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RESEARCH PAPER

Preparation and Comparative Clinical Evaluation of Liposomal Gel of Benzoyl Peroxide for Acne

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ABSTRACT

A novel topical benzoyl peroxide (BP) gel formulation containing liposomal BP was shown to significantly reduce local irritation relative to its nonliposomal BP gel (plain BP gel) preparation and also to improve clinical efficacy (almost twofold) in the treatment of acne. BP liposomes were prepared, optimized, and formulated into a carbopol 934 gel base. Drug release evaluated using dialysis membrane has repeatedly shown that a new topical gel formulation containing liposomal BP (liposomal BP gel) significantly reduced BP penetration. Clinical evaluation data were also compared with those obtained with liposomal tretinoin (TRE) gel in an earlier investigation of ours. The overall improvement in terms of percentage reduction in total number of skin lesions demonstrated almost similar results for both BP and TRE. However, variation was observed in the treatment of separate types of lesions in which liposomal TRE gel was found to be more effective in treating comedones and liposomal BP gel in treating papules and pustules. Also, the liposomal gel formulation of both the drugs significantly reduced the local adverse effects, thereby improving patient compliance.

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INTRODUCTION

Benzoyl peroxide (BP) is an effective topical agent in the treatment of acne (1) and has been widely available since the 1960s (2). The main mode of action of BP in acne is related to its antimicrobial activity against *Propionibacterium acnes* in the sebaceous follicle (3,4). The local irritation (1) and burning (5) are the major side effects of BP products, which often compromise the effective therapy by limiting patient compliance.

Liposomes have aroused considerable interest for utilization as carriers for targeting drugs into various layers of skin (6). According to many investigators (7–11), the liposomal drug formulations are able to reduce side effects and incompatibilities and to enhance the accumulation of drug at the administration site. Hence, an attempt was made to formulate liposomal BP gel with the aim to reduce local irritation and burning of BP therapy and to improve the therapeutic response, which in turn may improve patient compliance and therapeutic efficacy. It should be noted that, although liposomes are promising for potential use in pharmacotherapy, clinical studies are still scarce.

Therefore, in the present investigation, the liposomes of BP were prepared and optimized for drug entrapment efficiency. Liposomal BP gel was prepared using carbopol 934 gel base and was subjected to comparative in vitro drug diffusion studies along with the plain BP gel. An attempt was also made to evaluate clinically the developed liposomal BP gel against the plain BP gel in acne patients.

Tretinoin (TRE), also known as all-trans-retinoic acid, the acid form of vitamin A, is reported to be effective topically in the treatment of acne (12,13). Liposomal TRE gel has also been shown to improve clinical efficacy and to mask local adverse effects (14). Hence, the results of the clinical evaluation of BP were compared with those obtained with TRE.

EXPERIMENTAL

Materials

The BP (gift sample from SPARC, India); egg phosphatidyl choline (PC) (Centre for Biochemical Technology, India); cholesterol AR (CHOL) (S.D. Fine Chemicals Ltd., India); α-tocopherol (α-toco) and triethanolamine (Merck, USA); carbopol 934 (B.F. Goodrich, USA); phenyl mercuric nitrate (BDH, India); and dialysis membrane (molecular weight cutoff 12,000; Sigma Diagnostics, USA) were purchased and used as such without further purification. All other chemicals used were analytical reagent grade.

The pH 5.0 acetate buffer (ionic strength 0.261) was prepared as described in the Indian Pharmacopoeia 1996 (15).

Preparation of Liposomes and Liposomal Formulations

Liposomes of BP were prepared and optimized using the method already reported by us (14) using a rotary flash evaporation technique and 5 ml of chloroform : methanol (2 : 1) solvent system. Table 1 shows the composition of the liposomes.

Carbopol 934 (1% w/w) was dusted onto distilled water containing 0.001% phenyl mercuric nitrate while the mixture was stirred and left overnight to hydrate for 24 h. Triethanolamine (0.5 ml) was then added with gentle stirring to avoid inclusion of air (16). The pH of the gel base thus obtained was 5.6.

