doi: 10.1111/j.1472-8206.2009.00731.x

ORIGINAL ARTICLE

Efficacy of four insect repellents against mosquito bites: a double-blind randomized placebo-controlled field study in Senegal

Bernard Uzzan[a,b], Lassana Konate[c], Abdoulaye Diop[c], Patrick Nicolas[a,b], Ibrahima Dia[c], Yémou Dieng[d], Arezki Izri[b,e*]

[a]*Department of Pharmacology, AP-HP, Avicenne Hospital, 125 route de Stalingrad, 93009-Bobigny, France*
[b]*Paris-XIII University, 93009-Bobigny, France*
[c]*Dakar Faculty of Sciences, Dakar, Senegal*
[d]*Department of Parasitology-Mycology, Dakar Faculty of Medicine, Dakar, Senegal*
[e]*AP-HP, Avicenne hospital, Laboratory of Parasitology, 125 route de Stalingrad, 93009-Bobigny, France*

Keywords
field study,
insect repellents,
Latin-square design,
mosquito bite,
randomized controlled trial,
western Africa

Received 2 December 2008;
revised 10 March 2009;
accepted 7 April 2009

*Correspondence and reprints:
arezki.izri@avc.aphp.fr

The study sponsor was not involved in the study design and analysis of the study, writing the manuscript or the decision to submit the manuscript for publication.

ABSTRACT

Insect-borne diseases represent a worldwide threat. In addition to fight against vectors (insecticides) and disease prevention (vaccination against yellow fever, chemoprophylaxis against malaria), insect repellents applied on the skin could help reduce the heavy burden related to these diseases. In a field study performed in Senegal, we compared the efficacy of one skin application between 3 and 4 p.m. of four spray repellents [icaridine 20%, para-menthane-diol (PMD) 20% and 50% and DEET 50%] against placebo, among 100 healthy male and female volunteers experienced with mosquito capture. Double-blind randomized cross-over placebo-controlled study (Latin-square design) during five consecutive nights (7 p.m. to midnight) in two villages was conducted. To avoid residual effect, right or left leg was alternately exposed during consecutive nights and the exposed leg was washed before next night. The statistical model was random and mixed effects ANOVA. All four active repellents provided a significant and similar protection compared with placebo, lasting 8 h. However, there was a non-significant trend for a higher protection by DEET 50% than by PMD 20% ($P = 0.07$). Duration of protection was similar for all repellents. Their effects were similar among men and women, and against *Anopheles* or other species. No serious adverse drug reaction was noticed. Using a rigorous methodology and a large number of volunteers, our well-controlled study demonstrated an important and similar protective effect of all four repellents compared with placebo. Such field studies should be required before approval of any newly developed repellent.

INTRODUCTION

Insect-borne diseases represent a major cause of morbidity and mortality worldwide. Mosquitoes transmit infections to more than 700 million persons each year [1]. In Africa, insects transmit not only the *Plasmodium* species responsible for high prevalence of malaria but also filariases and the arboviruses causing dengue haemorrhagic fever and yellow fever. In addition to fight against vectors (insecticides) and disease prevention (vaccination against yellow fever, chemoprophylaxis against malaria), protection against insect bites relies mainly upon avoiding infested habitats, using impregnated bed nets [2,3], wearing protective clothes and using repellents. Repellents are indeed recommended by most travellers' health guides [4,5] and seem to be especially useful for prevention of chikungunya and dengue caused by arboviruses.

