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INSECT REPELLENT FORMULATIONS OF *N,N*-DIETHYL-M-TOLUAMIDE (DEET) IN A LIPOSPHERE SYSTEM: EFFICACY AND SKIN UPTAKE

ABRAHAM J. DOMB,[1] ALEXANDER MARLINSKY, MANOJ MANIAR[2] AND LIMOR TEOMIM

Department of Pharmaceutical Chemistry, School of Pharmacy—Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem 91120 Israel

ABSTRACT. Novel formulations for deet in lipospheres microdispersion in the form of lotion were prepared from natural solid triglycerides and phospholipids dispersed in buffer solution. The formulations containing 6.5, 10, and 20% deet were effective as a repellent against the common aggressive biting mosquitoes, *Aedes aegypti* and *Anopheles stephensi,* for up to 6 h. The acute dermal absorption of the 10% loaded formulation was conducted in rabbits using ^{14}C-labeled deet. ^{14}C-labeled deet, 10% in alcohol solution or in lipospheres microdispersion was applied to the intact rabbit skin under a porous nonirritating cover for 7 days. Plasma levels of radioactivity were determined for 24 h, and daily for a total of 7 days. The ^{14}C-deet blood levels following intravenous bolus administration were also measured. The bioavailability of deet from 10% ethanol solution was 45%, whereas the bioavailability of deet from lipospheres was 16%, a 3-fold reduction in the amount of deet absorbed. Examination of the rabbits during the experiment and after necropsy showed no evidence of toxicity or irritation. The 10% deet–liposphere formulation was stable at room temperature for at least 1 year.

INTRODUCTION

Insects such as mosquitoes, flies, and fleas are a significant factor in the spread of serious diseases including malaria and encephalitis. They are a general nuisance to humans and animals. Efforts to repel these insect pests, other than physical means, are generally effective for less than 3 h. A common means for repelling insects consists of applying the compound *N,N*-diethyl-m-toluamide (deet) to the skin. The commercially available liquid deet formulations contain between 15 and 100% deet and they are not recommended for use on children. The toxicity of deet has been extensively reported and related to its high skin absorption after topical administration (Reuveni and Yagupsky 1982, Snodgrass et al. 1982, Robbins and Cherniak 1986, Mody 1989, Mody et al. 1989, Lipscomb et al. 1992, Clem et al. 1993). Previous studies have demonstrated that as much as 50% of a topical dose of deet was systematically absorbed (Snodgrass et al. 1982, Robbins and Cherniak 1986, Mody 1989, Lipscomb et al. 1992). Several methods have been reported for increasing the duration of deet effectiveness (Gupta and Rutledge 1989, Raden 1989, Schreck and Kline 1989). These methods used polymer solutions that form a film on skin, complexes of deet with aromatic proton donor carriers, or acrylate-based microcapsules dispersed in oil in water emulsion. However, none showed a significant improvement over alcoholic solutions of deet (Gupta and Rutledge 1989, Schreck and Kline 1989).

A novel encapsulation system, referred to as liposphere, was developed for the delivery of bioactive compounds by parenteral injection or for topical application (Amselem et al. 1992a, 1992b; Domb 1993a, 1993b; Masters and Berde 1994). The system is an aqueous microdispersion of all-natural, solid, water-insoluble spherical microparticles of 0.2–100 μm in diameter. The liposphere microparticles are made of hydrophobic solid triglycerides having a layer of phospholipids embedded on the surface of the particle. The solid core contains the bioactive compound dissolved or dispersed in the solid fat matrix. The system has been effective in controlled delivery of antibiotics and anti-inflammatory agents for 3–5 days after a single injection (Domb 1993a, 1993b). Lipospheres have also been used for the delivery of vaccines (Amselem et al. 1992a, 1992b) and local anesthetics (Masters and Berde 1994).

In an effort to develop a new topical formulation for deet that possesses reduced skin absorption as well as an increase in the duration of repellency, we have encapsulated deet into lipospheres and studied its skin absorption dynamics and duration of action. We hypothesized that encapsulation of deet will reduce its contact surface area with skin and reduce its evaporation rate from the skin surface, resulting in reduced dermal uptake and extended repellent activity. We report the preparation of a deet-encapsulated liposphere system, its repellent activity, and the

[1] Corresponding author, affiliated with the David Bloom Center for Pharmacy, The Hebrew University of Jerusalem.