Plain BP gel (#PBG) and liposomal BP gel (#LBG) were prepared by incorporating plain BP or liposomal BP pellet, respectively, into carbopol gel base by trituration to obtain 2.5% w/w of BP-containing gels.

CHARACTERIZATION OF BENZOYL PEROXIDE LIPOSOMES

The liposomes were subjected to physical analysis to determine the size, shape, and lamellarity, and chemical analysis was performed to determine drug entrapment efficiency and PC and CHOL content as reported by us (14). Drug entrapment efficiency of the liposomes, expressed as percentage of the added drug actually entrapped in liposomes, was determined by estimation of BP by titrimetry (17). The liposomes were treated with acetone and 20% potassium iodide in the iodine flask, and the liberated iodine was titrated against 0.1 M sodium thiosulfate after allowing the solution to stand in the dark for 10 min. PC was estimated by the Stewart assay (18) and CHOL was estimated using the method of Zlatkis et al. (19) by measuring the absorption at 485 nm and 550 nm, respectively, against the reagent blank.

Table 1

Effect of Composition of Liposomes on Drug Entrapment Efficiency

Batch	PC : CHOL Molar Ratio	BP : PC : CHOL Molar Ratio	Entrapment Efficiency[a] Mean (SEM)	Free BP[b] Mean (SEM)
BP1	20.43 : 1	1 : 12.77 : 0.63	33.00 (0.044)	64.98 (0.048)
BP2	10.19 : 1	1 : 12.77 : 1.25	49.79 (0.51)	42.80 (0.339)
BP3	10.19 : 1	1 : 6.38 : 0.63	76.15 (0.10)	22.66 (0.035)
BP4	05.10 : 1	1 : 12.77 : 2.5	38.22 (0.16)	58.08 (0.24)
BP5	10.19 : 1	1 : 4.26 : 0.42	50.41 (0.46)	45.02 (0.52)
BP6	05.10 : 1	1 : 6.38 : 1.25	58.85 (0.29)	36.32 (0.33)

BP, benzoyl peroxide; CHOL, cholesterol AR; PC, phosphatidyl choline.
[a]Percentage of added BP actually entrapped into liposomes ($n = 6$).
[b]Percentage of added BP left unentrapped ($n = 6$).

Drug Retention in Liposomes

The potential liposomal batch, BP3, and its liposomal gel were sealed in 30-ml glass vials and stored at refrigerated temperature (2°C–8°C), room temperature (25°C ± 2°C), and body temperature (37°C). A sample from either batch at each temperature was taken at definite time intervals and centrifuged at $3.3 \times 10^6 \times g$ for 10 min, then the supernatant was analyzed for free drug concentration. The results were calculated in terms of percentage BP retained in liposomes (Fig. 1). The batches were also subjected to vesicle size determination immediately after preparation and after 3 months of storage; the results are shown in Table 2.

In Vitro Drug Diffusion Studies

The in vitro drug diffusion studies were carried out with plain BP gel and liposomal BP gel using a Franz static diffusion cell and dialysis membrane. The other conditions and the sampling conditions were the same as those reported earlier by us (14). The results are recorded in Fig. 2.

Clinical Studies

The study involved 30 human volunteers aged 19–26 years (16 female and 14 male) with mild to moderate acne, and the study was carried out for a period of 3 months in the Skin-VD Department of the S. S. G. Hospital, attached to the Faculty of Medicine, M. S. University of Baroda, India. The patients selected for the study were permanent visitors of the OPD in S. S. G. Hospital, and informed consent was obtained from the subjects after the nature and possible consequences of the studies were explained. Few patients had been suffering from acne for about 4 months to 6 years before

