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The sand tampan, *Ornithodoros savignyi,* as a model for tick–host interactions

B.J. Mans* **and A.W.H. Neitz***†

***Ornithodoros savignyi,* commonly known as the sand tampan, is of economic importance in southern Africa. Tampans kill lifestock, especially young calves and lambs, through secretion of toxic agents during feeding. The sand tampan has been used in our laboratory as a model to understand tick–host interactions, both at the level of haemostatic control by the tick and as regards pathogenic mechanisms. We review the important contribution that research on the sand tampan has made to recent developments in the understanding of tampan toxicoses, tick evolution and tick–host interactions. The pathogenic mechanisms of tampan toxicoses seem to be related to cardiac failure. This form of pathogenesis is distinct from other forms of toxicoses such as spring lamb paralysis. Furthermore, the absence of toxins in closely related tick species indicates that tampan toxicoses might be associated with only *O. savignyi.* The toxins form part of a group of tick proteins from the lipocalin family. The study of anti-haemostatic components secreted by the tampan indicates a close evolutionary relationship between different anti-haemostatic mechanisms. This has several implications for general tick biology, the most important being that the main tick families adapted independently to a blood-feeding lifestyle and that the different forms of tick toxicoses have distinct origins.**

Introduction

Those who have taken refuge from the sun underneath a camel thorn tree might have had a memorable encounter with a little appreciated member of the fauna of the Kalahari Desert, the sand tampan. Those who have, will attest to the ferociousness of its attack and the accompanying pain when bitten. The tick's notorious nature is well known and only the unwary will venture to rest unprotected in the shade of the acacia tree.[1] This spurned animal has, however, played an important role in the understanding of how ticks and their hosts interact.

The sand tampan (*Ornithodoros savignyi*) is grouped within the family Argasidae (soft ticks), which forms with the Ixodidae (hard ticks) the major families of the order Ixodida (ticks). The sand tampan occurs throughout the northwestern parts of South Africa and is also found in the semi-desert regions of North and East Africa, the Middle East, India and Ceylon.[2] Theiler published an extensive survey of the geographical distribution, habitat and host preference of the sand tampan in southern Africa.[2] The tampan lives in sandy regions where it resides below the surface near trees, corrals and other places likely to attract animals. It has been reported that the ticks prevail in areas where soil is less sand-like, for example in kraals, where it is probably attracted to domestic animals.[3]

Hoogstraal described the behaviour of the sand tampan in classic prose:

> At the Khartoum quarantine one may see a long, seething line of thousands of hungry tampans helplessly confined to the shade of a row of acacia trees. A few yards away, separated only by the hot, nine o'clock sun, newly arrived cattle tied to a post fence tempt the tampans to cross the glaring strip. The next morning, in the coolness of seven o'clock, those tampans under the trees are all blood bloated and resting comfortably in the sand, others dragging back from their hosts across the now nonexistent barrier, and the legs of the cattle are beaded with yet other podshaped ticks taking their fill of blood in a regular line just above the hoof.[4]

The ferociousness of these ticks can be attested to by personal experience. Using the dry ice (carbon dioxide) collection method to lure the animals from their hiding places,[5] we were surrounded by ticks in a twenty-metre radius around the dry-ice container.

*Department of Biochemistry, University of Pretoria, Pretoria 0002, South Africa.
†Author for correspondence. E-mail: albert.neitz@bioagric.up.ac.za

Ticks were running crazily this way and that, seeking animals they could scent but did not find (B.M., pers. obs., Upington, Western Cape province, 1995).

The use of acaricides to control the sand tampan is limited due to the nature of its distribution over a large area and the fact that it does not reside on the host. Places where the soil has been treated with chemicals are avoided by these ticks. The use of ivermectin, a systemic parasiticide for cattle, did, however, prove to be a viable control in a laboratory setting, where the tampan population was considerably reduced after feeding on cattle that were treated subcutaneously with ivermectin.[6] These ticks can have a lifespan of up to 15–20 years, even if they feed only occasionally (every 5–6 years).[7] Long-term biological control of these ticks would thus be a viable option. The use of domestic fowls in heavily infested areas has also been proposed as a means of biological control.[2]

Fig. 1. Morphology of the sand tampan. The external morphology of a female sand tampan is shown on the left. Note the uniform integument that folds in on itself and distends during feeding. Also note the position of the capitulum on the underside of the body and the genital pore. The integument and stomach were removed by dissection on the right, to display the position of the salivary glands that resemble two bunches of grapes, lying anterolaterally and extending posteriorly from both sides of the body. The length of this animal was approximately 10 mm.

