Enhanced Transdermal Delivery of Diazepam by Submicron Emulsion (SME) Creams

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Diazepam, a lipophilic drug with CNS activity, serves here as a model to investigate the efficacy of SubMicron Emulsion (SME) as a novel transdermal vehicle. Diazepam was formulated in various topical regular creams and SubMicron Emulsion creams of different compositions. The different formulations were applied topically and protection against Pentamethylenetetrazole induced convulsive effects in mice was monitored. The efficacy of Diazepam applied topically in emulsion creams strongly depends on the oil droplet size and to a lesser degree - on the formulation composition and the oil type. Processing medium-chain-triglyceride (MCT) emulsion with a high-pressure homogenizer causes a drastic reduction in the droplet size, thereby significantly increasing the transdermal activity of Diazepam. In this case both the high-pressure homogenization and the presence of lecithin, an efficient dispersant, contribute to the effective droplet size reduction of below 1 micron, usually between 100–300 nm. The SubMicron Emulsions as vehicles for transdermal delivery of Diazepam generate significant systemic activity of the drug as compared with regular creams or ointments. Transdermal delivery of Diazepam via SME formulations is very effective, and the activity may reach the range of parenteral delivery. A single application of Diazepam in SME cream to mice skin provides pronounced transdermal drug delivery and prolonged protective activity up to 6 hours.

KEY WORDS: submicron emulsions; transdermal delivery; penetration enhancement; diazepam; anticonvulsive activity; pentamethylenetetrazole toxicity protection.

INTRODUCTION

Transdermal drug delivery is comparable with continuous intravenous infusion for some cases of systemic medications when constant drug level in blood is desired. Transdermal systems of different types are widely marketed for controlled drug delivery. (1). However,this route is limited due to poor transport of many drugs through the unaffected human skin. To overcome this limitation either chemical enhancers (1, 10) or physical methods, such as iontophoresis or electroporation are suggested (1, 6, 10, 13-14). Another attempt is utilization of novel drug delivery systems, especially colloidal delivery systems such as liposomes (2).

Submicron Emulsions (SME) is a novel vehicle of the oil-in-water type dispersion which design is much different from a liposome bilayer structure. Our earlier studies indicated that drugs incorporated in SME can penetrate through the skin to a greater extent compared to usual topical compositions (3, 15). Improved efficacy of different steroidal and nonsteroidal anti-inflammatory drugs and local anesthetics has been observed for SME formulations. Mean droplet diameter in SME is below 1 micron, usually 100-300 nm. They are suitable for the transport of hydrophobic poorly water soluble drugs.

The present study concerns the CNS activities of the hydrophobic drug, Diazepam, delivered transdermally in different well defined topical vehicles and compared to the SME vehicle.

MATERIALS AND METHODS

Miglyol 812 (Medium Chain Triglyceride, MCT) and Polyoxyethylated Cetostearyl alcohol (Marlowet T-25, 25EO) were purchased from Huls, Germany. Purified egg phosphatidylcholine Lipoid E-80 from Lipoid, Germany. Cetostearyl alcohol, Isopropyl myristate (IPM), Pentamethylenetetrazole, mineral oil, soybean oil (LCT), α-Tocopherol succinate, Triethanolamine, and Tyloxapol, were obtained from Sigma (USA). Paraffin (melting point 51-53°C) and Sodium Dodecylsulfate were from Merck, Germany. Carbopol 940 was purchased from Goodrich, USA. Diazepam was a gift from Teva Pharmaceuticals, Israel.

Sub-Micron Emulsion Preparation

100 g of Miglyol 812, 15 g lecithin (Lipoid E-80) and 0.4 g α-Tocopherol succinate were mixed together and stirred slowly with magnetic stirrer at 40-45°C for about 2 hours until complete dissolution. 2% Tyloxapol was dissolved in water. When SME was prepared without lecithin, nonionic surfactant was added to the oil phase.

