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Clinical Assessment of the Combination Therapy with Liposomal Gels of Tretinoin and Benzoyl Peroxide in Acne

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INTRODUCTION

Acne has plagued humankind since antiquity. Acne vulgaris is a highly variable disease attracting a crisp social rebuttal. Various anti-acne preparations are available, each having different physiological effects on the condition. The basic lesion of acne is the comedone, a distention and impaction of the pilosebaceous unit with sebum and keratinous debris. Some comedones evolve into inflammatory papules, pustules, or nodules (facial eruptions) that are mainly due to the proliferation of *Propionibacterium acnes*, which results in the production of inflammatory compounds.

Tretinoin (TRE) is a unique topical medication used in the treatment of acne that allows the keratin plugs of microcomedones to be expelled; thus, fewer lesions are able to rupture and cause the papules, pustules, and nodules of inflammatory acne [1]. Benzoyl peroxide (BP), a mainstay of the dermatologist's treatment of acne, may act as mild comedolytic, but its main mode of action is to decrease the population of *P. acnes* in the sebaceous follicle and thus prevent inflammation. A combined assault against *P. acnes* with TRE and BP on both the processes of comedogenesis and bacteriostatic activity is a particularly rational approach to the disease [2]. However, the main obstacle to combination therapy with these topical agents is the undesirable side effects, such as erythema, itching, burning, scaling, and irritation [1].

Liposomal drug formulations have been reported to have good accumulation of drug at the administration site (thus improving therapeutic efficacy) and to have fewer side effects than plain formulations [3-8]. Although liposomal formulations are promising for potential use in pharmacotherapy, clinical studies are still scarce. Therefore, we attempted to clinically investigate the beneficial effects of simultaneous use of TRE and BP liposomal gels in terms of therapeutic efficacy and side effects. This investigation focused on comparative clinical evaluation of combination therapy with simultaneous use of liposomal gel formulations and plain drug gel formulations on 30 acne patients.

KEYWORDS: Liposome, tretinoin, benzoyl peroxide, acne

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MATERIALS AND METHODS

Preparation of gels for clinical studies

Carbopol 934 (1% wt/wt) was dusted onto distilled water containing 0.001% phenyl mercuric nitrate while the mixture was stirred; the mixture was then left to settle for 24 hours. Triethanolamine (.5 mL) was then added with gentle stirring to avoid inclusion of air. The pH of the gel base thus obtained was 5.6.

Plain TRE gel (PTG) and liposomal TRE gel (LTG) with a concentration of 0.025% wt/wt was prepared by incorporating plain TRE or liposomal TRE pellets, respectively, into the carbopol gel base by trituration [10]. Similarly, plain BP gel (PBG) and liposomal BP gel (LBG) with a concentration of 2.5% wt/wt was prepared by incorporating plain BP or liposomal BP pellets, respectively, into the carbopol gel base by trituration [11]. All 4 gels were filled separately into 20-g lacquered aluminium collapsible tubes and sealed securely. The tubes were coded as A, B, C, and D, not respectively, by a person not involved in the study.

Trial methodology

The study involved 30 volunteers aged 19 to 26 years (16 females, 14 males) with mild to moderate acne and was carried out in the Skin-VD department of the

S.S.G. Hospital attached to the faculty of medicine, MS University of Baroda, India, for 3 months. The study was approved by the institutional human experimentation committee in accordance with all applicable regulations, and informed consent was obtained after explaining the nature and possible consequences of the studies to all patients involved. All of the patients had been suffering from acne for about 4 months to 6 years before the study. Some had been undergoing treatment with different drugs before entering the study, but all previous treatments were discontinued at least 30 days before this study was begun.

In a double-blind design, a dose of about 0.5 g of BP gel was applied to the left side (PBG) and right side (LBG) of the face in the morning, and about 0.5 g of TRE gel was applied to the left side (PTG) and right side (LTG) of the face at bedtime to each patient at least 20 minutes after washing the face with lukewarm water. Care was taken to avoid contact with the corners of the nose, the eyes, angles of the mouth, mucous membranes, hair, and fabric. The patients were informed that some initial redness, irritation, and peeling and some exacerbations of acne conditions are frequent side effects of the therapy.

The treatment was continued for 3 months. All types of lesions (comedones, papules, and pustules) located on the facial area (4 cm^2) marked with a paper template were counted during the course of study.

