nerve terminal degeneration at the neuromuscular junction*

1450:  Dr Evelyne Benoit, CEA, France

*Venom toxin studies of the mouse neuromuscular system in vivo*

1510:  Dr Carol Trim, Canterbury Christ Church University, UK

*Targeting breast cancer signalling pathways with animal venoms*

1530:  Dr Edward Rowan, University of Strathclyde, UK

*Scorpion envenomation*

1550:  Close
Visions of improved antidotes against animal venoms

Robert A Harrison
Alistair Reid Venom Research Unit, Liverpool School of Tropical Medicine, Liverpool L3 5QA, UK

Snakebite is one of the most under-researched, under-resourced high mortality/high-morbidity emergency medical conditions that primarily afflicts rural subsistence farmers. These at-risk victims reside in some of the most disadvantaged communities in lower/middle income countries that are also populated with multiple species of snakes whose envenoming can cause diverse, potentially lethal systemic effects and often tissue necrosis. The ‘ideal snakebite antidote’ would therefore be (i) rapidly effective as a first aid and hospital tool in neutralising all venom-induced pathologies, irrespective of snake species and geography, (ii) free of adverse effects, (iii) affordable and (iv) readily accessible to at-risk communities. Antivenom is the mainstay treatment of the systemic effects of snakebite, and is IgG purified from horses (sheep and other animals) hyper-immunised with snake venom/s. My assessment of current monospecific and polyspecific antivenoms identifies some important aspects of antivenoms that fall short of the above target product profile of the ‘ideal snakebite antidote’. I will discuss my considerations of the downstream consequences and my thoughts on research that has promise, eventually, for outputting, for snakebite what paracetamol has done for headache.

In vivo neutralization of elapid neurotoxins by human IgG antibodies

Andreas H. Laustsen; Aneesh Karatt-Vellatt; Edward W. Masters; Saioa Oscoz; Peter Slavny; Alice M. Luther; Rachael A. Leah; Majken L. Olesen; Bruno Lomonte; José María Gutiérrez; John McCafferty

Snakebite remains a neglected tropical disease causing mortality and morbidity to hundreds of thousands of victims each year, particularly in poor, rural settings. Many snake species of the elapid family are notorious for their potent venom, causing systemic neurotoxicity in victims and prey. The venoms of two of the most feared species in their respective geographical regions of sub-Saharan Africa and Southeast Asia, Dendroaspis polylepis and Naja kaouthia, were analysed by toxicovenomics and their medically most important toxins were identified. Using a phage display selection approach, single-chain variable fragment (scFv) binders were identified against two of the most important toxins from these snakes, Dendrotoxin 1 and α-cobratoxin. The most promising binders were converted to human IgG format, transiently expressed in HEK293 cell, and tested in vivo in CD-1 mice. Several IgGs showed full protection (>24 hours) at low doses against both toxins and are being further investigated for their ability to cross-neutralize homologous snake venom toxins. These results represent the first report of human IgGs capable of neutralizing animal toxins, and the hope is that they will help pave the way for the development of recombinant antivenoms against animal envenomings.

Efficacy and safety of freeze-dried trivalent (Bothrops-Lachesis-Crotalus) antivenom for snakebites in Brazilian Amazon: a randomized controlled trial

Iran Mendonça da Silva, Antônio Magela Tavares, José Felipe Sardinha, Lilian de Amorim Zaparolli, Maria de Fátima Gomes dos Santos, Wuelton Marcelo Monteiro

1 Escola Superior de Saúde, Universidade do Estado do Amazonas, Manaus, Amazonas, Brazil
2 Dept de Ensino e Pesquisa, Fundação de Medicina Tropical Dr. Heitor Vieira Dourado, Amazonas, Brazil
Snake bite, a problem in Public Health in the tropical countries, occurs with large morbidity. *Bothrops*, *Crotalus*, *Lachesis* and *Micrurus* are the principal genera of poisoning for snake bite in Brazil. The lyophilized antivenom doesn’t need to be preserved in cold temperatures and it is very important (strategic) in rural areas and forests, without electric power to preserve the liquid antivenom. It was compared the efficacy between Freeze-Dried Trivalent Antivenom to *Bothrops-Lachesis-Crotalus* (FTDA) and Bivalent and Monovalent Liquid Antivenom of Brazilian Ministry of Health to *Bothrops*, *Bothrops-Lachesis*, *Bothrops-Crotalus* (BMMoHLA). The comparative analysis of Clinical Features, Adverse Events and Laboratorial Features like Clotting Time, International Normalized Ratio, Fibrinogen and others was done in snakebites victims, and it is considered the evolution time after the antivenom. The distribution of snakebite victims in accord of type of antivenom was: 57% (58/102) used FTDA and 43% (44/102) used BMMoHLA. There was no statistical difference in the Clotting Time (CT), International Normalized Ratio, Fibrinogen and others in Chi-square test between the two groups before the injection of FTDA and BMMoHLA. There was no difference in the efficacy between the antivenoms (FTDA and BMMoHLA). The FTDA can be used in the poisoning of snakebite with several economic and social advantages.

A national prospective study of ViperaTAb for the management of moderate-severe *Vipera berus* envenoming

Thomas Lamb, David Stewart, David Lupton, Sally M Bradberry, Euan A Sandilands, Simon HL Thomas, John P Thompson, Michael Eddleston

Pharmacology, Toxicology, & Therapeutics, University of Edinburgh, UK
National Poisons Information Service, Edinburgh, UK
National Poisons Information Service, Birmingham, UK
National Poisons Information Service, Newcastle, UK
National Poisons Information Service, Cardiff, UK

There are relatively few prospective data available on the administration of antivenom for *Vipera* envenoming in Europe. During 2016, we conducted a prospective study via the UK’s National Poison Information Service (NPIS) of patients treated with ViperaTAb after bites by *Vipera berus* in the UK. Clinicians telephoning the NPIS or accessing the NPIS database TOXBASE for the treatment of *V. berus* envenoming were prompted to discuss management with the NPIS toxicology consultant on call. Simple demographic data were recorded and advice given as to whether antivenom treatment should be initiated. Follow-up calls were made by a consultant toxicologist to offer further management advice, and to ascertain treatment response, adverse reactions and time of hospital discharge. Thirty-one patients received treatment with ViperaTAb (median age: 37 years [IQR 13-49.5]; 8 children; males 19/31 [61.3%]). Many cases occurred along the coast, with clusters occurring in North West Wales and Dorset. Median time to hospital presentation and to antivenom administration after envenoming was 2 [IQR 1.3-3.5] and 4 [2.6-14.8] hours, respectively. Four cases presented to hospital greater than 12 hours after envenoming, a further four cases received their first dose of antivenom greater than 12 hours after first presenting to hospital. The use of additional doses of antivenom was significantly more common in children (4/8) than adults (2/23) [p=0.03]. Two of 38 (5.3%) antivenom administrations were associated with adverse events (transient thrombocytopenia, urticaria). Median duration of hospital stay was 26h [18–68h] for adults and 47h [40-59h] for children. Treating clinicians reported sudden cessation of haemodynamic instability and cessation of limb oedema progression following administration of ViperaTAb. The comparatively short duration of hospital stay and few adverse reactions support previous experience in Scandinavia that ViperaTAb is both effective and safe.
Is antivenom always necessary to treat post-synaptic neurotoxicity in high care hospitals?

Marieke A Dijkman, Irma de Vries
Dutch Poisons Information Center, University Medical Center Utrecht, the Netherlands

A National Serum Depot (NSD) is operational in the Netherlands, guaranteeing rapid antivenom supply during medical emergencies. Antivenom from the NSD is available after contact with the Dutch Poisons Information Center (PIC). In case of foreign emergencies, the NSD has permission to deliver antivenom outside the Netherlands. In 2016, the DPIC was contacted by the Belgian PIC concerning a Naja kaouthia snakebite victim in Luxembourg requiring mechanical ventilation 7 hrs after a bite in the leg. The physician was advised to contact the DPIC directly. For unclear reasons this was not done immediately. During consultation the next day, it was decided that antivenom was not necessary as the neurological condition of the patient was already improving. Extubation was expected within the next 24 hrs. After this experience the question rose whether antivenom treatment is always necessary to treat post-synaptic neurotoxicity in high care hospitals. In 2015, a Naja kaouthia snakebite victim was treated in our hospital. The patient required mechanical ventilation within 2 hrs after a bite in the elbow. At the Intensive Care Unit (ICU) a total of 8 vials of Thai Red Cross Cobra antivenin were administered. Within 24 hrs after the bite, the patient was discharged to the medium care unit. Analysis of the available literature concerning Naja kaouthia bites shows that antivenom administration reduces mechanical ventilation duration with approximately 1.5 days. In conclusion: antivenom to treat (post-)synaptic neurotoxicity is life-saving when administered outside the hospital and in countries with poor medical facilities. In countries with high care facilities it seems that post-synaptic neurotoxicity can be treated without antivenom. At a certain point cost-benefit aspects are important as antivenoms vary in price from <100 Euro to >2500 Euro per vial and often many vials are necessary. These costs are not covered by health insurances, and paid by the hospitals.

The status of taxonomy and venom in sea snakes

Arne R Rasmussen1, Kate L Sanders2
1 The Royal Danish Academy of Fine Arts, School of Architecture, Design & Conservation, Copenhagen, Denmark
2 School of Earth and Environmental Sciences, University of Adelaide, Adelaide, South Australia 5000, Australia

Sea snakes form two aquatic groups of snakes with a flat, vertically paddle-form tail (sea kraits and viviparous sea snakes). Sea snakes belong to the same family Elapidae, which also includes the terrestrial mambas, cobra, kraits, taipan and brown snake. All elapids are characterized by the anterior position of the poison-fangs on the maxillary bone (proteroglyphous). Globally there are some 70 species of sea snake found in the tropical and subtropical waters of the Indian Ocean and the Pacific Ocean. Most species are found in the Indo-Malayan Archipelago, the China Sea, Indonesia, and the Australian region. Substantial morphological and molecular evidence has been found for recognizing two major clades within the viviparous sea snakes: An ‘Aipysurus’ lineage comprises ten species in the genera Aipysurus and Emydocephalus that are mostly restricted to the Australo-Papuan region, and a much more speciose ‘Hydrophis’ lineage contains about 50 species, many of which have very wide distributions across the Indo-Pacific. The Aipysurus group has experienced a relatively stable taxonomic history, and mitochondrial phylogenies of sampled taxa are well resolved. In contrast, Hydrophis group species have until recently been classified in 10–16 genera and/or subgenera, reflecting their confusing patterns of phenotypic diversity. The viviparous sea snakes are estimated (based on fossil calibrated molecular clocks) to have a divergence times from terrestrial elapids at around 7.8 million years before present, the Aipysurus group was separated from the other viviparous sea snakes at around 5.8 million years before present and in the Hydrophis lineage the Hydrophis group was separated from the three semi-marine lineages at around 4.4 million years before present. The venoms of sea snakes are rather simple, typically
containing a-neurotoxins and phospholipases A2 (PLA2s), and in terms of lethality are known to be more potent than the venoms from terrestrial snakes.

