Analysis of Insecticide Resistance in Mosquito Disease Vectors: From Molecular Mechanisms to Management

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Abstract

The intense use of insecticides has resulted in a degree of insecticide resistance in major mosquito vectors to such an extent that controlling them has become challenging in several cases. The investigation of insecticide resistance mechanisms, using a variety of approaches including transcriptomics and functional proteomics, improves our understanding of the role and contribution of individual genes and mutations and the physiological basis of the phenomenon. As a result, molecular diagnostics have been developed to monitor the spread and distribution of resistance alleles in field populations and to support decision-making on resistance management strategies, as well as tools for screening novel active ingredients and resistance-breaking compounds. An example from among our most recent studies includes the analysis of insecticide resistance mechanisms in the major arbovirus vector Aedes albopictus.

Zusammenfassung


1. Mosquitoes as Vectors of Disease

Mosquitoes cause severe global health problems as vectors of several important human diseases. Malaria, dengue, zika and chikungunya are just some examples of these diseases that threaten almost half of the world’s population and result every year in several thousand cases of mortality and morbidity, with a large socio-economic impact. Although the majority of these cases are reported in sub-Saharan Africa and in the tropical and sub-tropical areas of Latin America and Asia, Europe has also experienced recent outbreaks of mosquito-borne
diseases. For example, West Nile Virus (WNV), almost never present or diagnosed until recently, has caused epidemics with a series of fatalities in several European countries (Dimou et al. 2013, Gabriel et al. 2013). Furthermore, diseases that presented no threat in Europe are now presenting a high risk, as for example the recent chikungunya (CHIK) epidemic in Italy (Tomasello and Schlagenhaus 2013) and the few cases of autochthonous malaria that appeared in certain prefectures of Greece in 2009 (Danis et al. 2013). Finally, although the last large dengue epidemic in Europe was observed during 1927–1928, cases were reported in southern France and Croatia in 2010, while an outbreak was also detected in 2012 in Portugal (Madeira; Tomasello and Schlagenhaus 2013). Although these outbreaks are still occasional and on a small scale, their appearance could be intensified in the future by factors such as climate change and colonization of invasive vector species, as it is the case with Aedes albopictus, which was directly associated with the chikungunya outbreaks in Italy (2007) and Croatia/France (2012) (Enserink 2006). Other factors that might also facilitate the occurrence of local disease transmission are the large numbers of tourists, going annually into endemic areas, and immigration (Danis et al. 2013).

The most effective way to prevent vector-borne disease outbreaks remains the use of insecticides. However, their intense use in both public health and agriculture has resulted in the emergence of resistant mosquito populations.

2. Insecticide Resistance: Scale of the Problem

Insecticide resistance is at a critical tipping point in public health. Some mosquito populations are now showing resistance to all insecticide classes, and the strength, and hence the impact, of this resistance is escalating every year. There has been a large number of publications (Ranson et al. 2010, Vontas et al. 2012) reporting resistant mosquito populations all over the world, while extreme resistance phenotypes have also been identified. For example, some Anopheles mosquito populations from West Africa are now showing high levels of resistance to all registered insecticide classes used in control programmes (Ranson et al. 2011). As only a limited number of different types of insecticides are available on the market, and new ones are developed very slowly, it is getting increasingly important to manage insecticide resistance in order to ensure the sustainability of current vector control programmes.

3. Insecticide Resistance Mechanisms

Strategies for Insecticide Resistance Management (IRM) and the integration of chemical and non-chemical means of control (in the context of Integrated Pest/Vector Management – IPM/IVM) must be evidence-based. This requires an understanding of the mechanisms and dynamics of insecticide resistance, including cross-resistance between different insecticide classes. Insecticide resistance mechanisms involve mutations at the target site of insecticides that render them less sensitive to inhibition by decreasing their affinity for the insecticide molecules. Among the most prominent target site resistance mutations are those of the para sodium channel; these have been correlated with pyrethroid and DDT resistance in several mosquito species (Davies et al. 2007). For Anopheles gambiae the best-studied mutation is at residue L1014 with two variants: L1014F and L1014S, also known as the west and east kdr.
More recently, an additional mutation, N1575Y (Jones et al. 2012), was found in An. gambiae haplotypes possessing the L1014F mutation, conferring high levels of pyrethroid resistance. Mutations at residues V1016 and F1534, either alone (Brengues et al. 2003, Kasai et al. 2014) or in combinations (Kawada et al. 2014), have been identified in pyrethroid-resistant Aedes aegypti mosquito populations, and they have been shown to alter the sensitivity of the sodium channel against pyrethroids in in vitro electrophysiology studies (Hirata et al. 2014). Mutations linked to resistance have also been detected in acetylcholinesterase, the molecular target of organophosphate and carbamate insecticides (Weill et al. 2004) and the γ-aminobutyric (GABA) receptor, the target of organoclorines (Du et al. 2005). In addition to mutations at the target site, resistance can also be conferred by over-expression of certain enzyme families like P450s, esterases and glutathione S-transferases, which metabolize or sequester the insecticide molecules, keeping them away from their target. P450s are the best-studied category of detoxification enzymes, including members that have largely been associated with pyrethroid resistance. For example, Ae. aegypti CYP6BB2, CYP9J32 and CYP9J28 have been found up-regulated in pyrethroid resistant strains and also shown in in vitro studies to be capable pyrethroid metabolizers (Kasai et al. 2014, Stevenson et al. 2012). The An. gambiae cytochrome P450s CYP6M2 was shown to rapidly metabolize pyrethroids (Stevenson et al. 2011) and DDT (Mitchell et al. 2012), while CYP6P3 is also a very efficient metabolizer of pyrethroids (Muller et al. 2008) as well as being capable of metabolizing the carbamate bendiocarb (Edi et al. 2014). The glutathione transferase GSTe2 from An. gambiae (Ortelli et al. 2003) has very high DDT-dehydrochlorinase activity. Finally, esterases (CCEs) have been associated with resistance to organophosphates (OP), particularly in Culex sp mosquitoes. These enzymes usually do not act as quick metabolizers, but rather confer resistance through sequestration (Hemingway et al. 2004).