Clinical evaluations of the healing time of external lesions and the reduction in adverse effects of the drugs were carried out. The results of healing time of external lesions were graded at 2 levels.

Level 1: Progress was evaluated on a weekly basis on the same surface area (4 cm^2) marked with a paper template by a group of physicians in terms of percent reduction in the separate types (comedones, papules, and pustules) and total number of skin lesions. Percent reduction of lesions was calculated, and results are shown in Table 1.

Level 2: The overall improvement in skin condition of patients was evaluated on a monthly basis by a physician after 4, 8, and 12 weeks of treatment using evaluation indices [9] as discussed in the data analysis section. The results in terms of weighted means are recorded in Table 2.

Adverse symptoms of therapy: The skin condition of patients was evaluated in terms of adverse symptoms of therapy such as erythema, itching, burning, scaling, and irritation as explained in the data analysis section. The results in terms of weighted means are shown in Figure 1.

Data analysis

The results of the first-level study (ie, percent reduction in separate type and total number of skin lesions) obtained with liposomal drug gels (LBG and LTG) were compared with those obtained with plain drug gels (PBG and PTG) using analysis of variance (ANOVA); differences greater than $p < .05$ were considered significant.

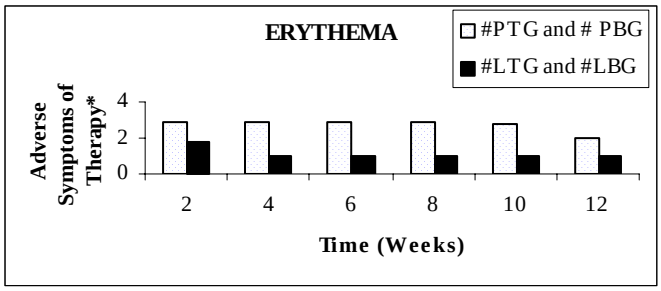
The results of the second-level study (overall improvement in skin condition) were graded on a monthly basis as evaluation indices, as reported by Skalko et al[9]. The initial index in all cases was 3. The grading of therapeutic efficacy was expressed as 0 = excellent, >66% improvement; 1 = very good, 33% to 66% improvement; 2 = good, <33% improvement; and 3 = no change. The weighted mean was calculated by dividing the sum of all individual patient indices by the total number of patients (n = 30).

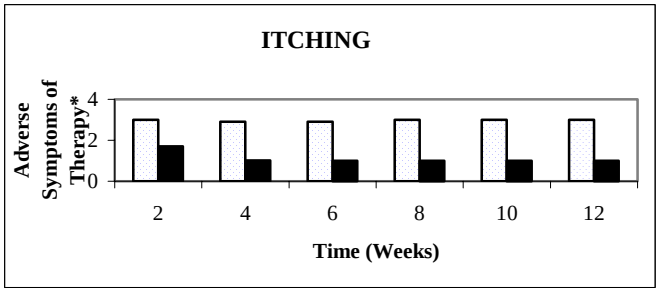
The adverse symptoms in terms of erythema, itching, burning, scaling, and irritation were graded as 1 = none, 2=less, and 3=high. The weighted mean was calculated by dividing the sum of all patients' degree of reaction (1, 2, or 3) by the total number of patients (n = 30).

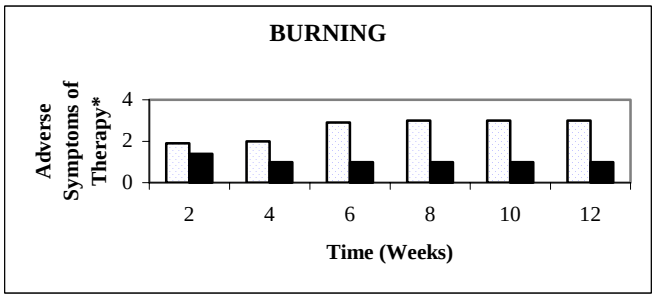
Table 1. Mean (± SD) Percent Reduction in Total Number of Different Types of Skin Lesions*