**Functional variation in the venom of desert vipers *Pseudocerastes* and *Eristicophis* (Viperinae: Viperidae)**

Bianca M op den Brouw1, Syed A Ali1,2, Nicholas R Casewell3, Behzad Fathina4, Parviz Ghezellou5, Bryan G Fry1

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2 HEJ Research Institute of Chemistry, International Centre for Chemical and Biological Sciences (ICCBS), University of Karachi, Karachi 75270, Pakistan
3 Alistair Reid Venom Research Unit, Liverpool School of Tropical Medicine, Liverpool L3 5QA, UK
4 Department of Biology, Faculty of Science, Yasouj University, 75914 Yasouj, Iran
5 Dept of Phytochemistry, Medicinal Plants & Drugs Research Inst, Shahid Beheshti University, Tehran, Iran

Middle Eastern desert vipers of the sister genera *Pseudocerastes* and *Eristicophis* represent a poorly studied clade of venomous snake. These ambush predators occupy an array of desert niches, and one species boasts a unique morphological adaptation used for prey capture. The spider-tailed viper, *P. urarachnoides*, has a modified caudal lure which closely resembles a spider and is used to attract birds. Little is known about the pathologies induced by these vipers’ venoms, though anecdotal reports indicate that envenomation can be severe. The venom composition of *P. urarachnoides* is thus-far undescribed in the literature and its medical significance is largely unknown. Variability in venom composition between the species *Pseudocerastes persicus*, *P. fieldi* and *Eristicophis macmahonii* has been previously demonstrated, yet the antivenom available to treat snakebite from these species is produced using the venom of *P. persicus* only. In this study, the venom composition of the four species belonging to this clade is investigated using a combined proteomics and activity-assay approach. The composition and coagulotoxic properties of *P. urarachnoides* venom are described for the first time. The coagulotoxicity of the venoms are compared and the neutralising capacity and cross-reactivity of antivenom assessed. These assays have revealed some substantial variability in venom bioactivity and composition which can be linked to the feeding ecology of these closely related snakes. The implications of this variability in relation to antivenom efficacy is also demonstrated and discussed.

**Coagulating colubrids: Evolutionary, pathophysiological and biodiscovery implications of venom variations between *Dispholidus typus* and *Thelotornis mossambicanus***

Jordan Debono1, James Dobson1, Nicholas R. Casewell2, Anthony Romilio3, Bin Li4, Simon P. Blomberg5, Hang Fai Kwok5, Nyoman Kurniawan6, Karine Mardon6, Vera Weisbecker3, Amanda Nouwens7, Bryan G. Fry1

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3 Vertebrate Palaeontology and Biomechanics Laboratory, The University of Queensland, St Lucia, Australia
4 Faculty of Health Science, University of Macau, Avenida da Universidade, Taipa, Macau SAR
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6 Centre for Advanced Imaging, University of Queensland, St. Lucia, Queensland 4072, Australia
7 School of Chemistry and Molecular Biosciences, University of Queensland, St Lucia, QLD 4072, Australia

Venoms can deleteriously affect any physiological system reachable by the bloodstream, including directly interfering with the coagulation cascade. Such coagulopathic toxins may be anti- or procoagulant. Snake venoms are unique in their use of procoagulant toxins for predatory purposes. The boomslang (*Dispholidus typus*) and the twig snakes (*Thelotornis* species) are iconic African snakes belonging to the family Colubridae and produce strikingly similar lethal procoagulant pathologies. Despite these similarities, antivenom is only
produced for treating bites by *D. typus*. The mechanism of action of both venoms have been understudied. In this study, we investigated the venom of *D. typus* and *T. mossambicanus* utilising a range of proteomic and bioactivity approaches, including determining the procoagulant properties of both venoms in relation to the human coagulation pathways. In doing so, we developed a novel procoagulant assay, utilising a Stago STA-R Max analyser, to accurately detect real time clotting in plasma at varying concentrations of venom. Clotting capabilities of the two venoms were assessed both with and without calcium and phospholipid co-factors. We found that *T. mossambicanus* produced a significantly stronger coagulation response compared to that of *D. typus*. Functional enzyme assays showed that *T. mossambicanus* also exhibited a higher metalloprotease and phospholipase activity, but had a much lower serine protease activity relative to *D. typus* venom. The neutralising capability of the available boomslang antivenom was also investigated on both species, with it being 11.3 times more effective upon *D. typus* venom than *T. mossambicanus*. In addition to being a faster clotting venom, *T. mossambicanus* was revealed to have a more complex venom than *D. typus*. CT and MRI analyses revealed significant internal structural differences in skull architecture and venom gland anatomy between the two species. This study increases our understanding of not only the biodiscovery potential of these medically important species but also increases our knowledge of the pathological relationship between venom and coagulation cascade.

**Tumescent Contravenom: Mouse Model for Pre-hospital Treatment for Snake Envenomation**

**Klein JA, Kim DP, Makdisi J, Klein PA**

Department of Dermatology, University of California, Irvine, Medical Sciences C, Irvine, CA, USA

Contravenom is any non-antivenom drug used for treating animal envenomation. Snake antivenom is often not available in rural settings. We propose the use of tumescent epinephrine consisting of a subcutaneous injection of a relatively large volume of dilute epinephrine in saline, as a contravenom for the pre-hospital treatment of neurotoxic snake envenomation. A murine model of for neurotoxic envenomation was developed as a pilot study using lidocaine as a surrogate for neurotoxic snake venom. A rescue treatment consisted of a tumescent infiltration of dilute epinephrine (2mg/L) in saline. Using the same technique, mice were injected with neurotoxic *Naja naja* cobra venom and then treated with tumescent epinephrine. The main end-point was survival time. In the pilot study, after a lethal dose of lidocaine was administered, none of the untreated controls survived. Following the tumescent epinephrine rescue, 80% of the treated mice survived. Following the treatment with LD50 doses of *Naja naja* venom, 50% of the untreated controls survived. Following tumescent epinephrine rescue, 94% of the treated mice survived (*P* = 0.0039). After treatment with LD100 doses of *Naja naja* venom, all mice died, however there was a significant (*p* < 0.0001) prolonged survival benefit in treated groups. **Conclusions:** Tumescent epinephrine contravenom delays systemic absorption of venom. Tumescent epinephrine improved both survival rates and survival time in mice injected with *Naja naja* venom. Subcutaneous tumescent delivery of contravenom has the potential to be a safe and effective pre-hospital treatment for snake envenomation. [Jeffrey Klein has a pending U.S. Patent application for the method of tumescent contravenom.]

**Clinical effects of snake-bite envenoming on the nervous system: lessons and challenges**

**David A Warrell**

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There is increasing evidence that more than 100,000 people die from snake-bite worldwide each year, including 46,000 in India alone. The three main causes of death are haemorrhagic or thrombotic strokes,
massive haemorrhage and shock after bites by vipers, pit-vipers and Australian and Oceanian elapids; acute kidney injury and the syndrome of generalised increase in capillary permeability after Russell’s viper bites; and respiratory paralysis and obstruction following bites by coral snakes, kraits, cobras, mambas, Australian and Oceanian elapids, and a few species of vipers. Snake venom toxins may affect the nervous system directly, by targeting voltage-gated ion channels in nerve endings and acetylcholine receptors at peripheral neuromuscular junctions, or indirectly by damaging cerebro-spinal blood vessels. The evolutionary significance of these mechanisms is to immobilise snakes’ natural prey. In human victims, neurotoxicity most commonly takes the form of descending flaccid paralysis, starting with weakness of external ocular muscles. Less well known neurotoxic features include persistent anosmia, pupillary abnormalities, autonomic nervous system disturbances, fasciculations and involuntary movements and agonising pain. Venom-induced coagulopathy and vascular endothelial damage can cause a variety of stroke syndromes and intracranial mass effects. If the venom contains predominantly post-synaptic toxins (cobras, some coral snakes, death adders Acanthophis), antivenoms may reverse paralysis within 1-2 hours, but in the case of predominantly pre-synaptic venoms (kraits, sea-snakes, Australian/Oceanian elapids), reversal is unlikely but early treatment might prevent or restrict paralysis. Airway maintenance and assisted ventilation are life-saving in severe neurotoxic envenoming. Ancillary pharmacological agents are of limited use, but anticholinesterases could delay progression to life-threatening respiratory paralysis, before the victim reaches medical care.

**Bungarus walli**

Ulrich Kuch¹ and John Harris²

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Abstract currently unavailable

**Snakebite Envenoming: Moving towards improved control**

Bernadette Abela-Ridder

Rabies Lead & Team Leader, Neglected Zoonotic Diseases, Department of the Control of Neglected Tropical Diseases, World Health Organization, Geneva, Switzerland

The WHO added snakebite envenoming (SBE) to the list of Neglected Tropical Diseases in July 2017, following recommendations from the NTD Department’s Strategic Technical Advisory Group after an application from several Member States coordinated by the Government of Costa Rica. The role of the WHO NTD Department and the process for initiating efforts to reduce the burden of SBE will be explained. A working group will be established to assist WHO in developing a road map plan for the control of SBE. The plan will be presented to a meeting of stakeholders in Geneva in the first half of 2018 and subsequently published. WHO hopes the plan will enable resources to be identified and secured to facilitate implementation of the plan. In parallel, Member States have announced the intention to present the WHO Executive Board (EB) with a draft resolution on SBE at the next meeting of the EB in January 2018. A new snakebite website has been developed and will be launched at this meeting. A call for expressions of interest in membership of the SBE-Working Group is also announced today.

**Recent WHO initiatives to ensure quality and efficacy of snake antivenoms**

C Micha Nübling

Essential Medicines and Health Products (EMP), World Health Organization, Geneva, Switzerland

Many of the territories with significant snakebite problems lack regulatory capacity to assess the quality and efficacy of the antivenoms used in their countries. In 2016 WHO started to assess antivenoms intended for
marketing in sub-Saharan Africa. The risk-based procedure includes evaluation of dossiers from manufacturers, lab testing of antivenom batches and inspection of production sites. Based on the experience gained the process might be expanded to other regions and to full pre-qualification of products. The assessment requirements are based on the “WHO Guidelines for the Production, Control and Regulation of Snake Antivenom Immunoglobulins” with its recent revision in 2016. Currently the WHO global database on venomous snakes distribution, specific antivenoms and manufacturers is being updated.

Building functional capacity for snakebite projects in LMIC settings

David Williams
Global Snakebite Initiative & University of Melbourne, Melbourne, Australia

Developing capacity to undertake snakebite projects focused on delivering tangible benefits to those impacted by this neglected tropical disease in low-middle income countries (LMICs) can be intensely challenging and fraught with a range of logistical, political, practical and operational obstacles that are unheard of elsewhere. Our experience includes developing rapid in-country assessment techniques for Cambodia and Papua New Guinea, establishing a long-term in-country research translation and engagement programme in Papua New Guinea, and activating a combined civil society engagement/applied research capacity building project in India. Each activity has presented unique opportunities and confronting issues. This short talk gives some examples of the hurdles that lay in the path of building functional capacity to successfully run projects in these difficult, but ultimately rewarding settings.

Reducing Snakebite Burden in the Developing World - Targets for the Next Decade!

Abdulrazaq Habib
Global Snakebite Initiative & Bayero University, ano, Nigeria

Current burden of snakebite morbidity and mortality is substantial and control efforts are sub-optimal. It is envisioned orchestrated strategies for improving healthcare workers’ education, training, utilization and expansion of access to antivenoms will result in at least halving bite morbidity, mortality and disability over the next decade. Large-scale preventive interventions - integrated within the Neglected Tropical Diseases framework - will also be needed in endemic societies to curtail bites. Considerable advocacy, policy formulations, funding, political goodwill and use of scientifically more potent and safer antivenoms with expanded venom-toxins coverage will need to be implemented to actualize these targeted goals.

Research activity at the Liverpool School of Tropical Medicine

Robert A Harrison
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I have been requested to briefly describe LSTM research activity relating to our objective of reducing the mortality and morbidity of tropical snakebite. As such, and on behalf of all current and previous members of the Alistair Reid Venom Research Unit and its many collaborators, I will describe the following research activity to:

- Improve the dose- and polyspecific-efficacy of antivenom
- Improve the treatment of venom-induced tissue necrosis
- Improve access of snakebite victims to effective health care
- Improve capacity to manage snakebite in sub-Saharan Africa
DNDi exploratory assessment of R&D needs and opportunities in snakebites

Isabela Ribeiro, Carolina Batista, Suman Rijal

Drugs for Neglected Diseases Initiative (DNDi), Geneva, Switzerland

Snake-bite is a priority neglected tropical disease, leading to over 95,000 deaths worldwide and significant permanent disability or disfigurement. Antivenoms, the only current treatment is often either scarce, not suitable for the snake species or of unassured quality. Limited efforts have been done to improve the quality and access of quality products nor have there been major global efforts to develop better tools. Drugs for Neglected Diseases Initiative is conducting an exploratory assessment of the current R&D landscape in different regions, identifying existing stakeholders, documenting the needs, gaps and opportunities for development of better tools for treatment and management.

