LABORATORY AND FIELD EVALUATION OF SPATIAL REPELLENCY WITH METOFLUTHRIN-IMPREGNATED PAPER STRIP AGAINST MOSQUITOES IN LOMBOK ISLAND, INDONESIA

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ABSTRACT. Spatial repellency of a new multilayer paper strip impregnated with metofluthrin, a newly synthesized pyrethroid, was evaluated in the laboratory and in the field at Kerandangan, Lombok Island, Indonesia, with the use of cow- and human-baited double nets. Spatial repellency was observed in both cow- and human-baited collections. Metofluthrin treatment reduced mosquito collection by >80% during the 1st 4 weeks. However, repellency seemed to reduce with the loss of metofluthrin by evaporation within 6 wk after treatment.

KEY WORDS Metofluthrin, spatial repellency, Anopheles balabacensis, Culex quinquefasciatus, Lombok Island

INTRODUCTION

Since the indoor residual spraying of insecticides lost its reliability because of the development of physiological or behaviorological resistance in mosquitoes, one of the major innovations in the field of malaria vector control during the past 2 decades has been insecticide-impregnated mosquito nets. Recent development of pyrethroids and its formulation technologies have accelerated the development of long-lasting insecticidal net (WHO 2000, N’Guessan et al. 2001). It will be the most promising measure for controlling malaria mosquitoes at low cost and high sustainability, as opposed to residual spraying. However, the exophagic behavior of mosquitoes to shift their biting preference to earlier in the evening still presents a challenge. In such cases, active people outside and inside of houses could still be exposed to the danger of malaria transmission. Mosquito coils, mats, and other formulations that prevent mosquito bites are broadly and successfully used inside houses in wealthy communities. Use of these devices is, however, limited in the poor communities because of lack of convention, electricity, and money. Moreover, their uses seem limited outside of houses because they sometimes are unable to maintain sufficient aerial concentration of active ingredient in outdoor conditions.

Metofluthrin, 2,3,5,6-tetrafluoro-4-methoxymethylbenzyl (E)-1(RS,3RS;1RS,3SR)-2,2-dimethyl-3-(prop-1-enyl) cyclopropanecarboxylate (S-1264) is a newly synthesized pyrethroid with demonstrated knockdown and lethal activity against mosquitoes (Shono et al. 2004, Sugano et al. 2004). The vapor pressure of metofluthrin (1.87 × 10^-3 Pa at 25°C) is ca. >2 times and >100 times larger than d-allethrin and permethrin, respectively. Metofluthrin vaporizes at normal temperature without heating, whereas the other conventional pyrethroids need heating for evaporation. High vapor pressure and insecticidal activity of metofluthrin could lead new mosquito controlling devices that need no external energy for vaporization with low cost and long time efficacy.

In this paper, we report the results of laboratory and field studies that evaluate the insecticidal activity of metofluthrin-impregnated paper strip against Anopheles balabacensis Baisas, which is a major malaria vector in Lombok Island, Indonesia. We also report on the possibility of the use of metofluthrin-impregnated paper strip in outdoor conditions against mosquitoes, including Anopheles spp. and Culex quinquefasciatus Say.

MATERIALS AND METHODS

Formulation of metofluthrin-impregnated paper strip: Metofluthrin multilayer paper strip devices were supplied by Sumitomo Chemical Co., Ltd. (Takarazuka, Hyogo, Japan). Metofluthrin (200 mg) diluted with acetone was uniformly applied to the paper strip device, which has a multilayer and foldable structure (Fig. 1), and acetone was allowed to vaporize under ambient conditions. The folded device was compact (9 × 7 cm, ~3 mm thick), and total surface area of the unfolded paper was ~2,000 cm².