[2] Present address: Houghten Pharmaceuticals, San Diego, CA 92121.

systemic absorption of topically administered deet–liposphere formulations.

MATERIALS AND METHODS

Materials: Deet was a gift from Morflex Inc. (Greensboro, NC) and contained 95% *N,N*-diethyl-m-toluamide and 5% related isomers. Radiolabeled deet (carbonyl C-14) 12.92 mCi/mmol with a radiochemical purity of >98.6% was purchased from New England Nuclear (Boston, MA). Hydrogenated vegetable oil (Sterotex®, melting point 65°C) was purchased from Capital City Products (Columbus, OH). Lecithin (Centrolex-D®) was purchased from Central Soya (Fort Wayne, IN). Dibasic sodium phosphate and monobasic potassium phosphate were purchased from EM Science (Cherry Hill, NJ). Propylparaben and methylparaben were obtained from Napp Chemicals Inc. (Lodi, NJ). All water used in this study was deionized. Absolute ethanol was purchased from Quantum (Newark, NJ). New Zealand white rabbits, 16 males weighing 4 kg, were purchased from Myrtle's Rabbitry (Jackson Station, TN).

Preparation of liposphere formulations: Formulations containing 10% deet were prepared by the addition of deet (10 g) and propylparaben (0.05 g) to 12.5 g of melted hydrogenated vegetable oil, followed by the addition of 72.5 ml phosphate buffer (pH 7.4, 0.05 M) containing methylparaben (0.10 g) and lecithin (5 g). The mixture was homogenized for 2 min using a Silverson L4 homogenizer (Silverson Machines, Waterside, England) to form a homogeneous dispersion and then cooled to room temperature to form a smooth, easy-to-apply lotion. Formulations containing 0 and 6.5% deet were prepared similarly; the formulation containing 20% deet was prepared using 20% hydrogenated vegetable oil.

Preparation of radiolabeled doses: The intravenous doses contained 160 μCi/80 mg of deet. The deet–liposphere formulation contained 20 μCi per gram of 10% formulation (20 μCi/100 mg deet). The radiolabeled deet liposphere formulation was prepared from deet (20 μCi/100 mg), hydrogenated vegetable oil, phosphate buffer, and phospholipid as described above. The alcoholic solution was prepared by the dilution of radiolabeled deet with ethanol to form a 10% solution containing 20 μCi/100 mg deet.

Characterization of lipospheres: Particle size of the liposphere formulations was determined using a Coulter LS100 particle size analyzer. The SEM analysis was conducted on a Philips 70 scanning electron microscope. The content of the phospholipid on the surface of lipospheres was determined by the trinitrobenzenesulfonic acid (TNBS) method using liposphere formulations containing phosphatidylethanolamine (PE) (New 1990). Deet content in lipospheres was determined by gas chromatography (GC) conducted on a Hewlett Packard 5890 GC with a HP 3396A integrator and a flame ionization detector. An HP Ultra2 (25 m × 0.2 mm × 0.11 mm), methyl phenyl silicone 5% column was used. The following conditions were used: detector and injector temperatures were 260°C, column temperature was 150°C, and helium gas at 40 psi. Viscosity measurements of formulations were conducted using an RTV Brookfield viscometer (Brookfield Labs., Stoughton, MA) using a Halipath spindle # B.

Storage stability: Ten percent deet–liposphere formulations (10 g) packed in 10-ml glass containers were stored at 4°C and 25°C in 60% humidity cabinets and the particle size, deet content, viscosity, and appearance were monitored for 12 months. Samples were analyzed in triplicate at 0, 1, 3, 6, and 12 months of storage.

Efficacy studies: The residual efficacy of liposphere formulations was evaluated on 3 volunteers who had given informed consent. The formulations were applied to the skin and exposed to mosquitoes. The time of 100% repellency (zero biting) was the index for determining the effectiveness of a formulation.