Snakebite survival priorities: case-management and mapping in Africa and Asia

Gabriel Alcoba

Geneva University Hospitals and Médecins Sans Frontières, Geneva, Switzerland

On behalf of colleagues at HUG and MSF

Since 2013, partners at Geneva University Hospitals (HUG) and Médecins Sans Frontières (MSF) have been collaborating to tackle snakebite, especially after a workshop with international experts held in Geneva in 2014. Increasing awareness, determining local burdens, preventing bites, and improving survival have been the priorities of HUG and MSF activities. Improved access to quality care, leading to improved survival, has been achieved in areas of Nepal and sub-Saharan Africa (South Sudan, CAR, Ethiopia MSF projects). Scientific partnerships of HUG with universities (in both endemic and non-endemic countries) and MoHs have focussed on epidemiology and access (Nepal, Cameroon), diagnosis (PCR and rapid tests in Nepal and Myanmar), and antivenom treatment (Nepal). To rapidly improve snakebite survival, the WHO roadmap should focus on access to quality antivenoms, but also on “decentralising” clinical skills (e.g. respiratory assistance) and effective transport in the most vulnerable sites. We welcome WHO’s recent classification of Snakebite as a priority NTD.

Snakes and Snakebite in the Digital Era: Neglected Opportunities for Innovation

Rafael Ruiz de Castañeda\textsuperscript{1}, Isabelle Bolon\textsuperscript{1}, Lester Genevieve\textsuperscript{1}, Gabriel Alcoba\textsuperscript{2,3}, Mohanty Sharada Prasanna\textsuperscript{4}, Faouzi Amrouche\textsuperscript{5}, Valeria Macalupu\textsuperscript{5}, Viki Zhang\textsuperscript{5}, Nicolas Ray\textsuperscript{6}, François Chappuis\textsuperscript{2}

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The recent recognition of snakebite as part of the WHO Neglected Tropical Diseases should bring a new political and scientific momentum to tackle this global health crisis. The complexity of snakebite, from the evolutionary ecology of snakes to the epidemiology of snakebite and the clinical management of snakebite victims, urges for interdisciplinary collaborations between health professionals, herpetologists, toxicologists...
etc., but also for innovative approaches and applications emerging from the field of data and computer sciences in the context of the current digital revolution. Spatial analysis and modelling, digital epidemiology, machine learning, citizen science and crowdsourcing, MOOCs etc. offer new and challenging opportunities for addressing old and emerging issues of the global snakebite crisis.

**Empowering Civil Society in Snakebite Prevention, Treatment and Advocacy in Sub Saharan Africa**

Benjamin Waldmann

Health Action International, Amsterdam, The Netherlands

Health Action International (HAI) is an NGO with 35 years’ experience in policy interventions that improve access to essential medicines. HAI is also secretariat to the Global Snakebite Initiative (GSI). The GSI is a registered non-profit, charitable organisation with global membership, founded to give a voice to the forgotten victims of snakebite in poor, mostly rural, communities around the world. Our work which is predominantly funded by the Dutch Government, also involves building capacity amongst civil society organisations and healthcare systems in snakebite-affected countries so they are better equipped to combat snakebite morbidity and mortality. Our Theory of Change (TOC) is focused on three specific elements:

1. Capacity building of CSOs in HAI partner countries to collect and analyse data, set up multi-stakeholder platforms and engage in lobby and advocacy at national, regional and international level
2. Evidence-based lobby and advocacy at the international level to reinstate snakebite on the international development agenda
3. Networking, learning and exchange: strengthening the Global Snakebite Initiative to become more effective

We believe that snakebite envenoming is a health emergency that demands a sustained, coordinated global response. We are urging the WHO and national governments to design and implement policies that improve snakebite data gathering and analysis, prevention and treatment. With civil society from Africa as an integral part of the advocacy strategy, both within target countries (Kenya, Uganda, Zambia) and at a global WHO level, we are currently propelling forward a programme to challenge the emergency and meet the urgent demand for action.

**Absolute venomics**

Juan J Calvete

Instituto de Biomedicina de Valencia, CSIC, Valencia, Spain

Venoms are integrated phenotypes that evolved independently in, and are used for predatory and defensive purposes by, a wide phylogenetic range of organisms. The same principles that contribute to the evolutionary success of venoms, contribute to making the study of venoms of great interest in such diverse fields as evolutionary ecology and biotechnology. Insights into the selective pressures that resulted in local adaptation and species-level divergence in venoms can shed light on the mutually enlightening relationships between evolutionary and clinical toxinology. Toxins bearing the highest mammalian prey incapacitation activity are often also the most medically important molecules in the context of a human envenoming. Therefore, identifying the molecular basis of venomous snake adaptation to their natural ecosystems may assist in the identification of those toxins that need to be neutralized to reverse the effects of venom, thereby guiding in the rational development of next generation snakebite therapeutics. The application of omics technologies and other disciplines have contributed to a qualitative and quantitative advance in the road map towards this goal. Recent significant developments and applications in the field of snake venom research include top-down MS
that allow achieving venom compositional resolution at the level of the protein species, and the absolute quantification of the venom proteome using hybrid element and molecular mass spectrometry. A straightforward translational application of the body of knowledge gained through venomics is the assessment of the extent of cross-reactivity of antivenoms against homologous and heterologous venoms, a field coined ‘antivenomics’. The conceptual and operational principles of top-down “absolute venomics” and third-generation antivenomics will be briefly discussed and illustrated through representative examples.

Retention of a compositionally diverse and functionally active venom phenotype in a pitviper produced by automictic parthenogenesis

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Facultative parthenogenesis is asexual reproduction in species that would otherwise reproduce sexually. Recent reports of facultative parthenogenesis occurring in natural vertebrate populations, combined with the recent demonstration that parthenogens can show reproductive competence, indicate that this mode of reproduction has important evolutionary consequences and implications. Facultative parthenogenesis results in progeny that are half-clones of the mother as the result of automictic development, resulting in dramatic reductions in heterozygosity. Consequently, the implications on key phenotypic characters seem likely to be significant, yet remain (almost) completely unstudied. Many lineages of snakes produce and use venom to subjugate prey; thus, this character is tightly linked to individual fitness. In this study, we assessed the phenotypic consequences of facultative parthenogenesis on the composition and function of venom collected from copperhead snakes (Viperidae: Agkistrodon contortrix), including a male parthenogen, its mother, and individuals from the same population. To do so, we used a variety of proteomic approaches, underpinned by venom gland transcriptome data, and a suite of in vitro biochemical assays. Our results demonstrate a degree of variation in both venom composition and function; however, overall they suggest that the apparent loss of genomic diversity resulting from facultative parthenogenesis does not result in a major loss of venom toxin complexity or functional activity, and consequently may not have a significant impact on the prey-capturing ability of venomous snakes produced via facultative parthenogenesis.

Exapted exochemicals – the role of co-option in the evolution of toxins

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Some molecules make better toxins than others. These molecules are exapted – suited for co-option as toxins. The significance of exaptation in toxin evolution reaches way back to the evolutionary origins of the cell membrane and the subsequent evolution of multicellularity. Ever since cell membranes first evolved, cells have had a need to transport chemicals across these boundaries in order to interface with the outside world.
These were the first *exochemicals*. When cells began to band together to form multicellular organisms, the need for cells to communicate in order to coordinate their activities and maintain the homeostasis of the organism as a whole drove the evolution of signalling pathways and regulatory systems. The molecules involved in these systems and pathways include channels, receptors, ligands and enzymes. These are the evolutionary substrate of all exochemicals, including toxins, and their targets. It is often claimed that toxin genes are special — that they evolve differently to genes that remain constrained by their regulatory functions. Toxin genes appear to duplicate more frequently than other genes and thus venom systems often exhibit high degrees of genetic redundancy, which facilitates neo- or sub-functionalisation. Is this really the case, or is it that genes which duplicate frequently are more likely to be recruited as toxins in the first place? Which of the characteristics of toxin genes predates “recruitment” (i.e. co-option) of the gene product as a toxin? Are some genes exapted, not just by the function of their products, but by their pattern of molecular evolution, for recruitment as toxins? Should any of this matter to toxinologists? All will be revealed.

**Genus-wide analysis of Mamba venoms, revealed by venom-gland transcriptomics, venom proteomics, toxicity and antivenomic profiling**

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Mambas (*genus Dendroaspis*) are among the most feared of the medically important venomous elapid snakes found in sub-Saharan Africa, but many facets of their biology, including diversity of venom composition, remain relatively understudied. Here, we present genus-wide venom gland transcriptomic and high-resolution top-down venomic analyses of all members of the genus *Dendroaspis*. Whereas the green mambas (*D. viridis, D. angusticeps, D. j. jamesoni* and *D. j. kaimosae*) express 3FTx-predominant venoms, black mamba (*D. polylepis*) venom is dominated by dendrotoxins I and K. The divergent terrestrial ecology of *D. polylepis* compared to the arboreal niche occupied by all other mambas makes it plausible that this major difference in venom composition maybe due to dietary variation. The pattern of intrageneric venom variability across *Dendroaspis* represented a valuable opportunity to investigate, in a genus-wide context, the variant toxicity of the venom, and the degree of paraspecific cross-reactivity between antivenoms and mamba venoms. To this end, the immunological profile of the five mamba venoms was assessed by antivenomics against a panel of commercial antivenoms generated for the sub-Saharan Africa market. This study provides a genus-wide overview of which available antivenoms may be more efficacious in neutralising human envenomings caused by mambas, irrespective of the species responsible.

**Venomics of remipede crustaceans reveals novel peptide diversity and illuminates biological role of the venom**

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We report here the first integrated proteomic and transcriptomic investigation of a crustacean venom. The venom glands of the predatory aquatic cave-dwelling remipede crustacean *Xibalbanus tulumensis* express a cocktail of enzymes and peptides. Serine peptidases are the most highly expressed venom proteins, followed by chitinases. This confirms our earlier study that was based solely on transcriptomic data. However, our proteomic profiling also revealed the presence of a previously unrecognized diversity of peptides in the venom. These peptides represent a variety of different scaffolds, including inhibitory cystine knot (ICK) peptides and double ICK peptides, as well as previously unknown cysteine-rich and cysteine-less scaffolds. Based on molecular phylogenetic analyses of selected venom protein families, and the functional analogy of remipedes and cephalopods, we speculate about the roles of these venom proteins and peptides in remipede predation and feeding.

*Steatoda nobilis* (Thorell, 1875): investigating Great Britain’s “most venomous spider”

John Dunbar, Michel M Dugon

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The araneomorph family Theridiidae (Sundevall, 1833) comprises several species of medically significant spiders, including “true widows” (genus *Latrodectus* Walckenaer, 1805) and “false widows” (genus *Steatoda* Sundevall, 1833). The Noble False Widow *Steatoda nobilis* (Thorell, 1875) has established thriving populations in urban centres throughout England, Wales and Ireland since it has been accidentally imported to southern Britain over a century ago. In the past three decades, *Steatoda nobilis* has been allegedly responsible for systemic envenomations in Britain, Chile and possibly France. Drawing from recent toxicity assays, morphological investigations, ecological studies and new envenomation reports, we discuss the potential medical and ecological impacts of *Steatoda nobilis* in Great Britain and Ireland.