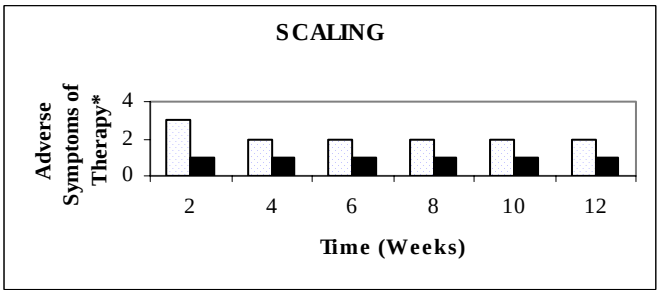
Time	Comedones		Papules		Pustules		Total	
(Weeks)	A	B	A	B	A	B	A	B
1	2.04 (4.16)	8.75 (9.26)	3.33 (5.64)	3.33 (6.13)	0.0 (0.0)	2.78 (6.30)	2.50 (2.52)	7.04 (5.31)
2	5.70 (4.98)	17.67 (12.98)	8.33 (5.97)	18.22 (15.94)	5.33 (5.10)	9.45 (9.31)	7.56 (2.57)	15.29 (10.74)
3	13.66 (9.69)	26.25 (13.75)	20.42 (15.56)	32.20 (13.09)	18.11 (13.04)	24.44 (7.50)	17.31 (7.50)	28.19 (8.16)
4	21.81 (7.72)	46.67 (15.12)	44.03 (8.05)	67.38 (8.05)	37.44 (12.65)	60.00 (13.86)	33.56 (4.60)	57.46 (10.79)
6	29.49 (12.00)	66.67 (16.10)	56.81 (13.75)	81.55 (17.03)	55.11 (19.88)	81.67 (15.77)	45.65 (8.27)	75.72 (12.10)
8	48.15 (2.68)	88.42 (7.01)	66.11 (17.03)	89.94 (11.45)	65.45 (11.28)	90.55 (12.87)	58.83 (4.77)	89.39 (7.12)
10	62.39 (10.74)	100.00 (.00)	71.25 (13.15)	100.00 (0.00)	72.78 (14.19)	100.00 (.00)	72.06 (2.57)	100.00 (.00)
12	75.09 (4.71)	-	78.75 (10.63)	-	79.22 (10.41)	-	80.60 (3.56)	-

***Study was conducted in 30 acne patients A indicates plain drug gels (PTG and PBG); B, liposomal drug gels (LTG and LBG).**









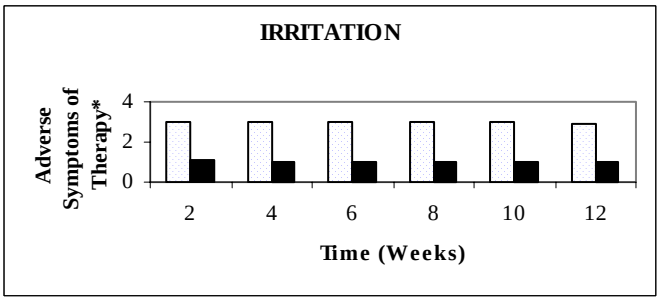


Figure 1. Evaluation of skin condition for adverse symptoms after concomitant therapy with tretinoin and benzoyl peroxide gels. *1 = none; 2 = less; 3 = high

Table 2. Improvement in Separate Types of Skin Lesions

Test Formulation	Evaluation Indices* (Weighted Mean)								
	Comedones			Papules			Pustules		
	A	B	C	A	B	C	A	B	C
Plain Drug Gels (PTG and PBG)	2.0	1.0	0.00	1.0	0.47	0.00	1.17	0.45	0.00
Liposomal Drug Gels (LTG and LBG)	1.2	0.0	0.0	0.37	0.0	0.0	0.67	0.033	0.00

***Initial index in all cases was 3. A indicates 4th week; B, 8th week; C, 12th week; 0, excellent (>66% improvement); 1, very good (33% to 66% improvement); 2 , good (<33% improvement); 3 = no change. The sum of all individual patient indices was calculated and divided by the total number of patients (n = 30).**

Table 3. Mean (± SD) Percent Reduction in Separate Type and Total Number of Skin Lesions* Using Tretinoin Gels and Benzoyl Peroxide Gels Separately on Different Patients