Application of an efficacious and safe repellent on the skin represents in some circumstances one of the best available protections against insect bites. As a single bite from an infested insect may transmit the disease, a total or near-total protection is required. Unfortunately, the clinical efficacy of insect repellents has been rather poorly documented. Elsewhere, the duration of protection varies between products. Insect repellents can be divided into two main categories: synthetic chemicals and plant-derived essential oils. *N*,*N* diethyl-3-methyl-benzamide (DEET) has the best documented efficacy [6–8]. First patented by US army in 1956, it was marketed in 1957. It has a broad spectrum and no other repellent has definitely shown superior efficacy. Several field studies showed that DEET was superior to other repellents, such as picaridin [9] or para-menthane-diol (PMD) plus lemongrass oil [10]. Higher concentrations of DEET provide longer-lasting protection, leading to a plateau for DEET concentrations exceeding 50% [2]. Products with 10–35% DEET (usually diluted in ethanol) provide adequate protection in most circumstances.

Clinical studies of repellents can be divided into laboratory and field studies [11]. In arm-in-cage studies, volunteers place their treated arms into a cage with a fixed number of unfed mosquitoes, with record of the time to first bite. Such laboratory studies can reduce potential confounding variables such as wind speed, temperature, hygrometry, density of the mosquito population, degree of hunger of mosquitoes and species. However, their results are less applicable to real life than field studies, which take place in an area highly infested by mosquitoes where volunteers are gathered and expose one limb to bites, the rest of their body being protected by clothes. Field studies need volunteers experienced in counting bites and catching mosquitoes, and investigators familiarized with this kind of research [11]. As susceptibility to mosquito bites is highly variable among individuals, one should always use each subject participating in a field study as his (her) own control.

We chose to place our field study in two villages (Keur M'baye and Mbilor) located at about 10 km west of Richard Toll, a small city north of Senegal, 370 km away from Dakar, for several reasons. First, there was a high prevalence of malaria in these villages, each inhabitant suffering from several crises each year, mainly during and just after the rain season (July–September). Secondly, their inhabitants were trained to catching of mosquitoes as we had already performed a field study of insect repellents in 1999 in that location [12]. The majority of population of these two villages is young and of Wollof ethnicity.

The aim of this study was to assess the protective efficacy of four insect repellents against mosquitoes in a field study, compared with a placebo, each product being applied on a single occasion. An ancillary goal was to assess local and general tolerance to these products. A more general objective was to establish such field studies as the standard for pre-marketing assessment of new repellents. The study protocol was approved by the Ethics committee of Senegal. It was not declared because, at that time, we deemed that this procedure only applied to drugs sensu stricto.

MATERIALS AND METHODS

Product selection

One hundred identical bottles for lotion use (sprays) for each of the five products (four commercially available repellents and a placebo) were provided by FULLTEC Laboratories (Zug, Switzerland). On the white pack only a letter, A–E, corresponding to the product was mentioned. Product A was icaridine 20% (Autan®; Bayer, Berlin, Germany); product B was naturally derived PMD 20% derived from lemon eucalyptus plant (Mosquito Protector® 20%); product C was a placebo lotion; product D was DEET 50% (InsectEcran®; Cooper, Melun, France); and product E was PMD at a higher concentration (Mosquito Protector® 50%; FULLTEC Laboratories). Thus, this study was a double-blind randomized placebo-controlled cross-over trial, with Latin-square design. Each product was applied on the skin of one leg, from knee to ankle (about 15 mL of product), at 3 p.m. by a physician from our team. We used the following sequences, each one corresponding to a group of 20 volunteers treated during five consecutive nights: ABCDE, BCDEA, CDEAB, DEABC and EABCD. Each volunteer received alternately on his right or left leg one product each night for five consecutive nights. During the following night, the opposite leg was exposed. Such a design reduced the risk of residual effect of the product applied the night before. In addition, before application of each product, volunteers were asked to wash carefully their exposed leg and then to dry it, to eliminate residual product.

Selection of volunteers

One hundred volunteers were recruited during a meeting under the talk tree. They were informed, in local language (Wollof) and in French, about the aim of the study, its conduct and its potential risks. They had a

succinct clinical examination by a physician from our team. They benefited from a financial compensation for inconvenience. Volunteers to be included were male or female adults, willing to participate and give a written informed consent. Women of childbearing age were included only if they had a negative pregnancy test. Volunteers were not included if they had used a dermatological therapy during the 2 weeks preceding inclusion, if they had a known hypersensitivity to any component of any product studied or had concomitant severe illness deemed incompatible with the study or dangerous for the volunteer. According to the time of his registration, each volunteer was attributed a file number from 1 to 100.