The calculated amount of diazepam is dissolved at 50-55°C in the oil phase which is then mixed thoroughly with the water phase to obtain 20% (w/w) coarse emulsion. The mixture is subjected to high speed homogenization (Polytron PT3000; Kinematika, Switzerland) at 20,000 rpm for a short time, and sizing is completed by treatment with High Pressure Homogenizer (Gaulin APV-70, Netherlands), 8 cycles at 800 bar. The resulting SME preparation is filtered through 0.45 micron Nylon filter (Schleicher & Shuell, Germany). Hard paraffin submicron suspension was prepared by the same method, with Marlowet T-25 as surfactant instead of lecithin, and treated at elevated temperature (65°C).

The droplet size was determined by photon correlation spectroscopy (N4MD, Coulter, USA). Diazepam content in SME was determined by UV-spectroscopy. SME (5-20 μl) was dissolved in absolute ethanol, and absorbance was measured at 316 nm. Final Diazepam concentration was 0.5% (w/v) in all formulations.

SME Cream Preparation

Drug loaded SME was mixed with preswallen 10% Carbopol-940 water gel to reach final Carbopol concentration 0.8% (w/w). The pH was adjusted to 5.5 - 8.0 by triethanolamine and the preparation was well mixed to obtain a homogenous cream, convenient for topical application (viscosity about 100,000 cP).

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Large Droplets Emulsion Cream

Emulsion with Large droplets and the same composition as for the SME cream, was prepared by mixing of the oil and water phases with Polytron at 5,000 rpm for 10 seconds. Coarse emulsion (mean droplet size about 5 microns) was immediately jelled by Carbopol and triethanolamine to obtain corresponding cream.

Regular Cream Preparation

Emulsifying wax was prepared by melting together cetostearyl alcohol (2.7 g) and sodium dodecylsulfate (0.3 g). Drug loaded regular cream was obtained by heating simultaneously Diazepam (0.175 g), Emulsifying wax (3.0 g) and mineral oil (5.0 g), followed by boiling water addition (27.0 g) and intensive stirring. After cooling a soft cream with irregular oil droplets (size 10-100 microns) is formed.

Pharmacological Tests

White mice (BALB/C, 23-25g) were obtained from Anilab, Israel. The anticonvulsive activity of Diazepam (4) serves here to evaluate its transdermal pharmacological availability. Diazepam protection against Pentamethylenetetrazole (PTZ) induced convulsions in mice was scored, according to the neurological severity score, presented in Table 1.

Hair on the backs of the mice were carefully clipped out, under light ether anesthesia, 1-3 days before the experiment. Diazepam (20 mg/kg) in the cream formulations was applied topically and gently rubbed into 4 cm^2 of the mice skin.

PTZ (150 mg/kg) was injected, according to the time schedule, intraperitoneally, and a behavior score was recorded. Monitored parameters are placed in Table 1 in accordance with their development and severity.

Score values above 37 indicate complete absence of protection, all animals died; 26 - 35 points - slight protection, severe convulsions, part of animals died; 20-26 points - medium protection, all symptoms are present except lethality; below 20 points -satisfactory protection, toxicity signs are not severe; score less than 12 points - only minor signs of PTZ poisoning.

RESULTS AND DISCUSSION

Droplet Size Influence

Our basic observation is that Diazepam in SME is much more effective compared with large droplets cream (Fig. 1).

The two cream formulations, applied topically, are identical in composition and differ only in their droplet size, 100 nm for SME cream and 5 microns for large droplets cream. However, Diazepam score for the preparation containing the submicron particles indicates much better activity. Thus, when changing from large droplets (5 microns) formulation to SME creams (100 nanometers), the Diazepam activity increases as is evident from the decrease in the PTZ induced score from 34.3 to 16.3 (Fig. 1). Figure 1 also illustrates that two hours after application the protective activity of topically applied Diazepam at a dose of 20 mg/kg in MCT-lecithin SME cream is comparable with parenterally delivered Diazepam at a dose of 10 mg/kg.

Time profiles of the protection activity of topical Diazepam formulations compared to parenterally injected Assival® are presented in Fig. 2. From the figure it is evident that transdermally delivered Diazepam in SME cream provides much greater protective activity than that furnished by the large droplet cream. Paired T-test showed that these groups are significantly ($p < 0.001$) different, while SME cream (20 mg/kg) and parenteral Diazepam groups are not statistically different ($p > 0.5$). A single topical application of Diazepam (20 mg/kg) in SME cream on the mice skin (4 cm^2) provides satisfactory drug delivery for up to 6 hours, comparable with parenteral Diazepam administration (10 mg/kg). It could be hypothesized that SME forms drug depot in the animal skin, which is accountable for slow, continuous and controlled systemic release of the drug (1).

