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Potential pharmacological use of salivary compounds from hematophagous organisms

Uso potencial farmacológico de los compuestos salivares de organismos hematófagos

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| Abstract |

Introduction: The saliva of hematophagous or blood-sucking insects contains different substances that allow obtaining and ingesting the blood of their vertebrate hosts without being detected.

Objective: To explore the salivary compounds of hematophagous insects which have vasodilator, anticoagulant, anti-inflammatory, immunomodulatory and anesthetic properties, and that can be exploited due to their high pharmacological potential.

Materials and methods: A non-systematic literature review was done in PubMed, EMBASE, and ScienceDirect OvidSP; data was not limited by date, language nor item type. Articles on salivary compounds of blood-sucking insects, whose main topic was the effects on hemostasis, immunomodulation and drug use, were sought. 59 articles met the criteria for inclusion in the review.

Conclusions: The saliva of hematophagous insects has a wide variety of molecules that constitute a source of research and have an incalculable potential for the discovery of compounds that could be pharmacologically useful.

Keywords: Saliva; Pharmacology; Anticoagulants; Vasodilators; Anti-inflammatory; Anesthetics (MeSH).

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| Resumen |

Introducción. La saliva de los artrópodos hematófagos contiene un arsenal de compuestos que les permite acceder a la sangre de sus hospederos vertebrados sin ser detectados.

Objetivo. Explorar los compuestos salivares de insectos hematófagos que tienen propiedades vasodilatadoras, anticoagulantes, antiinflamatorias,

inmunomoduladoras y anestésicas, las cuales se pueden aprovechar por su alto potencial farmacológico.

Materiales y métodos. Se realizó una revisión no sistemática de la literatura mediante búsqueda electrónica en las bases de datos PubMed, EMBASE, OvidSP y ScienceDirect; la búsqueda no se limitó por fecha, idioma ni tipo de artículo. Se buscaron artículos sobre los compuestos salivares de los insectos hematófagos, cuyo tema central fuese los efectos en la hemostasia, inmunomodulación y uso farmacológico. Se encontraron 59 artículos que cumplían con los criterios para ser incluidos en la revisión.

Conclusión. La saliva de los insectos hematófagos posee gran variedad de moléculas, lo que ofrece una fuente de investigación y un potencial incalculable para el descubrimiento de compuestos que podrían llegar a tener utilidad farmacológica.

Palabras clave: Saliva; Farmacología; Anticoagulantes; Vasodilatadores; Antiinflamatorios; Anestésicos (DeCS).

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Introduction

Hematophagy or feeding on blood has been seen in various taxa along the evolutionary tree. This is especially relevant in arthropods because they are vectors of different pathogens. Arthropods within the class Insecta or Hexapoda are among the most numerous and diverse of the animal kingdom, with almost 1 200 000 species described, which corresponds to 80% of all animals (1).

In general, the saliva of insects has several functions: digestion, water balance, antagonism of host defense systems, maintenance of the mouthparts, and secretion of pheromones; also, it is believed to have antimicrobial substances (2). Hematophagy is one of the many feeding habits that these species have, that is, they ingest vertebrate blood, including human blood.

Hematophagy is not unique to arthropods and occurs throughout the evolutionary scale; parasites such as *Entamoeba histolytica* and different species of *Plasmodium* consume blood or parts of its components. Leeches, *Annelida* phylum invertebrates and higher animals such as some species of bats of the *Desmodontinae* subfamily, better known by the name of vampires, also feed on blood.

Apparently, the ability to feed on vertebrate blood was developed during the Cretaceous period (3) in parallel to the early division of mammals and birds that replaced dinosaurs. One hypothesis of the evolution of hematophagy is that insects that lived very close to birds or mammals went from being detritivores to hematophagous, with a subsequent modification of the mouthparts, which means that the association between vertebrates and hematophagous insects induced coevolution.

Hematophagy appeared independently during the evolution of arthropods and was only developed by 14 000 species, distributed among 400 different genera from more than one million species of insects (4). These numbers suggest that hematophagy presented mechanical and physiological difficulties during its evolution (5), since it involved the modification of the mouthparts to penetrate, cut and suck, in addition to the challenges for digesting and using blood for different processes related to growth and development or for the maturation of eggs. The detoxification of the *heme* group was also very important, for which they had to chelate it and thus produce hemozoin (5).