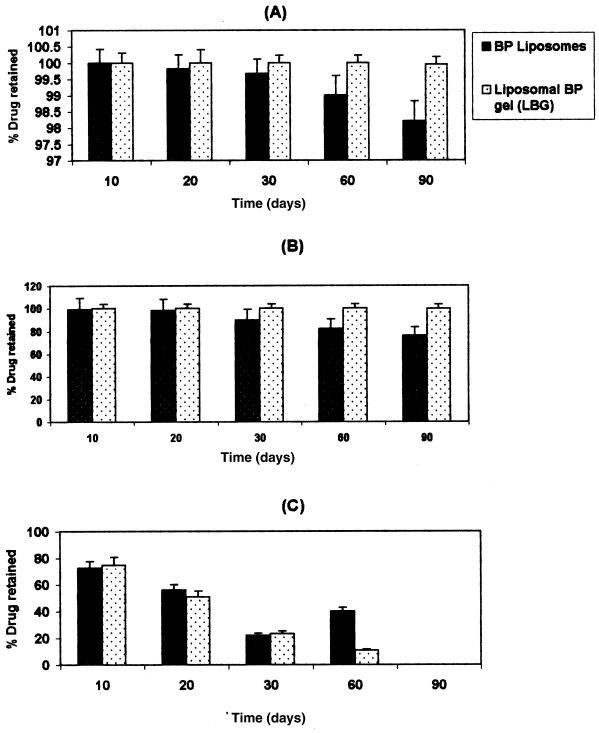

Figure 1. Mean percentage (±SEM) BP retention ($n = 6$) in liposomes at different storage conditions: (A), refrigeration temperature (2°C–8°C); (B), room temperature (25°C ± 2°C); (C), body temperature (37°C).

Table 2

Vesicle Size of Liposomes (Batch BP3) and Liposomal Gel (#LBG) on Storage for 3 Months at Different Conditions

	Vesicle Diameter (μm) Mean (% CV)			
	BP3		#LBG	
	A	B	A	B
Refrigeration temperature	2.52 (0.56)	2.55 (0.22)	2.52 (0.16)	2.53 (0.28)
Room temperature	2.52 (0.56)	2.59 (0.18)	2.52 (0.16)	2.53 (0.22)

A, immediately after preparation; B, after 3 months of storage.

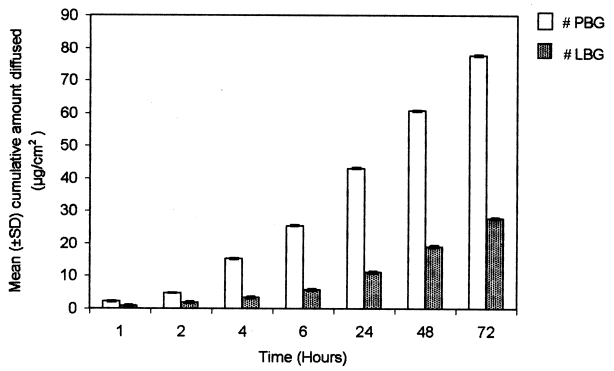

Figure 2. Mean cumulative BP amount diffused across the membrane.

the period of study. All previous treatments were discontinued at least 30 days prior to these studies.

The lesions located on the facial area were observed during the course of study, and the skin lesions consisted of different manifestations of acne, such as comedones, papules, and pustules. In a double-blind design, a dose of about 0.5 g of both the gels was applied to the left side (plain BP gel) and right side (liposomal BP gel) of the face once daily. The number of each type of lesion and the total number of lesions were counted initially and after each application of either formulation on a weekly basis on the same surface area (4 cm^2) and were marked with a paper template by the physician in charge in terms of percentage reduction in separate type and total number of skin lesions (Table 3). In addition, the overall improvement in skin condition of patients was evaluated on a monthly basis by an independent physician and was graded by evaluation indices using a three-point semiquantitative scale (20) as 0 = excellent (> 66% improvement), 1 = very good (33%–66% improvement), 2 = good (< 33% improvement), and 3 = no change. The initial index in all cases was 3. The sum of all individual patient indices was calculated and divided by the total number of patients ($n = 30$). The skin condition of patients was also evaluated in terms of adverse symptoms of therapy, such as irritation and burning, and the results in terms of weighted means are summarized in Fig. 3.