The dependence of the activity of transdermally delivered Diazepam on the diameter of the oil particles in various preparations is presented in Fig. 3. Transdermal activity of Diazepam in emulsions increases with the reduction of the droplet size. Drug penetration by SME vehicle with droplets of a mean diameter below 200 nanometers is apparently more efficient. It is possible that for SME vehicle, different pathways like hair follicles, sebaceous channels, pores or paracellular ways are much enhanced.

Recently Rolland and coworkers observed direct influence of the particles size on their penetration into the skin (5). It was found that the percutaneous penetration pathway of polymeric microspheres is size dependent. Particles below 3 microns were randomly distributed into the stratum corneum and hair follicles. The main penetration pathway of these microspheres was the transepidermal route since the outer surface of the follicular orifice is only 0.1% of the total skin surface area. The largest microparticles (>10 - 20 microns) did not penetrate the skin and remained on the outer surface of the stratum corneum. This observation is in a good agreement with our results.

Lecithin and Oil Type Influence

Lecithin significantly improve Diazepam protective activity in topical preparations. It is especially evident for nonpolar mineral oil based SME preparations (Score drops from 37.8 to 21.0, respectively). This effect could not be assigned to the increase of the drug solubility in the internal lecithin phase. We found (see Table 2) that addition of 15% of lecithin to oil phase does not change Diazepam solubility at 35°C (rat skin temperature). Therefore the surface activity of lecithin contributes only to droplet size reduction. This reduction is apparently the driving force for enhanced penetration. For polar MCT or less polar LCT formulations activities changes although not so dramatic, were however observed (see Fig. 4). In mineral oil SME, Diazepam solubility is low and the absence of precipitation suggests drug association with the lecithin layer at the droplets interface.

The oil type influence on Diazepam activity was examined in SME preparations, composed of various oils. The protection level at 2 hours post topical application was compared for SME cream formulations, prepared without lecithin. All preparations had a comparable droplet size (about

Table 1. PTZ Induced Convulsions—Neurological Severity Score

Scored parameter	Interval	Points
Tremor intensity	absence	0
	weak periodic	1
	mild constant	2
	strong	3
	very strong	4
Tail stand	absence	0
	weak	1
	moderate	2
	strong	3
Postural tonus	non disrupted	0
	slightly disrupted	1
	moderately disrupted	2
	strongly disrupted	3
Ataxia	absence	0
	weak	1
	moderate	2
	strong	3
Tail flick	absence	0
	weak	1
	moderate	2
	strong	3
CLONIC CONVULSIONS		
Latent time before convulsions	more than 10 minutes	1
	6 to 10 minutes	2
	3 to 6 minutes	3
	1 to 3 minutes	4
	0 to 1 minute	5
Intensity	absence	0
	weak	1
	moderate	2
	strong	3
CLONIC - TONIC CONVULSIONS		
Latent time before convulsions	more than 15 minutes	2
	10 to 15 minutes	4
	5 to 10 minutes	6
	2 to 5 minutes	8
	0 to 2 minutes	10
Intensity	absence	0
	weak	1
	moderate	2
	strong	3
Death (time after PTZ admistration)	no death	0
	10 to 15 minutes	6
	5 to 10 minutes	8
	2 to 5 minutes	10
	0 to 2 minutes	12
Maximal score, points		49

200 nm) and equal drug loading. The results obtained indicate (Fig. 5) that the polarity of the oil is an important factor for Diazepam activity in SME topical vehicle. Diazepam solubility in oils is proportional to the oil polarity (Table 2). Nonpolar mineral oil (liquid paraffin) in such formulation, where Diazepam solubility is minimal, demonstrates the lowest degree of protection, when compared to polar medium chain (capric/caprylic) or long chain (soya oil) triglycerides and isopropylmyristate with intermediate polarity.

