Evidence for Selection of Insecticide Resistance Due to Insensitive Acetylcholinesterase by Carbamate-Treated Nets in Anopheles gambiae s.s. (Diptera: Culicidae) from Côte d’Ivoire

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ABSTRACT  Pyrethroid-treated nets are an efficient tool for reducing malaria transmission and morbidity. The recent evolution of pyrethroid resistance in several Anopheles species represents a major threat for the future success of roll back malaria in Africa. The possible use of nonpyrethroid insecticides, such as carbamates, on nets is a promising alternative solution because these insecticides are effective against susceptible and pyrethroid-resistant populations of Anopheles and Culex mosquitoes. Unfortunately, carbamate resistance as a result of insensitive acetylcholinesterase has recently been detected in Anopheles gambiae s.s. populations from Côte d’Ivoire. Using biochemical assays on surviving Anopheles mosquitoes from an experimental hut trial, we showed evidence for selection for an insensitive acetylcholinesterase mechanism by carbamate impregnated bednets. However, no such selection has been found with nets treated with pyrethroid alone or pyrethroid/carbamate “two-in-one”-treated nets. Because pyrethroid-impregnated nets were suspected to select for the Kdr mutation in An. gambiae, we propose that use of two-in-one nets could be a promising alternative strategy for the management of insecticide resistance in malaria vectors.

KEY WORDS  insecticide-treated nets, insecticide combinations, insensitive acetylcholinesterase, Anopheles gambiae, Côte d’Ivoire

Malaria is by far the most important vector-borne disease, causing an estimated 300–500 million cases and 1.4–2.6 million deaths per year, 90% of them in Africa (WHO 1995). Insecticide-treated nets (ITNs) are strongly recommended against malaria vectors and nuisance insects, especially in sub Saharan Africa (Lines 1996). Pyrethroids are the only compounds currently recommended for impregnation because of high insecticide activity, low mammalian toxicity, and rapid degradation in the environment (Elliot et al. 1978). However, the evolution and selection of pyrethroid resistance in the major malaria vectors Anopheles gambiae s.s Giles (Elissa et al. 1993, Chandre et al. 1999) and Anopheles funestus Giles (Hargreaves et al. 2000) represent a threat for roll back malaria in Africa.

An alternative strategy to maintain the effectiveness of ITNs is to replace pyrethroids with insecticides having different modes of action. Organophosphate (OP) or carbamate-treated nets were found to be very effective in killing susceptible and pyrethroid-resistant Anopheles and Culex mosquitoes (Miller et al. 1991, Kolaczinski et al. 2000, Guillet et al. 2001). Unfortunately, carbamate resistance was recently detected in two populations of An. gambiae from Côte d’Ivoire (N’Guessan et al. 2003). Further investigations showed the existence of cross-resistance to OP (unpublished data), and biochemical analysis confirmed that the main resistance mechanism was an insensitive acetylcholinesterase (AChE).

The use of mixtures or mosaics of insecticides with different target sites has been demonstrated in theoretical models to be a potentially useful strategy for managing insecticide resistance (Mani 1985, Curtis et al. 1993, Barnes et al. 1995) especially if synergism is exhibited and there is no cross-resistance between components of the mixture (All et al. 1977, Denholm and Rowland 1992). Under laboratory conditions, a significant synergy was detected between bifenthrin (pyrethroid) and carbosulfan (carbamate) against susceptible An. gambiae mosquitoes (Corbel et al. 2002). Moreover, field studies in experimental huts have shown promising results with two-in-one combinations of bifenthrin and carbosulfan applied to the different parts of the same net (Guillet et al. 2001). The selection pressure exerted by carbamate-treated nets on the insensitive AChE resistance mechanism

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remains unknown. But just as pyrethroid-treated nets have been shown to select for the \( \text{Kdr} \) mutation in An. \textit{gambiae} (Fanello et al. 1999, Kolaczkinski et al. 2000), it is conceivable that carbosulfan-treated nets would select for carbamate resistance if deployed.

In the current study, we report on the frequency and level of acetylcholinesterase insensitivity of mosquitoes surviving nets impregnated with carbosulfan and bifenthrin, alone or in combination (mixture and mosaic) during an experimental hut trial in Côte d’Ivoire.

Materials and Methods

From August 2001 to December 2002, an experimental hut trial was conducted at M’bé Valley, Côte d’Ivoire, where An. \textit{gambiae} was shown to be susceptible to most of pyrethroids (Chandre et al. 1999) and partially resistant to carbamates via an insensitive \( \text{AChE} \) (N’Guessan et al. 2003). Net treatments allocated to huts were: carbosulfan (300 mg/m\(^2\)), bifenthrin (50 mg/m\(^2\)), a mosaic-containing carbosulfan (300 mg/m\(^2\)) on the roof and bifenthrin (50 mg/m\(^2\)) on each lateral side of the net, a mosaic one-thirds containing carbosulfan (300 mg/m\(^2\)) on the roof plus one-thirds upper side of the net and bifenthrin (50 mg/m\(^2\)) on the two-thirds lower part, a binary mixture (25 mg/m\(^2\) bifenthrin plus 6.25 mg/m\(^2\) carbosulfan) previously determined by Corbel et al. (2002), and an untreated net as a control. The netting material was knitted polyester 100 denier, 18-m\(^2\) area (SiamDutch Mosquito Netting Co., Bangkok, Thailand). The insecticides used were carbosulfan 2.5% microcapsule suspension and bifenthrin, 8% suspension concentrate, both provided by FMC, (Princeton, NJ). Every morning, all An. \textit{gambiae} mosquitoes were collected from each hut by the sleepers, identified according to classical entomological parameters (Darriet et al. 1984), and stored at \(-80^\circ\text{C}\) for biochemical analysis.

