SHORT REPORT: STUDY ON THE EFFICACY OF A NEW LONG-ACTING FORMULATION OF N, N-DIETHYL-m-TOLUAMIDE (DEET) FOR THE PREVENTION OF TICK ATTACHMENT

B. SALAFSKY, YI-XUN HE, JING LI, TAKESHI SHIBUYA, AND KALYANASUNDARAM RAMASWAMY
Department of Biomedical Sciences, College of Medicine, University of Illinois, Rockford, Illinois; Department of Pharmacology, Tokyo Medical College, Tokyo, Japan

Abstract. The beneficial effects of N, N-diethyl-m-toluamide (DEET) against biting insects of human and animals appear to last <6 hr after a single application to the skin. To prolong the repellent effects of DEET, recently we developed a new long-acting formulation of DEET called LIPODEET. This preparation was retained in the skin for a longer duration of time with minimal systemic absorption. In this study, we have evaluated the protective effect of three compounds (DEET, LIPODEET, and Morpel 220) against attachment of two species of ticks (Amblyomma americanum and Dermacentor variabilis) to rabbit ears. Results show that LIPODEET and Morpel 220 were highly effective in preventing tick attachment to the skin for a longer duration of time (up to 72 hr) than DEET after a single application. Moreover, LIPODEET was found to be acaricidal against both the species of ticks.

N, N-diethyl-m-toluamide (DEET) is a safe and broad spectrum insect repellent used extensively against both human and animal pests worldwide.1–3 We previously reported that DEET prevented infections due to the parasite Schistosoma mansoni when applied to the skin of mice.4 However, the longevity of DEET action on the skin was short-lived, possibly due to rapid absorption of DEET through the skin.5,6 Although experimental studies have confirmed that DEET-related toxicity is minimal after systemic absorption,5,6 there are a few reports of toxicity associated with the use of DEET in humans and animals especially, if the chemical is misused or the patients are more susceptible after systemic absorption of large quantities of DEET.7–10 Currently available preparations of DEET are contained in vehicles that inevitably increase the absorption of DEET through the skin.8 This potentially could increase the risk of DEET-induced toxicity. Therefore, any agent that may retard the systemic absorption of DEET can potentially decrease the DEET-induced toxicity. Several new vehicles have been tried to reduce DEET absorption through the skin with varying success.11–14 Recently, we reported a liposomal preparation of DEET (LIPODEET) that was exceptionally effective in retaining DEET on the superficial layers of the skin. Subsequent testing showed that LIPODEET prolonged the protective activity of DEET against S. mansoni for up to 72 hr after a single application.8 Several reports suggest that DEET has some repellency action against ticks. However, compared with other repellents such as permethrin or piperidine, DEET is less effective against ticks and the repellent action of DEET is short lived.15–21 Since ticks attach to the skin for feeding and LIPODEET is retained in the skin for a longer duration of time, in this study we wanted to evaluate the potential use of LIPODEET against tick attachment.

Adult stages of Amblyomma americanum and Dermacentor variabilis (obtained from Dr. Jerry Bowman, Department of Entomology, Oklahoma State University, Stillwater, OK) were used in these experiments. Three different formulations of repellents (20% DEET, 20% LIPODEET and 20% Morpel 220) were prepared for their effect on tick attachment. LIPODEET was prepared by mixing DEET (Morflex, Inc., Greensboro, NC) with positively charged liposomes (lot 47H8350; Sigma, St. Louis, MO) as described earlier.5 Morpel 220 was obtained from Morflex, Inc. Dilutions of DEET and Morpel 220 were prepared in isopropanol. Repellents were applied to the (0.2 ml to a 10 cm2 surface area) dorsal skin surface of the ears of male New Zealand white rabbits (Charles River Laboratories, Wilmington, MA) and allowed to dry for 30 min. Isopropanol was used as the control. To monitor tick attachment, a cell strainer (1 inch inner diameter, 70 μm sieve size, Falcon 2350; Fisher Scientific, Itasca, IL) was glued over the applied area to form a chamber (Figure 1a). Five adult ticks were introduced into the chamber after the first hour of the application (control or repellent). This was followed by an addition of a second group of 5 ticks after 24 hr, and finally the addition of a third group of 5 ticks after 48 hr. This resulted in a total of 15 ticks in the chamber close to the rabbit ear. Observations were made every 24 hr to determine how many ticks were attached to the rabbit ear skin. The values are shown in Table 1. Statistical significance of the means of values was tested by one-way analysis of variance using Sigmastat program (Jandel Scientific, San Rafael, CA). All pairwise multiple comparisons were done using the Student-Newman-Keuls method. Probability values of 5% or lower were considered significant. All values are expressed as the mean ± SD.

These studies showed that LIPODEET is a superior and long-acting repellent against tick attachment when compared with DEET. In control rabbits treated with isopropanol, nearly 69% of adult A. americanum ticks attached to the skin and started engorging. Although a few ticks failed to attach, they remained very active and their mouth parts were intact. Compared with adult A. americanum ticks, the D. variabilis ticks took longer time to attach to the skin. However, by 72 hr, attachment of D. variabilis (58% attached) to the skin was comparable to that of A. americanum (Table 1).