Insects ingest the blood in two different ways: some take the blood directly from channel blood vessels —solenophagy— and others break the tissues and produce multiple wounds forming a pool of blood —telmophagy (6). In both cases, insects had to develop an arsenal of molecules with different effects, such as vasodilators, anticoagulants, antiplatelets, anti-inflammatories, immunomodulators, analgesics and, apparently, local anesthetics in order to counteract defenses and other responses of the hosts against the damage inflicted by the hematophagous animal (7).

However, not all haematophagous insects use blood for the same purposes. For example, insects such as Triatomines need blood to cover all physiological requirements, whereas some species of the *Culicidae*, *Ceratopogonidae* and *Simuliidae* families need blood only to mature the eggs—in this case the composition and morphology of the salivary glands are different in both sexes (8).

Vertebrates have three efficient defense systems that make hematophagy potentially difficult, which are hemostasis, inflammation mechanisms and immunity. With all of these factors in mind, insects may be potentially beneficial for the discovery of new molecules and, perhaps, new drugs.

Below, different molecules that have been found in the salivary glands of hematophagous insects are described, and some research developed on their applicability in medicine are summarized.

Vasodilators

Vasoconstriction is one of the first reflex mechanisms against bleeding and capillary damage; for this reason, hematophagous insects have developed various vasodilator molecules to ensure blood intake. Some examples of this type of substances include nitroporins, which are molecules with a *heme* group that reversibly bind to nitric oxide and are then released into the host to exert a vasodilator and antiplatelet effect. Nitroporins have been identified in *Rhodnius prolixus* (9)—the main vector of *Trypanosoma cruzi* in Colombia—, are known as np1 and np4 and belong to the lipocalines group (10,11). They have also been identified in *Cimex lectularius*, or bed bug (12), but these nitroporins belong to the inositol phosphatases group (13).

In addition, ticks produce prostaglandins in large quantities; so far, PGE2 and PGF2 alpha have been identified (14,15). Diptera of the genus *Phlebotomus* produce adenosine, which is secreted by 75-80% after initiating blood ingestion, and its vasodilatory and antiplatelet activity has been demonstrated (16,17). Insects of the genus *Lutzomyia* have a potent vasodilator, maxadilan, which binds to the PACAP receptor and has 500-fold more vasodilatory activity than the peptide related to the calcitonin gene, the most potent vasodilator compound known to date (18,19). Other examples of isolated and characterized vasodilator molecules are type I and II sialokinins found in *Aedes aegypti* (20,21).

The species *Simulium vittatum* belongs to the *Simuliidae* family—known in Colombia as *fején*, *mosquito*, among others—and has a 15-kDa protein called *Simulium vittatum* erythema protein or SVEP in its saliva, which increases the perfusion of blood in the cutaneous capillaries and triggers the erythema associated with the insect bite. Apparently, its vasodilation mechanism relaxes smooth muscles, since it stimulates the opening of K⁺ channels that depend on ATP (22,23).

Finally, a peroxidase has been identified in the female *Anopheles albimanus*, one of the three main vectors of malaria in Colombia, which exerts its vasodilatory effect by destroying norepinephrine and serotonin (24).

Anticoagulants

Hemostasis, a mechanism used by animals as a response to tissue damage, involves three processes: platelet aggregation, coagulation and vessel constriction. This mechanism represents a major challenge for hematophagous animals, since thrombin inhibitors have multivalent reactions in common, which results in high specificity and affinity, besides of blocking interactions with possible substrates and cofactors. One of the best inhibitors of hematophagous animals is hirudin, extracted from the *Hirudo medicinalis* leech, although other serine proteases with the same function have also been found in other leeches (25).