Exposure to mosquito biting

The study was conducted during five consecutive nights (27th August–1st September, 2006), on five groups of 20 volunteers. In a previous field study conducted at the same places [12], 3025 mosquitoes were captured during 90 nights-subjects of exposure, including 660 *Anopheles* and 827 identified *Culicinae* (mainly *Mansoniae)*. Twenty volunteers were gathered in a courtyard from 3 p.m. to midnight. One of their legs was exposed from knee to ankle and the opposite leg was protected by clothes. They wore socks. Application of the products to all volunteers began at 3 p.m. and ended at 4 p.m. Volunteers were then allowed to attend to their usual affairs until 7 p.m. (thereafter, they were gathered in a courtyard), but they were not allowed to moisten, wash or dry their exposed leg. Indeed, preliminary testing showed the absence of mosquito bites during the first 3–4 h after application of the products. In addition, a too long period of capture would probably diminish its yield by decreasing volunteers' reaction. Consequently, mosquito catching began at 7 p.m. and ended at midnight. Volunteers had to catch all biting mosquitoes. Mosquitoes landing on the skin were not captured until they bit. Each volunteer was equipped with a torch, haemolysis tubes (to capture mosquitoes), cotton (to close the tube) and bags to be filled with tubes. Bags were collected hourly and transported to field entomologists who identified insects by means of binocular lenses.

Statistical tests

The statistical model used in this study was a random and mixed effects ANOVA. Fixed factors were product, period and sequence. Random factors were subjects (nested within sequence) and villages. A major random effect means that measured fixed effects depend largely on random factors. Extrapolation of results relying on fixed effects is more relevant in the absence of random effect. We used JMP statistical software version 5.1 (SAS Institute, Cary, NC, USA). A P value inferior to 0.05 was considered as statistically significant (two-tailed tests). The number of participating volunteers was not determined from power calculations but set empirically to 100 subjects, a number large enough to take into account all factors influencing clinical efficacy of products. We set an efficacy threshold for any repellent of at least 90% protection against mosquito bites.

RESULTS

Patient characteristics

As one volunteer dropped out from the second night of the last sequence (EABCD), the study continued with 99 subjects. Thus, only product E was assessed in 100 subjects. There were 67 men and 33 women. Their mean age ± SD was 25 ± 9 years (range: 16–62). Sixty volunteers came from Keur M'baye village, and 40 from Mbilor. A total of 1875 mosquitoes were captured in both villages, mainly *Anopheles* (32%, with *A. gambiae*, the main vector of *Plasmodium*, representing 11% of the total number of mosquitoes), *Culex* species (31%), and *Mansonia* species (27.5%). *Aedes* species was poorly represented (0.2%). The proportion of *Anopheles* captured increased during the last 2 h of the trial (10 p.m. to midnight). Men captured a total of 1265 mosquitoes and women 610, exactly reflecting sex ratio.

Protective effects of the four repellents

In our model, the variances of the subject effect and of the village effect only represented 7.2% and 8% of total variance respectively. This meant that the product effect we observed was highly consistent between subjects and villages. Product effect was highly statistically significant ($P < 0.0001$), as were period effect ($P = 0.015$) and sequence effect ($P = 0.02$). Period 5 was statistically significantly different from periods 3 and 4. Sequence EABCD was significantly different from sequence BCDEA.

As results were similar in both villages, we present only pooled data. Only 1868 captures were included in the statistical analysis. *Table I* shows the numbers of mosquitoes captured after each product was applied, with median and range. All four active products were statistically significantly superior to placebo (product C). Comparisons between the four active products showed no significant differences. However, protection afforded by product D (DEET 50%) tended to be superior to that of product B (PMD 20%) ($P = 0.07$). Protection against all

Table I Number of mosquito bites by product.

