FORMULATION OF BANZOCAINE LOADED PG-LIPOSOMES FOR ENHANCED SKIN DELIVERY

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Abstract

Present work investigates the enhanced dermal delivery of Benzocain (BNZ) from novel vesicular systems, "PG-liposomes" for topical anesthesia and pain relief. PG-liposomes were prepared from soya lecithin, propylene glycol and distilled water and were extensively characterized for shape and size, size distribution, surface charge and percent drug entrapment. The formed PG-liposomes were spherical in nature, nanometric in size and multilamellar vesicles. Zeta potential measurement reveals that PG imparted net negative charge on vesicles. Better percent drug entrapment (PDE) was found in PG- liposomes as compare to simple liposomes in which small aqueous core is available for BNZ solubilization.

Keywords*: anesthesia, liposomes, nanometric, banzocaine.*

Introduction

Benzocaine, a para-aminobenzoic acid ester, is a local anaesthetic used topically for pain relief act by blocking the influx of sodium ions and stop the propagation of the nervous impulse. It is used, often in combination with other drugs such as analgesics, antiseptics, antibacterials, antifungals and antipruitics for the temporary local relief of pain associated with dental conditions, oropharyngeal disorders, hemorrhoids, anal pruritus and ear pain. Still systemic absorption which can can cause toxic effects together with rapid but short acting efficacy required the effective drug delivery of this drug (Covino and Vassallo, 1976; Barclay and Vega, 2004).

Lately, different drug delivery approaches have been successfully explored to achieve effective drug transport into and across the skin. Among these, liposomes have shown promising results, by virtue of their unique physicochemical character and diversity in their constructs. Liposomes have proved to be a very effective means of delivery, especially for topical administration. (Mezei and Gulasekharam, 1982; Fresta and Puglisi, 1996) Characteristic merits of liposomes, to get a more conducive milieu within the unique amphiphillic interiors of vesicular compartments, favoring its partitioning and transport across the skin barrier. Mura et al (2007) recently proposed the liposomal formulation of benzocaine for improving its clinical effectiveness in topical anaesthesia (Mura et al., 2007). Propylene glycol (PG) has been widely used as a stand-alone penetration enhancer and as a vehicle for application of accelerants (Yamane et al., 1995). It is a well-accepted excipient in topical formulations and its enhancing effect on skin permeation, arising from structural changes, is marginal. As a vehicle, PG works synergistically with many enhancers (Yamane et al., 1995). 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. 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.

The development of a new effective topical drug delivery system intended to suitably modulate the benzocaine release rate, thus prolonging its anaesthetic effect, and to enhance its localization in the skin, thus reducing its systemic toxicity, could be particularly useful. In Present study, aim was to develop a novel vesicular formulation of

benzocaine, intended to improve its clinical effectiveness in topical anaesthesia in terms of both enhanced intensity and prolonged duration of action.

2. Materials and Methods

2.1. Materials

Benzocaine (benzocaine (BNZ) (ethyl-4-aminobenzoate, pKa = 2.8, log P = 1.44) and Lipoid S 100 (phosphatidylcholine (PC) from soybean lecithin) was a kind gift from Cipla Limited, Mumbai and Lipoid GmbH (Ludwigshafen, Germany), India respectively. Cholesterol (CHOL), and Sephadex G-50 medium (bead size range 50–150 μm), were procured from Sigma Chemical Co. (St. Louis, MO, USA). Methylcellulose (viscosity grade: 3000–5000 mPas, 2%/20°C) was procured from LOBA-Chemie (India). All other materials and solvents obtained from commercial sources were of analytical grade. Double-distilled water was used throughout the experimental studies.

2.2. Preparation of lipid vesicles

The PG embodying liposomes systems investigated here were composed of 2–5% Soya lecithin (SPC), 20–50% PG, BNZ and water to 100% w/w. SPC was dissolved in PG. Aqueous solution of BNZ (1% in PBS pH 7.4) was added slowly in a fine stream with constant mixing at 700 rpm by mechanical stirrer (Remi, India) in a well-sealed container designed for this preparation (fabricated in house). Mixing was continued for an additional 5 min. The system was kept at 30□C throughout the preparation and was then left to cool at room temperature. Simple liposomes were also prepared by lipid–hydration method (Bangham et al., 1965) using PC and Chol in mass ratio of 3:2 Briefly, a chloroform solution of SPC and CHOL and BNZ (10 mg) were first dried in a rotary evaporator (Super fit, Ambala, India) to form a lipid film on a wall of a round bottomed flask, until the solvent was completely evaporated. The resulting lipid film was then rehydrated with aqueous solution of (in PBS pH 7.4).