Anti-hemostatic molecules show the great diversity of compounds derived from the saliva of hematophagous animals. For example, apyrase belongs to a family of enzymes that hydrolyze ATP and ADP to yield AMP, and that may have an anti-inflammatory and antihemostatic effect (26-27). These molecules can be found in various insects such as *Aedes aegypti* (28), vector of some flaviviruses including dengue, Zika and Chikungunya in Colombia; *Lutzomyia longipalpis*, vector of *Leishmania infantum* in many Latin American regions (29); *Phlebotomus papatasi*, vector of *Leishmania donovani* in the Old World and of bedbugs (30), and *Cimex lectularius* (31) and *Ayadualina* extracted from the salivary glands of *Lutzomyia ayacuyensis*, whose function is to inhibit collagen-induced platelet aggregation and ADP (32). Higher concentrations of heparin have been found in the midgut of *Aedes togoi*, which is related to the salivary glands of females who have ingested blood (33). An isolated Factor Xa inhibitor, characterized and known as anticoagulant Factor Xa (AFXa), belongs to the superfamily of serpins and can be found in *Aedes aegypti* (34,35).

Several thrombin inhibitor compounds such as Mandanin-1 and Mandanin-2—competitive inhibitors of thrombin and extracted from the saliva of the tick *Longicornis haemaphysalis*— (36) have been recently discovered. Other examples of thrombin inhibitors include the Americanin molecule, obtained from the salivary glands of the tick *Amblyomma americanum* (37); the Anopheline molecule from the *Anopheles albimanus* mosquito (38,39), and the CE5 protein found in *Anopheles gambiae* (40), and AaTI or *Aedes aegypti* thrombin inhibitor (41,42).

Anti-inflammatories and immunomodulators

Studies and isolations of these types of compounds have been carried out, for the most part, with the saliva of ticks, since these arthropods remain close to the host for several days and have developed potent anti-inflammatory and immunomodulatory compounds. By means of these compounds, ticks manage to delay the immune response of the host vertebrate and, thus, prolong their feeding.

The most studied compounds are anti-complement compounds such as the saliva of *Ixodes scapularis* or Isac (43), the IRAC I and IRAC II proteins found in *Ixodes ricinus*, which inhibit the alternative complement pathway (44), and the salivary protein 20 found in *Ixodes scapularis* or Salp20, which acts by dissociating C3 convertase (45).

Another site of action is the effect on cells of the immune system. For example, the saliva of *Ixodes dammini* inhibits the functionality of neutrophils (46), whereas *Dermacentor reticulatus* and *Ixodes ricinus* suppress the functions of NK cells, the production of interferon and interleukins (47,48), and the proliferation of lymphocytes (49,50). Salivary extracts of *Amblyomma americanum* and *Dermacentor variabilis* ticks are also capable of affecting the proliferation, migration and phagocytosis of macrophages (51,52). In addition, the maturation of dendritic cells is inhibited by the saliva of *Rhipicephalus sanguineus* (53).

On the other hand, the saliva of some ticks has shown an important effect on chemotaxis, the production of various cytokines and the modification of the Th1 and Th2 response (54,55). It also has the ability to interfere in healing since it hinders fibroblast migration, decreases the production of growth factors and the formation of the cellular cytoskeleton (56,57), and alters angiogenesis—which is product of the effect of the calreticulin protein (54) found in the saliva of *Ixodes Scapularis* and *Amblyomma americanum* (58).

The *Trypanosoma brucei* vector—producer of American trypanosomiasis and found in the saliva of *Glossina morsitans*—has a peptide named Gloss 2 which inhibits the secretion of TNF- α factor, IFN- γ and IL-6, apart from affecting the humoral immune response by inhibiting the production of IL-10 (59).

Anesthetics

Little has been studied about the anesthetic effect of the saliva of hematophagous insects. However, the presence of mechanisms through which pain is blocked have been proposed, since many of such insects are big, produce a non-painful sting and can suck blood for long periods of time without being perceived.

Regarding this hypothesis, there is a study that shows that the saliva of *Triatoma infestans*, the main vector of *Trypanosoma cruzi* in the southern cone of South America, has an inhibitory effect on nerve transmission, as it achieves a progressive reduction of the action potential amplitude on a rat sciatic nerve model. In the same study, an inhibition of sodium-dependent voltage channels in cultures of neuronal GH3 cells was demonstrated, leading to hypothesize that the saliva of these insects may decrease the generation and conduction of nerve action potential, similar to local anesthetics currently used (60).

Pharmacological usefulness

One of the best examples of pharmacological use of compounds derived from the saliva of hematophagous insects is the development of direct thrombin inhibitors: bivalirudin, argatroban and desirudin, derivatives of the Hirudin anticoagulant peptide obtained from the saliva of the leech *Hirudo medicinalis* (61).