Comparison of D peptide and huwentoxin-IV as potent ial antinociceptive agents targeting the NaV1.7 subtype of voltage-gated sodium channels

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Over the last two decades, animal venom toxins have been explored as an original source of new antinociceptive drugs targeting different ion channels. To increase the number of positive hits, although the gold standard remains the time-consuming manual patch-clamp, automated patch-clamp platforms were developed to study drug effects on ion channels, such as the first marketed Ion-Works Quattro (Molecular Devices), the QPatch HT and its evolution, the Qube (Sophion Biosciences). These technologies are complementary approaches with their own advantages and drawbacks. Our target of interest Na$_V$1.7 is a target of choice for antinociception that has been validated by human genetic evidence such as congenital indifference to pain and paroxysmal extreme pain disorder. High throughput screening of Sanofi’s collection of venom toxins, using QPatch HT (Sophion Biosciences) on HEK cells overexpressing human Na$_V$ subtypes, pointed out a new toxin, the D peptide, sharing the same inhibitory cysteine knot (ICK) motif with toxins reported to date and originating from the same theraphosids spider family, such as huwentoxin-IV (HwTx-
IV), protoxin-II (ProTx-II) and Grammostola porteri toxin-I (GpTx-I). The D peptide, as HwTx-IV, showed nanomolar range affinity for Na\textsubscript{v}1.7, Na\textsubscript{v}1.6, Na\textsubscript{v}1.1, Na\textsubscript{v}1.2 and Na\textsubscript{v}1.3 and micromolar range affinity for Na\textsubscript{v}1.5, Na\textsubscript{v}1.4 and Na\textsubscript{v}1.8. Moreover, in DRG neurons isolated from adult mice, the two peptides preferentially inhibited tetrodotoxin (TTX)-sensitive Na current, flowing mainly through Na\textsubscript{v}1.7, with high affinity, compared with TTX-resistant Na current flowing through Na\textsubscript{v}1.8 and Na\textsubscript{v}1.9. However, compared with HwTx-IV, the D peptide had less marked \textit{in vivo} side-effects as detected by analyzing the excitability properties of the mouse neuromuscular system, mediated by Na\textsubscript{v}1.4 and Na\textsubscript{v}1.6. In conclusion, the pharmacological profile of D peptide paves the way for further engineering studies aimed to optimize its potential antinociceptive capability.

**Physiological activity of venom from the Eurasian water shrew \textit{Neomys fodiens}**

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Venomous mammals are rare and their venoms have not been comprehensively investigated. Among shrews, only venom of \textit{Blarinia brevicauda} has been analyzed so far and blarina toxin has been proven to be its main toxic component. It is assumed that \textit{Neomys fodiens} employs its venom to hunt on larger prey. However, toxic profile, properties and mode of action of its venom are largely unknown. Therefore, we analyzed cardio-, myo- and neurotropic properties of \textit{N. fodiens} venom and saliva of non-venomous \textit{Sorex araneus} in vitro in physiological bioassays carried out on two model organisms: beetles and frogs. For the first time we fractionated \textit{N. fodiens} venom and \textit{S. araneus} saliva by performing chromatographic separation. Then, properties of selected compounds were analyzed in cardiotropic bioassays on \textit{Tenebrio molitor} heart. Venom of \textit{N. fodiens} caused a high decrease in the conduction velocity of frog sciatic nerve, as well as significant decrease in the force of frog calf muscle contraction. We recorded significant decrease in the frog heart contractile activity as well. Most of selected compounds from \textit{N. fodiens} venom displayed a positive chronotropic effect on the beetle heart. However, one fraction caused a strong decrease in the \textit{T. molitor} heart contractile activity coupled with a reversible cardiac arrest. We did not observe any responses of insect heart and frog organs to the saliva of \textit{S. araneus}. Preliminary Mass Spectrometry analysis revealed that neuropeptide K and calmodulin are present in venom of \textit{N. fodiens}, whereas thymosin \textbeta}4 in \textit{S. araneus} saliva. Our results showed that \textit{N. fodiens} venom has stronger paralytic properties and lower cardioinhibitory activity. Therefore, it is highly probable that \textit{N. fodiens} might use its venom as a prey immobilizing agent. We also confirm that \textit{S. araneus} is not a venomous mammal as its saliva did not exhibit any toxic effects.

**The discovery of nicotinic acetylcholine receptor antagonists from ladybird defensive secretions: leads for new insecticidal compounds?**

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Ladybird beetles represent a diverse group of chemically defended predators. Toxicity of whole beetles and extracts has been demonstrated towards passerine birds, other ladybirds and Daphnia. Structurally unique alkaloids, found in high concentrations within the haemolymph, are thought to be responsible. Ecological data suggests that ladybirds are intraguild predators, regularly predated upon by insects but rarely consumed by vertebrates. As defensive toxins are often honed to the taxa most regularly encountered as predators; we hypothesize that ladybird alkaloids may show selectivity for insect over vertebrate targets. We hypothesized
that ladybird alkaloids target nicotinic acetylcholine receptors (nAChRs) to achieve toxicity. We used patch-clamp electrophysiology of human muscle (TE671) and insect (Schistocerca gregaria) neuronal cells, both expressing nAChRs. We show that all ladybird alkaloid extracts antagonised vertebrate and insect nAChR in a concentration dependent manner, but some compounds, showed differential toxicity. For example, (-)-adaline, produced by the 2-spot ladybird (Adalia bipunctata) was between 15-19 fold more potent to the insect nAChR with IC\(_{50}\) values of 24.4 and 1.28 µM for human and locust cells respectively at V\(_{H}\)-75 mV. We also used two-electrode voltage clamp of Xenopus oocytes expressing recombinant nAChRs to compare the selectivity further; the acetylcholine response of recombinant rat α4β2 nAChRs was also inhibited by (-)-adaline with an IC\(_{50}\) of 13.2 µM at V\(_{H}\)-75 mV. Because of the apparent selectivity of (-)-adaline for insect nAChR we conducted bioassays against some insect pests. These showed strong insecticidal activity against tobacco whitefly and mustard beetles, as well as anti-feedant properties against diamondback moth larvae. Given the results of this study, we propose that ladybird alkaloids represent a large and, as yet, untapped source of novel nAChR antagonists which show promise as insecticide lead structures.

**Are domestic cats poisonous animals? The evidence so far**

Rodrigo Ligabue-Braun

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Found in at least half the households worldwide, the domestic cat (*Felis domesticus*) is a pet of increasing popularity. Part of a complex process of urbanization, indoor pets have been associated with a rise in allergic diseases. With special emphasis on IgE-mediated ones, these allergies are strongly related to cats. Up to 15% of the adult population are sensitizes, with symptoms that may reach lethal asthmatic complications. Cat dander elicits IgE response in humans, especially via the major cat allergen, Fel d 1, a secretoglobin with variable degrees of glycosylation. Secreted in salivary, perianal, and sebaceous glands, this protein has no clear physiological function. Its glycovariants and putative calcium-binding properties have been identified as the source of allergy-like responses, but recent observations dispute these conclusions. Fel d 1 has two asymmetrical internal cavities, which are involved in binding of undefined ligands. Its similarity to the slow loris (*Nycticebus* spp.) brachial gland protein, an agent of anaphylactic shock, raises the provocative possibility of cats being poisonous animals. As observed in the prosimians, Fel d 1 is able to bind multiple hydrophobic ligands, while undergoing conformational changes. These observations, along with the confirmed presence of Fel d 1 in larger felines (e.g. lions, servals) and the well-known cat behaviour of fur licking and chin scrubbing points to an intra-specific communication role. On the other hand, male, handling-aversive domestic cats shed larger amounts of the allergen when handled, pointing towards a putative defensive role. As observed in the slow loris, Fel d 1 seems to have a dual role, acting as both an intra-specific communication agent and an inter-specific allergen/toxin. This proposal leads to new paths in basic science, such as feline chemo-communication, and biotechnology, allowing for the development of Fel d 1-neutralizing agents or truly Fel d 1-negative cats.

**Studies on Anticoagulant Potential of Barrel Jellyfish (*Rhizostoma pulmo*) Tentacle Extract**

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Cnidarians may negatively impact human activities and public health but concomitantly their venom represents a rich source of bioactive substances. This study was aimed at the assessment of anticoagulant and platelet aggregation inhibiting activities in the tentacle extracts of one of the commonly found jellyfish species on the Goan coast i.e. Barrel jellyfish (*Rhizostoma pulmo*). The tentacle extract was capable of inhibiting the blood coagulation cascade at three different levels: 1) Platelet aggregation 2) Fibrinogen digestion 3) Fibrin
degradation. Barrel jellyfish tentacle extract (BJFTE) could digest fibrinogen in dose and time dependent manner. It could digest clots, made with fibrinogen but not the ones made with essentially plasminogen free fibrinogen. Fibrinogenolytic activity as well as fibrinolytic activity of BJFTE was significantly reduced on treatment with EDTA and on exposure to heat. BJFTE showed dose and time dependent hemolytic activity against human RBCs. It could also inhibit ADP dependent platelet aggregation in a dose dependent manner. BJFTE exhibited strong proteolytic activity on fibrinogen, casein, gelatin and azocaesin, which was significantly lost on treatment with EDTA. As a result of a combination of ammonium sulphate precipitation and SE-HPLC, a semi-pure anticoagulant fraction was obtained, which showed strong fibrinogenolytic activity but no fibrinolytic activity. A 95 kDa metalloproteinase was identified in this fraction and was named Rhizoprotease. Protein mass fingerprinting analysis revealed that Rhizoprotease is a novel protein. This work reveals, for the first time, the anticoagulant potential of jellyfish tentacle extract.

Enjoying a toxic meal

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Plants are serving as a primary food source for heterotrophic organisms such as insects. To avoid or at least limit the deleterious effects of herbivory, plants developed a huge number of secondary metabolites, among them toxins exhibiting a wide variety of chemical structures and mode of action. On the other hand, insects entered this arms race by evolving mechanisms to overcome toxicity. For instance, caterpillars of butterflies when feeding on poisonous host plants are sorting out the harmful food components by preventing their resorption from the midgut. Herbivorous organisms may also achieve resistance by modifying receptors or ion-channels, which efficiently prevents binding of the toxic dietary compounds. Predatory animals face similar problems when feeding on toxic prey, such as praying mantids devouring caterpillars containing toxic products from their host plant or snakes swallowing poisonous toads, frogs or salamanders. Again, avoiding the uptake of toxins and developing mechanisms of resistance are important measures to enjoy such a toxic meal. This often provides access to an exclusive food source. Birds and primates even detoxify dietary toxins by consuming soil. Geophagy is a method which is widely used by numerous other vertebrates including man.

Equine Grass Sickness is associated with enhanced exocytosis, synaptic vesicle depletion and nerve terminal degeneration at the neuromuscular junction

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Equine Grass Sickness (EGS) is a disease of unknown aetiology, afflicting young, grazing equids. It is seen most commonly in UK but is also well recognised across Western Europe, and South America. Affected horses exhibit signs attributable to gastrointestinal ileus, generalised muscle weakness, tachycardia, sweating and ptosis. Survival is uncommon. On autopsy there is distension of the stomach and small intestine and impaction of the colon. Autonomic and enteric neurodegeneration is characterised by chromatolysis, perikaryon swelling and disruption of Golgi and RER, the accumulation of the SNARE proteins SNAP 25 and synaptophysin within the neuronal perikarya and depletion of synaptic vesicles from enteric synapses. To date there have been no formal studies of the pathology of the neuromuscular junction in EGS. A provisional EM study of synaptic vesicle density in the terminals of neuromuscular junctions in 6 EGS horses and 3 control horses showed a consistent and statistically significant reduction in the volume fraction of the terminal boutons occupied by
synaptic vesicles (24±4.3% n=58 in EGS v. 40±5.3% n=39 in control horses p>0.05). More detailed studies showed that of 73 junctions from a total of 6 EGS horses 18% could be classified as indistinguishable from controls, 32% showed unmistakeable evidence of accelerated exocytosis, 27% showed severe depletion of synaptic vesicles and 10% were in early stages of terminal degeneration. Failure to replenish the synaptic vesicle pool as a result of Golgi disruption probably contributes to vesicle depletion. Though currently unknown, the aetiology of EGS may involve an environmental neurotoxin, such as a pasture mycotoxin, that targets peripheral synapses in both the autonomic and somatic (skeletal) nervous systems to cause severe exocytosis leading to the depletion of synaptic vesicles and neuro-degeneration.