2.3. Vesicular shape and surface morphology

Transmission electron microscope (Philips CM12 electron microscope, Eindhoven Netherlands) was used as a visualizing aid for PG-embodying liposomes. Vesicles were stained with 1% Phosphotungstic acid (PTA) before visualization.

2.4. Vesicular size distribution measurements

The size distribution of elastic liposomes was measured in two sets of triplicates, in a multimodal mode, by dynamic light scattering (DLS) technique using a computerized Malvern Zetamaster ZEM Autosizer 5002 inspection system (Malvern, UK). For vesicles size measurement, vesicular suspension was mixed with the appropriate medium [phosphate buffer saline (PBS), pH 7.4] and the measurements were conducted in triplicate.

2.6. Entrapment efficiency

Prepared PG-embodying liposomes were taken and separated from the free (un-entrapped) drug by a Sephadex G-50 minicolumn centrifugation technique (Fry et al. 1978). The vesicles were lysed by Triton X-100 (0.5% w/w). The entrapped/ loaded drug was estimated spectrometrically assayed at 282 nm (UV-VIS Spectrophotometer-1800,, Shimadzu, Tokyo, Japan).

3. Results

3.1. Preparation of PGLs

Different ratios of soya lecithin to propylene glycol were used to prepare PG-embodying liposomes, the drug concentration in each formulation was kept constant (Table No. 1). It is clearly observed that formulation code PGL 02 (SPC 2 % and Propylene glycol 25 %) had highest entrapment efficiency.

3.2. Vesicular shape and surface morphology

For an initial characterization of the vesicles, PG-embodying liposome preparations were examined by negative stain electron microscopy (TEM). PGLs prepared from 2% SPC, 25% Propylene glycol and water appeared as nano-size, multi-lamellar vesicles (Figure No.1).

3.3. Vesicular size distribution measurements

PGL size and charge, effect of phospholipid and Propylene glycol concentration on the size distribution of PGL vesicles was investigated using DLS. For PGLs prepared with 25% propylene glycol and 2% SPC, the data showed a narrow particle size distribution with an average size of 174 nm. In the Propylene glycol concentration range of 15–30%, the size of the vesicles increased with decreasing propylene glycol concentration, with the largest particles in preparations containing 20% propylene glycol and the smallest in preparations containing 30% propylene glycol (Table No.1). By comparison, conventional liposomes, made from the same phospholipid by the film forming method and containing no Propylene glycol, had an average size of 612 ± 59 nm (Table No.2). The dependence of vesicle size on phospholipid content was determined for PGLs containing 25% Propylene glycol and SPC concentrations ranging from 1.5 to 3%. It can be seen in Table No.1 that PGL size exhibits a limited dependence on phospholipid concentration. The charge of the PG-embodying lipid vesicles is an important parameter that can influence both vesicular properties such as stability, as well as skin–vesicle interactions. While liposomes (SPC 2%, no Propylene glycol) exhibited a zeta potential of -4.9 ± 0.45 mV, the addition of 25% Propylene glycol induced a transition in the charge of the vesicles to more negative (-7.2 ± 1.3 mV) (Table No.2.)

3.5. Entrapment efficiency

The entrapment efficiency BNZ in PGLs was found 31% of initial concentration of BNZ used. It was slightly higher as compared to liposomes (27%) (Table No. 2). Drug loading in to PGLs neither significantly increase size nor melting temperature of phospholipids. Increase in both the concentration of lecithin or propylene glycol enhances the drug loading up to certain ratio.

4. Discussion

PG-embodying liposomes, novel permeation-enhancing lipid carriers embodying propylene glycol, contain vesicles with interdigitated fluid bilayers. One characteristic of PGLs is their small size relative to liposomes, when both are obtained by preparation methods not involving any size reduction steps such as sonication or filtration. In this study, we evaluated PGLs as carriers for the topical application of BNZ. We prepared some formulations at different propylene glycol and soybean phosphatidylcholine percentages to evaluate the effect of the composition on the mean size distribution of PGLs. various formulations were submitted to light scattering analysis to choose the most suitable formulation to be tested in vitro. As shown in Table No. 1, PGLs showed a narrow particle size distribution, in particular the formulation prepared with the greater amount of propylene glycol. In the interval of propylene glycol concentration investigated in this paper, the size of PGLs decreased with increasing propylene glycol concentration. While, the concentration of lecithin used for PGL preparation influenced the vesicle mean size in a different way, namely the higher the lecithin concentration the larger the PGL mean size. These data are in agreement with previous findings reported for ethosmes (Touitou et al. 2000). Photon correlation spectroscopy analysis was also carried out on BNZ loaded PGLs/liposomes. In both cases, at the concentrations investigated in this paper, no significant ($P > 0.005$) size variation with respect to unloaded PGLs was observed, thus showing that the presence of the drugs was not a determinant factor capable of influencing the mean size of the various PGL colloidal suspensions. Drug entrapment within a vesicular carrier is an important parameter to be defined to really evaluate the delivery potentiality of the system. For this reason, the entrapment efficiency of BNZ within the formulations was evaluated in an attempt to investigate the influence of PGL composition, i.e., the quantity of propylene glycol and lecithin, on the drug loading

