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PG-liposomes: novel lipid vesicles for skin delivery of drugs

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Abstract

A novel type of lipid vesicles, propylene glycol-embodying liposomes or PG-liposomes, composed of phospholipid, propylene glycol and water, is introduced. The new lipid vesicles were developed and investigated as carriers for skin delivery of the model drug, cinchocaine base. PG-liposomes showed high entrapment efficiency and were stable for at least one month of storage at 5 ± 1°C. Preliminary in-vivo skin deposition studies, carried out using albino rabbit dorsal skin, showed that PG-liposomes were superior to traditional liposomes, deformable liposomes and ethosomes, suggesting that PG-liposomes, introduced in the current work, are promising carriers for skin delivery of drugs.

Introduction

Propylene glycol (PG) has been widely used as a stand-alone penetration enhancer and as a vehicle for application of accelerants (Williams 2003). It is a well-accepted adjuvant in topical formulations and its enhancing effect on skin permeation, arising from structural changes, is marginal (Yamane et al 1995). As a vehicle, PG works synergistically with many enhancers (Williams 2003). Therefore, it was speculated that incorporation of PG in liposomal structures may result in a possible synergistic effect that leads to a promising vesicular formulation, similar to ethosomes but lacking the drastic effect of ethanol on skin (El Maghraby et al 2000) that limits the use of ethanol-containing topical formulations in some skin disorders. Additionally, incorporation of PG is expected to improve vesicular entrapment efficiency of several drugs, as a result of improved solubility of several drugs in PG. Improved stability of vesicular formulations is also expected as a result of increased viscosity. However, due to the solubilizing and the interdigitation effect of PG on lipid bilayers, it was doubted whether vesicles can coexist with PG in the suggested formulations.

Cinchocaine is a topical amide local anaesthetic. It is one of the most potent long-acting local anaesthetics (Sweetman 2003). However, it is also one of the most toxic local anaesthetics and is now generally only used for surface anaesthesia (Sweetman 2003). Foldvari et al (1993) attempted to formulate liposomal cinchocaine. They concluded that cinchocaine is difficult to be formulated in a physically stable liposomal formulation with high entrapment efficiency and without crystal growth (Foldvari et al 1993).

The aim of this study is to introduce, develop and evaluate novel lipid vesicles, the propylene glycol-embodying liposomes or PG-liposomes, as carriers for skin delivery of drugs. Cinchocaine base is used as a model problematic drug in liposomal formulations. Characterization and evaluation include determination of vesicle size and entrapment efficiency, stability testing and a preliminary study of in-vivo skin deposition behaviour, using albino rabbit dorsal skin, in comparison with traditional liposomes, deformable liposomes and ethosomes.

Materials and Methods

Lipoid S 100 (phosphatidylcholine (PC) from soybean lecithin) was a kind gift from Lipoid GmbH (Ludwigshafen, Germany). Cinchocaine base (Orgamol, Switzerland) was kindly supplied by Pharaonia Pharmaceuticals (Alexandria, Egypt). Sodium cholate was kindly

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supplied by New Zealand Pharmaceuticals Limited (Palmerston North, New Zealand). All other chemicals were of analytical grade and used as received.

During preliminary studies that were conducted, under non-buffered conditions, to prepare cinchocaine liposomal formulations containing a total cinchocaine concentration of 0.5% w/w (the minimum concentration required for anaesthesia (Foldvari et al 1993)), excessive drug crystallization was observed just after addition of the hydration medium or a few minutes after preparation when formulations were left to cool at ambient temperature. Trials were initiated to overcome drug crystallization and to allow preparation of a stable liposomal formulation of the potent long-acting local anaesthetic, cinchocaine, containing a total drug concentration of 0.5% w/w. Improving the stability was based on using buffers of finely tuned pH instead of distilled water. Reducing pH was expected to improve the drug solubility (increase the ionized form proportion) in the external phase, which would subsequently reduce the possibility of crystallization. Unfortunately, it was expected also to result in reduction of entrapment efficiency. Therefore, buffers of gradually reduced pH (so as to attain the highest pH that could produce stable vesicles while maintaining the maximum possible entrapment efficiency) were examined, in each liposomal formulation, until stable crystal-free formulations were obtained. Optimum buffer pH values were pH 6.0 for ethosomes and PG-liposomes and pH 5.5 for deformable and traditional liposomes. This difference could be explained by the presence of either PG or ethanol, which improves the drug solubility in the case of PG-liposomes or ethosomes, while in the case of deformable and traditional liposomes, containing the same total drug concentration, further reduction in pH was necessary to avoid crystallization. Details of final stable formulations are shown in Table 1.

PG-Liposomes were prepared as follows. Lipoid S 100 and the drug were dissolved in PG at 60°C. Phosphate buffer (0.1 M, pH 6.0) was added slowly in a fine stream, at constant rate, in a well-sealed container, with constant mixing at 700 rev min^{-1}. Mixing was continued for an additional 30 min. The system was kept at 60°C throughout the preparation. A milky white dispersion was formed. Traditional and deformable liposomes were prepared by the conventional mechanical dispersion method, as described previously (El Maghraby et al 1999; Elsayed et al 2007). Ethosomes were prepared as described previously (Dayan & Touitou 2000; Elsayed et al 2006).