The Maxadilan peptide, found in the saliva of *Lutzomyia longipalpis*, is a potent agonist vasodilator specific for the PACAP type I receptor, which is widely distributed in the brain. In a study with rabbits, this compound was useful for the management of cerebral spasms secondary to subarachnoid hemorrhage (62). In addition to its vasodilatory effect, its influence on the metabolic level has also been observed, since prolonged administration in murine models increases insulin sensitivity and lowers basal plasma glucose (63). Likewise, Maxadilan has been shown to prevent apoptosis of human pluripotent cells by regulating caspases 3 and 6, without affecting the karyotype or pluripotent state of insect cells (64).

Two molecules are involved in clot formation. One of them is simlagrafin, found in *Simulium nigrimanum*, which inhibits the interaction of the von Willebrand factor with type III collagen by specifically and completely blocking platelet adhesion under high flux conditions—it has been proven useful in inhibiting the formation of carotid thrombi in mice (65). The other is aegeptin, which binds to collagen and inhibits platelet aggregation to soluble or fibrillar collagen and the interaction of the Von Willebrand factor (66).

Considering the properties of tick saliva to inhibit cell migration and healing, a study on osteosarcoma tumor cells and breast cancer was performed, finding that saliva had an inhibitory effect on the migration and metastatic invasion of these cells (67).

Conclusions

This review has explored how hematophagous insects have an important variety of molecules capable of acting on hemostasis, immunity and response to vertebrate pain, thus ensuring their engorging. Similarly, different compounds are being investigated for pharmacological use in circulatory, metabolic and even oncological pathologies.

All this leads to conclude that the saliva of hematophagous insects offers a great source of research and incalculable potential for the discovery of new compounds that could become pharmacologically useful and even provide valuable medical alternatives for humanity.

Conflict of interests

None stated by the authors.

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References

1. Mora C, Tittensor DP, Adl S, Simpson AG, Worm B. How many species are there on earth and in the ocean? *Plos Biol*. 2011;9(8):e1001127. <http://doi.org/fpr4z8>.
2. Musser RO, Kwon KS, Williams SA, White CJ, Romano MA, Holt SM, et al. Evidence that caterpillar labial saliva suppresses infectivity of potential bacterial pathogens. *Arch Insect Biochem Physiol*. 2005;58(2):138-44. <http://doi.org/fjq262>.
3. Lukashovich ED, Mostovski MB. Hematophagous insects in the fossil record. *Paleontological Journal*. 2003;37(2):153-61.