Initial studies of sand tampan toxicoses

The sand tampan is a problem in areas where it is endemic. Cattle succumb if kept overnight in pens with large numbers of tampans. Domestic animals are also vulnerable, especially young calves and lambs. The death of domestic animals was thought to be due to excessive blood-loss caused by the tampans, but a toxic component in tick salivary gland secretions proved to be responsible.[8] It was realized that a deeper study of tampan toxicoses could eventually make a contribution to the control of these animals.[8] What made the tampan an attractive subject for the study of tick biology is the relative ease with which it can be collected. The animal burrows beneath the sand and large numbers can be found around cattle pens, where they can be sifted from the soil or collected using dry ice.[5] Tampans require little care and can be kept alive for several months in a container of sand.

Initially, research focused on the pathological nature of the saliva of *O. savignyi*. For this purpose, saliva was collected by injection of the cholinergic parasympathetic stimulant, pilocarpine, into the haemocoele of the tick via its genital orifice.[8] This was the first report of stimulated salivary gland secretion in ticks and yielded approximately 10 μl of secretion per animal. Obtaining salivary gland secretion was optimized to such an extent (200 females daily) that enough fluid was obtained to allow extensive investigation into its toxic properties as related to its pathogenicity.[3,9] During this period it became clear that further biochemical studies into the toxic principles was necessary. The Department of Biochemistry at the University of Pretoria was approached in early 1969 to collaborate in determining the molecular basis of sand tampan toxicoses. The first proteinaceous toxin was soon purified to homogeneity and had a molecular mass of 15 400 Da as determined by sedimentation equilibrium ultracentrifugation.[10] This was the first bio-active component that was purified from tick salivary gland secretions and showed that the interaction between the tick and its host could be studied on a molecular level. An elaborate series of characterization experiments then followed, which included the purification of a closely related, non-toxic protein and the determination of partial N-terminal amino acid sequences.[11] These were the first amino acid sequence data reported for tick salivary gland proteins.[12] This work also provided new data on the toxin, including that it was a glycoprotein of 43 amino acid residues with 11% carbohydrate content and a molecular mass of 6346 Da, which upon removal of the carbohydrate moieties changed to 4652 Da. The toxic activity was lost after removal of the carbohydrate moiety.[12]

An important result of this study was that tick toxicoses did not occur in isolation, but were probably assisted synergistically by other bio-active molecules secreted by the tick, that assist in transmission and uptake of toxins in the host.[13] The presence of hyaluronidase, an enzyme involved in the degradation of the extracellular matrix and thus important for the enlargement of the feeding site of the tick and for enhanced of toxin delivery, was also identified in this study.[11,13] This was the first time such a molecule had been identified in a tick species.

Tick toxicoses as a tangible example of host interaction

Tick toxicosis is one of the clearest phenotypic examples to demonstrate that ticks affect their hosts during feeding and thus is the most logical place to start when investigating tick–host interactions. After the initial characterization of sand tampan toxicosis, the next question was whether this had any relationship with other forms of tick toxicoses. This led to the investigation of pathological conditions, especially tick paralysis, the main manifestation of tick toxicoses and toxins found in tick eggs. Valuable contributions on the pathogenicity of various tick toxins were made in this period.[14,15] That ticks interact with their hosts, not only in a pathological way but also in a specific orchestrated manner to obtain a blood-meal, became apparent as more data on tick salivary gland components were gathered.[13] This prompted a return to investigating the salivary gland components of *O. savignyi*.