Vesicle size and size distribution were determined using the particle size analyzer CILAS 1064 (Cilas, USA), which works on a laser diffraction principle. Filtered distilled water was used as dilution medium. Entrapment efficiency was measured by the ultracentrifugation method. Experiments were run in at least triplicate. For assessment of stability, vesicles were stored in sealed tubes refrigerated at 5 ± 1°C. Sampling was performed at intervals of 15 and 30 days or only after 30 days. Stability was assessed through optical microscopical investigation to ensure absence of crystallization. Assessment of vesicular size over time was also carried out.

In-vivo skin deposition studies employed male albino rabbits (University of Alexandria, Egypt), 1.00–1.25 kg, and were conducted in accordance with the institutional animal care ethical guidelines, adhering to the Guide for the care and use of laboratory animals (published by the U.S. National Academy of Sciences, 1996) and to the Guide to the care and use of experimental animals (Published by Canadian Council on Animal Care, 1993). Rabbits were prepared, one day before topical application, by manually trimming the dorsal hair, to a maximum length of 2 mm, using an electrical razor. The skin was then wiped gently with a cotton swab moistened with 50% v/v ethanol in distilled water. A general anaesthetic (Thiopental (Sandoz, Austria), i.p., at an initial dose of 30 mg kg^{-1}, followed by 10 mg kg^{-1} every 30 min) was used to keep test rabbits stress-free during manipulations. The precise application site (3.14 cm^2) on the rabbit's back was marked and the appropriate amount (100 μL) of the formulation was applied with a micropipette on the skin. The applied formulation was uniformly spread on the application site area (using the side of the micropipette tip) and allowed to dry. Application sites on the same rabbit were separated from each other by at least 2 cm. Three hours after application of the formulation, rabbits were killed by overdose of the anaesthetic agent and the treated skin areas were carefully excised. Excised skin areas were wiped free of the formulation on the skin surface with a cotton swab moistened with 50% v/v ethanol in distilled water. Samples were stored in a freezer until assay. An HPLC analytical method that was previously developed and validated in our laboratory (Elsayed 2007) was used, with slight modifications, for determination of cinchocaine in rabbit skin after in-vivo application of different cinchocaine formulations. The method was validated for extraction recovery, selectivity, linearity, accuracy and precision as described previously (Elsayed 2007).

Table 1 Formulation code and composition of final stable cinchocaine liposomal formulations

Code	Type	Composition[a]			
		Lipid/surfactant	Ethanol	PG	Aqueous phase
PGL-10	PG-liposomes	PC	—	10% (w/v)	0.1 M pH 6.0 phosphate buffer
PGL-20	PG-liposomes	PC	—	20% (w/v)	0.1 M pH 6.0 phosphate buffer
DL	Deformable liposomes	PC–sodium cholate; 86:14 (w/w)	7% (w/v)	—	0.1 M pH 5.5 phosphate buffer
ES	Ethosomes	PC	30% (w/v)	—	0.1 M pH 6.0 phosphate buffer
TL	Traditional liposomes	PC	—	—	0.1 M pH 5.5 phosphate buffer
TL-Chol	Traditional liposomes (membrane-stabilized)	PC–cholesterol; 1:1 (molar ratio)	—	—	0.1 M pH 5.5 phosphate buffer

[a]Concentration of phosphatidylcholine and cinchocaine base in all formulations was 4.0 and 0.5% w/v, respectively.

All reported data are expressed as mean ± s.e.m. Statistical significance was examined using one-way analysis of variance followed by Bonferroni and Tukey multiple comparison post tests (GraphPad Prism 4.02, GraphPad Software Inc.) with $P < 0.05$ as a minimal level of significance.

Results and Discussion

Due to the solubilizing and the interdigitation effect of PG on lipid bilayers, it was doubted whether vesicles could coexist with PG in the suggested formulations. In this study, using 10–20% PG concentration, the formation of a persistent milky white dispersion indicated the formation of vesicles, which was verified by optical microscopical investigation. Vesicular size analysis further verified the formation of vesicles. Mean vesicle size over time and entrapment efficiency of each formulation are shown in Table 2.

Regarding vesicle size, deformable liposomes had significantly ($P < 0.001$) lower mean vesicle size relative to traditional liposomes, which could be explained by inclusion of the surfactant, in addition to 7% v/v ethanol used (Elsayed et al 2007). Ethosomes had also significantly ($P < 0.001$) lower mean vesicle size relative to traditional liposomes, a characteristic of ethosomes (Dayan & Touitou 2000) that was previously explained by Touitou et al (2000) and López-Pinto et al (2005). Incorporation of 10% PG also resulted in vesicles with significantly lower mean vesicle size relative to traditional liposomes ($P < 0.001$). PG-liposomes with 10% PG were not significantly different from deformable liposomes and ethosomes. Increasing PG concentration to 20% did not significantly affect vesicle size.

