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Cross-induction of detoxification genes by environmental xenobiotics and insecticides in the mosquito *Aedes aegypti*: Impact on larval tolerance to chemical insecticides ☆

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Abstract

The effect of exposure of *Aedes aegypti* larvae to sub-lethal doses of the pyrethroid insecticide permethrin, the organophosphate temephos, the herbicide atrazine, the polycyclic aromatic hydrocarbon fluoranthene and the heavy metal copper on their subsequent tolerance to insecticides, detoxification enzyme activities and expression of detoxification genes was investigated. Bioassays revealed a moderate increase in larval tolerance to permethrin following exposure to fluoranthene and copper while larval tolerance to temephos increased moderately after exposure to atrazine, copper and permethrin. Cytochrome P450 monooxygenases activities were induced in larvae exposed to permethrin, fluoranthene and copper while glutathione S-transferase activities were induced after exposure to fluoranthene and repressed after exposure to copper. Microarray screening of the expression patterns of all detoxification genes following exposure to each xenobiotic with the *Aedes Detox Chip* identified multiple genes induced by xenobiotics and insecticides. Further expression studies using real-time quantitative PCR confirmed the induction of multiple *CYP* genes and one carboxylesterase gene by insecticides and xenobiotics. Overall, this study reveals the potential of xenobiotics found in polluted mosquito breeding sites to affect their tolerance to insecticides, possibly through the cross-induction of particular detoxification genes. Molecular mechanisms involved and impact on mosquito control strategies are discussed.

Keywords: Mosquitoes; *Aedes aegypti*; Induction; Resistance; Insecticides; Xenobiotics; Detoxification; Cytochrome P450 monooxygenases; Glutathione S-transferases; Carboxylesterases

1. Introduction

Mosquitoes transmit a wide range of human and animal pathogens, and insecticides are widely employed in their control. The efficacy of these insecticides is influenced by the mosquitoes' history of past exposure. Long-term exposure to a toxicant will eventually select for mutations conferring a level of resistance to that toxicant and indeed, insecticide-resistant populations of mosquitoes are now threatening the success of control programmes. Extensive research efforts are aimed at elucidating the molecular basis of this resistance in order to facilitate the management of insecticide resistance in the field. Less attention has been paid to the short-term effect of exposure to insecticides or other xenobiotics on the mosquitoes' tolerance to insecticides and yet this could also have a significant impact on the efficacy of mosquito control.

☆ The description of the microarray '*Aedes Detox Chip*' can be accessed at ArrayExpress (http://www.ebi.ac.uk/arrayexpress) accession no. A-MEXP-623. All experimental microarray data can be accessed at VectorBase (http://VectorBase.org) and ArrayExpress database accession no. E-TABM-353.

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For example, we have shown that *Aedes aegypti* larvae exposed to the herbicide atrazine become more tolerant to the organophosphate temephos (Boyer et al., 2006). Similarly, exposure of *Aedes albopictus* larvae to benzothiazole (a major leachate compound of automobile tires) and pentachlorophenol (a wood-protecting agent) can increase their tolerance to insecticides such as carbaryl, rotenone and temephos (Suwanchaichinda and Brattsten, 2001, 2002). This increase in tolerance in *Ae. albopictus* is correlated with an induction of cytochrome P450 activity. This enzyme family, together with the carboxylesterases and glutathione transferases, play a central role in the detoxification and in the metabolism of insecticides and other xenobiotics (Feyereisen, 2005; Hemingway et al., 2002, 2004).