Anti-haemostatic mechanisms of *Ornithodoros savignyi*

Pilocarpine stimulation yielded small quantities of saliva per tick; moreover, a high degree of technical capability is needed in order not to kill the tick during injection.[8] Pilocarpine is also neurotoxic and as such is undesirable for use in a student setting. Engorged female ticks, on the other hand, are quite large (1–2 cm in diameter) and can be easily dissected to obtain their salivary glands (Fig. 1). The salivary glands themselves are quite large (2.5 mm in length), thus allowing the gathering of adequate quantities of starting material to purify bio-active components from the glands. Not all salivary gland components are secreted during stimulation of ticks with pilocarpine or during feeding.[16,17] Secretory components are rather stored in salivary gland granules that are secreted once feeding starts (Fig. 2). This makes salivary glands a suitable starting material for research on the

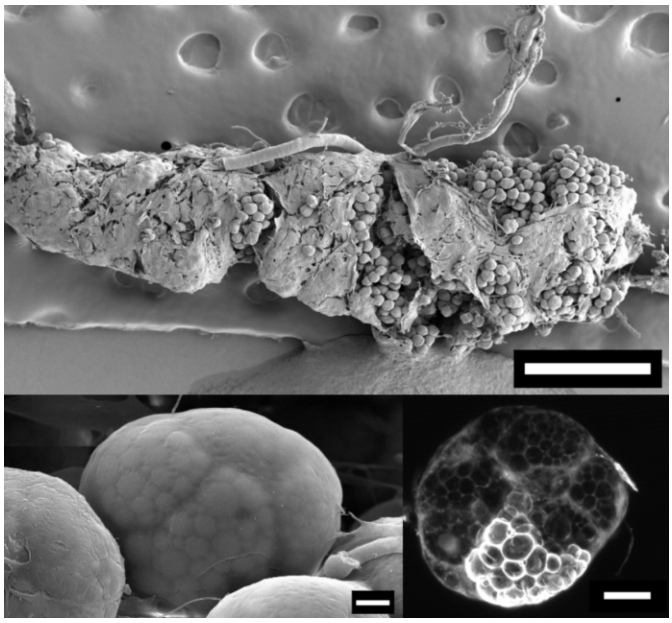

Fig. 2. Morphology of the salivary glands of the sand tampan. (**Top**) A view of a salivary gland at low magnification using scanning electron microscopy. The myo-epithelial sheath that surrounds soft tick salivary glands is present and individual acini can be observed where the sheath is torn. The scale bar is 500 µm. (**Left**) A granular acinus at higher magnification and higher electron voltage (25 kV), showing at least four salivary gland cells packed with salivary gland granules. The scale bar is 10 µm. (**Right**) Localization of savignygrin, a platelet aggregation inhibitor stored in the secretory granules of a specific salivary gland cell acinus. The inhibitor is absent in at least three other salivary gland cells that are shown in the micrograph. The picture is a composite image of 17 sections of 2 µm each obtained during immunofluorescent localization using a confocal microscope. The scale bar is 10 µm.

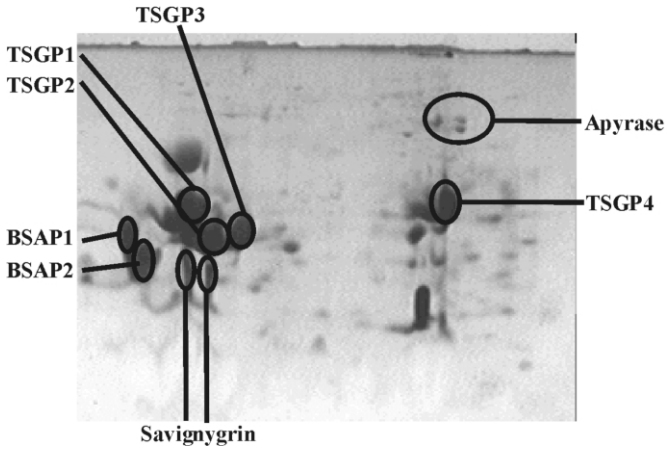

Fig. 3. The proteome of tick salivary gland extract from *Ornithodoros savignyi* and various proteins identified. Indicated are apyrase (an ATP-diphosphohydrolase enzyme), savignygrin (a platelet integrin antagonist), the BSAPs (barium sulphate adsorption proteins) and the TSGPs (tick salivary gland proteins).

chemicals secreted during feeding. Dissection of salivary glands was found to be a suitable alternative to pilocarpine stimulation and was used to isolate the first anti-haemostatic factor from this tick.[18,19] Since then *O. savignyi* has proved to be a rich source of bio-active components (Table 1 and Fig. 3).