Despite the progress made in understanding insecticide resistance mechanisms, several questions remain to be answered in the future. Recent molecular studies demonstrated that the co-evolution of multiple mechanisms can lead to high levels of resistance; for example cuticular resistance may substantially contribute to the most striking resistance phenotypes recorded today (Balabanidou et al. 2016), while additional enzyme systems such as ABC transporters and UGTs may also dramatically enhance the phenotype (Ahn et al. 2012, Dermauw and Van Leeuwen 2014).

4. Insecticide Resistance in Aedes albopictus: Current Research

Our current work has focused on insecticide resistance in Aedes albopictus – one of the most invasive species (Bonizzoni et al. 2013) – which has recently also expanded in Europe and is a potent vector of several arboviral diseases like dengue, chikungunya, and zika. Despite its importance as both a vector of disease and a major pest, as it is an aggressive feeder, little is known about its specific molecular mechanisms of resistance. In a recent study (Grigoraki et al. 2015), we investigated the molecular basis for resistance against temephos in an Ae. albopictus population from Greece (Tem-GR). Temephos is an organophosphate larvicide being widely used in many parts of the world, especially in Asia and Latin America. In Europe, although it was being used for several decades, its use is currently banned. Nevertheless, it remains an important backup solution, in case of failure of the limited alternative larvicides, or of emergencies. Biochemical data indicated that resistance to temephos was associated...
with elevated levels of esterase activity. In order to find which specific esterase genes were over-expressed, we followed a transcriptomic approach, comparing the transcript levels of the resistant strain to those of a standard susceptible laboratory colony, through next generation sequencing. Two CCE genes, CCEae3a and CCEae6a, were among the most highly up-regulated genes (27-fold and 12-fold respectively, compared to the reference susceptible strain) and this up-regulation was shown to be at least partially due to gene amplification. The link between CCEae3a/CCEae6a gene amplification and temephos resistance was further supported by genetic crosses, which demonstrated a strong association between survival to temephos exposure and gene copy numbers in the F2 generation.

Subsequently, we functionally expressed CCEae3a using the baculovirus expression system and investigated its interaction with temephos. Tissue localization analysis on paraffin sections and whole mounts of resistant Aedes albopictus larvae using a specific antibody for CCEae3a revealed the specific expression of those esterases in malpighian tubules and nerve tissue (Grigoraki et al. 2016). We are currently investigating the distribution of this temephos resistance mechanism in Ae. albopictus populations from several countries.

We have also screened Ae. albopictus populations from around the globe for target site pyrethroid resistance mutations. More specifically, we are searching for mutations on the para sodium channel at residues V1016 and F1534, associated with pyrethroid resistance, and also for mutation A302S (Tantely et al. 2010) on the GABA receptor, linked to dieldrin resistance. As yet we have not detected any known functionally characterized pyrethroid resistance mutation on the sodium channel, but individuals possessing the A302S mutation have been found in Europe and the United States.

5. New Tools to Tackle Insecticide Resistance

Understanding the mechanisms responsible for insecticide resistance can drive the development of new tools to overcome this resistance, such as new synergists and new formulations of insecticides. For example, the identification and characterization of detoxification enzymes that metabolize or sequester insecticides provide the basis alongside screening tools for the rational design of enzyme inhibitors (synergists) and/or improved active ingredients.

In addition, the identification and validation of markers that are associated with insecticide resistance allow the development of molecular diagnostics, such as PCR, Taqman (Ranson et al. 2000) and immunodiagnostic assays (Nauen et al. 2015), which are used to screen field populations for the presence of specific insecticide resistance alleles. This allows accurate monitoring of molecular mechanisms present in a specific area, which facilitates decision-making on the use of the most appropriate insecticides (Bregues et al. 2003, Mitchell et al. 2012, Nauen et al. 2013). However, the implementation of effective interventions also requires contemporary data on mosquito species composition and infection status, which can be achieved with additional separate diagnostic tools.

A fully automated platform (LabDisk) is now being explored for application with An. gambiae (www.dmc-malvec.eu), as well as other major arbovirus vectors subsequently, to simultaneously screen for mosquito species ID, pathogen infection and insecticide resistance alleles (including levels of detoxification genes), in a sample-to-answer approach (including nucleic acid extraction). Such automated multiplex diagnostics can be interfaced with Disease Data Management Systems (DDMS), i.e. custom-made data management software,
which will collate and manage data from routine entomological monitoring activities, providing information in a timely fashion based on user needs and in a standardized way (CHANDA et al. 2012). Data generated using these tools can be readily integrated into existing alternative malaria decision-making support tools to facilitate the selection of the most appropriate combinations of interventions to control malaria vectors.

References

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