Pharma-entomology: when bugs become drugs

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Life on Earth has been evolving for more than three billion years and has produced a fantastic biological diversity. Every component of this, including insects, has contributed to the survival of Earth's life forms. At present, the estimated figure for total living species on Earth is 10 million, of which a mere 3% are vertebrates and over 60% are insects. Insects are by far the most diverse group of organisms on Earth.

This huge diversity of insect species forms an intrinsic part of the Earth's ecosystems, and is even considered by some to be what makes the ecosystems function [1]. When estimates of local diversity were extrapolated to include all the rain forests in the world, a figure of 30 million species was obtained, most of which are insects [2]. Yet the worldwide scientific community has yet to give this major and crucial component of Earth's biodiversity the attention it deserves and scientific knowledge about tropical ecosystems remains incomplete. Researchers have estimated that at least three quarters of tropical species still await discovery and naming, and the actual numbers might well be significantly greater.

What has made insects so successful?

Insects have colonized most parts of the globe and, as such, have confronted the vast majority of micro-organisms during their existence. To survive in this wide variety of environmental conditions and to combat these micro-organisms, insects have evolved powerful defence systems. These rely mainly on the synthesis of peptides and organic small molecules with predefined biological activities.

Although this immune response does not exhibit the same range of different immune cells that are employed by humans, insects have had much longer to finely tune their innate immune response. Insects have existed for around 500 million years; in comparison mammals appeared around 200 million years ago, with human ancestors *Homo sapiens sapiens* only arriving ~120,000 years ago.

The role of innate immunity

At first glance, the insect innate immune system might appear to be inferior to its human equivalent because it is unable to produce either antigen-specific lymphocytes or antibodies like the human adaptive immune response. However, templates from innate immunity have been conserved from primitive life forms to humans and the two systems are not as different as people might think. The human innate immune system is far from obsolete, but rather works in tandem with the adaptive system. The example of patients with severe burns can be used to illustrate its importance in humans. Not only is their skin barrier disrupted, but their skin's antimicrobial peptides and cells such as macrophages are also lost, making the patients much more vulnerable to infection. In addition, although insects rely on innate immunity alone, the molecules involved have been selected to perform specific functions by half a billion years of evolution.

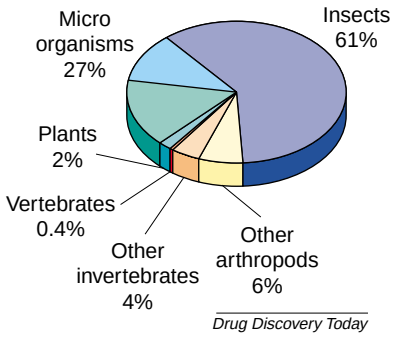

Figure 1. Insects are the largest and the most diverse group of living animals on earth. They have been colonizing most parts of the globe and have potentially been confronting all micro-organisms during the last 500 million years. As a result, they have evolved powerful defence systems that rely mainly on the synthesis of peptides, as well as small organic molecules, thus representing a unique and vast source of novel potential therapeutics.

Insects as a source of new drugs

Based on the number of species, insects represent more than twice the biodiversity of plants and micro-organisms put together (see Fig. 1). When it is taken into account that almost half the drugs currently on the market are derived from plants or micro-organisms, insects represent a significant untapped source of novel therapeutics such as antimicrobials.

To isolate promising molecules, insects are challenged with a variety of microbes to trigger their immune systems. This results in the release of peptides into the haemolymph (the insect equivalent of blood), which can then be separated using HPLC and screened for their efficacy against different targets.

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Since the first antibacterial peptide was induced in this way and isolated from an insect in 1981 (cecropin from the pupae of the moth *Hyalophoria cecropia* [3]), more than 170 antimicrobial peptides have been found in insects. Insect antimicrobial peptides exhibit a high level of structural diversity and could prove to be excellent candidates to solve the increasing problem of microbial drug resistance. Current antibiotics work by, for example, inhibiting protein synthesis or nucleic acid synthesis or disrupting the cell wall. To achieve this, they target specific receptors, enzymes or proteins, which can make them vulnerable to the development of resistant strains. Insect-derived peptides use modes of action that should restrict the development of resistant strains, because they have a much broader target – the microbe cell membrane. In addition, they are active against a wide spectrum of micro-organisms, kill them rapidly and should prove particularly suitable for immunosuppressed patients. This is because insect antimicrobial peptides that are produced systemically have demonstrated no toxicity in mammalian cell lines *in vitro* or during *in vivo* studies in-house at Entomed (http://www.entomed.com). For example, ETD-151, an antifungal peptide derived from a species of moth, has been administered to animal models by continuous intravenous infusion at doses up to 600 mg kg^{-1} with no signs of toxicity (S. Dawe, unpublished).