Comparative studies related to *Ornithodoros moubata*

Much of our research has been focused on complementing work on *O. moubata,* a related tick commonly known as the African hut tampan. Extensive studies on anti-haemostatic components from this animal have been conducted.[20–25] A study of a closely related tick, like *O. savignyi,* could thus prove useful to gain comparative data, as there are a few notable differences between these species. Morphologically they are similar, although *O. savignyi* has two pairs of eyes situated in its supracoxal folds, which are lacking in *O. moubata.* Whereas *O. savignyi* burrows in sandy areas, *O. moubata* is endophilous (nest-dwelling). During its lifetime, *O. moubata* is generally confined to a limited set of hosts that inhabit its burrow. *Ornithodoros savignyi* is more predatory and will feed on any mammal in its proximity.[4] *Ornithodoros moubata* transmits *Borrelia duttoni,* which causes relapsing fever in humans and also transmits African swine fever virus. In contrast, no transmission of pathogenic organisms has yet been recorded for *O. savignyi,* although natural infection with *Borrelia* spirochetes has been reported.[26] *Ornithodoros savignyi* does, however, secrete toxic substances that can be lethal, especially in young animals. One adult tick or the salivary gland extract equivalent of half of a salivary gland can kill an adult mouse (weighing 20 g) within 20 minutes.[27] *Ornithodoros moubata,* however, does not secrete any toxins, as shown by the non-lethal feeding of five adult females, 10 males and 100 nymphs on an adult mouse to generate an immune response.[28]

Blood-clotting inhibitors from *Ornithodoros savignyi*

Anti-haemostatics from the genus *Ornithodoros* target key components of the blood coagulation and platelet aggregation cascades. For blood coagulation this includes inhibition of the blood-clotting enzymes fXa and thrombin. For platelet aggregation this includes removal of the platelet aggregation agonist ADP by the enzyme apyrase, targeting of the main platelet receptor involved in aggregation via fibrinogen binding, and targeting collagen-mediated platelet aggregation. For a detailed description of the molecular mechanisms behind blood coagulation and platelet aggregation, see ref. 29.

Research into the anti-haemostatic components of *O. savignyi* started off by investigation of inhibition of both the intrinsic and extrinsic pathways of the blood-clotting cascade.[18] The fact that both pathways were inhibited suggested that the central blood-clotting enzymes, fXa and thrombin, which are involved

Table 1. Salivary gland proteins characterized from *Ornithodoros savignyi.*

Protein name*	Protein family	Function/target	Physiological process	M_r (Da)	References
TSGP1	Lipocalin	Granule biogenesis?		18 424	9, 27, 45
TSGP2	Lipocalin	Toxin, Granule biogenesis	Cardiac system	15 970	3, 9, 10, 11, 12, 27, 44
TSGP3	Lipocalin	Granule biogenesis		15 878	9, 11, 27, 44
TSGP4	Lipocalin	Toxin, Granule biogenesis	Cardiac system	17 141	9, 27, 44
fXaI	BPTI	FXa inhibitor	Blood clotting	7 183	18, 19, 30, 43
Savignin	BPTI	Thrombin inhibitor	Blood clotting	12 430	18, 33, 43
Savignygrin	BPTI	$\alpha_{IIb}\beta_3$ antagonist	Platelet aggregation	6 966, 6 808	39, 40, 42, 43, 44
BSAP1	?	Extrinsic pathway	Blood clotting	9 333	34
BSAP2	?	Extrinsic pathway	Blood clotting	9 173	34
Apyrase	?	ATP, ADP hydrolysis	Platelet aggregation	~67 000	35, 36, 38

*Abbreviations are TSGPs (tick salivary gland proteins), BSAPs (barium sulphate adsorption proteins) and BPTI (basic pancreatic trypsin inhibitor).