**A non-invasive method to appraise time-dependent effects of venom toxins on the mouse neuromuscular excitability *in vivo***

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The conventional methods, usually performed *in vivo*, provide limited information regarding neuromuscular excitability properties. In the present work, we review the new minimally invasive methods that have been improved to supplement the conventional electrophysiological methods. In particular, the automated sequence of multiple excitability tests developed by Professor Bostock (Qtrac®, Institute of Neurology, London, UK) to assess neuromuscular excitability in clinical neurophysiology, has been adapted to animal models *in vivo*, initially in rats and, more recently, in mice. This method is particularly appropriate for venom toxin injections and repeated recordings from the same animal cohort, making it a promising tool to appraise the progression of toxin effects, and that of an eventual given treatment, on the neuromuscular excitability of animal models in vivo. The *in vivo* efficiency in mice and long-term follow-up of the effects of sub-lethal doses of spider and *conus* venom toxins will be emphasised. It is indubitable that this method brings specific information regarding changes in ion transfer and membrane properties induced by a given venom toxin which, thereby, will help to a better characterization of toxin action on the neuromuscular excitability properties, *in vivo*.

**Targeting breast cancer signalling pathways with animal venoms**

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In the UK breast cancer is the most common type of cancer and new treatments are being developed but current options have limitations. These new drugs are mainly small molecule and antibody cancer therapies which are expensive and not always effective. In addition, these drugs can cause off-target effects and patients show resistance to the treatment over time. There is a need for new approaches to cancer therapy. Venoms from Araneidae, Scorpionidae, Viperidae, Elapidae and Apidae have been shown to have potential as peptide therapeutics in the treatment of diseases including cancer (Provencio *et al*. 2009; Huh *et al*. 2010; Gomes *et al*. 2010). Previous research by our laboratory using epithelial cancer cell lines overexpressing Epidermal Growth Factor Receptor (*EGFR*) demonstrated that sixteen invertebrate venoms block binding of Epidermal Growth Factor (EGF) to its target receptor EGFR. In this study we screened a venom diversity set (Venomtech Ltd) at a range of concentrations for their effect on phosphorylation of the EGFR. These were investigated using SDS PAGE and Western blotting of cell line proteome. Kinome blots were also undertaken to investigate venom effects on a large number of kinases and pathways involved in cancer. We are currently fractionating promising candidate venoms to identify the active components for the specific kinases.
Scorpion envenomation

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On an annual basis there are more than one million recorded cases of scorpion envenomation causing significant morbidity in adults and the risk of death in children. This presentation will highlight the main clinical features of scorpion envenomation with the emphasis on the likely mechanism of action of the venom components on the various physiological targets that are seen to be important in the expression of the venoms pathophysiology. For example: systemic envenomation often cause localised pain, erythema, oedema, paresthesias, muscle fasciculation and numbness can occur at the site of the sting. Generalized neuromuscular disturbances are attributed to alterations in cholinergic transmission and are likely due to changes in the properties of neuronal sodium channels. A further feature of scorpion envenomation is a profound activation of the autonomic nervous system that has been coined the “autonomic storm”. Both branches of the autonomic nervous system are affected and the effects are driven by the release of acetylcholine, noradrenaline, adrenaline and other peripheral neurotransmitters and hormones. Effects on the central nervous system are uncommon as the blood brain barrier limits the ability of the toxins in venoms from gaining access to the brain and spinal cord.
POSTER ABSTRACTS

Neuroinflammation and oxidative stress induced by Kv channel-blocker: Neuroprotective effect of Coenzyme Q10

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Kaliotoxin (KTx) is a neurotoxin purified from the venom of *Androctonus australis hector* scorpion, it blocks specifically the voltage-dependent potassium channel (Kv). These channels contribute to the regulation of the potential of action in neurons, where they are involved in the repolarization phase. Dysfunction of Kv channels has been associated to seizures. Neuroinflammation, oxidative stress and mitochondrial dysfunction play an important role in the pathophysiology of seizures. The present study has been designed to investigate the role of coenzyme Q10 an essential cofactor of the electron transport chain in mitochondrial against disorders caused by KTx. NMRI mice were injected with KTx by i.c.v route with or without pretreatment using CoQ10 administered by o.p route. Results showed that KTx was detected by immunofluorescence in cerebral cortex area due to its binding to the specific receptors the Kv channel. KTx induced seizures and an activation of inflammatory response and oxidative stress characterized by an increase of NF-κB and COX-2 expression and NO, MDA levels associated to a decrease of GSH level. The neuroinflammatory response is accompanied by cerebral alterations and blood–brain barrier (BBB) disruption. The use of CoQ10 prior to the KTx exerts preventive effect to the described disorders characterized by a decrease of NF-κB and COX-2 expression and NO, MDA concentrations accompanied by an increase of anti-oxidant markers (GSH level and the catalase activity). Results showed that CoQ10 prevents also the BBB disruption and the altered tissues. In conclusion, the obtained results showed that KTx is able to induce neurological disorders by blocking the Kv ion channel. The CoQ10 seems to have a neuroprotective effect against the induced disorders by KTx by enhancing the mitochondrial function. CoQ10 treatment could be a new therapeutic approach in brain alterations due to seizures.

Involvement of cytokines in neuroinflammatory disorders induced by Kaliotoxin

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Scorpion venom is a rich source of various peptides that are useful for biological research. Kaliotoxin (KTx), a neurotoxin isolated from *Androctonus australis hector* venom, acts on voltage-gated potassium (Kv) channels. The binding of KTx to its targets in the brain, is able to alter neuronal excitability leading to neurological disorders, accompanied by inflammatory response activation and immunological mediator release. The pro-inflammatory cytokines are known to induce local and systemic responses. In the present study, we investigated the contribution of IL-6 and TNF-α in the modulation of neuroinflammatory disorders induced by KTx. Biomarkers of inflammatory response and tissue damage were analyzed at 24 h after injection of a sublethal dose of KTx by intra-cerebroventricular route. Obtained results revealed that KTx injection induced significant increase of Matrix Metalloproteinases (MMPs), Myeloperoxidase (MPO) and Eosinophil Peroxidase (EPO) activities in tissue homogenates accompanied by severe alterations in cerebral cortex, myocardium and lung parenchyma. Furthermore, evaluation of blood–brain barrier (BBB) permeability by Evans Blue extravasation showed that KTx significantly affected the BBB, inducing higher permeability. Cytokine antagonists (Anti-IL-6, Anti-TNF-α) injected 30 min prior to KTx allowed to a significant reduction of MMPs,
MPO, EPO activities. These antagonists protect a BBB integrity and prevent tissue damage induced by KTx.

The binding of KTx to Kv channels in the central nervous system seems to induce local brain tissue damage and a change in the permeability of the BBB resulting in activation of systemic inflammatory response caused by indirect mechanism involving activation of the immune system and the massive release of IL-6 and TNF-α. The use of KTx, as Kv channel blocker, could be potential pharmacological tools to elucidate and to better understand neurological mechanisms in pathogenesis related to immune-inflammatory process.

Serological profiling of novel anti-neurotoxic and anti-haemotoxic antivenoms designed to treat snakebite in sub-Saharan Africa

Jaffer Alsolaiss, Robert Harrison

Snakebite is a Neglected Tropical Disease. The most economically-productive and educationally-vulnerable 15-30-year-old Africans suffer disproportionately high rates of snakebite-induced mortality (32,000 deaths) and disability. The very limited availability of polyspecifically effective and affordable antivenom is the most important contributor to this disease burden. This study is part of a MRC-funded project to use a novel ‘antivenomic’ approach to develop a single antivenom for sub-Saharan Africa. The first phase of the project is serological profiling of IgG from sheep immunised with venoms exerting the greatest neurotoxic or hemotoxic effects. Five groups of sheep (2/group) were immunised (monthly) with distinct mixtures of venoms/adjuvants for 20 weeks. Sera samples were collected at monthly intervals. Sera and caprylic acid-isolated IgG from the venom-immunised sheep were assessed by titration ELISA (IgG-sensitivity), chaotropic ELISA (IgG-avidity) and immunoblotting (IgG-specificity). Conclusions: each venom-immunisation protocol induced rapid seroconversion; some venom mixtures induced higher venom-specific IgG titres than others; the experimental glucan particle adjuvant stimulated lower IgG titres than Freunds adjuvants.

Preliminary study of Vipera ursinii macrops venom composition and biological activity

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Meadow vipers (Vipera ursinii) form a group of five subspecies, but their distribution area is highly fragmented and covers Europe, Western and Central Asia. Karst viper, Vipera ursinii macrops (Vum) inhabits high mountain grasslands in Croatia, Bosnia and Herzegovina, Serbia, Macedonia, Montenegro and northern Albania. In Croatia it is a highly threatened viper species that appears only in five isolated localities. Scientific literature on V. ursinii deals only with its morphology, ecology and distribution range due to the species’ conservation problems, while its venom composition and properties have not been investigated. Meadow vipers are medically less significant than other Vipera species. A majority of envenoming generally displays mild and negligible local symptoms only, that spontaneously resolve for a couple of days without any medical treatment or antivenom therapy. This may be associated with the short length of their flanges and a very low amount of injected venom, which cannot cause serious systematic symptoms. Here we investigate for the first time the composition and biological activity of the Vum venom composition and biological activity. The Vum venom is less lethally toxic in mice than the V. a. ammodytes venom, however the pattern of mice dying indicates the presence of a strong neurotoxic component. Interestingly, a two-dimensional gel electrophoresis revealed a lack of basic phospholipases, which are known neurotoxic components of Vipera venoms. Western blot of non-reduced Vum venom with anti-ammodytoxin antibodies (anti-Atx) gave no signal. Mass spectrometric
Identification of SDS-PAGE bands showed the lack of ammodytoxins, as well. This was finally proven by ELISA, in which Vum venom coated wells were not recognized by anti-Atx antibodies. Taken together, Vum venom might be a good starting material for the discovery of a novel neurotoxic component in Vipera venoms.

The DoubleBARR Restraining Frame for handling venomous snakes

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The production of snake venoms is fundamental to the manufacture of snake antivenom immunoglobulins, since these complex mixtures of proteins and peptides provide the immunogens necessary for driving the production of IgG antibodies by host animals. Venoms are also used for basic research and for biodiscovery applications by many institutions and companies around the world. Conventional snake handling techniques often expose handlers to unnecessary risk of accidental envenoming. In order to collect venom samples, snakes must be physically restrained and either encouraged to bite so that venom is released into a suitable collection vessel, or manipulated so that a collection tube can be placed over the fangs to obtain venom. Common techniques used to handle and restrain snakes such as the tailing/pinning method leave the snake able to lunge and bite during the process. Likewise, coaxing snakes into plastic tubes can lead to bites, and the wearing of puncture-resistant gloves may not prevent envenoming. Unfortunately, many snake handlers are reluctant to explore new techniques which could improve safety. Our laboratory in Port Moresby, Papua New Guinea produces snake venoms for use in both basic research and antivenom production, and this requires staff to work with a number of species of venomous snakes, including Papuan taipans Oxyuranus scutellatus, Papuan black snakes Pseudechis papuanus and New Guinea small-eyed snakes Micropechis ikaheka. These large elapid snakes present a very high risk to handlers so in order to improve safety we have developed a new technique that completely contains specimens during the process. This method incorporates see-through plastic holding bags and a novel restraining frame to provide a means for keepers to safely confine snakes during routine husbandry activities, and for venom extraction. We present an overview of our technique and its use, and discuss advantages and disadvantages compared to traditional methods.