The formulations were applied on 4 locations on the arm of volunteers at a concentration of 2.5 mg/cm^2 on a total area of 12 cm^2 skin surface. Mosquitoes were placed in a screen-bottomed (18 mesh netting, 10-cm^2 exposure area) 1-oz. cylindrical cup, made of clear polymethacrylate, containing 15 5–15-day-old female mosquitoes displaying host-seeking behavior with access to the skin through the netting. The forearm was placed on the mosquito netting for 10 min every 30 min and the number of biting mosquitoes (evident by a blood meal) was recorded. Prior to any efficacy experiment the mosquitoes were tested on untreated skin to confirm their host-seeking behavior. Two mosquito species were tested: *Aedes aegypti* (Linn.) and *Anopheles stephensi* Liston, both aggressive biters.

Skin absorption studies: The pharmacokinetic profile of deet in rabbits was determined after intravenous administration (Group IV) and topical administration of 10% deet in ethanol (Group A) and 10% deet in lipospheres (Group B). Sixteen rabbits were used in the study; 8 rabbits received intravenously 20 μCi/10 mg/kg of radiolabeled deet in alcohol (Group IV). After a washout period of 3 days, 8 of the 16 rabbits received a 1-g/kg topical dose of 10% deet in ethanol (Group A) and 8 rabbits received a 1-g/kg topical dose of 10% deet in lipospheres (Group B). A summary of the experimental groups is shown in Table 1.

Preparation of the rabbits for the study: The

Table 1. Experimental groups used in the rabbit skin absorption study.

	Experimental group		
	Intravenous (IV)	A	B
Administration	IV—blood levels	topical—liposphere	topical—alcohol
No. of rabbits	8	8	8
Formulation	10% in alcohol	10% in liposphere	10% in alcohol
Dose administered (formulation)	0.1 ml/kg	1 g/kg	1 g/kg
Dose administered (deet)	10 mg/kg	100 mg/kg	100 mg/kg
Dose administered (μCi)	20 μCi	20 μCi	20 μCi

animals were housed in metabolic cages for the 7 days of the study and allowed free access to food and water. Prior to application of the topical doses, the back and neck of the rabbits were shaved. On the day of the study, the administration area was outlined with a 0.25-in.-high strip of polystyrene tape (secured to the animal with paper tape) in order to form a barrier for the dose.

Administration of radiolabeled doses: Intravenous doses containing one-tenth of the deet of the topical dose, but the same amount of radioactivity, were administered via the marginal ear vein over 15 sec and flushed with 0.9% NaCl. Topical administration was accomplished by application of the material to a 64-cm^2 square area of rabbit skin, which approximates 10% of the body surface area in a 4-kg rabbit. Rabbits received deet–liposphere formulation dose of 1 g/kg over a 8 × 8-cm^2 skin area. A nonocclusive cloth was then secured to the top of the barrier wall in order to collect evaporated deet, and the body of the rabbit wrapped to prevent removal of the patch. An Elizabethan collar was placed on the rabbit to inhibit animal access to the administration area. The residual dose was removed from the skin by washing and quantitated after 24 h.

Collection of samples: A heparin lock was placed in the ear of the rabbit for collection of the first several blood samples after administration of the intravenous and topical doses. Before drawing the samples, the lock was cleared of heparinized saline. Samples of 1.0 ml of blood were drawn at 0, 5, 10, 15, 30, 45, 60, and 90 min, and at 2, 3, 4, 6, 6.5, 7, 7.5, 8, 9, and 10 h for the intravenous doses. A sample was drawn immediately before administration of the topical dose for validation of the washout period. After topical administration, blood samples were drawn at 0, 5, 15, 30, 45, 60, 90, 120, 150, 180, and 210 min, and at 4, 5, 6, 8, 12, 16, and 24 h, and daily for a total of 7 days.

Urine was collected 24 h after intravenous administration and after 1, 2, and 3 days in each of the rabbits administered a topical dose. The rabbits were sacrificed by isoflurane overdose after 7 days. The application sites of the skin were collected and frozen at −70°C until analysis.