in both pathways, might be targeted. Specific chromogenic assays for these enzymes showed that both were indeed inhibited by gland extracts from *O. savignyi*.[18] The first inhibitor characterized was the fXa inhibitor (fXaI); this is closely related to tick anticoagulant peptide (TAP), the inhibitor from *O. moubata* with similar competitive, slow, tight-binding kinetics.[18,19,30] The cloned product of fXaI showed an amino acid sequence identity of 46% and similarity of 78%; it was used to construct a molecular model based on the structure of TAP that classified this protein as a member of the basic pancreatic trypsin inhibitor (BPTI) protein family.[30] The high level of structural similarity suggested that the mechanism of inhibition of fXa should be similar to that of TAP. The structure of the TAP–fXa complex was recently solved and shows that TAP inhibits fXa by first binding to the exo-site of fXa via its C-terminal α-helix before inserting its N-terminal residues into the fXa active site.[31]

The thrombin inhibitor savignin was purified next and was shown to be like fXaI, a competitive, slow, tight-binding inhibitor that also interacted with thrombin's fibrinogen binding exo-site.[32] It is closely related to its homologue ornithodorin from *O. moubata,* that is based on the high identity (63%) and similarity (89%) of their amino acid sequences.[33] A structural model of savignin–thrombin interaction was constructed using the recently derived ornithodorin–thrombin crystal structure.[25] Whereas TAP and fXaI are composed of a single BPTI domain, savignin and ornithodorin possess two BPTI domains arranged in tandem. In this case the C-terminal BPTI domain of savignin/ornithodorin binds to thrombin's substrate binding exo-site via its α-helix, whereas the N-terminal residues from the N-terminal BPTI domain binds within thrombin's active site.[33] This mechanism is similar to that of TAP and fXaI. Although the structure and mechanism of action of ornithodorin have been elucidated, no information on its kinetic mechanism have yet been reported. However, the kinetic data obtained for savignin supports the structural mechanism quite nicely.[32]

Many of the host's blood-clotting enzymes possess modified glutamic acid residues (γ-carboxy-glutamic acid) that allow specific targeting to negatively charged surfaces, such as activated platelets, where they bind to membrane-bound cations and are localized at the site of haemorrhage. This targeting mechanism is important in the activation of the extrinsic pathway of the blood coagulation cascade. The γ-carboxy-glutamic acid residues also confer on the clotting factors the ability to bind to barium sulphate, which has been used as a means of purification. Adsorption of tick salivary gland proteins to barium sulphate was investigated to see whether tick proteins might mimic this targeting phenomenon.[34] Two small (9 kDa), highly acidic barium sulphate adsorption proteins (BSAPs) have been identified by this method. They do not contain any γ-carboxy-glutamic acid residues but bind to membranes. Preliminary evidence suggests that they might target the extrinsic pathway.[34] The nature of their mechanism of action and their identities are still to be elucidated.

Platelet aggregation inhibitors from *O. savignyi*

Research into the ability of *O. savigny* to inhibit platelet aggregation began with an investigation of the inhibitory capability of crude salivary gland extract.[35] Platelet aggregation induced by ADP, collagen and thrombin was inhibited and aggregated platelets were disaggregated.[35] Inhibition of ADP-induced platelet assemblage could be explained by the presence of an ATP-diphosphohydrolase activity (apyrase).[36] Inhibition of collagen-induced platelet aggregation was assumed to be due to an inhibitor similar to moubatin, isolated from *O. moubata*.[22] Inhibition of thrombin-induced platelet assemblage could be assigned to the presence of savignin.[32] It was suggested that the observed disaggregation of clustered platelets was due to fibrinogenolytic activity.[35] Such activity does indeed exist in salivary gland extracts,[37] but this specific type of activity was also found for apyrase and an inhibitor named savignygrin, which indicates that disaggregation of clustered platelets could be a function performed by salivary gland secretions.[38,39]

Apyrase was the first platelet aggregation inhibitor characterized.[36,38] It exhibited properties known for other apyrases and is the only tick apyrase yet purified to any extent.[36] It appears to use a catalytic metal ion for the hydrolysis of ATP and ADP.[36] Its most intriguing feature to date is its ability to disassemble platelets aggregated by ADP.[38] Disaggregation is accompanied by extensive shape change of the spherical aggregated platelet back to a discoid shape, similar to that of unaggregated, unactivated platelets, although the platelets have secreted their granular contents.[38] In contrast, savignygrin, an inhibitor of platelet assemblage that targets the fibrinogen receptor, also disaggregates aggregated platelets, but does not cause any shape changes.[40]