4. **Ribeiro JM.** Blood-feeding arthropods: live syringes or invertebrate pharmacologists?. *Infect Agents Dis.* 1995;4(3):143-52.
5. **Graça-Souza AV, Maya-Monteiro C, Paiva-Silva GO, Braz GR, Paes MC, Sorgine MH, et al.** Adaptations against heme toxicity in blood-feeding arthropods. *Insect Biochem Mol Biol.* 2006;36(4):322-35. <http://doi.org/d7wg9n>.
6. **Marcondes CB.** Entomologia médica e veterinária. 2nd ed. São Paulo: Editora Atheneu; 2011.
7. **Ribeiro JM, Francischetti IM.** Role of arthropod saliva in blood feeding: sialome and post-sialome perspectives. *Annu Rev Entomol.* 2003;48:73-88. <http://doi.org/d9jw37>.
8. **Nascimento P, dos Santos-Malafronte R, Marinotti O.** Salivary gland proteins of the mosquito *Culex quinquefasciatus*. *Arch Insect Biochem Physiol.* 2000;43(1):9-15. <http://doi.org/bm7fwd>.
9. **Ribeiro JM, Hazzard JM, Nussenzweig RH, Champagne DE, Walker FA.** Reversible binding of nitric oxide by a salivary heme protein from a bloodsucking insect. *Science.* 1993;260(5107):539-41. <http://doi.org/c38shz>.
10. **Champagne DE, Nussenzweig RH, Ribeiro JM.** Purification, partial characterization, and cloning of nitric oxide-carrying heme proteins (nitrophorins) from salivary glands of the blood-sucking insect *Rhodnius prolixus*. *J Biol Chem.* 1995;270(15):8691-5. <http://doi.org/ftm43m>.
11. **Montfort WR, Weichsel A, Andersen JF.** Nitrophorins and related anti-hemostatic lipocalins from *Rhodnius prolixus* and other blood-sucking arthropods. *Biochem Biophys Acta.* 2000;1482(1-2):110-8. <http://doi.org/b6pfz8>.
12. **Valenzuela JG, Walker FA, Ribeiro JM.** A salivary nitrophorin (nitric-oxide-carrying hemoprotein) in the bedbug *Cimex lectularius*. *J Exp Biol.* 1995;198(Pt 7):1519-26.
13. **Valenzuela JG, Ribeiro JM.** Purification and cloning of the salivary nitrophorin from the hemipteran *Cimex lectularius*. *J Exp Biol.* 1998;201(Pt 18):2659-64.
14. **Dickinson RG, O'Hagan JE, Schotz M, Binnington KC, Hegarty MP.** Prostaglandin in the saliva of the cattle tick *Boophilus microplus*. *Aust J Exp Biol Med Sci.* 1976;54(5):475-86. <http://doi.org/dcw5gs>.
15. **Ribeiro JM, Evans PM, MacSwain JL, Sauer J.** Amblyomma americanum: characterization of salivary prostaglandins E2 and F2 alpha by RP-HPLC/bioassay and gas chromatography-mass spectrometry. *Exp Parasitol.* 1992;74(1):112-6. <http://doi.org/bbvts5>.
16. **Ribeiro JM, Katz O, Pannell LK, Waitumbi J, Warburg A.** Salivary glands of the sand fly *Phlebotomus papatasi* contain pharmacologically active amounts of adenosine and 5'-AMP. *J Exp Biol.* 1999;202(Pt 11):1551-9.
17. **Ribeiro JM, Modi G.** The salivary adenosine/AMP content of *Phlebotomus argentipes* Annandale and Brunetti, the main vector of human kala-azar. *J Parasitol.* 2001;87(4):915-7. <http://doi.org/c77rvr>.
18. **Lerner EA, Ribeiro JM, Nelson RJ, Lerner MR.** Isolation of maxadilan, a potent vasodilatory peptide from the salivary glands of the sand fly *Lutzomyia longipalpis*. *J Biol Chem.* 1991;266(17):11234-6.
19. **Moro O, Lerner EA.** Maxadilan, the vasodilator from sand flies, is a specific pituitary adenylate cyclase activating peptide type I receptor agonist. *J Biol Chem.* 1997;272(2):966-70. <http://doi.org/bdb27v>.
20. **Champagne DE, Ribeiro JM.** Sialokinin I and II: vasodilatory tachykinins from the yellow fever mosquito *Aedes aegypti*. *Proc Natl Acad Sci USA.* 1994;91(1):138-42. <http://doi.org/d2zw5x>.
21. **Beerntsen BT, Champagne DE, Coleman JL, Campos YA, James AA.** Characterization of the Sialokinin I gene encoding the salivary vasodilator of the yellow fever mosquito, *Aedes aegypti*. *Insect Mol Biol.* 1999;8(4):459-67. <http://doi.org/fp244b>.
22. **Cupp MS, Ribeiro JM, Champagne DE, Cupp EW.** Analyses of cDNA and recombinant protein for a potent vasoactive protein in saliva of a blood-feeding black fly, *Simulium vittatum*. *J Exp Biol.* 1998;201(Pt 10):1553-61.