Insect antimicrobial peptides have several common features, including low molecular weight (less than 5 kDa), a positive net charge at physiological pH, along with structural characteristics, such as amphiphilic α-helices or hairpin-like β-sheets. Together with sequence data, these structural features enable them to be loosely categorized into three classes (see Fig. 2):

- Linear peptides that form α-helices without cysteine residues
- Peptides that contain disulfide bridges
- Proline- and/or glycine-rich peptides.

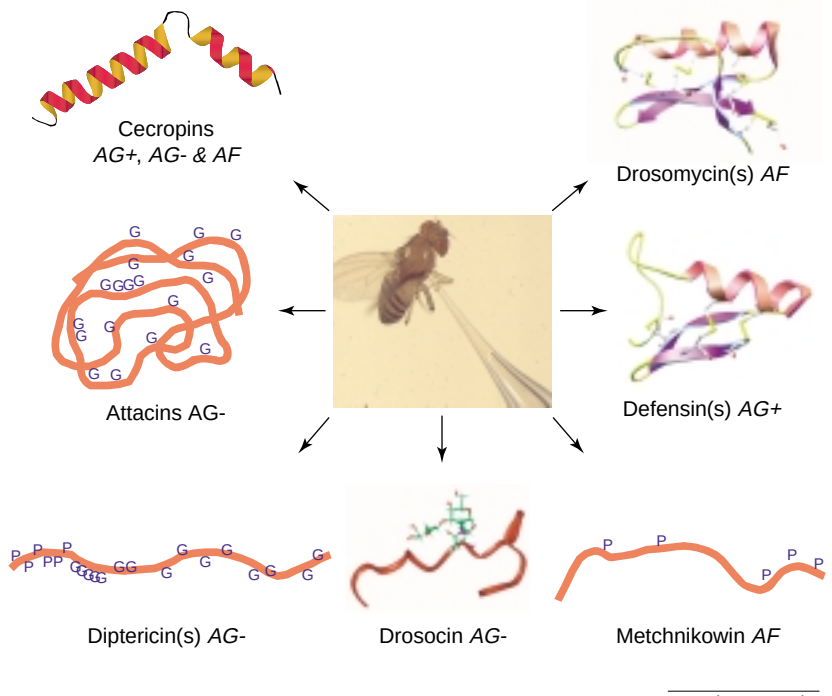

Figure 2. *Drosophila* nicely illustrates the diversity of the antimicrobial peptide repertoire that is produced by an insect in response to an infection. These molecules share a number of common features including small size, positive net charge and amphipathic properties. However, they have different structures and spectra of activity against bacteria and fungi. Antimicrobial peptides can be loosely classified into three major families: helical peptides [9], proline and/or glycine-rich peptides [11] and peptides containing disulfide bridges [4,9]. In view of drug development, cysteine-rich peptides are particularly interesting because of their remarkable stability.

Despite all falling into one of these categories, their biological activity and modes of action vary considerably. For this reason, here we will concentrate on cysteine-rich examples of insect antimicrobials.

Cysteine-rich antimicrobials

The first example group, that of insect defensins, is active against a wide range of Gram-positive bacteria, exerting an almost immediate lytic effect. A one-minute exposure to the peptide is sufficient to kill the bacteria [4]. Studies performed on a recombinant version of *Phormia terranovae* defensin demonstrated that the peptide instantly disrupts the permeability of the bacterial cytoplasmic membrane in *Micrococcus luteus*, resulting in loss of cytoplasmic potassium and, ultimately, inhibition of respiration [5].

This cysteine-rich peptide family also includes examples with antifungal properties. One of these, drosomycin, was the first inducible antifungal peptide to be isolated from insects and, to date, has only been reported in the fruitfly *Drosophila melanogaster* [6]. Compared to defensins, drosomycin includes an additional disulfide bond between the first and the C-terminal cysteine residues, giving the peptide a highly compact shape. This provides the molecule with a remarkable level of resistance to degradation by proteases (a property that is shared by many other insect immune peptides), both *in vitro* [5] and *in vivo* [7]. Drosomycin is active against both human and plant

fungi at concentrations often below 5 μM, delaying fungal hyphae growth at low concentrations and inhibiting spore germination at high concentrations. However, its mode of action has yet to be determined [4].

Thanatin was the first broad-spectrum insect peptide to be reported, with activity against both Gram-positive and Gram-negative bacteria as well as filamentous fungi. It exerts bactericidal and fungicidal effects at minimal concentrations, often below 2.5μM and is highly specific (with no side effects on red blood cells for example). Although the mode of action for thanatin is not yet understood, data suggest that it differs depending on the micro-organism being targeted [8].

These molecules serve to illustrate just a few of the different antimicrobials that can be induced in, and isolated from, insects. However, insects should not be thought of solely as a source of antimicrobials – ~25% of the peptides involved in the immune response are antimicrobial, with the other 75% performing an array of different biological roles; for example, anti-inflammatory, anti-proliferative and antiviral properties, as well as ion channel modulators (J. Dimarcq, unpublished).