Recently, microarray-based approaches have been used to investigate the effect of xenobiotic exposure on the expression of detoxification genes in *Drosophila*. Phenobarbital and the herbicide atrazine induced the expression of multiple P450s (*CYP* for genes) and *GST* genes in adult flies including genes previously linked to insecticide resistance (Le Goff et al., 2006). In mammals, a causal link between the induction of particular detoxification enzymes by xenobiotics and their ability to metabolize them has been demonstrated and successfully utilized to identify drug metabolizing enzymes (Luo et al., 2004; Waxman, 1999). This approach was also used to identify two *CYP* genes (*CYP6B1* and *CYP6B3*) in the black swallowtail *Papilio polyxenes* induced by and metabolizing furanocoumarins, toxins produced by their host plant (Petersen et al., 2001; Wen et al., 2003). Likewise, other *CYP* genes able to metabolize xanthotoxin were characterized in the cotton bollworm *Helicoverpa zea* (Li et al., 2000; Sasabe et al., 2004). Hence, studying the induction profile of insect detoxification enzymes has been suggested as a means to identify the major enzymes involved in insecticide detoxification. In *Drosophila*, exposure to high concentrations of insecticides induced the expression of few detoxification genes while two known inducers (phenobarbital and caffeine) and piperonyl butoxide induced multiple detoxification genes, including those involved in insecticide metabolism (Willoughby et al., 2006, 2007). In mosquitoes, insecticides have also been shown to induce detoxification enzymes. By using a microarray representing more than 11,000 unique ESTs, Vontas et al. (2005) identified *Anopheles gambiae* detoxification genes induced by the insecticide permethrin.

Little is known about regulatory elements controlling the induction of detoxification genes by chemicals in mosquitoes. In vertebrates, xenobiotics such as planar aromatic hydrocarbons and polychlorobiphenyls (PCB) can trigger the induction of *CYP* genes via the intracellular aryl hydrocarbon receptor (AhR) (Goksoyr and Husoy, 1998). Korashy and El-Kadi (2005) have suggested the possible involvement of this nuclear receptor in the induction of *CYP* genes by heavy metals. Upon binding of a ligand, AhR migrates to the nucleus where it dimerizes with 'ARNt' (AhR nuclear translocator) before binding to specific DNA sequences called xenobiotic response elements (XREs) located in the upstream region of *CYP* genes (Backlund and Ingelman-Sundberg, 2005; Petrulis and Perdew, 2002). AhR–XRE pathway was shown to be conserved in insects as the black swallowtail caterpillar (*P. polyxenes*) responds to xanthotoxin by inducing *CYP6B1* via XRE-like binding sites (Brown et al., 2005; McDonnell et al., 2004). Putative XRE binding sites were also found upstream of *An. gambiae CYP6* genes induced by permethrin (David et al., personal communication). Other regulatory elements such as the ecdysone response element (EcRE) (Gilbert et al., 2002), the constitutive androstane receptor family (CAR) (King-Jones et al., 2006) and the antioxidant response element (ARE) (Li et al., 2002) have been involved in insect response to xenobiotics and may participate in the cross-induction of detoxification genes in mosquitoes.

Here, we investigate the capacity of various xenobiotics to modify the tolerance of *Ae. aegypti* larvae to two chemical insecticides through the induction of detoxification enzymes. We exposed mosquito larvae for 24 h to sublethal doses of three different xenobiotics likely to be found in highly polluted breeding sites (the herbicide atrazine, the polycyclic aromatic hydrocarbon fluoranthene and the heavy metal copper) and two chemical insecticides (the pyrethroid permethrin and the organophosphate temephos). After exposure to each xenobiotic, larval tolerance to insecticides and detoxification enzyme activities were compared. Expression patterns of detoxification genes following exposure to xenobiotics and insecticides were compared by using the microarray '*Aedes Detox Chip*' (Strode et al., 2007) and real-time quantitative PCR. To investigate the role of particular regulatory elements in cross-induction mechanisms, a comparative analysis of the 1000 bp upstream region of selected detoxification genes was performed. Overall, this study suggests that the cross-induction of detoxification enzymes involved in insecticide metabolism by environmental xenobiotics may enhance the tolerance of mosquito larvae to chemical insecticides.

2. Materials and methods

2.1. Mosquitoes and xenobiotics

A laboratory strain of *Ae. aegypti* (Bora-Bora strain, susceptible to insecticides) was reared in standard insectary conditions (26 °C, 8 h/12 h light/dark period, tap water) and used for all experiments. Larvae were reared in similar conditions with controlled amount of larval food (hay pellets) for 5 days (fourth stage) before exposure for 24 h to five different xenobiotics belonging to various chemical classes likely to be found in highly polluted mosquito larvae habitats: the pyrethroid insecticide permethrin (Chem Service, USA), the organophosphate insecticide temephos (Abate 500 E, Bayer, France), the herbicide atrazine (Cluzeau, France), the polycyclic