Time	Comedones		Papules		Pustules		Total	
(Weeks)	A	B	A	B	A	B	A	B
01	-9.33 (6.85)	8.33 (5.64)	.00 (.00)	.48 (2.63)	.00 (.00)	.00 (.00)	-4.24 (3.07)	3.72 (2.58)
02	-12.28 (8.88)	12.42 (7.73)	2.44 (6.36)	5.48 (7.34)	0.83 (4.55)	3.33 (7.56)	-4.47 (4.44)	7.85 (3.78)
03	7.38 (8.82)	19.83 (5.91)	5.00 (8.49)	9.53 (9.86)	2.50 (7.62)	5.33 (8.99)	5.56 (6.79)	12.98 (5.48)
04	18.57 (9.41)	34.17 (6.69)	11.00 (16.00)	18.49 (8.33)	05.00 (10.19)	11.33 (10.08)	13.62 (8.44)	23.44 (5.10)
06	27.00 (7.01)	53.33 (4.82)	14.89 (16.00)	30.95 (13.37)	06.67 (11.23)	16.00 (8.17)	19.17 (8.33)	36.91 (8.00)
08	40.97 (8.66)	75.83 (4.33)	25.56 (10.69)	41.27 (12.22)	10.56 (13.32)	23.33 (7.56)	29.82 (7.23)	51.68 (7.45)
10	50.95 (4.11)	83.75 (8.27)	35.55 (8.23)	52.94 (4.27)	21.67 (13.75)	36.00 (8.16)	39.31 (4.16)	62.03 (6.63)
12	62.36 (5.37)	94.17 (5.97)	49.33 (8.27)	69.76 (5.59)	36.39 (8.06)	55.33 (11.40)	53.50 (3.29)	76.64 (9.97)
	C	D	C	D	C	D	C	D
01	.22 (.04)	.75 (1.11)	.48 (.26)	2.53 (6.74)	.00 (.00)	.56 (.31)	1.24 (2.40)	3.05 (4.05)
02	3.24 (2.79)	7.24 (3.57)	3.81 (9.54)	7.25 (11.49)	1.67 (5.09)	3.67 (7.50)	3.79 (4.63)	8.17 (4.57)
03	5.07 (5.05)	10.41 (4.60)	11.24 (9.67)	20.88 (14.39)	5.56 (8.00)	16.95 (15.17)	8.73 (8.16)	16.72 (6.60)
04	12.76 (8.21)	20.97 (6.83)	21.62 (18.78)	34.04 (16.10)	13.33 (13.60)	31.78 (15.65)	15.88 (11.62)	28.54 (7.74)
06	21.43 (6.61)	30.07 (15.91)	32.86 (14.12)	52.36 (19.14)	30.00 (22.71)	43.72 (20.02)	27.36 (7.21)	13.81 (13.81)
08	27.62 (9.88)	40.21 (9.83)	42.95 (21.37)	61.98 (19.12)	36.94 (23.34)	55.39 (21.27)	34.11 (12.31)	52.76 (11.12)
10	34.76 (13.13)	51.45 (12.23)	49.62 (27.34)	71.94 (18.22)	43.61 (18.74)	66.89 (17.45)	42.07 (17.09)	62.98 (9.81)
12	42.29 (14.68)	65.21 (17.25)	52.43 (10.87)	82.75 (18.49)	51.44 (12.17)	82.89 (15.21)	54.71 (12.65)	80.40 (12.21)

***Study was conducted with 30 acne patients. A indicates plain TRE gel (PTG); B, liposomal TRE gel (LTG); C, plain BP gel (PBG); D, liposomal BP gel (LBG).**

RESULTS AND DISCUSSION

In our earlier investigations, we had carried out work on liposomal TRE gel [10] and liposomal BP gel [11] separately and compared the results with those of the respective plain drug gels. The results of our earlier investigations are shown in Tables 3 and 4. It is evident from the results that the liposomal TRE gel is significantly more effective in treating comedones and liposomal BP gel is more effective in treating papules and pustules. It was also observed during our study that the adverse effects-erythema, itching, burning, scaling, and irritation-that were found with plain drug gels were not as common with liposomal TRE and BP gels.

The sum of all individual patient indices was calculated and divided by the total number of patients (n = 30).

Hence, it was thought worthwhile to investigate the beneficial effects of simultaneous use of liposomal TRE and liposomal BP gels in acne patients with a view to improving therapeutic response and reducing the possible accumulation of side effects, which is reported to be the main drawback of this beneficial combination [1].

The lesions located on the facial area were selected for the study because the physician could count them easily and because the facial area generally has more lesions that would require treatment with the formulations under investigation. The patients were instructed to avoid contact of the formulation with the corners of the nose, eyes, angles of mouth, and mucous membrane because the mucosae are much more sensitive than the skin to the irritant effects of drugs [12]. The patients were also instructed to avoid contact of the gels with hair and fabric because of the possible bleaching action of BP.