Determination of radioactivity in specimens: The specimens (blood, urine, skin, patch, and washings from dose removal) were homogenized and a known aliquot treated with tissue solubilizer (Soluable®, New England Nuclear) and decolorized with 30% hydrogen peroxide (Sigma, St. Louis, MO) according to the manufacturer's directions. The samples were then suspended in scintillation cocktail and the radioactivity quantitated by liquid scintillation counting. Scintillation counting was performed according to standard operating procedures on a Beckman LS60001C liquid scintillation counter (Beckman Instruments, Colombia, MD) equipped with internal quench and efficiency adjustments. Each sample was analyzed in triplicate and the mean taken as the true value.

Data analysis: The recovery of the radioactivity administered was calculated based upon the radioactive content of the organs tested, skin, patch removed, and urinary excretion. The absolute bioavailability was computed from the area under the curve (AUC) of a plot of radioactive content in the blood stream versus time, using piecewise trapezoidal integration. The mean AUCs of the group of rabbits receiving the topical applications were compared to the AUCs of the group of animals receiving the intravenous administration (each was corrected for dose) using the following formula:

$$\%\ \text{absolute bioavailability} = \{(\text{AUC [topical]})/(\text{AUC [IV]})\} \times 100.$$

RESULTS AND DISCUSSION

Preparation and characterization of deet-loaded lipospheres: The liposphere microdispersions containing deet incorporated in solid triglyceride particles were prepared by the melt method using common natural ingredients in one step without

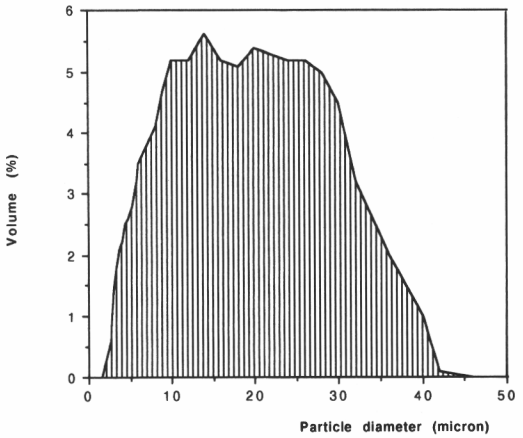

Fig. 1. Typical particle size distribution of deet–liposphere formulation. Obtained from Coulter LS100 particle size analyzer.

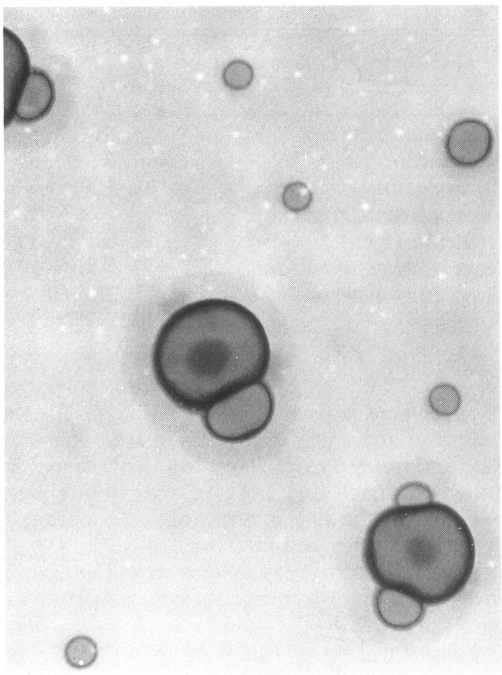

Fig. 2. Scanning electron microscope picture of a typical deet–liposphere formulation (33,000×).

the use of solvents. The formulation was preserved by parabens, propylparaben in the oil phase and methylparaben in the aqueous phase.

A unimodal particle size distribution was observed (Fig. 1). The average particle size was in the range of 15 μm, with less than 2% of particles greater than 100 μm.

In order to determine the structure of the liposphere particles, a macroscopic examination of a typical liposphere formulation and the surface phospholipid content was conducted. The microscopic examination showed spherical particles (Fig. 2). Determination of the surface phospholipid using the TNBS method showed that more than 90% of the phospholipid polar heads are in the surface of the liposphere particles. These data suggest that lipospheres are spherical with a monolayer of a phospholipid coating where the hydrophobic chains of the phospholipids are embedded onto the surface of the spherical triglyceride core containing the deet.