Topically applied lipids modify the fluidity of the intercellular lipid bilayers in *stratum corneum* (6,7). The type of the lipids comprising the topical vehicle influence the degree of such modification and therefore the magnitude of drug transport. In the lipid regions at least two types of disorder can be distinguished: the disorder of the alkyl chains inside one lipid bilayer (a shorter range disorder) and the disorder in the lipid bilayer arrangement (a longer range disorder) (8). The structural features of compounds that induce changes in

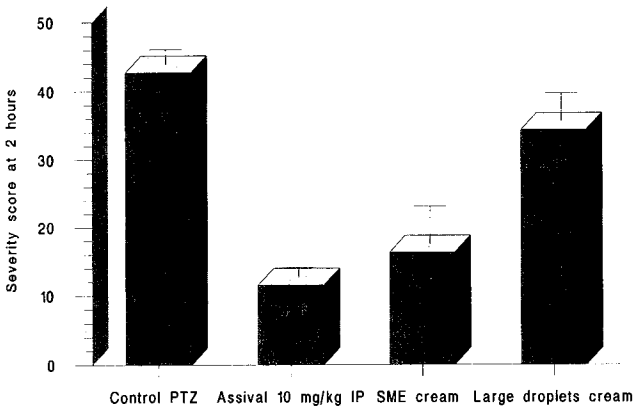

Figure 1. PTZ (150 mg/kg IP) toxicity protection by transdermal Diazepam (20 mg/kg topically) in various emulsion creams. Assival® 10 mg/kg (Diazepam for injection, Teva, Israel) was used as positive control. All animals were injected with PTZ 2 hours after Diazepam application. Digits in columns - number of died mice in group of 6.

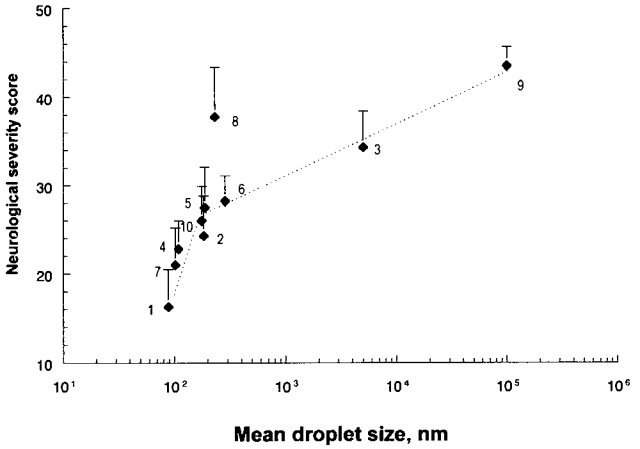

Figure 3. Influence of the droplet size on the anticonvulsive activity of transdermally delivered Diazepam (20 mg/kg, topically) in different emulsions. PTZ (150 mg/kg, IP) was injected 2 hours after the application of the cream. Numbers near the points correspond to the formulation number in Table 3.

the skin permeability include an alkyl chain of around 8-16 carbon atoms and a polar head group (9-10). Capric/caprylic triglycerides (MCT) incorporate into structured skin lipid more readily than less polar long chain triglycerides (LCT), isopropylmyristate (IPM) or mineral oil. Accordingly, Diazepam activity in SME vehicles is correlated to the oil type and its influence on the skin lipid arrangement. Very interestingly, this phenomena is not observed at all for creams with large sizes of oil droplets, which manifested very low activity.