Clinical evaluations of the healing time of external lesions (Tables 1 and 2) and reduction in adverse symptoms of the drugs (Figure 1) were carried out.

First level of study for healing time of external lesions: It is evident from the results shown in Table 1 that all manifestations of acne were equally responsive to the concomitant therapy of TRE and BP in the form of both liposomal and nonliposomal drug gels. A combination of the comedolytic action of TRE and the bacteriostatic action of BP may be responsible for this additive effect [1]. However, with concomitant therapy of liposomal forms of these drugs, almost complete cure (100% reduction in skin lesions) was seen within 10 weeks, whereas with the plain drug gels, 75% to 80% reduction in skin lesions was achieved even after prolonging the application of preparations for up to 12 weeks. Cure of the disease requires longer duration of treatment, and in many cases the lesions are not responsive to the plain drug therapy beyond certain a percent reduction in skin lesions. The results clearly suggest that liposomal drug gels require shorter duration of therapy and achieve complete remission of symptoms, perhaps due to the higher skin retention of TRE and BP with liposomal encapsulation, as previously reported [10,11]. The statistical analysis of the data using ANOVA showed significant (1.5-fold) ($p < .05$) improvement in the therapeutic response with the liposomal drug gels compared with that of the plain drug gels at all evaluation time points.

Second-level of study for healing time of external lesions: The results shown in Table 2 reveal that the liposomal form provides better clinical improvement according to physicians' evaluation of skin status before and after treatment. The liposomal drug gels showed excellent results in separate types of skin lesions within 8 weeks, whereas the same could not be attained even after 12 weeks treatment with plain drug gels.

A comparison of concomitant treatment with TRE and BP gel (Tables 1 and 2) with either TRE therapy or BP therapy alone (Tables 3 and 4) clearly revealed significantly better (1- to 1.5-fold) therapeutic response of concomitant therapy in treating all types

Table 4. Improvement in Separate Types of Skin Lesions Using TRE gels (1) and BP gels (2) Separately on Different Patients

Test Formulation	Evaluation Indices* (weighted mean)								
	Comedones			Papules			Pustules		
	A	B	C	A	B	C	A	B	C
(1)									
PTG	2.17	1.13	.63	2.47	1.70	1.00	2.80	2.50	1.10
LTG	1.33	.067	.00	2.10	1.03	.00	1.57	1.83	1.07
(2)									
PBG	2.27	1.67	1.27	2.07	1.17	.47	1.3	1.03	.2
LBG	1.93	1.1	.54	1.5	.47	.2	1.5	.37	.1

***Initial index in all cases was 3. A indicates 4th week; B, 8th week; C, 12th week; 0, excellent (> 66% improvement); 1, very good (33% to 66% improvement); 2, good (<33% improvement); 3, no change.**

of acne lesions along with a reduction in duration of therapy. These factors in turn improved patient compliance. This is consistent with the report of Cohen et al [1], which stated that unplugging of the follicle by TRE makes the remaining *P. acnes* accessible to the action of a topical antibacterial agent (BP).

Adverse symptoms of therapy: The main obstacle to the combination therapy with topical agents is the possible accumulation of adverse symptoms of TRE and BP. Hence, the skin of patients was examined for these adverse symptoms; the results are shown in Figure 1. As seen in Figure 1, until the 12th week of treatment with plain drug gels, the severity of all adverse symptoms were high except scaling and erythema (both of which were reduced). Although liposomal drug gels caused no adverse symptoms from the 4th week of treatment, some symptoms were observed in the initial phase of treatment. This agrees with the findings of Korting et al [13] that liposomal formulation of betamethasone reduced erythema and scaling more than a conventional gel and did so more rapidly. This can be further correlated to less free drug being available with liposomal encapsulation [14], which produced better results and thus improved patient compliance to a larger extent.

CONCLUSIONS

The findings of this investigation thus conclusively demonstrate the promising role of concomitant therapy of liposomal TRE and BP gels in treating acne patients. Although their use improved the therapeutic response, more benefits of the liposomal form of drugs were observed in terms of reduction in adverse effects of therapy, thus fostering better patient compliance. It can be concluded that this study underscores the potential utility of concomitant therapy with liposomal TRE and BP gels in the treatment of acne. However, the role of liposmal formulations may only be established after clinical evaluation of a large number of patients, with a special focus on the adverse symptoms of therapy.

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