Deformable liposomes significantly ($P < 0.001$) improved entrapment efficiency over traditional liposomes, ethosomes and PG-liposomes with 10% PG. However, deformable liposomes were not significantly superior to PG-liposomes with 20% PG. Incorporation of an anionic edge activator is expected to produce negatively charged vesicles. This may give an explanation for the observed higher entrapment efficiency of deformable liposomes based on a possible ionic interaction between the cationic drug and the negatively charged vesicular lipid bilayer components. Ethosomes failed to significantly improve the entrapment efficiency over traditional liposomes. Cinchocaine PG-liposomes with 10% PG showed significantly improved entrapment efficiency over traditional liposomes and ethosomes. This may be explained by the presence of PG, which allows for better solubility of cinchocaine. Increasing PG concentration to 20% significantly improved entrapment efficiency. These findings suggest that PG-liposomes and deformable liposomes are promising cinchocaine carriers that could provide improved drug entrapment efficiency.

All final liposomal formulations showed continued absence of crystallization during 30 days storage at 5 ± 1°C. No significant change in vesicle size was observed during 30 days of storage, suggesting successful preparation of stable cinchocaine liposomal formulations with high entrapment efficiency.

Results of in-vivo skin deposition studies are shown in Table 3. Statistical examination using one-way analysis of variance revealed significant difference ($P < 0.0001$) among different formulations. Incorporation of cholesterol in traditional liposomes did not significantly affect cinchocaine skin deposition. Deformable liposomes and ethosomes also did not significantly improve cinchocaine skin deposition over traditional liposomes. However, in the case of deformable liposomes, a possible improvement might be expected after prolonged exposure, based on the idea that more time (lag period) might be necessary for complete drying of the formulation, which would subsequently initiate improved vesicular penetration into the stratum corneum (Cevc & Blume 1992).

PG-liposomes with 10% PG significantly improved ($P < 0.01$) cinchocaine skin deposition relative to all other liposomal formulations, even relative to deformable liposomes and ethosomes, having reported promising properties as carriers for skin delivery of drugs. Skin-deposited cinchocaine was 3.0-, 2.1-, 2.6- and 2.0-fold higher from PG-liposomes with 10% PG, relative to traditional liposomes, cholesterol-containing traditional liposomes, deformable liposomes and ethosomes, respectively. Improved skin delivery is expected to be partially due to the penetration-enhancing effect of PG. However, cinchocaine solution in 10% v/v PG in 0.1 M pH 6.0 phosphate buffer showed only slight and non-significant increase (1.3 fold, $P > 0.05$) in cinchocaine skin deposition relative to solution in 0.1 M pH 5.5 phosphate buffer (Elsayed 2006), while PG-liposomes with 10% PG resulted in significant improvement (3.0 fold, $P < 0.01$) in cinchocaine skin deposition relative to traditional liposomes, suggesting that

Table 2 Entrapment efficiency and vesicle size, over time, of different formulations

Formulation code	Entrapment efficiency (%)	Mean vesicle size (nm)		
		1 day	14 days	30 days
PGL-10	64.229 ± 0.994	78.7 ± 2.1	73.0 ± 0.8	68.4 ± 0.5
PGL-20	76.914 ± 2.609	93.8 ± 3.9	89.3 ± 7.3	85.4 ± 11.5
DL	84.293 ± 0.257	88.7 ± 3.4	—	88.5 ± 7.3
ES	52.867 ± 2.398	75.9 ± 2.3	—	90.0 ± 6.2
TL	47.466 ± 0.879	231.9 ± 5.7	—	217.9 ± 3.6
TL-Chol	37.365 ± 3.356	178.6 ± 1.6	—	176.5 ± 0.0

Data are means ± s.e.m. Absence of crystallization was verified for all formulations, during 30 days storage at 5 ± 1°C.

Table 3 In-vivo skin-deposited cinchocaine, in rabbit dorsal skin, after 3 h of non-occlusive application, from different liposomal formulations

Formulation code	Cinchocaine deposited (%)
PGL-10	9.818 ± 1.339 (n = 6)
PGL-20	9.221 ± 1.000 (n = 6)
DL	3.765 ± 0.300 (n = 7)
ES	4.903 ± 0.281 (n = 5)
TL	3.313 ± 0.167 (n = 4)
TL-Chol	4.718 ± 0.488 (n = 4)

Data are means ± s.e.m.

the penetration-enhancing effect is not the only factor operating. A possible synergistic effect may exist. PG-liposomes with 20% PG were not significantly different ($P > 0.05$) from PG-liposomes with 10% PG.

Conclusion

Results of this work suggest successful preparation of a novel type of lipid vesicle, the propylene glycol-embodying liposome or PG-liposome. PG-liposomes of cinchocaine base, a model problematic drug in liposomal formulations, showed high entrapment efficiency and were stable for at least one month of storage at 5 ± 1°C. Preliminary in-vivo skin deposition studies confirmed that PG-liposomes are promising as carriers for skin delivery of drugs. Studies are encouraged to further investigate the effectiveness of this newly developed system and the possible mechanisms by which these vesicles could improve skin delivery of drugs.

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