Disagregin, a platelet aggregation inhibitor from *O. moubata,* was shown to target the fibrinogen receptor, integrin $\alpha_{IIb}\beta_3$,[23] which lacked the RGD integrin recognition motif, normally present in integrin antagonists. This was of interest, because a $\alpha_{IIb}\beta_3$ antagonist called variabilin that possess an RGD motif, was previously described for the hard tick, *Dermacentor variabilis*.[41] This raised the question as to whether the homologue of disagregin, which we called savignygrin (to indicate its ability to target the integrin $\alpha_{IIb}\beta_3$), would possess an RGD motif. Characterization of savignygrin revealed that it did possess an RGD motif.[39] *Ornithodoros savignyi* expresses two isoforms of savignygrin, whereas disagregin is expressed as a single isoform by *O. moubata.* Savignygrin is also expressed at significantly higher concentrations than disagregin. This suggested that multiple copies of savignygrin might be maintained by *O. savignyi* specifically to control platelet aggregation in the diverse range of hosts it might encounter.[42]

Savignygrin and the blood-clotting inhibitors

fXaI and savignin belong to the BPTI family, but do not operate via the mechanism of the canonical BPTI inhibitors. These inhibitors function by targeting their respective serine proteases by insertion of a substrate-binding presenting loop into the enzyme active site.[25,31] An answer as to why the mechanism of the tick inhibitors differs from that of the other BPTI inhibitors emerged only after the fibrinogen receptor antagonist savignygrin was identified. This showed that the RGD motif is presented on the substrate binding-presenting loop of the BPTI fold.[39] It suggested that an evolutionary relationship might exist between the various tick BPTI-like proteins. Comparison of tick proteins and other members of the BPTI family indicated that tick proteins share a common ancestor within the tick lineage.[43] Furthermore, the results suggested that the tick BPTI proteins evolved their functions in a specific order that explains the differences between the mechanisms of the tick-derived proteins and their canonical BPTI counterparts. In this scheme the C-terminal BPTI domain of the thrombin inhibitors evolved thrombin-targeting capabilities first. This was followed by a gene duplication event and a tandem fusion to produce the double BPTI-domain protein that evolved the novel active site-targeting mechanism observed for the tick proteins. A duplication of the N-terminal domain alone resulted in a protein that could target the active site of fXa using this novel mechanism. Another gene duplication event involving this fXa inhibitor eventually resulted in the platelet aggregation inhibitors that

used the original substrate-binding loop of the BPTI fold as a scaffold to present the RGD motif to integrin $\alpha_{IIb}\beta_3$.[43] In addition to the RGD motif, a binding loop is involved in savignygrin–$\alpha_{IIb}\beta_3$ interaction.[44] The regions involved in the interaction are distinct from the active regions of the blood-coagulation inhibitors. This allowed the BPTI fold to evolve integrin-targeting capabilities.[44] This specific evolutionary pathway is not present in hard ticks and implies that hard and soft ticks adapted separately to a blood-feeding environment.[29] Many other features of their biology that are intimately involved in the blood-feeding process differ between hard and soft ticks.[29] The search for universal anti-tick feeding vaccines, that will target both tick families at the same time, is thus probably a futile exercise.[29]

A return to sand tampan toxicoses

Initial characterization of savignygrin indicated the presence of two isoforms with molecular masses close to 6346 Da. Purification patterns obtained after size exclusion chromatography followed by anion exchange chromatography were similar to those obtained for the original toxin.[10,11] We were optimistic that the toxin had been rediscovered with a new biological activity. However, no toxicity as demonstrated by biological assay was found associated with savignygrin. An elaborate series of experiments followed which convinced us that savignygrin was not the toxin; N-terminal amino acid sequencing confirmed this. These experiments were, however, enough to rekindle our interest in the native toxin and we proceeded to re-purify it. This was partly because we found another toxic activity residing in the salivary glands.[44] Attempts to obtain the complementary DNA sequence of the original toxin over the years were not successful. We suspected that we were missing something important in the native toxin that would allow cloning of its cDNA. This was later confirmed when several specific amino acid residue differences were found between the toxin described in 1969 and that described in 2001.[27,45]