Laboratory evaluation against An. balabacensis: Knockdown activity of metofluthrin-impregnated paper strips was evaluated in an office of NTB Dinás Kesehatan Propinsi (Lombok, Indonesia). Room dimensions were 2.7 m wide, 3.9 m deep, and 3.2 m high. Field-collected larvae of An. balabacensis were reared in the laboratory and emerged adults were used in the test. Five females (3–7 days old) were released into stainless steel cages (210 × 170 × 150 mm, 11 mesh). Four pairs of cages were hung in a diagonal line at 1 m and 2.4 m (in the corner of the room) from the center of the room at a height of 130 and 30 cm, respectively, from the floor. A multilayer paper strip was hung at the center of the ceiling such that the bot-
Fig. 1. Multilayer paper strip device impregnated with 200 mg of metofluthrin.

tom end of the strip was 160 cm from the floor. Knockdown of mosquitoes was observed for 1 h, and mortality was recorded at 24 h. There was no air-conditioner or ventilation system in the room, and temperature and relative humidity (RH) were recorded at 28–30°C and >70%, respectively.

Field collection of mosquitoes in Kerandangan: Weekly collection of mosquitoes was carried out at Kerandangan, a coastal area about 15 km northwest of Mataram, Lombok Island, Indonesia. A house was used for indoor human-baited collection (HBI). A bed net was hung in a room with 2 human volunteers inside, and collection of mosquitoes was made outside the bed net. Outdoor human-baited collection was carried out with the use of a double net (HBO). Collection of mosquitoes was made inside of the outer net (3 × 3 × 2 m) and outside of the inner net (1 × 1 × 2 m) in which 2 humans lay. Outdoor cow-baited collection also used a double net (CBO). Collection of mosquitoes was made inside of the outer net (6 × 6 × 2 m) and outside of the inner net (4 × 4 × 2 m) in which a cow lay. Half-night (1800–2400 h) collection of mosquitoes by aspirator was carried out at 1-h intervals. Collection time was 45 min for HBI and HBO (e.g., 1800–1845 h) and 15 min for CBO (e.g., 1845–1900 h). Field collection was carried out once a week from November 12, 2002, to February 11, 2003. Species and number of collected mosquitoes were recorded the day after collection.

Evaluation against mosquitoes in the field: Evaluation was carried out at the same place as mosquito collection in Kerandangan. Trial design is shown in Fig. 2. A room of similar size (volume 15–18 m³, floor area 5.5–8.5 m²) in 3 different houses was used for the indoor human-baited collection (the HBI-1 house was used for the 2nd, 3rd, and 4th tests and the HBI-2 house was used only for the 1st test; the HBI-3 house was used for the 1st, 2nd, 3rd, and 4th tests). The door and windows of each room were kept open during the test. Two sites were used for outdoor human-baited collection with a double net. Two sites were used for outdoor cow-baited collection with a double net. Test strips were set before the test started (1800 h). Strips were hung 10–30 cm below the ceiling of the house outside the bed nets for HBI (Fig. 3A) and in a space between the inside net and outside net of the double nets for HBO and CBO (Fig. 3B, 3C), respectively. The number of test samples hung were 1 for HBI, 2 for HBO, and 4 for CBO. The number of test samples in HBO and CBO was roughly decided according to the sizes of both nets. Half-night (1800–2400 h) collection of mosquitoes by aspirator was carried out at 1-h intervals in the same manner as in the weekly collection. In every test, 1 site was used for the treatment and the other site was left untreated. Each test was repeated 2 times on successive days at a different site. The 1st test (just after unfolding of strips) was carried out on December 22, 2002 (1800–2400 h), and December 24, 2002 (1800–2400 h); the 2nd test (after 2 wk) on January 7, 2003 (1800–2400 h), and January 8, 2003 (1800–2100 h; the test was stopped at 2100 h because of rain); the 3rd test (after 4 wk) on January 21, 2003 (1800–2400 h; replication could not be carried out because of rain); and the 4th test (after 6 wk) on February 4, 2003 (1800–2400 h), and February 6, 2003 (1800–2400 h). Test strips were preserved in unfolded condition in the room in Mataram city for the next test. Average temperature and relative humidity recorded ranged from 25 to 30°C and 70 to 90% RH, respectively. The rainy season started at the end of October 2002 and lasted throughout the test period. The number of collected mosquitoes and species identified was recorded every test day.

RESULTS

Laboratory test: evaluation of metofluthrin-impregnated paper strip against An. balabacensis: Knockdown activity of a metofluthrin-impregnated paper strip against female adult An. balabacensis is shown in Table 1. All insects were knocked down within 30 min after treatment of the strip, and mortality at 24 h was 100% irrespective of the height and position of cages. Knockdown seemed to be faster in the cages at a distance of 2.4 m (in the
corner of the room) than those in the cages 1 m from the strip.