Product	No. mosquitoes captured	Mean (/night/subject)	Median	Range
Icaridine 20% (A)*	143	1.43	1	0–17
Para-menthane-diol 20% (B)*	235	2.37	1	0–14
Placebo (C)	1241	12.5	10	0–47
DEET 50% (D)**	128	1.25	0	0–12
Para-menthane-diol 50% (E)*	128	1.29	0	0–10

*Statistically significant superiority over placebo at $P < 0.05$; **statistically significant superiority over placebo at $P < 0.05$, and a non-significant trend for superiority over product B at $P = 0.07$.

Anopheles species provided by all four active products was similar to protection against all other species of mosquitoes (*Culex*, *Mansonia*).

Duration of protection was similar for all four products (7–12 p.m.). *Table II* shows the number of catches by product and by hour. The number of catches increased substantially from 7 p.m. to midnight for all products. However, the protective effect of repellents was almost the same, in proportion, at midnight as at 8 p.m.

Women caught as many mosquitoes as men. The protective effect of repellents was similar in men and women. There were important individual differences in the numbers of mosquitoes captured by volunteers, with 'good catchers' and 'poor catchers', as the range of mosquitoes caught varied from 0–10 for product E (PMD 50%) to 0–17 for product A (Icaridine 20%) and even to 0–47 for placebo. No serious adverse drug reaction was recorded during the trial.

DISCUSSION

In a double-blind randomized placebo-controlled cross-over study involving 100 volunteers, we found that all four active repellents applied once were statistically significantly superior to placebo, showing a rather similar efficacy, with a trend favouring DEET 50% over PMD 20%. This trend would have translated into a statistically significant difference, had we set a lower threshold for efficacy (90%), but we required a near-total protection.

Table II Number of mosquito captures by product and by time.

Product	7–8 p.m.	8–9 p.m.	9–10 p.m.	10–11 p.m.	11–12 p.m.	Total
A	4	31	34	25	49	143
B	7	35	44	65	84	235
C (%)	90 (82)	197 (67)	268 (69)	320 (68)	366 (63)	1241
D	3	18	30	29	48	128
E	5	15	37	34	37	128
Total	109	296	413	473	584	1875

A = Icaridine 20%; B = para-menthane-diol 20%; C = placebo; D = DEET 50%; E = para-menthane-diol 50%. Each product was applied to volunteers between 3 and 4 p.m.

We chose to perform our field study in Senegal for several reasons. Senegal belongs to the Sahelian area south of Sahara, highly affected by arthropod-borne diseases. It represents one of the main African destinations for European tourists. In addition, many people from this country migrate to Europe. The choice of Richard Toll district relied upon its high infestation by mosquitoes and hence high prevalence of insect-borne diseases, and the presence in both villages of people trained to catch mosquitoes, an essential condition to evaluate the efficacy of repellents accurately. We conducted this study at the end of the rain season as the density of mosquitoes is the highest at this time.

Our trial represents one of the largest field studies of repellents ever published. We found only two repellent studies, which included more volunteers. The older included 1148 individuals from an Afghan refugee camp in Pakistan and showed that DEET 20% plus permethrin protected against *falciparum* malaria significantly more than placebo [13]. Recently, another double-blind randomized controlled trial involving 4008 individuals in 860 households in Bolivian Amazon compared the protection against malaria afforded by lemon eucalyptus, applied at dusk each evening, and placebo, all subjects receiving in addition bed nets treated with a pyrethroid insecticide. This was an area where vectors bite during the early evening. There was a highly significant 80% reduction in episodes of *Plasmodium vivax* in the group that used both bed nets and repellent [14]. Numbers of *Plasmodium falciparum* cases during the study were small and although a similar protective effect was observed, it was not statistically significant. This study has major consequences for improvement of malaria vector control programmes outside Africa and fully justifies the advocated use of insect repellents combined with impregnated bed nets for tourists travelling to high-risk areas. The insect repellent was chosen based on field evaluations of several plant-based repellents and a DEET standard, showing a high protection (>98%) against *Anopheles darlingi*, the malaria vector, for up to 4 h [15]. Obviously, our study has a lesser clinical impact than these two trials, as it did not assess comparatively the numbers of malaria crises, but it may bring new information to the clinical evaluation of insect repellents.