Topical application of 20% DEET was partially effective in preventing attachment of adult A. americanum ticks to rabbit ears (Table 1). In the case of D. variabilis, DEET conferred no protection compared with controls (Table 1). However, a single topical application of 20% LIPODEET was 100% effective in preventing attachment of adult A. americanum ticks for up to 72 hr (Table 1). LIPODEET was also highly effective against D. variabilis (Table 1).
Figure 1. Effect of LIPODEET on tick attachment to the skin. Tick attachment to rabbit ear skin was monitored inside a chamber formed by gluing a cell strainer cup to the skin (a). Ticks could be easily visualized through the sieve screen. An experiment was designed where half the surface area of skin within the sieve cups was treated with 20% N, N-diethyl-m-toluamide (DEET) or 20% LIPODEET and the other half was left untreated. Five ticks each were introduced into the cup at 1 hr and 24 hr after repellent application and observations were made at 48 hr. In animals treated with DEET, ticks were seen attached equally to both the treated and untreated skin surfaces (b), whereas, in animals treated with LIPODEET, ticks were found attached only to the untreated half of the skin area (c).
LONG-ACTING DEET FORMULATION AGAINST TICKS

TABLE 1
Effect of various insect repellents on tick attachment*

<table>
<thead>
<tr>
<th>Hours after application</th>
<th>Isopropanol control</th>
<th>20% DEET</th>
<th>20% LIPODEET</th>
<th>20% MORPEL 220</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ambyalomma americanum</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>24</td>
<td>3.33 ± 0.58</td>
<td>1.33 ± 1.53</td>
<td>0 ± 2†</td>
<td>0 ± 0†</td>
</tr>
<tr>
<td>48</td>
<td>7.00 ± 1.73</td>
<td>2.00 ± 1.00</td>
<td>0 ± 2†</td>
<td>0 ± 0†</td>
</tr>
<tr>
<td>72</td>
<td>10.33 ± 3.06</td>
<td>4.67 ± 0.58†</td>
<td>0 ± 0†</td>
<td>0 ± 0†</td>
</tr>
<tr>
<td><em>Dermacentor variabilis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>24</td>
<td>1.00 ± 1.00</td>
<td>2.00 ± 1.00</td>
<td>1.00 ± 0</td>
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</tr>
<tr>
<td>48</td>
<td>3.00 ± 1.00</td>
<td>2.67 ± 0.58</td>
<td>0.33 ± 0.58†</td>
<td>2.00 ± 0</td>
</tr>
<tr>
<td>72</td>
<td>8.67 ± 1.53</td>
<td>9.00 ± 1.73</td>
<td>2.33 ± 2.5†</td>
<td>5.00 ± 0†</td>
</tr>
</tbody>
</table>

*DEET = N,N-diethyl-m-toluamide. Values are the mean ± SD number of ticks attached to the rabbit ear skin at each time point. A total of 15 ticks were introduced into the experimental area.† Significantly (P < 0.05) different from the isopropanol controls.

A single application of Morpel 220 was equally effective as LIPODEET in preventing attachment of *A. americanum* adult ticks to the skin (Table 1). However, the effect of Morpel 220 against *D. variabilis* adult ticks was variable. Thus, these results suggest that Morpel 220 is also a highly effective agent against ticks.

Surprisingly more than 75% of ticks used in our experiments were dead after contact with LIPODEET. Although we have used a well-aerated cell strainer cup, we initially thought that the tick mortality might be due to possible DEET vapor trapped within the strainer cup. Therefore, we designed experiments where half the surface area of skin within the sieve cup was treated with 20% LIPODEET and the other half was left untreated. Five adult ticks of *A. americanum* were then introduced into the sieve cups every 24 hr as before. After 48 hr, the sieve cups were removed and tick attachment was evaluated. A similar experiment was designed with 20% DEET and the results are presented in Figure 1b and c. Similar results were also obtained with *D. variabilis*. These results suggested that tick mortality was not due to DEET vapor released into the cup. It is possible that the ticks might have tried to feed onto the skin and this physical contact might have been lethal. Thus, LIPODEET was found to be acaricidal for both the species of ticks tested.

These preliminary studies thus show that LIPODEET and Morpel 220 may be superior to DEET in preventing tick attachment to the skin for a longer duration of time and thus may be important in field condition for preventing tick-borne infections. It is possible that other biting insects that feed similarly through the skin may also be affected by LIPODEET. Further field studies, will determine whether LIPODEET or Morpel 220 is an efficacious, dependable, and cost-effective agent for human or veterinary application against tick attachment.

Financial support: This study was supported by Morflex, Inc. (Greensboro, NC).

Authors’ addresses: B. Salafsky, Yi-Xun He, Jing Li, and Kalyansundaram Ramaswamy, Department of Biomedical Sciences, College of Medicine, University of Illinois, Rockford, IL 61107. Takeshi Shibuya, Department of Pharmacology, Tokyo Medical College, Tokyo, Japan.

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