Data Analysis

The data obtained for all the experimental investigation were evaluated using analysis of variance (ANOVA), and the differences were considered significant at $P < .05$.

RESULTS AND DISCUSSION

The lipid film hydration technique yielded spherical and multilamellar liposomes, as confirmed by microscopy and light-scattering laser diffraction analysis, with the vesicle size ranging from 0.75 to 6.00 μm. The α-toco was added to prolong the characteristic induction phase of autooxidation and thus the shelf life of liposomes (21,22).

The data shown in Table 1 reveal that increasing the CHOL concentration with the same PC and BP concentrations (BP1 to BP2) led to increased entrapment efficiency of dissolved BP. Further increase in CHOL concentration (BP2 to BP4) resulted in a significant decrease in drug entrapment efficiency. Also, a marked decrease in entrapment efficiency was observed with increasing BP concentration (BP3 to BP5). The drug retention study shown in Fig. 1 indicated maximum drug retention at refrigeration temperature, and the liposomal drug gel showed a reduction in drug leakage compared to the liposome drug suspension. Also, the mean vesicle diameter increased only slightly after storage, as indicated in Table 2.

In Vitro Drug Diffusion Studies

The in vitro permeation of BP was calculated in terms of the mean cumulative amount diffused Q

Table 3

Mean (±SEM) Percentage Reduction in Separate Type and Total Number of Skin Lesions[a]

Time (Weeks)	Comedones		Papules		Pustules		Total	
	A	B	A	B	A	B	A	B
1	0.22 (0.008)	0.75 (0.020)	0.48 (0.048)	2.53 (1.23)	0.00 (0.00)	0.56 (0.056)	1.24 (0.438)	3.05 (0.739)
2	3.24 (0.510)	7.24 (0.651)	3.81 (1.174)	7.25 (2.097)	1.67 (0.929)	3.67 (1.369)	3.79 (0.844)	8.17 (0.834)
3	5.07 (0.922)	10.41 (0.840)	11.24 (1.765)	20.88 (2.625)	5.56 (1.459)	16.95 (2.765)	8.73 (1.489)	16.72 (1.205)
4	12.76 (1.499)	20.97 (1.247)	21.62 (3.427)	34.04 (2.938)	13.33 (2.482)	31.78 (2.855)	15.88 (2.120)	28.54 (1.412)
6	21.43 (1.206)	30.07 (2.904)	32.86 (2.577)	52.36 (3.492)	30.00 (4.145)	43.72 (3.471)	27.36 (1.315)	13.81 (2.521)
8	27.62 (1.803)	40.21 (1.794)	42.95 (3.900)	61.98 (3.489)	36.94 (4.260)	55.39 (3.881)	34.11 (2.246)	52.76 (2.003)
10	34.76 (2.396)	51.45 (2.231)	49.62 (4.989)	71.94 (3.325)	43.61 (3.419)	66.89 (3.184)	42.07 (3.119)	62.98 (1.790)
12	42.29 (2.862)	65.21 (3.147)	52.43 (1.984)	82.75 (3.374)	51.44 (2.220)	82.89 (2.776)	54.71 (2.309)	80.40 (2.229)

A, plain BP gel (#PBG); B, liposomal BP gel (#LBG).
[a]Study was conducted in 30 acne patients.

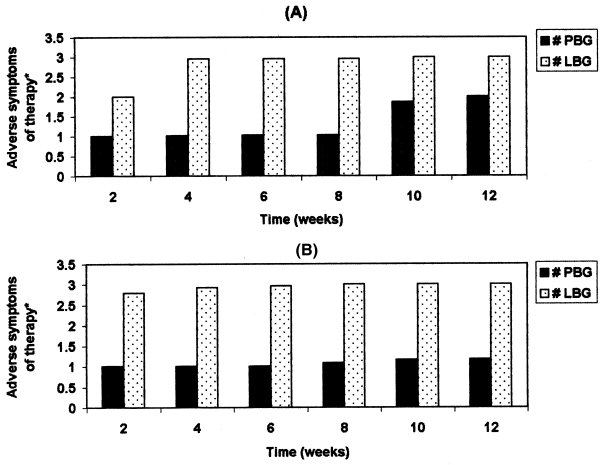

Figure 3. Evaluation of skin condition for adverse symptoms of therapy: (A) irritation; (B) burning. *1 = high, 2 = less, 3 = no.