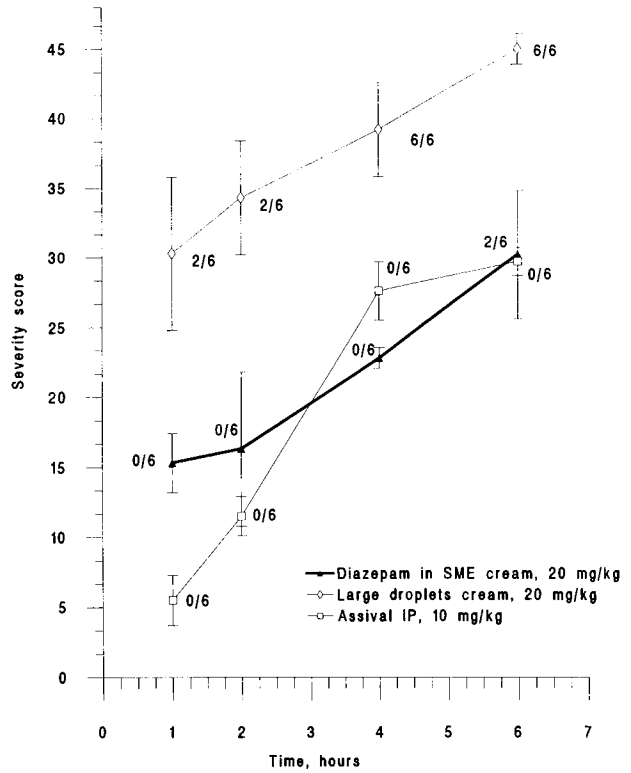

Figure 2. Time profile of Diazepam protection against PTZ toxicity. Diazepam (20 mg/kg, topically) or Assival® (10 mg/kg, IP) as a reference, were administered at T = 0 hours. PTZ (150 mg/kg, IP) was given to the different groups of mice (N = 6 for each group) at each point of time and the score measured immediately. Digits near the points denotes the number of died mice in a group of 6.

Extrinsic and Intrinsic SME Preparations with Hard Paraffin

Two types of paraffin oil were tested for SME Diazepam preparations - liquid paraffin (mineral oil) and solid paraffin (melting point 51-53°C). Solid paraffin nanoparticles are known to form an occlusive layer after topical application of the suspension (11), in contrast with liquid oils. Actually, the solid paraffin SME with Diazepam demonstrates higher protective activity than SME with mineral oil and similar droplet size (Formulations 8 and 10 respectively, Table 3). It is contributed to the elevated entrapment of the drug into the molten paraffin droplets. Similar phenomena has been described for hydrophobic drug incorporation into lipid nanoparticles: after cooling, drug forms crystallic core inside the lipid phase (12). Solid particles obtained by this way are loaded with the drug in higher quantities than liquid mineral oil droplets. However, solid paraffin nanoparticles are not able to dissolve Diazepam while not melted. This fact enabled us to prepare two identical Diazepam SME compositions where Diazepam is either dissolved in the internal oil phase or dispersed in the external phase. Thus, extrinsic or intrinsic addition of Diazepam allows to control the drug location in different SME regions. Mixing of the Diazepam powder with a plain submicron hard paraffin cream (extrin-

Table 2. Diazepam Solubility in Different Types of Oil in Presence or Absence of Egg Lecithin at 35°C

Diazepam solubility, mg/ml, in:	MCT	LCT	Mineral oil	IPM
Pure oil	28.5 ± 1.2	15.5 ± 2.0	3.0 ± 0.2	15.6 ± 1.0
Oil phase with 15% Lecithin	24.5 ± 2.8	14.6 ± 2.2	3.5 ± 0.5	15.0 ± 1.5

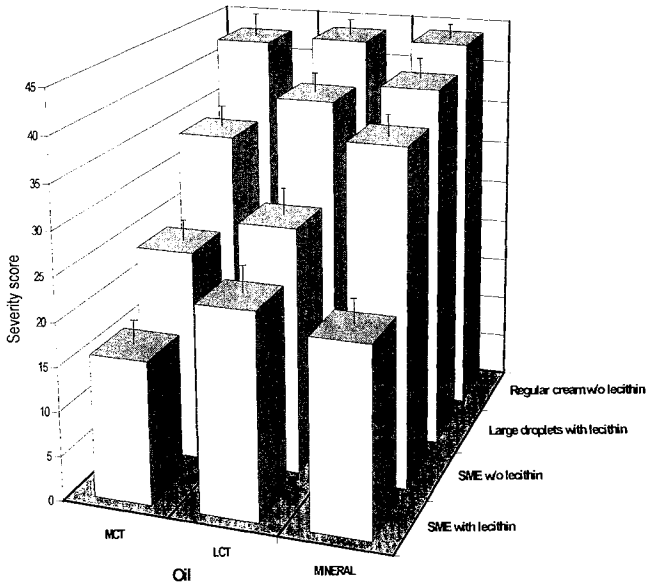

Figure 4. Lecithin influence on topically applied Diazepam. All animals were injected with PTZ (150 mg/kg, IP) 2 hours after Diazepam application (20 mg/kg, topically); for each experiment N = 6 mice.

