REVIEW PAPER

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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 nitroporines 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 *jején, 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 dependend 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 *Leshmania 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 SaIp20, 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 *Trypansoma 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 simplagrin, 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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