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

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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 to 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 In this work, we investigated the systemic absorption of DEET on the skin. Subsequent experimental studies have confirmed that DEET-related toxicity is minimal after systemic absorption.6–8 Therefore, any agent that may retard the systemic absorption of DEET can potentially decrease the 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.9–14 Currently available preparations of DEET are contained in vehicles that inevitably increase the absorption of DEET through the skin.15–17 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.15–17 Therefore, new formulations that might reduce the absorption of DEET are being developed.

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 to both the species of ticks.

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).

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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 2

<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>
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</thead>
<tbody>
<tr>
<td>Amblyomma americanum</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 ± 0†</td>
<td>0 ± 0†</td>
</tr>
<tr>
<td>48</td>
<td>7.00 ± 1.73</td>
<td>2.00 ± 1.00</td>
<td>0 ± 0†</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>Dermacentor variabilis</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>
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<td>72</td>
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</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).

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