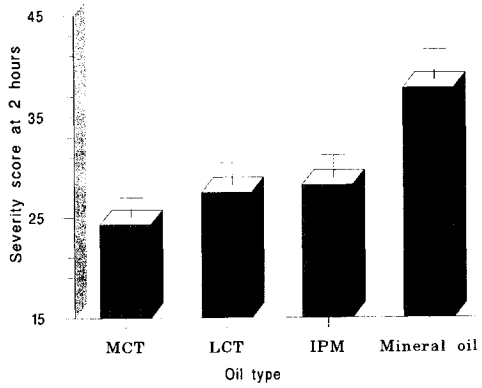

Figure 5. Diazepam protection against PTZ toxicity as a function of the oil type in topical SME formulations. All animals were injected with PTZ (150 mg/kg, IP) 2 hours after Diazepam (20 mg/kg, topically) application. For each experiment N = 6 mice. MCT - medium chain triglyceride, LCT - long chain triglyceride (soya oil), IPM - isopropylmyristate. Lecithin is absent in all formulations.

sic addition) leads to a slight protective effect due to improved drug absorption under occlusive paraffin layer (11), however intrinsic submicron preparation does provide significant and much more enhanced effect (Table 3, Formulations 10 and 11, respectively).

CONCLUSIONS

The data shown in this study demonstrate that incorporation of a hydrophobic drug such as Diazepam into Submicron Emulsion is an efficient way for significant improvement of drug penetration through the *stratum corneum*. A pronounced systemic activity could be achieved without irritative permeation enhancers and organic solvents, such as dimethylsulfoxid, Azone® (N-dodecylcaprolactam), oleic acid, ethyl and isopropyl alcohol etc. Decrease of the droplet diameter below 200 nm significantly increases the transdermal penetration of Diazepam. For the most effective SME formulation, comprised of polar oil with droplet size about 100 nm, the activity level was comparable to that obtained with parenteral delivery. A single topical application of Diazepam in the SME cream onto mice provided satisfactory drug deliver for up to 6 hours. Submicron emulsion is a promising vehicle for enhanced transdermal delivery of hydrophobic drugs with systemic activity, as compared to other emulsion preparations.

Table 3. Topical Formulations for Transdermal Delivery of Diazepam and Their Protective Activity (Diazepam Dose 20 mg/kg, Cream was Applied 2 Hours Before PTZ (150 mg/kg, IP) Injection)

#	Oil type	Lecithin[a]	Droplet size, nm ± S.D.	Score at 2 hours ± S.D.
1	MCT	+	88 ± 25	16.3 ± 5.6
2	MCT	−	183 ± 36	24.3 ± 6.5
3	MCT	+	5μm Large[b]	34.3 ± 4.1
4	LCT	+	109 ± 28	22.8 ± 3.2
5	LCT	−	188 ± 49	27.5 ± 4.6
6	IPM	−	285 ± 54	28.2 ± 2.9
7	Mineral oil	+	102 ± 42	21.0 ± 6.1
8	Mineral oil	−	231 ± 68	37.8 ± 5.6
9	Mineral oil	−	100 μm Regular[c]	43.5 ± 2.1
10	Paraffin + DZ intrinsic[d]	−	176 ± 53	26.0 ± 6.9
11	Paraffin + DZ extrinsic[e]	−	171 ± 60	34.8 ± 5.1
12	Assival IP 10 mg/kg			11.6 ± 2.1
13	CONTROL PTZ 150 mg/kg			43.8 ± 2.2

[a] Lecithin - Presence or absence of 15% of Lipoid E-80 in the lipid phase of the SME.
[b] Large - coarse emulsion with droplets about 5 micrometers.
[c] Regular - Emulsifying wax based cream, droplets larger than 100 micrometers.
[d] DZ intrinsic - Diazepam dissolved in molten paraffin.
[e] DZ extrinsic - Diazepam powder added to plain submicron paraffin suspension.

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