Field test: population density and species composition of mosquitoes during the test period: Species composition at each untreated test site from November 12, 2002, to February 11, 2003, are shown in Table 2. Species compositions were almost the same in HBI and HBO; >80% of the mosquitoes collected by human bait were Culex spp., among which, Cx. quinquefasciatus was dominant (>90%), and the 3-species complex of the Pyretophorus series (i.e., Anopheles vagus Doenitz, Anopheles indefiniteis (Ludlow), and Anopheles subjunctus Grassi) ranked next after Culex spp. The proportions of An. balabacensis and An. sundaicus, however, were low, as in the human-baited collections. Mosquito density was highest in CBO (461 per night), which was ~4 times and 20 times more than in HBO and HBI, respectively. Anopheles spp. and Culex spp. peaked in December 2002 and then gradually decreased by the end of February 2003.

Spatial repellency of metofluthrin-impregnated paper strips against mosquitoes: The number of Anopheles spp. and Culex spp. collected in metofluthrin-treated and untreated collection sites and overall changes in the total number of mosquitoes collected per hour in metofluthrin-treated and untreated sites by 3 different collections are shown in Table 3 and Fig. 4, respectively. A significant reduction in mosquito density in metofluthrin-treated sites was maintained in HBI (2-way ANOVA, F = 7.42, 1 df, P < 0.011). Mosquito density was >6 times higher in HBO than in HBI, which indicates the exophagy of mosquitoes. Species composition in the cow-baited collection, on the other hand, was different from those in the human-baited collections; ~70% of the collection comprised the 3-species complex of Pyretophorus series, and the proportion of Culex spp. was lower than in the human-baited collections. The proportions of An. balabacensis and An. sundaicus, however, were low, as in the human-baited collections. Spatial repellency was prominent in the cow-baited collection, in which higher numbers of mosquitoes were collected than in human-baited collections. Percent reduction of mosquito density in the
Field test scene of each test site. (A) Indoor human-baited collection (HBI; a strip was hung 10–30 cm below the ceiling of the house outside the bed net). (B) Outdoor human-baited collection with a double net (HBO; 2 strips were hung in a space between the inside and outside nets). (C) Cow-baited collection with a double net (CBO; 4 strips were hung in a space between the inside and outside nets).

metofluthrin-treated CBO sites versus the untreated controls was 90.7% for Anopheles spp. and 92.6% for Culex spp. at the initial test. Percent reduction was low for Anopheles spp. after 2 wk (58.0%), but it recovered to 90.9% after 4 wk. For Culex spp., on the other hand, percent reduction was >90% until 4 wk after treatment. Percent reduction decreased after 6 wk for both groups of mosquitoes. The difference between percent control in metofluthrin-treated sites at 6 wk and at 0, 2, and 4 wk were significant by the chi-squared test on the basis of the number of mosquitoes collected in CBO. For

Table 1. Knockdown activity of metofluthrin-impregnated paper strip against caged female adults of Anopheles balabacensis at 0, 10, 30, and 60 min.

<table>
<thead>
<tr>
<th>Distance from strip (m)</th>
<th>Height (m)</th>
<th>Replicate</th>
<th>No. knocked down</th>
<th>% mortality at 24 h</th>
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<td>%</td>
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<td></td>
<td>%</td>
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<td>90</td>
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</table>
Tsetse" sp."t" mosquitoes at each collection site from November 12, 2002, to February 11, 2003.