Storage stability: The formulations were stable for at least one year when stored at 4 and 25°C in a closed glass container, the deet content, particle size, and viscosity remained almost constant (Table 2).

Efficacy studies: The results are summarized in Table 3. The results are an average of 3 tests. The formulations were repellent for a minimum of 2.5, 3.5, and 6.3 h for the 6.5, 10, and 20% deet-containing lipospheres, respectively. The deet-free formulation (control) and the untreated groups did not show any activity against *Ae. aegypti,* and the 10% deet solution in alcohol was repellent for a minimum of 1.5 h.

Skin absorption: Figures 3 and 4 represent the blood concentrations after intravenous dosing (IV) and topical administration of 10% deet in ethanol (Group A) and topical administration of

Table 2. Stability of a formulation containing 10% deet–liposphere stored at 25°C.[1]

Storage time (months)	Deet content (%)	Particle size (μm)	Viscosity (× 10^{-3} cps)	Appearance
0	10.15 ± 0.20	15 ± 4	25 ± 1.5	white lotion
1	9.95 ± 0.25	15 ± 4	26 ± 2.3	white lotion
3	9.90 ± 0.22	17 ± 5	27 ± 2.4	white lotion
6	10.21 ± 0.10	14 ± 6	27 ± 1.6	white lotion
12	10.05 ± 0.15	16 ± 5	28 ± 2.3	white lotion

[1] Deet content, particle size, and viscosity were determined using GC analysis, Coulter LS100 particle size analyzer, and Brookfield viscometer, respectively. Results are average of 3 samples.

Table 3. Repellency effectiveness of deet–liposphere formulations.[1]

Time after application (min)	Number biting *Aedes aegypti* and *Anopheles stephensi*					
	Untreated[2]	Control[2]	% deet in formulation 6.5	10	20	Reference[3]
15	2 (5)	4 (0)	0 (0)	0 (0)	0 (0)	0 (0)
30	5 (4)	5 (0)	0 (0)	0 (0)	0 (0)	0 (0)
60	3 (5)	5 (3)	0 (0)	0 (0)	0 (0)	0 (0)
90	3 (5)	5 (3)	0 (0)	0 (0)	0 (0)	0 (0)
120	5 (5)	5 (5)	0 (0)	0 (0)	0 (0)	1 (1)
150	5 (3)	5 (5)	0 (0)	0 (0)	0 (0)	0 (1)
180	3 (5)	4 (4)	1 (1)	0 (0)	0 (0)	1 (2)
210	4 (4)	5 (3)	1 (1)	0 (0)	0 (0)	1 (1)
240	5 (3)	3 (4)	1 (2)	0 (2)	0 (0)	2 (1)
270	3 (4)	4 (3)	2 (2)	0 (2)	0 (0)	
300	4 (6)	4 (5)	3 (2)	1 (2)	0 (0)	
380	5 (6)	5 (5)			0 (0)	

[1] Cups with mosquitoes were placed for 10 min every 30 min on the skin of volunteers treated with formulation (2.5 mg/cm^2), and the number of bites were recorded. The results are average of 3 independent tests (cup placements), the results in parentheses are of *An. stephensi*.

[2] Untreated test was without any treatment, Control was a deet-free liposphere formulation.

[3] Reference formulation was 10% by weight of deet in ethanol.

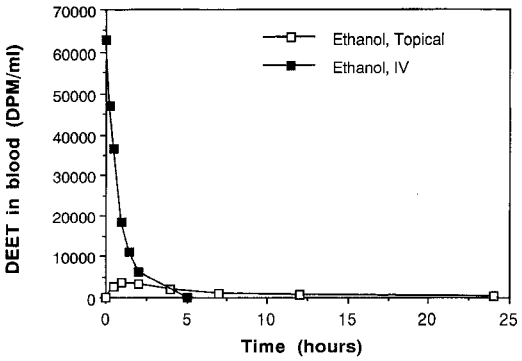

Fig. 3. Blood concentrations (DPM/ml) after intravenous and topical administration of 10% deet in ethanol solution. Ten percent deet solution (1 ml, 20 μCi/ml) administered intravenously (IV) or topically (surface area 64 cm^2) to rabbits. Results are average of 8 rabbits.