Usually, field studies involve at best 20 volunteers [10,16]. It is essential to include more volunteers, to take into account the important individual variability in susceptibility to mosquito bites. In addition, we included both male and female volunteers, in contrast with other studies involving men only. One study included pregnant Karen women [17]. The two recent randomized studies included 50% women [13,14]. In this study, women were not more exposed than men to mosquito bites. Application of the products by physicians guaranteed a correct technique, avoiding variability in effect related to the amount of product applied, but somewhat limited the extrapolation of our results to normal conditions of use.

Even if our Latin-square design was not balanced, product B always following product A and so on, this mistake was unlikely to influence the results because products were alternately applied on one leg or the other, minimizing the consequences of a hypothetical residual effect of any product, and because each volunteer had to wash his exposed leg before each new session. Among one-to-one comparisons following ANOVA, only three reached statistical significance. For example, sequence EABCD differed from sequence BCDEA, probably a chance finding.

The small proportion of *Aedes* captured during our main trial was not surprising considering the absence of larvae sites in both villages. Therefore, we performed a small study in Dakar following a similar design including 10 volunteers (eight men and two women) who captured 632 mosquitoes: 322 *Aedes aegypti* (51%), 50 *A. gambiae* (8%) and 258 *Culex* species. This ancillary study also showed that products A, B, D, and E were statistically significantly superior to product C (placebo), and that product D (DEET 50%) was significantly superior to product B (PMD 20%) (data not shown).

The number of mosquitoes captured during the main study increased steeply from 7 p.m. to midnight. We chose to limit the observation period because the demonstration of earlier efficacy of repellents (during the first 4 h) was already provided in their drug marketing files and because a longer period would not have allowed volunteers to maintain their top catching capacity. Thus, in this study, the precision of the estimation of the duration of protection is limited. However, the protection provided by the active products was similar in proportion. Only half of the volunteers had a complete protection (no mosquito bite), but the second half had a 90% protection. We think that repellents alone are a very useful but insufficient mode of protection against insect-borne diseases. There were large individual differences in the numbers of captures by 'good catchers' and 'poor catchers'. The cross-over design of our trial could control for this phenomenon. These individual differences in the capacity of captures might be related to variable susceptibility to mosquito bites or skilfulness of volunteers in catching mosquitoes. In future field studies, exclusion of 'poor catchers' (by a run-in phase of one night duration without repellent) would probably improve the sensitivity of such trials and allow a more precise comparative evaluation of various active repellents. In this study, DEET, PMD, and icaridine exerted a similar and high protection against mosquito bites compared with placebo.

As insect repellents are not considered as drugs but as medical products, their clinical development is not ruled by the same guidelines. For approval of any newly developed insect repellent, a field study such as this one should be needed and should compare two different concentrations of the new product with DEET 50%, the reference product, and placebo, to establish its efficacy and its best active concentration.

ACKNOWLEDGEMENTS

We thank FULLTEC AG (Zug, Switzerland) for partly granting the study and providing us with all products.

CONFLICTS OF INTEREST

None declared.

AUTHORS CONTRIBUTIONS

All authors have signed and approved the final version of the manuscript. AI wrote the protocol, recruited physicians, coordinated the study and participated in the writing of the manuscript, BU wrote the first draft and final version of the manuscript, PN made the statistical analysis, LK, AD, and ID were the field entomologists who identified insects, and YD coordinated the study locally.