23. **Cupp MS, Ribeiro JM, Cupp EW.** Vasodilative activity in black fly salivary glands. *Am J Trop Med Hyg.* 1994;50(2):241-6.
24. **Ribeiro JM, Valenzuela JG.** The salivary purine nucleosidase of the mosquito, *Aedes aegypti*. *Insect Biochem Mol Biol.* 2003;33(1):13-22. <http://doi.org/c2tbck>.
25. **Corral-Rodríguez MA, Macedo-Ribeiro S, Barbosa-Pereira PJB, Fuentes-Prior P.** Leech-derived thrombin inhibitors: from structures to mechanisms to clinical applications. *J Med Chem.* 2010;53(10):3847-61. <http://doi.org/fcjt7t>.
26. **Ribeiro JM, Endris TM, Endris R.** Saliva of the soft tick, *Ornithodoros moubata*, contains anti-platelet and apyrase activities. *Comp Biochem Physiol A Comp Physiol.* 1991;100(1):109-12. <http://doi.org/d4bpcm>.
27. **Bergillos-Gasion F, Rivas Fernández M.** Picaduras y mordeduras de animales: Tratado de toxicología clínica. Tomo I. Barcelona: Elsevier; 2012.
28. **Champagne DE, Smartt CT, Ribeiro JM, James AA.** The salivary gland-specific apyrase of the mosquito *Aedes aegypti* is a member of the 5'-nucleotidase family. *Proc Natl Acad Sci USA.* 1995;92(3):694-8. <http://doi.org/ft95ff>.
29. **Charlab R, Valenzuela JG, Rowton ED, Ribeiro JM.** Toward an understanding of the biochemical and pharmacological complexity of the saliva of a hematophagous sand fly *Lutzomyia longipalpis*. *Proc Natl Acad Sci USA.* 1999;96(26):15155-60. <http://doi.org/bcbewf>.
30. **Valenzuela JG, Belkaid Y, Rowton E, Ribeiro JM.** The salivary apyrase of the blood-sucking sand fly *Phlebotomus papatasi* belongs to the novel Cimex family of apyrases. *J Exp Biol.* 2001;204(Pt 2):229-237.
31. **Valenzuela JG, Charlab R, Galperin MY, Ribeiro JM.** Purification, cloning, and expression of an apyrase from the bed bug *Cimex lectularius*. A new type of nucleotide-binding enzyme. *J Biol Chem.* 1998;273(46):30583-90. <http://doi.org/bn7tsp>.
32. **Kato H, Gómez EA, Fujita M, Ishimaru Y, Uezato H, Minori Y, et al.** Ayadualin, a novel RGD peptide with dual antihemostatic activities from the sand fly a vector of Andean type cutaneous leishmaniasis. *Biochimie.* 2015;112:49-56.
33. **Ha YR, Oh SR, Seo ES, Kim BH, Lee DK, Lee SJ.** Detection of heparin in salivary gland and midgut of *Aedes togoi*. *Korean J Parasitol.* 2014;52(2):183-8. <http://doi.org/b436>.
34. **Stark KR, James AA.** A factor Xa-directed anticoagulant from the salivary glands of the yellow fever mosquito *Aedes aegypti*. *Exp Parasitol.* 1995;81(3):321-31. <http://doi.org/bh5cr3>.
35. **Stark KR, James AA.** Isolation and characterization of the gene encoding a novel factor Xa-directed anticoagulant from the yellow fever mosquito, *Aedes aegypti*. *J Biol Chem.* 1998;273(33):20802-9. <http://doi.org/bk8ds6>.
36. **Figueiredo AC, de Sanctis D, Pereira PJ.** The tick-derived anticoagulant madanin is processed by thrombin and factor Xa. *Plos One.* 2013;8(8):e71866. <http://doi.org/b437>.
37. **Zhu K, Bowman AS, Brigham DL, Essenberg RC, Dillwith JW, Sauer JR.** Isolation and characterization of americanin, a specific inhibitor of thrombin, from the salivary glands of the lone star tick *Amblyomma americanum* (L.). *Exp Parasitol.* 1997;87(1):30-3. <http://doi.org/bztkw m>.
38. **Francischetti IM, Valenzuela JG, Ribeiro JM.** Anophelin: kinetics and mechanism of thrombin inhibition. *Biochemistry.* 1999;38(50):16678-85. <http://doi.org/dnz328>.
39. **Valenzuela JG, Francischetti IM, Ribeiro JM.** Purification, cloning, and synthesis of a novel salivary anti-thrombin from the mosquito *Anopheles albimanus*. *Biochemistry.* 1999;38(34):11209-15. <http://doi.org/czxsnp>.
40. **Ronca R, Kotsyfakis M, Lombardo F, Rizzo C, Currà C, Ponzi M, et al.** The *Anopheles gambiae* cE5, a tight- and fast-binding thrombin inhibitor with post-transcriptionally regulated salivary-restricted expression. *Insect Biochem Mol Biol.* 2012;42(9):610-20. <http://doi.org/f35s6n>.

