'Bacterial Growth Inhibition' – A novel treatment strategy for Acne

Janani S., Gayathri Rajagopal
Dr. JRK's Siddha Research and Pharmaceuticals Pvt., Ltd, Chennai, India

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Summary

The present study reports a novel treatment strategy for the treatment of acne. The strategy is the effective use of topical agents to inhibit the bacterial growth. This was achieved through increased skin residency and water resistance capacity. The study provides scientific evidence for the above claim.

Riassunto

Il presente studio riporta i risultati di una nuova strategia utilizzata nel trattamento dell'acne. Il trattamento si basa sulla verifica dell'attività svolta da agenti topici nell'inibire la crescita batterica, sul controllo, sul tempo di permanenza sulla cute e sulla resistenza al risciacquo con acqua.
Lo studio riporta alcune evidenze scientifiche a supporto.

INTRODUCTION

Propionibacterium acnes is considered the principal cause of pimples (acnes) (1). Therefore, the most anti-acne preparations targeted towards *P. acnes* have a high bactericidal activity.

Among them, clindamycin is the widely used anti-acne agent (2). Even though Clindamycin has high anti-microbial activity, its residency on skin alone can ensure the treatment success. Therefore, beyond the spectrum of high anti-microbial activity, high residency of the agent(s) on the skin alone could prevent the bacterial growth.

We have evolved the inhibition of bacterial growth as a new strategy in the treatment of acne.

The present study reports the effect of an anti-acne pack* as an agent in inhibiting the bacterial growth of *P. acnes in vitro*. The above finding was shown by studying the water resistance capacity of this anti-acne pack controlled on volunteers.

The present study assumes significance in the treatment of acne in the light of the prolonged residency of anti-acne pack along with the effective inhibition of bacterial growth.

Findings are presented in this paper.

MATERIALS & METHODS

The standard culture of *Propionibacterium acnes* (MTCC 1951) was procured from MTCC, Chandigarh. The culture was revived as per the standard procedures and was sub cultured onto Propionibacter isolation agar (Hi-media, India) with supplement. 48 hours grown culture was used for preparing inoculum.

Description of the test material

The anti-acne pack tested for the study is a cosmetic formulation of Dr. JRK's Siddha Research & Pharmaceuticals, Pvt. Ltd. containing Bentonite, Zinc oxide, Calamine, Titanium dioxide, Salicylic acid, *Aloe vera, Ocimum sanctum*.

Preparation of sample

The sample was weighed accurately and dissolved in normal saline to achieve the following concentrations viz. 100, 500 & 1000 mg/ml respectively.

Determination of contact time versus death of Propionibacterium acnes[3]

The 48 hours grown *P. acnes* culture in normal saline was adjusted to an absorbance of 0.6 at 450 nm.

One hundred (100) microlitre of the standardized inoculum was inoculated into the sample suspension (100 mg/ml, 500 mg/ml & 1000 mg/ml) in triplicate and was incubated for 10 minutes.

After 10 minutes, 0.1 ml of sample was drawn from each tube and was plated onto Propionibacter isolation agar and incubated anaerobically for 48 hrs. Untreated inoculum was maintained as control.

Determination on inhibition of bacterial growth

The concentrations such as 100 mg, 300 mg, 500 mg, 800 mg & 1000 mg of anti-acne pack were weighed separately in petri dishes and then dissolved in 1 ml of normal saline. Then, 15 ml of the media at molten stage was added to each plate, mixed well to ensure uniform dispersion of the sample with the media. The plates were then allowed to solidify.

* *Trade name: Verdura anti-acne pack by Dr. JRK's Siddha Research and Pharmaceuticals Pvt., Ltd., Chennai.*

After solidification, 0.1 ml of the standardized inoculum of *P. acnes* was inoculated onto the plate and incubated for 48 hours in an anaerobic environment. Triplicate sets were maintained. Alongside, control plates inoculated with 0.1 ml of the inoculum was also maintained.

Water resistance as established by skin adhesion post wash – Mexameter-based study[4]

To establish the effective adhesion and *water resistance capacity* of anti-acne pack, a study was conducted by evaluating the erythema index, post exposure to sun.

In the volar forearm region, varying concentrations of anti-acne pack was applied evenly on 2 cm^2 areas. After 15 minutes, the skin area was washed with distilled water. Then the cm^2 area of the skin was exposed to sun for 15 minutes by using window patch made by a thick black sun impermeable cloth. Similarly the control site, without application of anti-acne pack was also exposed to sun by the same method.

The erythema reading was taken using Mexameter. The erythema value of the skin on exposure to sun with and without the application of the cream was measured and compared.