($\mu g/cm^2$) at each sampling time during 72 h of study. The results are shown in Fig. 2. It is evident from the results that cumulative permeation of BP was significantly greater from plain BP gel ($P < .05$) at all time points. This agrees with the findings of many investigators (10,23) that liposomal entrapment of BP significantly prolongs the drug release across the membrane. The 0.993 value of the correlation coefficient r for the plain BP gel and the 0.990 value for the liposomal BP gel suggest a linear relationship between Q and $t^{1/2}$ and suggest that the release follows the pattern delineated in Higuchi's diffusion-controlled model (24). The mean flux values (Fig. 2) of 1.041×10^{-1} μg/min for plain BP gel and 2.573×10^{-2} μg/min for liposomal BP gel show a significantly ($P < .05$) higher flux (almost 4.05 times) of BP from plain BP gel. This further confirms the reduction in the rate of diffusion of the drug after liposomal entrapment.

Comparative Clinical Evaluation

A comparative double-blind clinical evaluation of plain BP gel and liposomal BP gel was carried out over a period of 3 months on 30 patients suffering from mild to moderate acne. The lesions located on the facial area were selected for the study because of ease of counting by the physician and because the number of lesions on the facial area was sufficient to justify the role of the formulations under

investigation. The patients were instructed to avoid contact of the formulations with the corners of the nose and eyes, angles of the mouth, and mucous membrane because the mucosae are much more sensitive than the skin to the irritant effects of the drug (5). The patients were also instructed to avoid contact with hair and fabric because of the possible bleaching action of BP.

The data for mean percentage reduction in separate type and total number of skin lesions (Table 3) reveal that all the manifestations of the disease were not equally responsive to BP therapy. Papules and pustules were most effectively treated, followed by comedones, which may be attributed to the antibacterial effect of BP (3,4). Liposomal BP gel showed significantly improved therapeutic response (about twofold) of BP at all evaluation time intervals compared to the plain BP gel at $P < .05$. These results were also analyzed in terms of evaluation indices (20), and it was seen that liposomal BP gel showed results toward excellence as compared to the plain BP gel. At the end of the 12th week, the evaluation indices for comedones, papules, and pustules for the plain BP gel were 1.27, 0.47, and 0.2, respectively, whereas the same for liposomal BP gel were 0.54, 0.2, and 0.1, respectively. This further confirms the significantly better skin condition of patients ($P < .05$) with liposomal BP gel treatment.

The adverse symptoms of BP therapy regarding the irritation and burning that mainly hamper the effectiveness of BP therapy by considerably decreasing patient compliance were evaluated at the end of 2, 4, 6, 8, 10, and 12 weeks and graded as 1 = high, 2 = less, and 3 = no. As seen in Fig. 3, plain BP gel showed high irritation until the eighth week of treatment and gradually decreased thereafter, whereas high burning was seen throughout the study, being more severe in the initial period of therapy and thus tempting patients to discontinue the therapy. Liposomal BP gels showed much less irritation until the second week of treatment, when it completely disappeared, and there was no burning throughout the studies. This significant decrease in adverse symptoms may be correlated to the decrease of free drug availability on liposomal entrapment, therefore inducing neither irritation nor burning (6).

The findings of this investigation conclusively demonstrate a vital role of liposomally entrapped BP in improvement of therapeutic response and a marked reduction in adverse symptoms in treating acne patients.

In our earlier findings (14), the liposomal TRE gel was shown to have better response in treatment of comedones, whereas the liposomal BP gel of this investigation showed a predominant response in the treatment of papules and pustules. Hence, concomitant therapy with liposomal TRE and liposomal BP gel is expected to give more effective treatment of acne.

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