The aim of the present work was to determine and to compare the frequency distributions of acetylcholinesterase inhibition rates of mosquitoes surviving exposure to each net treatment. For each treatment, 30 mosquitoes were analyzed for their \text{AChE} activity, using microplate assays described by Hemingway (1998), adapted from Brogdon and Barber (1987). Mosquitoes were individually ground in 200 \( \mu \text{L} \) distilled water. For each mosquito, 25 \( \mu \text{L} \) of homogenate were placed in two wells of a microplate. 145 \( \mu \text{L} \) of phosphate buffer (0.1 M, pH 7.8) containing 1\% Triton and 10 \( \mu \text{L} \) DTNB (0.01 M) was added to each well. For each mosquito, 25 \( \mu \text{L} \) of substrate acetylthiocholine iodide (0.014 M) was added to the first well (inhibited activity), whereas 25 \( \mu \text{L} \) of substrate acetylthiocholine iodide (0.014 M) plus propoxur (3.10\(^{-5}\) M) was added to the second well (inhibited activity). The activity rates in each well were determined using Kinetic reading (420 nm) for 5 min with a spectrophotometer (Biotek ELX808) and then analyzed using the KC4 software (1997). The percentage of \text{AChE} inhibition by propoxur was calculated for each mosquito as \( 1 - \text{(activity rate in propoxur inhibited well/activity rate in uninhibited well)} \). Statistical analysis were performed using Mann–Whitney \text{U} \text{test to test for variation between the distributions of \text{AChE} inhibition rates.}

Results

The frequency distributions of \text{AChE} inhibition rates for each treatment are shown in Fig. 1. With the untreated net, 54\% of mosquitoes displayed <50\% \text{AChE} inhibition by propoxur, confirming there was a fairly strong proportion of carbamate-resistant mosquitoes in the sample from M’bé Valley. Statistical analysis showed that the frequency distributions of \text{AChE} inhibition rates were similar between samples issued from the control and bifenthrin (Mann–Whitney \text{U} \text{test, } Z = 0.28, P > 0.05), mixture (\( Z = -0.11; P > 0.05 \)), mosaic (\( Z = -0.70; P > 0.05 \)), and mosaic one-thirds (\( Z = -0.72; P > 0.05 \)) treatments. Such results indicate that there was no selection for insensitive \text{AChE} by either the bifenthrin or the bifenthrin plus carbosulfan combined treatments. With carbosulfan, significant lower levels of \text{AChE} inhibition were recorded relative to the untreated net (\( Z = 4.15; P < 0.001 \)) as well as all other treatments (\( Z > 3.99; P < 0.001 \)). No individuals showed >50\% \text{AChE} inhibition, indicating that all susceptible individuals were killed by carbosulfan. The mean values of the \text{AChE} inhibition rates with carbosulfan (66\% ± 3\%) was significantly lower than the control (79\% ± 4\%), bifenthrin (79\% ± 2\%), mixture (78\% ± 4\%), mosaic (80\% ± 3\%), and mosaic “one-thirds” (82\% ± 5\%) treatments, indicating a strong selection by the carbosulfan treated net for insensitive acetylcholinesterase in An. \textit{gambiae}.

Discussion

Biochemical assays were performed to investigate the effect of nets treated with bifenthrin and carbosulfan, alone or in combination (mixture and mosaic) in the selection for carbamate resistance in An. \textit{gambiae}. The results clearly indicate there was selection for insensitive \text{AChE} by the carbosulfan treated net as compared with the untreated one. Conversely, no such selection was found either with bifenthrin alone or bifenthrin plus carbosulfan “two in one” treated nets. In view of the high level of resistance usually conferred by \text{AChE} insensitivity, such resistance may be an obstacle to the use of OP and carbamate insecticides for net treatments (N’Guessan et al. 2003). Although carbosulfan has been suggested as an alternative for pyrethroids to delay or overcome pyrethroid resistance problem (Kolaczkinski et al. 2000), the selection by carbosulfan of mosquitoes with high levels of insensitive \text{AChE} suggested that an urgent alternative strategy needs to be found. In fact, the lack of alternative insecticide families prioritize an optimal use of compounds already available (Poirié and Pasteur 1991). Mixtures and mosaics of insecticides with different modes of action have been proposed for a better control of mosquitoes with different susceptibilities to each component of the association (Curtis
In the current study, the mosaic configurations, containing bifenthrin and carbosulfan at both the recommended dosage did not select for an insensitive AChE in contrary to carbosulfan alone at the same concentration (300 mg/m²). Such results indicated that even when carbosulfan is present on the one-thirds upper side of the net, the pyrethroid insecticide (bifenthrin) could eliminate efficiently most of the mosquitoes strongly resistant to carbamates, thus preventing the selection of insensitive AChE mechanism in mosquitoes. The same observation was made with the mixture, with the advantage that mixture required significantly lower concentrations of both insecticides.

Further investigations will be done to test for or against selection for pyrethroid resistance based on the Kdr mutation by the two-in-one–treated nets in An. gambiae. Because combined pyrethroid and carbamate treated nets proved to have no impact on the insensitive AChE selection, multiple attacks of insecticides may be considered as a promising strategy for the management of insecticide resistance in malaria vectors.

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