Role of the non-toxic fraction (F1) purified from Androctonus australis hector venom on early hepatocarcinoma induced by Fumonisin B1

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Scorpion venoms are mixture of bioactive components which are known by their high toxicity, but their use as tools in therapeutic is in progress. This study was undertaken to investigate the ability of the purified non toxic fraction of Androctonus australis hector (Aah) venom to restore carcinogenic effect of fumonisin B1 on the liver. An evaluation of oxidative marker levels (pro-oxidative and anti-oxidative mediators), enzymatic activities, DNA quantification and tissue analysis were assessed. Obtained results showed that carcinogenesis initiated by fumonisin B1 was characterized by biomarker disturbance (unbalance of oxidative status and DNA alteration) which is associated to tissue alterations (apparition of muffled nucleus, karyo and cytomegaaly and upnormal and large nuclei into hepatocytes). The tissular alterations induced by fumonisin B1seem to be restored by the purified non toxic faction of Androctonus australis hector venom. Decreased levels of oxidative and anti-oxidative mediators were also observed. DNA in hepatocytes returned also to the physiological values. Structure of hepatic tissue showed a restoration of some alterations such as karyo- and cyto-megaly; decrease of polyploidy hepatocytes induced by FB1. The non-toxic fraction purified from Androctonus australis hector venom seems to contain bioactive components endowed with anti-tumoral activity. Purification of this activity from non-toxic fraction F1 could be of interest to identify the anti-tumoral components.
The Presynaptic Activity of *Tityus bahiensis* (brown scorpion) venom

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Scorpion envenomation produces local and systemic effects predominantly increasing neurotransmitter release by venom-containing neurotoxic peptides, which exhibit high selectivity for ion channels. Despite *Tityus bahiensis* (*T. bahiensis*) being responsible for the majority of scorpion stings in Brazil, its venom remains poorly studied especially on the somatic nervous system. Here, we investigated the neurotoxicity of *T. bahiensis* venom using myographic and electrophysiological approaches. In mouse phrenic nerve-diaphragm preparations (PND), *T. bahiensis* venom at a low concentration (1 µg/mL) caused persistent neuromuscular facilitation within 120 min-incubation; at higher concentration (30 µg/mL), the initial facilitation was followed by complete blockade (~40 min). In curarized PND, venom (1-30 µg/mL) caused concentration- and time-dependent neuromuscular blockade. Low-concentrations of venom (0.3 µg/mL) increased the frequency of the miniature end-plate potentials (mEPPs) but did not affected their amplitude. On the other hand, end-plate potential (EPP) amplitude was increased by venom, multiple EPPs per stimuli and spontaneous EPPs were observed. *T. bahiensis* venom also affected the mouse sciatic nerve compound action potential (CAP) reducing its amplitude and delaying the repolarization-phase. Higher concentrations of venom abolished the CAP. These results indicate that *T. bahiensis* venom at low concentrations stimulates the spontaneous release of ACh (mEPPs) and delays repolarization of the CAP both of which can facilitate acetylcholine release resulting in an increased EPPs amplitude and consequently the nerve-evoked twitches. However, higher concentrations abolish the CAP, inhibits the acetylcholine release and blocks the indirectly stimulated contractions.

Inflammatory mediators in victims of *Botrhops* snakebites in Brazilian Amazon Region

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A prospective cohort study was conducted to evaluate inflammation in *Botrhops atrax* snakebite in Dr Vieira Dourado Tropical Medicine Foundation – Manaus – Amazonas State (FMT-HVD), for the period from August 2014 to February 2016. It was performed laboratorial analysis like bloodstream venom race, blood clotting and inflammation markers like erythocyte sedimentation rate, C-reactive protein, creatine kinase, dehydrogenase, alanine aminotransferase, aspartate aminotransferase and several serum cytokines at the times after snakebite (0-12 hours, 12-24 hours, 24-48 hours, 48-72 hours and after 7 days). There were included 103 individuals admitted to hospital within 24 hours after snakebite who signed an Informed Consent Form and Committee on Ethics in Research of FMT-HVD adopted the project. Mean age was 35.3, median age was 44.5. Prevalent age group 11-40 years old in 55%, male in 82%, moderate accidents in 49%, rural area origin in 85%, secondary infection in 33%, time between the snakebite and the use of antivenom was before 6 hours in 91%, the local pain based on visual analysis scale was light in 25%, moderate in 37% and severe in 38%. The victims brought the snakes in 25%, systemic manifestations were observed in 50% and the more common were headache in 82.7%, dizziness in 51.7%, vomiting in 31% and gingivorrhagia in 31%. In 87.4% of victims, the blood was collected in the first eight
hours after the snakebite. In most of patients, there was a significant reduction of edema in the area around the bite after seven days. IL2, IL4, IL6, IL10, IFN-y, IL17-A, MCP-1 and MIG were increased in the first 12 hours. In moderate and severe bites, there were more significant with IL2, IL4, IL6 (highest increase), IL10, IFN-y and IL17-A. Considering the limitation of anti-inflammatories and the complex inflammatory process, studies could be carried out using anti-inflammatory cytokines, as pentoxyfilline, for example, in the therapeutic approach.

Efficacy of Amoxicillin Clavulanate in Preventing Secondary Infection - Bothrops Snakebites in Brazilian Amazon: Randomized Controlled Clinical Trial

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The aim of this work was to estimate the efficacy of amoxicillin clavulanate for reducing the secondary infection incidence in patients bitten by Bothrops snakes and identify risk factors for secondary infections from snakebites in the Western Brazilian Amazon. This was an open-label, two-arm individually randomized superiority trial to prevention secondary infection from Bothrops snakebites. The antibiotic chosen for this clinical trial was oral tablet amoxicillin clavulanate per seven days. A total of 345 patients were assessed for eligibility in the study period. From this total, 187 accomplished the inclusion criteria and were randomized, with 93 in the interventional group and 93 in the untreated control group completing the follow-up. Enzyme immunoassay confirmed Bothrops envenoming diagnosis in all included patients. Primary outcome was defined as secondary infection (abscess and/or cellulitis) until day 7 after admission. Survival analysis has shown that time from patient admission to 7 days of follow-up evidenced no differences between amoxicillin clavulanate treated and control groups (p=0.789). Secondary infection incidence until 7 days after admission was 35.5% in the intervention group and 44.1% in the control group [RR=0.80 (95%CI=0.56-1.15; p=0.235)]. Secondary infections incidence in 7 days of follow-up was independently associated to fibrinogen >400mg/dL [AOR=4.78 (95%CI=2.17-10.55; p<0.001)], alanine transaminase >44IU/L [AOR=2.52 (95%CI=1.06-5.98; p=0.037)], C-reactive protein >6.5mg/L [AOR=2.98 (95%CI=1.40-6.35; p=0.005)], moderate pain [AOR=24.30 (95%CI=4.69-125.84; p<0.001)] and moderate snakebites [AOR=2.43 (95%CI=1.07-5.50; p=0.034)]. Preemptive amoxicillin clavulanate was not effective for preventing secondary infections from Bothrops snakebites. Laboratorial markers, such as high fibrinogen, alanine transaminase and C-reactive protein levels, and severity clinical grading of snakebites, may help to accurately diagnose secondary infections.

Discovery and engineering of recombinant antibodies targeting Naja nigricollis toxins

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Snakebite envenomation is a major public health issue and it is one of the top 20 most neglected tropical diseases defined by the World Health Organization. Snakebite envenomation is an environmental and occupational health hazard that not only causes more than 100,000 deaths per year, but also economic catastrophe for victims and families living in impoverished communities of Africa, Central and South America, and Asia. The only effective treatment against the snakebite envenomation is the use of heterologous serum obtained after hyper-immunization of horses with selected snake venoms. Due to the production method, existing antivenoms are immunogenic, often causing adverse reactions in patients upon administration. Furthermore, existing antivenoms are expensive to be manufacture, and they are therefore unavailable to many snakebite victims. Accordingly, there is a current need for a new generation of antivenoms that are cost-effective, safer, and efficacious. In the current project, phage display selection was employed with the aim of discovering novel human single chain variable antibodies fragments (scFv) against the medically most relevant venom toxins from Naja nigricollis, the African black-necked spitting cobra. Venom obtained from N. nigricollis was fractionated to separate the key toxins responsible for tissue necrosis, which is one of the clinical hallmarks of N. nigricollis envenoming. Thereafter, the IONTAS phage library was panned against the selected toxins, and polyclonal phage DNA was subcloned into the expression plasmid pSANG10-3F for successful selections (as judged by ELISA and plate tests). Resultant ligations were transformed into E. coli BL21(DE3) for scFv expression. Further characterization of the subcloned human scFvs is pending. The hope is that the results of this research will provide promising scFv binders that can be converted to toxin-neutralizing human IgG antibodies.

Purification and pharmacological characterization of β-neurotoxins from *Micrurus lemniscatus lemniscatus* (South American coral-snake) venom

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*Micrurus* venoms are known for their neurotoxicity, which is mediated by α-neurotoxins (three-finger toxins–3FTx) and β-neurotoxins (phospholipases–PLA₂). Here, we have purified and characterized the activity of two toxins from *M. l. lemniscatus* venom. The crude venom was fractionated in HPLC-RP system using a column C-18 and the fractions subjected to enzymatic assay to determine those with PLA₂ activity. The neuromuscular activity of the fractions-PLA₂ was investigated through measurement of miniature end-plate potentials (MEPPs) in mouse phrenic nerve-diaphragm (PND) preparation followed by measurement of perineural currents in mouse triangularis sterni nerve-muscle (TSn-m) preparation and calcium imaging in SK-N-SH neuroblastoma cell line. The chromatograph profile of *M. l. lemniscatus* venom revealed more than forty peaks, some of which exhibited high PLA₂ activity, i.e., peaks P₃₀⁻₃₄, P₃₇ and P₃₈. Peaks P₃₀ and P₃₇ caused changes in the frequency of MEPPs; P₃₅ exhibited a triphasic effect on the neurotransmitter release [MEPPs/min: 42±5 (t₀), 17±1* (t₁), 51±8 (t₁₅) and 12±3* (t₆₀ min); *p<0.05] whereas P₃₇ caused progressive decrease in frequency of MEPPs [MEPPs/min: 57±9 (t₀), 44±9 (t₁), 27±3* (t₁₅) and 6±1* (t₆₀ min); *p<0.05]. In SK-N-SH cells, both of toxins increased intracellular calcium although P₃₀ has shown to be more potent [ΔF/F₀ (AU): 1.1±0.1 (P₃₀), 0.6±0.1 (P₃₇) and 1.3±0.2 (β-btx)]. In TSN-m preparations, P₃₀ caused significant decrease in the waveform associated with Ca²⁺ current [mV: 1.09±0.21 (t₀) vs. 0.33±0.06 (t₆₀ min); p<0.05] while P₃₇ did not affect the influx of Ca²⁺ in the motor nervous terminal [mV: 1.04±0.25 (t₀) vs. 0.63±0.12 (t₆₀ min)]. The toxins P₃₀ and P₃₇ from *M. l. lemniscatus* venom exhibit PLA₂ activity and induce motor neurotransmitter release changes. In addition, P₃₀ exhibits a classical presynaptic profile seen in other Elapidae β-neurotoxins and appears to affect the calcium ion modulation in motor nerve terminals.
Hemodynamic and vascular responses to *Micrurus lemniscatus* lemniscatus (South American coral-snake) venom

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*Micrurus lemniscatus lemniscatus* is a coral snake found in the northern region of Brazil and occasionally causes envenomation in humans. In this study, we have investigated the hemodynamic and vascular responses to *M. l. lemniscatus* venom in anesthetized male Wistar rats and in rat isolated thoracic aorta preparation. Samples of heart and lungs from anesthetized rats were dissected for histological analysis. Venom caused immediate hypotension that was maximal within the first minute (AP: 98±3 vs 63.6±4 mmHg (35±1.5% decrease) and 103±4 vs 65.6±4 mmHg (46±3.2% decrease) for 0.1 and 0.3 mg/kg, respectively (n=4, p<0.05); both doses were lethal after 20-40 min. There were no significant changes in ECG, heart or respiratory rates nor morphological damages in heart and lungs under light microscopy. Venom alone did not contract aortic strips nor did it affect the maximal responses to pre-contraction with phenylephrine (PE, 0.0001–30 µM) in strips with (E⁺) and without (E⁻) endothelium (E⁺: 4.0±0.9 vs 2.7±0.7 mN; E⁻: 10.4±2.2 vs 7.1±1.3 mN in the absence and presence of venom, respectively; n=6). However, in strips pre-contracted with PE, venom produced relaxation in E⁺ strips [13.3±1.8% (vehicle) vs 67.3±8.1% (venom) of relaxation; n=6; p<0.05] without affecting relaxation induced by sodium nitroprusside (a nitric oxide donor) in E⁻ strips (94.7±6.8% vs 99.9±1.2% relaxation in the absence and presence of venom, respectively; n=6). *M. l. lemniscatus* venom caused hypotension with no changes in ECG, heart or respiratory rates. This finding suggested a predominantly vascular action. In addition, venom caused endothelium-mediated relaxation but did not affect vascular smooth muscle reactivity. The hypotension caused by *M. l. lemniscatus* venom in anesthetized rats in and the venom-induced relaxation in rat isolated thoracic aorta preparation suggested a possible muscarinic action.

