Mechanisms of DDT and Permethrin Resistance in *Aedes aegypti* from Chiang Mai, Thailand

by

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Abstract

Two strains of *Aedes aegypti*, one resistant to DDT but susceptible to permethrin (RdSp), and the other resistant to both DDT and permethrin (RdRp), were established in mosquitoes collected from Chiang Mai province, northern Thailand. Comparisons with a susceptible reference strain indicated that DDT resistance in both RdSp and RdRp strains was mainly due to an increase in DDTase activity. Similar moderate increases in cytochrome P450 levels were observed in the two resistant strains, hence this enzyme family may also play a role in DDT resistance. Glutathione S-transferase and esterase activities in the two resistant strains were similar and slightly higher than those of the susceptible strain, suggesting that neither enzyme group has a major role in permethrin resistance. The lack of an evident metabolic basis for the pyrethroid resistance in the RdRP strain suggests that nerve insensitivity may be present in this strain. The two mutations at residues reported to produce *kdr* resistance in other insects were not present, but some individuals from the permethrin resistant strain had an amino acid mutation at position 106 involving a valine to glycine mutation in the same segment 6 of domain II of the para sodium-channel gene, which may confer *kdr*-like resistance.

Keywords: *Aedes aegypti*, DDT, permethrin resistance, Chiang Mai, Thailand.
Introduction

*Aedes aegypti*, the primary vector of yellow fever, dengue and dengue haemorrhagic fever (DHF), is insecticide-resistant in numerous locations throughout the world\(^1\). DDT resistance is now widely distributed in *Aedes aegypti* throughout northern Thailand and it is also resistant to the pyrethroids, permethrin and deltamethrin, and the neo-pyrethroid etofenprox in many areas (P. Somboon, unpublished data).

The major mechanisms involved in DDT resistance in insects are increased metabolism of DDT by the glutathione S-transferase (GST) enzyme family and the insensitivity to inhibition of the voltage-gated sodium channel (known as *kdr*)\(^2\). As both DDT and pyrethroids act on the nervous system by modifying the gating kinetics of voltage-sensitive sodium channels, mutation in specific regions of this sodium channel can lead to binding failure with DDT and pyrethroids; therefore; cross-resistance between DDT and pyrethroids is common. In this paper, we applied biochemical and molecular assays to elucidate the resistance mechanisms in DDT-resistant and DDT/permethrin cross-resistant strains of *Aedes aegypti* from Northern Thailand.

Materials and methods

**Mosquitoes**

One-day old females, emerging from field-collected larvae of *Aedes aegypti* from Ban Pang Mai Dang, Mae Tang district, Chiang Mai province, Thailand, were exposed to 4% DDT-impregnated papers for 30 minutes in the WHO standard exposure tubes. These females had <1% mortality, whereas those exposed to 0.25% permethrin for 60 min. gave 70% mortality. Single-family selections for several generations produced two strains of *Aedes aegypti*, one fully resistant to DDT (R\(^{SD}\)) but susceptible to permethrin and the other resistant to both DDT and permethrin (R\(^{DP}\)). These two strains were maintained under insecticide pressure for at least 10 generations before the resistance ratios were determined and the insects were harvested for biochemical and molecular assays. *Aedes aegypti* Rockefeller (Rock) strain was used as a reference-susceptible for comparisons.

**Bioassay and determination of LT\(_{50}\)**

For all bioassay, four replicates (25 females per replicate) of 4 and 10 different exposure time periods for 4% DDT and 0.25% permethrin respectively were undertaken. Percentage mortalities were calculated for each exposure time and the mortality data were analysed on a log-time probit mortality regression using a computer programme provided by Dr C J Schofield, WHO, Geneva.

**Enzyme assays**

One-day old females of each strain were used for enzyme or molecular assays. Each batch was homogenized in an appropriate buffer at 4°C and the 10,000 g supernatant was then determined for enzyme activities. The GST and DDTase activity were determined using the methods of Prapanthadara et al. 1996\(^3\). The esterase
activity was measured as described by Peiris & Hemingway 1990[4] with p-nitrophenyl acetate as the substrate[4]. The method for determination of insensitive acetylcholinesterase (IAChE) was as from firench-Constant & Bonning 1989[5]. Mono-oxygenase activity was indirectly determined by measuring the different spectra of cytochrome P450 in microsomal fraction by using a carbon monoxide trap.

Protein was assayed using the Bio-Rad protein reagent with bovine serum albumin as the standard protein.

The PCR fragments of the segment 6 domain II region of the sodium channel gene were obtained as described by Martinez-Torres et al. 1998[6].