deet 10% in lipospheres (Group B). Figure 4 represents the blood concentration following topical administration data from Fig. 3 so that they may be compared directly on an expanded concentration axis. The AUC after topical dosing was 24,494 DPM/ml for Group A (10% alcohol solution) and 8,444 DPM/ml for group B (liposphere formulation). Therefore, the absolute bioavailability of deet from a 10% ethanol solution was 45%, whereas the bioavailability from lipospheres was only 16%, a 3-fold reduction in the amount of deet absorbed. This result is based on the assumptions that the absorption of deet from lipospheres and ethanol vehicles is a comparison of absorption only. The conclusion is also based on the assumption that the rate of elimination is not dose dependent and that elimination after bolus intravenous administration is the same as elimination after topical administration.

Table 4 summarizes the total urinary content of ^{14}C after intravenous and topical administration of deet formulations. About 74% of the IV-administered dose was collected in the urine, and 39 and 19% of the topically administered doses were collected for the alcoholic and liposphere formulations, respectively. Optimal collection of urine was difficult in the metabolic cages used in this experiment as evident from the large variation in urine collection (74 ± 23). Assuming that the error in urine collection is similar in all experiments, the difference in radioactivity contained in the urine after topical administration of the liposphere dosage form is about 50% that of the alcoholic dose, which corresponds to the blood bioavailability calculations. The total amount of deet recovered from skin (washing of residual dose and extraction from skin) was similar for both formulations, indicating that both formulations were similarly exposed to the skin

Table 4. Recovery of ^{14}C-deet in urine and skin of rabbits after intravenous (IV) and topical administration of alcoholic or liposphere formulation.[1]

Dose type	Percent recovery ± SD		
	Urine	Skin[2]	Total
IV—alcohol	74 ± 23	—	74 ± 23
Topical—alcohol	39 ± 5 (53)	12 ± 3	51 ± 3 (65)
Topical—liposphere	19 ± 8 (26)	12 ± 9	31 ± 8 (38)

[1] Results are % of administered dose, the numbers in parentheses are the corrected values for 74% recovery after intravenous administration.

[2] Combined radioactivity from washings and skin tissue content.

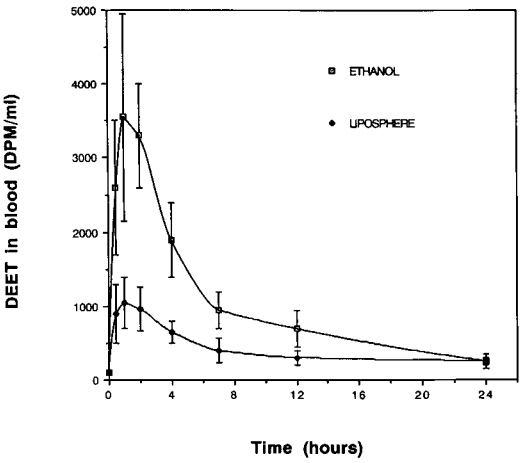

Fig. 4. Blood concentrations (DPM/ml) after topical administration of 10% deet in ethanol or liposphere formulation. Formulations 10% containing deet (1 ml, 20 μCi/ml) administered topically (surface area 64 cm^2) to rabbits. Results are average of 8 rabbits.

and thus the results are comparable. The nonrecovered deet after topical administration is probably due to evaporation of deet from the nonocclusive patches to the environment. The lower deet recovery of the liposphere formulation can be interpreted as more deet is released to the environment resulting in more repellent activity.

Conclusions: Encapsulation of deet in a novel stable liposphere microdispersion system resulted in improved efficacy and reduced dermal absorption. Deet-containing lipospheres (10%) were effective against mosquitoes for at least 3.5 h. The deet absorption through skin from these formulations was a third of that from alcoholic solution for the same concentration. The improved efficacy and reduced dermal absorption of deet, in addition to its composition of only 10% deet content encapsulated in common natural ingredients and the simple method of preparation, make this formulation attractive for consumer use.

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