Design of scFab-based chimeric antibodies against snake venom metalloproteinase from *Bothrops asper*

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Snakebite envenoming is one of the world’s most neglected tropical diseases, responsible for the death of 125,000 people each year. The only current treatment is antivenom based on antiserum derived from the blood of immunised mammals. This antiserum is costly to produce and carries a high risk of causing adverse effects in human recipients because of its heterologous origin. Furthermore, due to poor tissue permeability, antivenom against locally acting snake toxins targeting peripheral tissue is less effective. Consequently, an impending need exists for improvement of the efficacy, safety, and affordability of today’s antivenom. *Bothrops asper* is a medically relevant snake from the lowland regions of Central America, north-western South America and Mexico, where it is responsible for 50% to 80% of all snakebites and 60% to 90% of all fatalities related to snakebite. Phospholipase A₂s and snake venom metalloproteinase (SVMPs), found in the venom of *B. asper*, both play a major role in the development of local tissue damage. The aim of this project was to convert a recombinant scFv antibody against the BaPI toxin (an SVMP) from *B. asper* venom into a chimeric scFab-BaPI antibody. An scFv-BaPI antibody based on the original scFv scaffold was designed and used as control. The
scFab-BaPI, scFv-BaPI, and primers were designed and optimised using bioinformatic tools and analytical methods. To allow subcloning into the pSANG10-3F expression vector, genes of interest were amplified using PCR. The pSANG10-3F-scFab-BaPI and pSANG10-3F-scFv-BaPI vectors were then transformed into BL21 (DE3) E. coli cells. To verify the success of the experiments, gel electrophoresis was performed continually. More experiments to assess the efficacy of scFab-BaPI are pending. The hope is that this antibody format will contribute to the treatment of snakebite victims in the future.

The Cytotoxic Effect of Slow Loris (Nycticebus) Venom on Human Epidermal Carcinoma Cells

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Within the Kingdom Mammalia, venom evolution is rare, occurring in only six orders. Arguably the most cryptic, and academically neglected venom occurs within primates among slow lorises (Nycticebus spp.). Venoms comprise novel biological compounds with a potential plethora of proteins and peptides available for utilisation in bio-medical research. We collected samples of slow loris saliva from eight captive-bred pygmy slow lorises (N. pygmaeus) at Paignton Zoo and Shaldon Wildlife Trust UK, given voluntarily as slow lorises chewed on Salimetrics children’s swabs. From January to March 2017, we employed MTT assays, and microscopy assessments to determine cell survival on human epidermal carcinoma cells (A431 line) after the application of concentrations of slow loris salivary venom. Cell survival from both male and female derived saliva was half that of untreated cells. Cytotoxic action is demonstrated in concentrations as low as 0.1% venom. Results demonstrate a cytotoxic effect with ensuing physiological damage on human cancer cells, demonstrating the cytotoxic action of slow loris saliva only, without the admixture of brachial gland exudate. We show that even captive-bred slow loris saliva harbours potentially dangerous substances, with functional applications towards slow loris husbandry. Knowledge of slow loris salivary venom increases understanding of the novel salivary composition and supports discussions of slow loris conservation by proposing a functional narrative to oppose the illegal pet trade, by contradicting their ‘cute and cuddly’ appeal. Evidence of salivary venom shows that cytotoxic effects can result even in the absence of a bite puncturing the skin, and further demonstrates their inappropriateness as pets.

Preliminary study of the cytotoxicity of king cobra (ophiophagus hannah) l-amino acid oxidase (lao) in cell lines of acute lymphoblastic leukaemia

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King Cobra (LAAO) has a wide range of potential therapeutic applications and has previously demonstrated cytotoxicity in cell line models of breast, lung and prostate cancer. Acute Lymphoblastic Leukaemia (ALL) is a haematological malignancy in which abnormal lymphoblasts replace the bone marrow. Untreated this disease is rapidly fatal and despite progress with chemotherapy, immunotherapy and molecularly targeted drugs 5yr survival remains poor at approximately 40% in adults. Further advances in treatment are necessary. The amino acid asparagine is essential for lymphoblast metabolism and L asparaginase is an integral component of treatment. Recently there has been increased interest in amino acid metabolism in malignant disease and we sought to explore this further studying the effects of King Cobra LAAO in B Cell ALL. The proposed mechanism of cytotoxicity of LAAO is hydrogen peroxide production subsequent on oxidative deamination of amino acids.
NALM-6 and REH cell lines were used as a model of ALL. For dosing experiments cells were incubated with concentrations of LAAO from 0.25µg/mL to 5µg/mL. The cell titre-glo luminescent cell viability assay (CTG®) was used to determine cell viability, in comparison to vehicle control (storage buffer that venom was solubilised in). No effect of the venom was seen on the cells at early time points of 1-4 hours. However, by 48 hours efficacy was demonstrated at higher concentrations with IC_{50} NALM-6 76µg/mL and REH 128µg/mL. Testing at higher concentrations was limited with this LAAO preparation due to toxic effects of the storage buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0, 50% glycerol). In summary we have demonstrated cytotoxic effects of King Cobra (Ophiophagus Hannah) LAAO in B Cell ALL Cell lines as evidenced by reduced cell viability.

An improved technique for the assessment of venom-induced haemorrhage in mice

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Snakebite envenomings pose a significant risk to public health globally, causing mortality and morbidity to hundreds of thousands of victims each year. Currently, the only specific treatment for systemic envenoming is the intravenous administration of antivenom. However, the efficacy of antivenoms to neutralize toxicity has to be demonstrated through preclinical testing before being used clinically. The assessment focuses on the antivenoms’ efficacy in neutralising venom-induced lethality, but also includes other key toxic activities such as myotoxicity, dermonecrosis and haemorrhage. Particularly haemorrhage is of importance, since it is one of the most common clinical signs in victims of snakebite and can cause prominent local tissue damage and cardiovascular disturbances. To date, the most widely used method for analysing an antivenom’s capability to neutralise haemorrhage involves the intradermal injection of mice with venom and antivenom and a following measurement of the area of the haemorrhagic lesion. The minimum haemorrhagic dose is then determined as the amount of venom that results in a haemorrhagic lesion of 10 mm diameter. The main drawback of this procedure is that haemorrhagic lesions having a similar diameter might still vary in their depth and in the intensity of haemorrhage. The aim of this study was to improve and expand the rodent skin haemorrhage methodology through the application of computational and image analysis tools, allowing a more efficient and accurate analysis of venom induced haemorrhage. Therefore, we developed a method that improves the precision of the lesions’ area measurements and, more importantly, also assesses intensity. This allows a significantly more precise evaluation of haemorrhagic lesions and specifically variations in their intensities. Together with the minimal cost involved and its ease of use, this new method will aid the better assessment of the neutralising capabilities of antivenoms and consequently increase their reliability.

Comparative Transcriptomic Analysis of Venom Gland Tissues from Spitting and Non-spitting Cobras

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Cobras are highly venomous elapid snakes that inhabit Asia and Africa and cause thousands of deaths each year. Some cobras have specialised fangs that allow them to eject venom as a spray into the eyes of an aggressor, causing immediate pain, opthalmia and, occasionally, blindness. Spitting cobras have therefore evolved a novel, defensive use of their venom that is not observed in other venomous snakes. The aim of this study was to assess whether the venom composition of spitting cobras has changed in response to the
evolution of this defensive adaptation. Using the venom glands from 16 species of cobra, transcriptomic libraries were generated, sequenced, annotated and the identified venom toxin protein types aligned. In all cobra species, the dominant toxin families identified were three-finger toxins (3FTXs), phospholipase A2s (PLA2) and snake venom metalloproteinases (SVMPs), although these are expressed variably among species. Early analyses find significant differences in the expression of PLA2s between the African spitters, African non-spitters and Asian cobras (p = 0.0055) and between the African spitters and African non-spitters (p = 0.0014), suggesting this toxin type may be associated with defensive venom spitting, in African lineages at least. Additionally, preliminary phylogenetic analyses reveal that these PLA2 toxins have diversified via gene duplication events restricted to African spitting cobras. We also find, using a fluorescent enzymatic PLA2 assay, that spitting cobras exhibit stronger PLA2 activity than non-spitting cobras. Future work will focus on identifying whether other toxin families are also associated with defensive venom-spitting in this medically-important lineage of venomous snakes.

**A new generation of antivenoms to treat Dendroaspis polylepis bites**

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Snakebite is one of the major health concerns in rural, tropical parts of the world. Each year, 5 million bites occur, leading to 2.5 million cases of envenoming, 125,000 deaths, and 400,000 amputations. Survivors, who are often agricultural workers and children, frequently lose their limbs and thus their ability to work, underlining the socio-economic issues related to snakebite. The current treatment consists of animal-derived antibodies, which are expensive to produce and which due to their heterologous origin are likely to induce immunologic side-effects, such as serum-sickness and anaphylactic shock. The goal of the research presented here is to develop a safer, cheaper, and more efficacious snakebite antivenom based on a cocktail of specifically selected human recombinant IgGs. As part of this goal, focus was directed on one of the deadliest snakes in Sub-Saharan Africa, the black mamba (*Dendroaspis polylepis*). The venom from this species derives its potency from the combined action of dendrotoxins and neurotoxins that cause involuntary muscle contractions and flaccid paralysis, respectively. In severe cases, this leads to paralysis of the respiratory muscles, causing suffocation and death of the patient due to hypoxia. Untreated bites are almost certain to cause death. In this project, phage display selections were employed to discover human scFv fragments capable of binding the most medically relevant toxins from *D. polylepis*. Currently, these antibody fragments are being converted to IgGs, which will be tested for cross-reactivity to homologous toxins and their ability to neutralise mamba venom in vivo. Such IgGs could be produced cost-effectively by mammalian cell cultivation, economically allowing them to be manufactured and distributed to the poorest segments of the afflicted areas in Sub-Saharan Africa. It is the hope that this research will help pave the way for a new generation of antivenoms.