41. Watanabe RM, Tanaka-Azevedo AM, Araujo MS, Juliano MA, Tanaka AS. Characterization of thrombin inhibitory mechanism of rAaTI, a Kazal-type inhibitor from *Aedes aegypti* with anticoagulant activity. *Biochimie*. 2011;93(3):618-23. <http://doi.org/bt4cz7>.
42. Hildebrandt JP, Lemke S. Small bite, large impact-saliva and salivary molecules in the medicinal leech, *Hirudo medicinalis*. *Naturwissenschaften*. 2011;98(12):995-1008. <http://doi.org/bnbp6g>.
43. Valenzuela JG, Charlab R, Mather TN, Ribeiro JM. Purification, cloning, and expression of a novel salivary anticomplement protein from the tick, *Ixodes scapularis*. *J Biol Chem*. 2000;275(25):18717-23. <http://doi.org/fm7z2x>.
44. Schroeder H, Daix V, Gillet L, Renauld JC, Vanderplasschen A. The paralogous salivary anti-complement proteins IRAC I and IRAC II encoded by *Ixodes ricinus* ticks have broad and complementary inhibitory activities against the complement of different host species. *Microbes Infect*. 2007;9(2):247-50. <http://doi.org/d5zc2s>.
45. Tyson K, Elkins C, Patterson H, Fikrig E, de Silva A. Biochemical and functional characterization of Salp20, an *Ixodes scapularis* tick salivary protein that inhibits the complement pathway. *Insect Mol Biol*. 2007;16(4):469-79. <http://doi.org/frwgmK>.
46. Ribeiro JM, Weis JJ, Telford SR III. Saliva of the tick *Ixodes dammini* inhibits neutrophil function. *Exp Parasitol*. 1990;70(4):382-8. <http://doi.org/dksjkt>.
47. Kopecký J, Kutheřlová M. Suppressing effect of *Ixodes ricinus* salivary gland extract on mechanisms of natural immunity in vitro. *Parasite Immunol*. 1998;20(4):169-74.
48. Kubes M, Fuchsberger N, Labuda M, Zuffová E, Nuttall PA. Salivary gland extracts of partially fed *Dermacentor reticulatus* ticks decrease natural killer cell activity in vitro. *Immunology*. 1994;82(1):113-6.
49. Kovár L, Kopecký J, Říhová B. Salivary gland extract from *Ixodes ricinus* tick polarizes the cytokine profile toward Th2 and suppresses proliferation of T lymphocytes in human PBMC culture. *J Parasitol*. 2001;87(6):1342-48. <http://doi.org/d5v9tt>.
50. Macaluso KR, Wikel SK. *Dermacentor andersoni*: effects of repeated infestations on lymphocyte proliferation, cytokine production, and adhesion-molecule expression by BALB/c mice. *Ann Trop Med Parasitol*. 2001;95(4):413-27. <http://doi.org/c6rq4f>.
51. Jaworski DC, Jasinskas A, Metz CN, Bucala R, Barbour AG. Identification and characterization of a homologue of the pro-inflammatory cytokine Macrophage Migration Inhibitory Factor in the tick, *Amblyomma americanum*. *Insect Mol Biol*. 2001;10(4):323-31. <http://doi.org/bdzv62>.
52. Kramer C, Nahmias Z, Norman DD, Mulvihill TA, Coons LB, Cole JA. *Dermacentor variabilis*: regulation of fibroblast migration by tick salivary gland extract and saliva. *Exp Parasitol*. 2008;119(3):391-7. <http://doi.org/fjkq2n>.
53. Oliveira CJ, Carvalho WA, Garcia GR, Gutierrez FR, de Miranda-Santos IK, Silva JS, *et al*. Tick saliva induces regulatory dendritic cells: MAP-kinases and Toll-like receptor-2 expression as potential targets. *Vet Parasitol*. 2010;167(2-4):288-97. <http://doi.org/cwgvcs>.
54. Barriga OO. Evidence and mechanisms of immunosuppression in tick infestations. *Genet Anal*. 1999;15(3-5):139-42. <http://doi.org/czm8wv>.