REFERENCES

1 Taubes G. A mosquito bites back. New York Times Magazine (August 24, 1997) 40–46.

2 Henry M.C., Assi S.B., Rogier C. et al. Protective efficacy of lambda-cyhalothrin treated nets in *Anopheles gambiae* pyrethroid resistance areas of Côte d'Ivoire. Am. J. Trop. Med. Hyg. (2005) **73** 859–864.

3 Carnevale P., Bitsindou P., Diomandé L., Robert V. Insecticide impregnation can restore the efficiency of torn bed nets and reduce man-vector contact in malaria endemic areas. Trans. R. Soc. Trop. Med. Hyg. (1992) **86** 362–364.
4 Bradley D.J., Bannister B. Guidelines for malaria prevention in travellers from the United Kingdom for 2003. Commun. Dis. Public Health (2003) **6** 180–199.
5 Goodyer L.I. Bite avoidance, in: Goodyer L.I. (Ed.), Travel medicine for health professionals, Pharmaceutical Press, London, 2004, pp. 139–171.
6 Fradin M.S. Mosquitoes and mosquito repellents: a clinician's guide. Ann. Intern. Med. (1998) **128** 931–940.
7 Fradin M.S., Day J.F. Comparative efficacy of insect repellents against mosquito bites. N. Engl. J. Med. (2002) **347** 13–18.
8 Lalloo D.G., Hill D.R. Preventing malaria in travellers. Br. Med. J. (2008) **336** 1362–1366.
9 Frances S.P., Waterson D.G., Beebe N.W., Cooper R.D. Field evaluation of commercial repellent formulations against mosquitoes (Diptera: Culicidae) in Northern Territory, Australia. J. Am. Mosq. Control Assoc. (2005) **21** 480–482.
10 Moore S.J., Darling S.T., Sihuincha M., Padilla N., Devine G.J. A low-cost repellent for malaria vectors in the Americas: results of two field trials in Guatemala and Peru. Malaria J. (2007) **6** 101; doi: 10.1186/1475-2875-6-101.
11 Schreck C.E. Techniques for the evaluation of insect repellents: a critical review. Ann. Rev. Entomol. (1977) **22** 101–119.
12 Izri A., Konate L., Dieng Y. et al. Efficacité de l'association DEET (20%) et EHD (15%) contre les piqûres de moustiques. Résultats d'une étude de terrain effectuée au Sénégal. Bull. Soc. Pathol. Exot. (2001) **94** 280–283.
13 Rowland M., Downey G., Rab A. et al. DEET mosquito repellent provides personal protection against malaria: a household randomised trial in an Afghan refugee camp in Pakistan. Trop. Med. Inter. Health (2004) **9** 335–342.
14 Hill N., Lenglet A., Arnez A.M., Carneiro I. Plant based insect repellent and insecticide treated bed nets to protect against malaria in areas of early evening biting vectors: double blind randomised placebo controlled clinical trial in the Bolivian Amazon (2007) (Published Online in Br. Med. J. (2007) **335**, 1023); doi: 10.1136/bmj.39356.574641.55.
15 Moore S.J., Lenglet A., Hill N. Field evaluation of three plant-based insect repellents against malaria vectors in Vaca Diez Province, the Bolivian Amazon. J. Am. Mosq. Control Assoc. (2002) **18** 107–110.
16 Naucke T.J., Kröpke R., Benner G. et al. Field evaluation of the efficacy of proprietary repellent formulations with IR3535® and Picaridin against *Aedes aegypti*. Parasitol. Res. (2007) **101** 169–177.
17 Lindsay S.W., Ewald J.A., Samung Y., Apiwathnasorn C., Nosten F. Thanaka (*Limonia acidissima*) and deet (di-methyl benzamide) mixture as a mosquito repellent for use by Karen women. Med. Vet. Entomol. (1998) **12** 295–301.