**Development of recombinant human antivenom against forest cobra toxins**

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Every year around 5 million people are bitten by venomous snakes, resulting in 125,000 deaths and 400,000 amputations. The only effective treatment against snake envenoming is antivenom, which has been based on animal-derived antibodies for more than 100 years. Due to the heterologous nature of the antivenom, treatment against snakebite envenoming can inflict severe side effects, such as serum sickness and anaphylaxis, which in some cases may lead to death. Due to the severity of snakebite combined with the lack of research in novel antivenom, the World Health Organization (WHO) characterizes snakebite envenoming as one of the world’s most neglected tropical diseases. The forest cobra, *Naja melanoleuca*, is the largest cobra species in Africa and has high medical importance according to the WHO. *N. melanoleuca* venom derives its potency from the type I and II α-neurotoxins. α-neurotoxins target the nicotinic acetylcholine receptor, which causes inhibition of neuromuscular transmission. Symptoms of envenoming by *N. melanoleuca* include flaccid paralysis, which without treatment may result in death by hypoxia due to the victims inability to ventilate. Here, we report the most recent results of our ongoing work towards the identification of human antibodies with neutralizing effects against the medically most relevant toxins from *N. melanoleuca* venom. Employing phage display selection, we have discovered different human scFv antibodies from the IONTAS phage display library. Through determination of binding capacity and cross-reactivity to other toxins, the most promising candidates will be converted into the human IgG format and assessed in preclinical studies. It is our hope that this work will be a step in the right direction towards development of a cost-effective recombinant antivenom with better safety and efficacy for treatment of envenoming by the forest cobra.

**Effects of two Phospholipases A2 (Asp-49 and Lys 49) isolated from *Bothrops pauloensis* venom on isolated kidney of rat**

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Acute renal failure (ARF) which is one of the most serious complications of *Bothrops* snakebites, is one of the main causes of death and consequences for victims. However, its pathogenesis remains unclear. Considering that isolated toxins are relevant tools for understanding the actions of the whole venom, we studied properties of phospholipases A2 (Asp-49 and Lys-49) isolated from *Bothrops pauloensis* (from State of São Paulo, Southeast region of Brazil),on isolated perfused kidney of rat. The control group perfused with modified Krebs–Henseleit solution alone. Asp-49 increased the perfusion pressure (PP), renal vascular resistance (RVR), urinary flow (UF) and decreased the percent sodium and chloride tubular transport (%TNa), the glomerular filtration rate (GFR) was unchanged. The treatment with Lys-49 caused decrease in PP and GFR. Oxidative stress in kidney tissue was evaluated by TBARS (thiobarbituric acid reactive substances), Nitrite and reduced Glutathione (GSH) level measurement. A significant increase was observed in TBARS (Asp-49) and Nitrito (Asp-49 and Lys-49) and a decrease in GSH levels (Lys-49). The determination of the myeloperoxidase (MPO) activity was analyzed and Asp-49 increased the level of MPO. The levels of inflammatory cytokines were measured. We analized the inflammatory cytokines, pro-inflammatory interleukin 1 (IL-1), tumor necrosis factor-α (TNF-α) and anti-inflammatory interleukin 10 (IL-10). The results showed high levels of both cytokines. Increased MPO activity, TBARS, Nitrito level and decreased of GSH activity might be implicated in the presence of oxidative stress in patients with snake bite envenomation. These findings demonstrated that PLA2s caused nephrotoxicity in isolated kidney. The characterization of the effects in the isolated kidney gives strong evidences that the acute renal failure induced by these PLA2s is a result of the direct nephrotoxicity which may involve oxidative and inflammatory mechanisms.
Discovery of human IgG antibodies against key venom toxins from the Central American coral snake (*Micrurus nigrocinctus*)

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Snakebite envenoming remains a major public health issue in Latin America and other rural tropical regions of the world, causing mortality and morbidity to hundreds of thousands. Particularly in Central America, the species *Micrurus nigrocinctus* is the most abundant and clinically relevant coral snake from the Elapidae family. The venom of *M. nigrocinctus* is predominantly composed of phospholipases A2 (PLA2s) and three-finger toxins (3FTxs), constituting 48% and 38% of the venom proteins, respectively. Snakebites from this species predominantly induce neurotoxic effects, although myotoxicity has been shown in animal models and may occur in humans. Myotoxicity is caused by PLA2s, while neurotoxicity is mainly caused by 3FTxs, with contribution of some PLA2s. Existing coral snake antivenoms are based on serum from immunized horses. These are complicated to manufacture due to difficulties in procuring scarce *M. nigrocinctus* venom and due to the poor immunogenicity of the venom toxins, making them less effective in the immunization process. Additionally, existing antivenoms may cause undesirable adverse reactions as they contain heterologous proteins that are not compatible with the human immune system. In this project, we employ phage display technology to discover human antibodies able to neutralize the medically most important toxins of *M. nigrocinctus* venom. After identification of scFv antibody fragments using the IONTAS phage display library, the most promising scFvs will be converted to the IgG format to gain improved half-life to provide prolonged protection against snake toxins in the circulatory system. We hope that with these initial steps we will pave the way for the development of a next-generation recombinant antivenom for *M. nigrocinctus* envenomings with improved efficacy and safety.

Venom in furs: pelage as an aposematic signal in slow lorises (Primates, *Nycticebus*)

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The evolutionary function of colouration in mammals includes concealment, communication, and interspecific signalling including the use of aposematism as a warning signal. Several mammals use aposematism to warn predators of their dangerous odour or unpalatability. The slow loris is the only venomous mammal that has a coat pattern with distinctive dark and light markings, suggesting a potential aposematic role. To help understand if slow lorises use conspicuous aposematism to advertise their venom to potential predators or slow loris competitors, we measured colouration in 54 known wild individuals (37 adults, 17 juveniles) from photographs taken in the field under similar lighting conditions. Using ImageJ, we extracted RGB colour values of 12 characters of the contrasting facial mask of Javan slow lorises (*Nycticebus javanicus*) from trichromatic, dichromatic and monochromatic variations of the same photo. We conducted canonical function analysis within each colour variation, and found that individuals were significantly allocated to their age class for each variation (monochromatic - 82.9%, p< 0.0001; dichromatic - 92.7%, p<0.001; trichromatic - 90.0%, p<0.01). The distinguishing characters making up the first three components, however, differed. Trichromes were distinguished by interocular stripe, crown and circumocular patch bottom; dichromes were distinguished by interocular strip, patch bottom and eye rim; monochromes were distinguished by circumocular patch top, circumocular patch bottom and crown. Slow lorises have monochromatic vision but are faced with both dichromatic and trichromatic predators. These differences suggest a role for age class recognition amongst conspecifics, allowing adult slow lorises to recognise young individuals quickly. Predators too can distinguish...
age classes and we discuss the possibility if this may be related to the ability of slow lorises of different age
classes to produce different amounts of venom.

In vivo neutralization potential of monoclonal human IgGs against elapid neurotoxins

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The Black mamba (Dendroaspis polylepis) and the monocled cobra (Naja kaouthia) are two notorious
venomous snakes belonging to the elapid family, causing a considerable share of the severe envenomings
occurring in sub-Saharan Africa and Southeast Asia, respectively. In this study, monoclonal fully human IgGs
were discovered by a combined toxicovenomics and phage display selection approach and assessed for their
ability to neutralize medically relevant toxins from these snakes in vivo. Upon expression in mammalian Expi-
293 cells, the monoclonal human IgGs were assessed using two different routes of administration in CD-1
mice. First, IgGs and their target neurotoxins (α-neurotoxins and dendrotoxins) were incubated for 30 min at
37°C in different IgG to toxin molar ratios (mol toxin : mol IgG of 1:3 to 1:8). Thereafter, incubated solutions
were either administered intracerebroventricularly (i.c.v.) (in the case of dendrotoxins) using a toxin dose of
0.5 µg or intravenously (i.v.) (in the case of α-cobratoxin) using a toxin dose of 4 µg to assess neutralization
potential of IgGs. The monoclonal human IgGs significantly prolonged survival of mice administered with lethal
doses of the elapid toxins. Here, we thus report for the first time the discovery of monoclonal fully human IgGs
that can neutralize snake toxins in vivo. Moreover, one of the discovered human IgGs targeting α-cobratoxin
from N. kaouthia displayed prolonged survival when tested both against the toxin itself and when tested
against whole venom. This signifies the importance of α-cobratoxin in N. kaouthia venom, and underlines the
applicability of using the Toxicity Score, central for the toxicovenomics approach, for target selection.

Identification of Cobra Venom Actives as Potential Novel Pancreatic Cancer Therapeutics

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Pancreatic cancer is an aggressive form of cancer which has a particularly poor prognosis. Currently only 3% of
patients survive more than five years and just 1% of patients survive for more than ten years after diagnosis.
This highlights the urgent need for development of novel treatments for pancreatic cancer and the importance
of early diagnosis. Animal venom contains a complex mixture of proteins, peptides, enzymes and small
molecules. In addition to their negative effects on human health, components from venoms have been utilised
as treatments for conditions such as hypertension, angina and even cancer. This research investigates
potential use of cobra venom peptides as a treatment for pancreatic cancer. Resazurin, a blue weakly
fluorescent redox dye was used as an indicator of cell viability. Conversion of resazurin to resorufin, a pink,
highly fluorescent dye is proportional to cell viability and may be seen visually or measured through changes in
fluorescence values. A panel of 19 cobra venoms were screened against MIA PaCa-2 and BxPC-3 pancreatic
cancer cell lines at different venom concentrations in order to identify selectively toxic venoms. Following
tenom exposure, fluorescence values were measured, allowing assessment of cell viability. Venoms from five
phylogenetically and geographically related cobras were identified to have selective toxic activity at low concentration against MIA PaCa-2 cells. These five venoms were fractionated using RP-HPLC to separate out the venom components and the fractions were screened for activity. The identified active fractions were further fractionated using size exclusion chromatography in order to identify the single entities responsible for cell toxicity.

A compact and effective procedure for antivenom downstream processing

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Antivenoms obtained from hyperimmune animal plasma, mostly equine or ovine, are the only specific therapeutics effective for rapidly counteracting post-snakebite pathophysiological manifestations. There are various well established refinement processing strategies that have been implemented into commercial scale antivenom production. However, optimisation of manufacturing process yielding safe, effective and available immunotherapeutics is still of great concern. Here, we report simple, feasible and economically viable fractionation protocol for preparation of V. a. ammodytes-specific antivenom. Hyperimmune equine plasma pool was fractionated in only few simple and easily scalable purification steps. The purification process can be stopped at different steps, depending on the desired final antivenom product: whole IgG molecules or F(ab')2 fragments, both fulfilling regulatory requirements for product purity. Particular emphasis was put on quantification of each purification step in terms of IgG or F(ab')2 yield determined by several in vitro methods. Throughout the procedure, IgG molecules or F(ab')2 fragments were constantly kept in the solution, preventing their precipitation and binding to chromatography columns which can lead to aggregation. Firstly, unwanted plasma proteins, mostly albumin, were precipitated by caprylic acid (CA). Ultrafiltered IgG-rich and CA-depleted supernatant was used for pepsin digestion. The final product, F(ab')2 fragments, was polished by ion-exchange chromatography on CIM QA disk under conditions which prefer binding of pepsin and byproducts of enzymatic digestion exclusively.
MicroPharm Limited is a developer and manufacturer of therapeutic polyclonal antibodies for human and veterinary use. The Company’s core expertise lies in the raising of ovine antisera containing high levels of specific antibodies directed against antigens such as toxic molecules or viruses and the subsequent purification and modification of such antibodies to produce a range of immunotherapeutic products for clinical use. All are designed to treat acute, life-threatening emergencies, have been developed at the request of the medical profession and are required urgently either because no alternative exists or because any alternative is ineffective and/or unsafe. MicroPharm currently produces two antivenoms, ViperaTab® for the treatment of envenomation by the European adder and EchiTabG™ for the treatment of the carpet viper, Echis ocellatus. The Company has a number of products in development including: OraCAb for the treatment of mild and recurrent C. difficile infections, PolyCAb for the treatment of severe C. diff.; ColchiBIND for the treatment of colchicine poisoning; and ViperaVet for the treatment of dogs envenomed by one of the four medically important species of Vipera (adder) found throughout Western Europe. MicroPharm’s collaborators include Public Health England, University of Oxford, University of Leeds and the University of Edinburgh.

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