Species composition (Zo)'

- An. vagus
- An. indefinitus
- An. subpictus
- An. tessellatus
- An. annularis
- An. barbirostris
- An. maculatus
- An. Flavirostris
- An. sundaicus
- An. kochi

Aedes spp.  1.3
Armigeres spp.  0.3
Culex spp.  87.8

Totals  100 (20.2)

*HBI, indoor human-baited collection; HBO, outdoor human-baited collection; CBO, cow-baited collection.*

Anopheles spp., $\chi^2 = 106.3$ for week 0 versus week 6 (1 df, $P < 0.0001$), $\chi^2 = 17.4$ for week 2 versus week 6 (1 df, $P = 0.00003$), and $\chi^2 = 19.6$ for week 4 versus week 6 (1 df, $P = 0.00001$). For Culex spp., $\chi^2 = 32.3$ for week 0 versus week 6 (1 df, $P < 0.0001$), $\chi^2 = 38.6$ for week 2 versus week 6 (1 df, $P < 0.0001$), and $\chi^2 = 12.7$ for week 4 versus week 6 (1 df, $P = 0.00037$).

DISCUSSION

Metofluthrin and its impregnated multilayer paper strip showed promising spatial repellency against mosquitoes in the laboratory and in field conditions. Spatial repellency is thought to be caused by high knockdown activity and an intrinsic sublethal effect that also is present in other pyre-
The laboratory test showed that mosquitoes were affected by airborne metofluthrin vapor and not by direct contact with the strip. The long-lasting effect of more than 4 wk in open field conditions is outstanding, even though the double net could have interfered with natural air circulation under our test conditions. Spatial repellency seemed to decrease with the loss of effective metofluthrin by evaporation within 6 wk of treatment. According to net area and number of strips treated in this study, the effective area affected by a metofluthrin-impregnated strip was estimated to be >5.5 m² for HBI, >4.5 m² for HBO, and >9 m² for CBO. A higher amount of active ingredient, improvement of slow-release devices, and additional protection for the active ingredient against degradation by external factors, such as oxidation and light irradiation, are required for the practical and successful use of metofluthrin in the field.

Our preliminary investigation on the ecology and biting habit of mosquitoes in Meninting County, Lombok Island, including the test area of this study, has shown that mosquitoes are exophagous and their biting seems to peak in the early night (Mae-kawa et al., unpublished data), and it is likely that malaria transmission occurs in the early night when most people are active outside. The people of Lombok Island, like other people in tropical areas, have arbor huts made of palm leaves, called “berugas,” which have no walls or have only simple screens. People use berugas to nap, pray, and converse in the evening with neighbors. Control or prevention of mosquitoes in berugas, therefore, seems to be ideal for malaria control. Use of traditional and inexpensive practices, such as mosquito coils and mats, insecticide-impregnated or untreated bed nets, and curtains have mainly been focused on the prevention of mosquito bites inside houses (Yap et al. 1990, Aikins et al. 1994, Hewitt et al. 1996). There are very few reports of the efficacy of antimosquito products in the outdoors. Jensen et al. (2000) reported that only mosquito coils and N,N-diethyl-3-methylbenzamide (deet) products significantly reduced mosquito landing rates relative to untreated controls in the field among the several commercial antimosquito products, including mosquito attractant, mosquito coils, ultrasonic repellent, citronella candles, and mosquito plant. Pates et al. (2002) reported unique attempts with a kerosene oil lamp to vaporize transfluthrin, a new volatile pyrethroid. They modified the lamp by mixing transfluthrin with vegetable oil and heating it to 120°C in a tin can held just above the flame to avoid decomposition of the insecticide by heat. More than 90% protection was achieved with a higher concentration of transfluthrin (0.5%) in the vegetable oil, whereas 0.1% transfluthrin gave <75% reduction in typical houses of Dar es Salaam, Tanzania. Their device will offer a cost-effective alternative to mosquito coils and mats because kerosene lamps are cheap and used widely in tropical areas. Both kerosene lamps and mosquito coils could be applicable for outdoor use. The above 2 devices, however, need heat by burning of solvents or coil materials for evaporation and diffusion of active ingredients, which have the potential danger of fire, trouble in handling, and occasional side effects for human health by smoke.

The multilayer paper strip used in this study is thought to be an ultimate device that needs no external energy for heating and no other materials for evaporation and diffusion of active ingredients with low cost and high convenience. It is noteworthy that the metofluthrin-impregnated paper strips showed significant spatial repellency against mosquitoes in open field conditions for a month at 200 mg concentration. Further evaluation in more practical conditions, such as berugas, and further studies to enhance the duration of activity will promote development of the devices for the suppression of mosquito-borne diseases.
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