Developing insect-derived drug candidates

Despite the potential of insects as a source of new drugs, exploiting the field and the subsequent process from insect molecule to new drug is far from simple. Triggering the insect immune response, extracting the relevant compounds and optimizing them for use in humans all require substantial expertise and technical know-how. In addition, companies obviously need to have considerable entomological expertise and access to a wide range of insect species.

Entomed's solution to the above problem is based on ENTOWEB™, a network of exclusive collaborations with several selected entomology centres. These are situated around the equator, where much of the world's insect diversity is concentrated, giving the company access to a huge variety of novel insect-derived molecules. Other companies are using insects in slightly different ways. Evolutec (http://www.evolutec.co.uk) aims to identify molecules that are secreted in the saliva of blood-sucking arthropods. In particular, the Evolutec selection process is aimed at proteins and peptides that modulate the inflammatory, haemostatic and immune responses of the host, rather than those involved in the insects' own immune response. Arthropods use several different molecules that enable them to feed without disturbing their host, such as local anaesthetics and anticoagulants.

Alternative approaches have resulted in insect-derived peptide drugs targeted to localized indications, such as infected diabetic foot ulcers, oral mucositis, central venous catheter-related infections and acne [9]. However, unlike systemic peptides, many of these are topical drugs that are limited to localized use for specific indications. For example, MBI226, a peptide drug product from Micrologix (http://www.mbiotech.com), is currently in clinical trials in the USA for the prevention of central venous catheter-related bloodstream infections.

Entomed uses peptides with systemic activity; these potentially have a greater range of use and, additionally, like drosomycin, are naturally resistant to proteases. Once insects have been obtained via the ENTOWEB™, they are challenged with a variety of microbes as previously mentioned. Once peptides have been separated using HPLC, they are screened for their efficacy against a selection of seven different microbes as well as other targets. The fractionation and purification process is also adapted to identify small molecules that are submitted to the same screening battery. The most effective hits can then be structurally characterized and modified using molecular evolution or chemical lead optimization to improve their activity profile, for example, against specific human pathogens.

Emerging drug candidates for the future

All of these methods are identifying new compounds, with Entomed having characterized over 175 novel molecules from around 100 different species of insects. Despite a highly conserved immune response among all the insects studied, the same compound, whether it is a peptide or small molecule, has yet to appear in more than one species. Perhaps more importantly, these compounds are already resulting in promising lead compounds being taken forward into clinical development. One example, ETD151, is an antifungal 44 amino acid peptide analogue based on a naturally occurring peptide from the lepidopteran *Heliothis virescens*. It has been optimized by genetic manipulation of the native peptide and is now in advanced preclinical development for the treatment of life-threatening hospital acquired fungal infections in immunosuppressed patients. Other lead compounds are based on native defensin peptides, from a variety of insect species, which have been modified to improve their biological profiles against Gram-positive bacteria. In addition to these peptide products, insect studies are yielding small-molecule candidates with antimicrobial and anti-proliferative properties, which are shortly due to enter preclinical development [10].

The future of drugs from bugs

Insect-derived molecules have a huge variety of potential uses for treating human disease. From antimicrobial peptides that use completely novel

modes of action, to anti-proliferative small molecule drugs, via anti-inflammatory, anti-viral and ion channel modulator molecules, the full scope and breadth available is yet to be realized. However, to exploit this field requires not just drug development technical know-how, but also substantial entomological expertise. Companies are rising to this challenge, with drug candidates already emerging from company pipelines, making sure that the field of 'pharma-entomology' lives up to its promise.

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Structural pharmacogenomics: the answer to antimicrobial drug resistance? ▼

The 20th century saw great advances in our understanding of the aetiology, transmission and prevention of infection. The introduction of chemotherapeutics in the 1930s, the advent of mass production of penicillin in 1943, rapid advances in the discovery and development of new classes of antibacterial drugs, and the introduction of safe and effective vaccines gave rise to the belief that bacterial infectious diseases could be controlled and, eventually, mastered. Although antibiotics have saved countless lives and transformed the practice of medicine, the initial widespread optimism has proven premature [1] and multiple drug resistance threatens our capacity to treat many infections [2].

Antiviral chemotherapy arrived much later; the nature of the viral lifecycle led to the belief that antiviral drugs would inevitably be toxic to the host. However, with improved understanding of viral replication, new drug candidates have been emerging in recent years, with much effort focused on the treatment of HIV infection. The problem of antiviral resistance is acute and, in this context, the surveillance of resistance genotypes is an important component in the overall strategy to keep ahead of the microbes.

Rapid sequencing of bacterial and viral genomes has created the opportunity to identify new drug targets. In a recent review in *Drug Discovery Today*, Edward Maggio and colleagues detail the use of structural pharmacogenomics as a means of rational drug design to overcome the problems of organisms that are resistant to anti-infectives [3]. By sequencing thousands of gene variants coding for HIV1 protease and reverse transcriptase, and mapping the deduced amino acid changes to the 3D structure of the proteins, inferences are made about the positions of conserved areas where mutation frequencies are low. These areas are deemed to be essential for

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