Salivary gland biogenesis and the toxins

At the time we were purifying savignygrin we were also characterizing the most abundant tick salivary gland proteins (TSGPs).[27] Our rationale for this was that highly abundant proteins might be involved in the formation of secretory granules within the salivary glands. We characterized four abundant proteins with molecular masses ranging from 15 800 to 18 400 Da as determined by mass spectrometry. We realized only when we obtained their N-terminal amino acid sequences that we had been investigating both toxins and the non-toxic homologue previously described.[27] Characterization of these proteins proved to be serendipitous when we discovered that they also belonged to the same lipocalin protein family.[45,46] Determination of the complementary DNA sequences of the TSGPs showed that the toxins had masses corresponding to that of the recently purified native proteins and no evidence of glycosylation was found.[45] The hypothesis that the proteins might be involved in salivary gland granule biogenesis is supported by the immunolocalization of the TSGPs to all known types of secretory granules in argasid salivary glands.[47] In contrast, the platelet aggregation inhibitors apyrase and savignygrin are localized to specific granule types. This study concluded that salivary granules cannot be classified based on granule morphology alone.

Sand tampan toxicoses and their relationship to other forms of pathology

A central issue in the study of tick toxicoses was whether they are related and what their origin might be. The rediscovery of the toxin and other related molecules allowed us to reconsider these questions. The earlier purification of the toxin was performed under conditions that yielded a preparation with relatively low toxicity, so that more than 400 μg of pure toxin was needed to kill a 10-g mouse within 90 minutes.[10] New high-performance liquid chromatography procedures allowed the rapid purification of the toxins and yielded products with a much higher toxicity, so that ~20 μg of purified toxin would kill a 24-g mouse within 30 minutes[9] Purification of the toxin permitted further investigation of the pathological mechanism of sand tampan toxicoses. Both toxins affected the cardiac system of the host, probably by antagonism of cardiac ion channels.[9] This together with data on the clinical pathology of this form of toxicosis suggested that there is no relationship between sand tampan and other toxicoses, especially tick paralysis.[9,15] This was supported by toxin sequences that showed that the TSGPs all belong to the lipocalin protein family.[45] These were the first toxic lipocalins described as well as the first tick toxins for which full-length sequences were elucidated. The TSGPs are related to histamine-binding proteins from the hard tick, *Rhipicephalus appendiculatus,* to the platelet aggregation inhibitor moubatin, specific for collagen-induced platelet aggregation and various other tick proteins. The TSGPs, however, do not possess moubatin-like activity and salivary gland extracts from *O. moubata* do not contain toxins. This implies that the toxins evolved relatively recently, after the divergence of *O. moubata* and *O. savignyi.*[45] Thus, tick toxins do not have a common origin.

Future research on the sand tampan

The discovery of both the BPTI and lipocalin families of the sand tampan opens up several avenues for future research. The evolutionary relationships between the blood-coagulation and platelet aggregation inhibitors, and the fact that they all use the same protein scaffold for diverse functions, could allow the construction of chimeric proteins that encode all of these functions at once. Such a molecule could be useful for therapeutic purposes such as the control of thrombosis. Construction of a variety of these chimeric molecules may also be useful for investigating this specific evolutionary pathway further and might provide new insights into the evolution of new protein function. A central question not resolved yet is whether these anti-haemostatics contribute towards sand tampan toxicoses in a synergistic way. As for the toxicoses themselves, the specific pathogenic mechanism of action of each toxin is still not resolved at a molecular level. We have not identified their specific targets. The toxins and the non-toxic TSGPs are related to other tick lipocalins that have been shown to be potential vaccine targets.[45] The time is thus ripe to investigate their usefulness as vaccine targets to control the sand tampan. In addition, the toxins themselves should be targeted for vaccination purposes to generate a neutralizing response. Even if the sand tampan burden cannot be controlled by vaccination, a neutralizing agent directed against the poison involved might counter death due to toxicosis. The sand tampan will undoubtedly remain a good model for investigations into tick biology with many mysteries still left to be solved.

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