capacity. As shown in Table No. 1, both propylene glycol and lecithin amounts, used for PGL preparation, positively influenced the entrapment efficiency of the colloidal carrier. Namely, the higher the amount of propylene glycol and lecithin, the greater the BNZ entrapment within the PGLs. In agreement with previously reported findings (Touitou et al. 2000; Elsayed et al., 2006), the values of entrapment efficiency for BNZ are higher than those expected for conventional vesicle formulations. This fact can be explained by the presence of propylene glycol, which increases the BNZ solubility in the polar phase of the colloidal formulations of PGLs. Both vesicle lamellarity and solubility of the drug in the medium influence the entrapment capacity of PGLs. The loading capacity and the mean size of a colloidal carrier are important parameters that are able to influence the percutaneous permeation of an incorporated drug. For this reason, we chose formulation PGL 2, which is characterized by small vesicle size, a homogenous vesicle size distribution (Table No. 1) and a high entrapment efficiency of BNZ (Table No. 1) for further studies.

5. Conclusion

The dimensions of empty and BNZ/ PGLs depend on their composition, and in particular the mean size decreases with increasing propylene glycol concentrations whereas it increases as phospholipid concentrations decrease. These findings are very encouraging and confirm that PGLs are a very promising carrier for the topical administration due to the enhanced delivery of drugs through the skin thus prompting various opportunities for the development of suitable therapeutic strategies through the topical route.

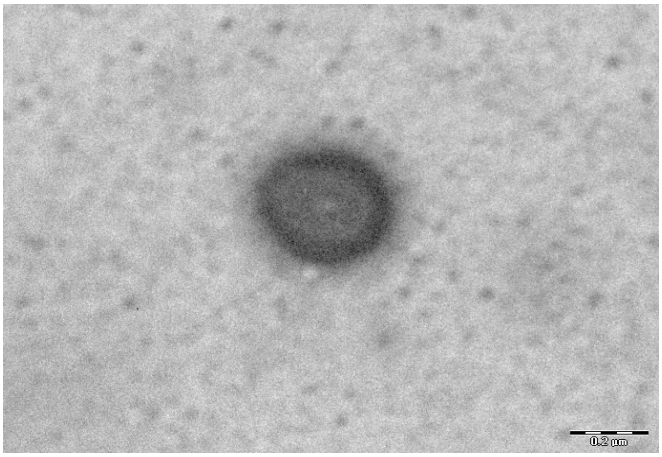

Figure 1: Transmission electron photomicrograph of PG Liposomes (X 10,000)

Table No.1 Optimization of BNZ formulations

S. No.	Formulation Code	Composition (%)				Particle Size	Entrapment efficiency
		Soya Lecithin	Ethanol	Water	BNZ		
1.	PGL 1	1.5	25	73.5	1	137 ± 21	28 ± 2.3
2.	**PGL 2**	**2.0**	**25**	**73**	**1**	**174 ± 31**	**31 ± 3.2**
3.	PGL 3	2.5	25	72.5	1	215 ± 37	21 ± 2.2
4.	PGL 4	3.0	25	72	1	247 ± 34	19 ± 1.9
5.	PGL 5	2.0	15	83	1	219 ± 28	29 ± 4.2
6.	PGL 6	2.0	20	78	1	192 ± 23	23 ± 3.1
7.	PGL 7	2.0	30	68	1	134 ± 17	27 ± 4.4

Table 2 Characteristics of BNZ vesicular formulations

S. No.	Formulation (s)		Particle Size (PI) (nm)	Entrapment efficiency (%)	Zeta potential (mV)	Thermal Analysis (Tm, ^{o}C)
1.	PG-liposomes	(without BNZ)	164 ± 27 (0.15)	NA	-4.2 ± 0.6	-8.5 ± 2.3
	(2 % SPC, 25 % Ethanol)	(with BNZ)	174 ± 31 (0.29)	31 ± 3.2	-7.2 ± 1.3	-11.7 ± 1.7
2.	Liposomes	(without BNZ)	557 ± 41 (0.21)	NA	-3.9 ± 0.5	12.2 ± 3.2
	(2% SPC)	(with BNZ)	612 ± 39 (0.31)	27 ± 4.6	-2.9 ± 0.3	7.4 ± 2.9

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