55. Oliveira CJ, Cavassani KA, Moré DD, Garlet GP, Aliberti JC, Silva JS *et al*. Tick saliva inhibits the chemotactic function of MIP-1 α and selectively impairs chemotaxis of immature dendritic cells by down-regulating cell-surface CCR5. *Int J Parasitol*. 2008;38(6):705-16. <http://doi.org/b3rp76>.
56. Kramer CD, Poole NM, Coons LB, Cole JA. Tick saliva regulates migration, phagocytosis, and gene expression in the macrophage-like cell line, IC-21. *Exp Parasitol*. 2011;127(3):665-71. <http://doi.org/dx2h4t>.
57. Slovák M, Štibrániová I, Hajnická V, Nuttall PA. Antiplatelet-derived growth factor (PDGF) activity in the saliva of ixodid ticks is linked with their long mouthparts. *Parasite Immunol*. 2014;36(1):32-42. <http://doi.org/f5jw5k>.
58. Pike SE, Yao L, Jones KD, Cherney B, Appella E, Sakaguchi K, *et al*. Vasostatin, a calreticulin fragment, inhibits angiogenesis and suppresses tumor growth. *J Exp Med*. 1998;188(12):2349-56. <http://doi.org/dsk9cd>.
59. Valenzuela JG, Francischetti IM, Pham VM, Garfield MK, Mather TN, Ribeiro JM. Exploring the salivary glands of the tick *Ixodes scapularis*. *J Exp Biol*. 2002;205(Pt 18):2843-64.
60. Bai X, Yao H, Du C, Chen Y, Lai R, Rong M. An immunoregulatory peptide from tsetse salivary glands of *Glossina morsitans morsitans*. *Biochimie*. 2015;118:123-8. <http://doi.org/f7zvtw>.
61. Dan A, Pereira MH, Pesquero JL, Diotaiuti L, Beirão PS. Action of the saliva of *Triatoma infestans* (Heteroptera: Reduviidae) on sodium channels. *J Med Entomol*. 1999;36(6):875-9. <http://doi.org/b44j>.
62. Schiele F, Vuilleminot A, Kramarz P, Kieffer Y, Anquenet T, Bernard Y, *et al*. Use of recombinant hirudin as antithrombotic treatment in patients with heparin-induced thrombocytopenia. *Am J Hematol*. 1995;50(1):20-5. <http://doi.org/b59v4g>.
63. Kaminuma T, Shimizu H, Ahmad I, Ochiai N, Ehama R, Ohnuma M, *et al*. Prevention of cerebral vasospasm by vasodilatory peptide maxadilan following subarachnoid hemorrhage in rabbits. *J Control Release*. 1998;52(1-2):71-80. <http://doi.org/czvcxh>.
64. Yu R, Yi T, Xie S, Hong A. Long-term administration of maxadilan improves glucose tolerance and insulin sensitivity in mice. *Peptides*. 2008;29(8):1347-53. <http://doi.org/bnbn9h>.
65. Zhao Z, Yu R, Yang J, Liu X, Tan M, Li H, *et al*. Maxadilan prevents apoptosis in iPS cells and shows no effects on the pluripotent state or karyotype. *PLoS One*. 2012;7(3):e33953. <http://doi.org/b44k>.
66. Chagas AC, McPhie P, San H, Narum D, Reiter K, Tokomasu F, *et al*. Simiplagin, a platelet aggregation inhibitor from *Simulium nigricans* salivary glands specifically binds to the Von Willebrand factor receptor in collagen and inhibits carotid thrombus formation in vivo. *PLoS Negl Trop Dis*. 2014;8(6):e2947. <http://doi.org/f59ck6>.
67. Calvo E, Tokomasu F, Mizurini DM, McPhie P, Narum DL, Ribeiro JM, *et al*. Aegiptin displays high-affinity for the von Willebrand factor binding site (CRGQOGVMGF) in collagen and inhibits carotid thrombus formation in vivo. *FEBS J*. 2010;277(2):413-27. <http://doi.org/cb5pjw>.
68. Poole NM, Nyindodo-Ogari L, Kramer C, Coons LB, Cole JA. Effects of tick saliva on the migratory and invasive activity of Saos-2 osteosarcoma and MDA-MB-231 breast cancer cells. *Ticks Tick Borne Dis*. 2013;4(1-2):120-7. <http://doi.org/f4s95d>.