### Results

The LT50 of the RdSp and RdRp strains for DDT (Table 1) were 23- and 30-fold higher, respectively, than the LT50 of the Rock strain. Log time-probit mortality lines for both RdSp and RdRp (Figure) were not linear for DDT (Chi square analysis P<0.005), indicating that resistance had not been selected to homogeneity. In contrast, the Chi square value for permethrin in both the RdSp and RdRp strain were non-significant which suggested that resistance to this insecticide had been selected to homogeneity.

One-day old adult female were used to determine all enzyme activities except cytochrome P450, where larvae were used in order to minimize pigment interference. Activities in each strain were determined using at least 20 batches of 10 mosquitoes and the mean activities ± SD were calculated.

<table>
<thead>
<tr>
<th>Strains</th>
<th>DDT</th>
<th>Permethrin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LT50</td>
<td>χ²</td>
</tr>
<tr>
<td>ROCK</td>
<td>17 (15-19)</td>
<td>36.9</td>
</tr>
<tr>
<td>RdSp</td>
<td>390 (352-427)</td>
<td>27.9</td>
</tr>
<tr>
<td>RdRp</td>
<td>513 (466-559)</td>
<td>24.5</td>
</tr>
</tbody>
</table>

The enzyme activities are presented in Table 2. There was an ~10-fold increase in DDTase activity in the two resistant strains and a 4-fold increase in cytochrome P450 activity compared to the Rock strain. The GST and esterase activities were slightly increased. There was no change in the sensitivity of acetylcholinesterase to inhibition by bendiocarb, in either resistant

<table>
<thead>
<tr>
<th>Strains</th>
<th>GST (µmole/min/mg)</th>
<th>DDTase (nmole/mg)</th>
<th>EST (µmole/min/mg)</th>
<th>Cytochrome P450 (nmole/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rock</td>
<td>0.22±0.05</td>
<td>3.23±1.23</td>
<td>0.2±0.03</td>
<td>0.07±0.02</td>
</tr>
<tr>
<td>RdSp</td>
<td>0.38±0.09</td>
<td>31.03±13.88</td>
<td>0.34±0.07</td>
<td>0.24±0.02</td>
</tr>
<tr>
<td>RdRp</td>
<td>0.37±0.04</td>
<td>26.85±5.12</td>
<td>0.26±0.04</td>
<td>0.28±0.04</td>
</tr>
</tbody>
</table>

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strain compared to the Rock susceptible. The levels of increased DDTase and cytochrome P<sub>450</sub> activities were very similar in the two resistant strains. This is in contrast to the difference in the DDT-resistance ratios. Sequencing of the S6 domain II region of the para-sodium channel gene demonstrated that the standard leucine to phenylalanine or leucine to serine mutation, which confers kdr-resistance in An. gambiae<sup>60</sup>, was not present in either resistant strain of *Aedes aegypti*. However, there was a valine to glycine mutation at position 106 in this domain in the 4 R<sup>R</sup>P individuals sequenced. This mutation was not detected in the 4 R<sup>S</sup>P or 20 Rock individuals sequenced.

**Discussion**

We found that DDT resistance in both R<sup>S</sup>P and R<sup>R</sup>P strains was due to increased DDTase activity and cytochrome P<sub>450</sub> content whereas permethrin resistance in the R<sup>R</sup>P strain probably involved a non-metabolic kdr mechanism. This kdr should also generate cross-resistance to DDT and would explain the greater resistance ratio to DDT in R<sup>R</sup>P as compared to the R<sup>S</sup>P strain.

In earlier reports of DDT and pyrethroid resistance in *Aedes aegypti*, selection of a resistant strain with DDT generated moderate resistance to pyrethroids, whereas selection with permethrin resulted in strong resistance to both permethrin and DDT<sup>7</sup>. Our results suggest that DDTase/cytochrome P<sub>450</sub>-based DDT-resistance does not confer cross-resistance to pyrethroids. However, the kdr-based pyrethroid resistance mechanism probably increases the DDT-resistance levels.

In conclusion, our study suggested that DDT resistance was due to multiple factors. DDT selection, as shown by our results and others<sup>20</sup>, often generates GST-based metabolic resistance which does not usually confer cross-resistance to other insecticides. This should be considered before a policy of abolishing DDT use for susceptible mosquito vectors is implemented. Although the persistence of DDT residues in the environment are well documented, making it unsuitable for large-scale spraying, limited use of this insecticide as an indoor spray may still be a good method of mosquito vector control in many countries.
Acknowledgements

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