RESULT

Irrespective of the concentration, the anti-acne pack ranging from 100 to 1000 mg/ml, did not affect the survival of *P. acnes*. Further, the CFUs (Colony Forming Units) of *P. acnes* remain practically with reference to control (Table I).

TABLE I

Contact time versus death.

S.No.	Concentration of the test material (mg/ml)	Number of colony forming units (CFUs) in average
1.	Control	4000
2.	100	3920
3.	500	3860
4.	1000	3779

When the organism was allowed to grow in media plate containing varying concentrations of anti-acne pack and incubated for 48 hours, there was a small decline in the CFUs of *P. acnes* from the concentration of 100 mg/ml to 1000 mg/ml and the number of CFUs was 3300 for 100 mg/ml and 250 CFUs for 1000 mg/ml. Whereas, the CFUs of the control was 4000 (Table II).

When the control plates were incubated for a further period of 72 hours to 5 days, the growth of *P. acnes* turns to TNTC (bacteria too numerous to count).

On the contrary, the CFUs of *P. acnes* in 100 mg i.e. (the least concentration of the sample) showed only a marginal increase in the CFUs. The trend was almost the same in other concentrations as well up to a maximum period of 5 days (Table II).

After sun exposure to sun for 15 minutes, significant increase in erythema formation was observed in control skin region (Table III).

The cumulative % difference in erythema value found decreased with increased dose of anti-acne pack from 5 mg/cm^2 to 15 mg/cm^2. The effect of anti-acne pack post wash in reducing erythema formation suggests the water resistance capacity of the anti-acne pack (Table III).

The cumulative erythema index in control was -6.6 as against 3.7, 4.1 & 4.9 respectively for 5, 10 & 15 mg/cm^2 of anti-acne pack (Table III).

TABLE II

Effect on bacterial growth inhibition.

S.No.	Concentration of the test material (mg/ml)	Number of colony forming units (CFUs) in average			
		24 hours	48 hours	72 hours	5 days
1.	Control	4000	7300	TNTC	TNTC
2.	100	3300	4200	4560	5020
3.	300	2540	2230	1890	1640
4.	500	1754	1520	1289	946
5.	800	645	530	478	382
6.	1000	250	218	194	156

TABLE III

Reading of erythema value post exposure to sun by Mexameter.

Volunteer	Control			5 mg			10 mg			15 mg		
	Before	After	% diff	Before	After	% diff	Before	After	% diff	Before	After	% diff
1.	492	510	-4	430	410	5	455	435	5	440	420	5
2.	399	432	-8	310	305	2	399	381	5	350	322	8
3.	375	399	-6	370	366	1	385	375	3	355	330	7
4.	452	468	-4	488	460	6	475	425	12	456	424	7
5.	463	475	-3	394	390	1	415	402	3	390	378	3
6.	489	508	-4	488	465	5	456	440	4	490	473	3
7.	466	480	-3	470	434	8	449	445	1	460	440	4
8.	309	325	-5	472	445	6	389	375	4	412	400	3
9.	390	412	-6	415	410	1	411	400	3	432	418	3
10.	425	470	-11	469	459	2	447	440	2	470	455	3
11.	445	480	-8	475	469	1	447	460	4	517	490	5
12.	395	464	-17	457	440	4	429	406	5	455	410	10
13.	459	485	-6	424	395	7	442	408	8	430	400	7
14.	418	450	-8	459	445	3	460	450	2	475	455	4
15.	491	525	-7	501	479	4	470	455	3	492	489	1
Sum	6468	6883	-98.3	6622	6372	54.8	6559	6297	61.6	6624	6304	73.75
Average	431.2	458.9	**-6.6**	441.5	424.8	**3.7**	437.3	419.8	**4.1**	441.6	420.3	**4.9**

DISCUSSION

The present study has brought a new strategy for the treatment of acne.

The acne treatment is always approached from the angle of killing the causative agent (5). Even the effective microbicidal agents are proven to be less effective in treating acne, this phenomenon could not be due to a lack of efficacy of such agents, but may be due to their poor residency over the skin. Further, any preparations, when applied over the face, would likely to occlude the sweat glands activity. Hence, such occlusion may accelerate profuse sweating (6). Due to the consequent profuse sweating, the possibility of anti-acne agents to be washed off is quite high (7). Therefore, if the residency of the anti-acne preparations is increased along with the water resistance capacity, such products may offer an effective answer to acne problems.

If the residency of the products could be increased, even with topical agents of *P. acnes* also, we can achieve greater treatment success.

Our present study clearly shows that the anti-acne pack is quite effective in preventing the bacterial growth. However the above product did not show great microbicidal activity at short contact period.

The reason we wish to attribute to the obtained effect of anti-acne pack in inhibiting the bacte-

rial growth, is its high residency over skin combined with its good water resistance.

The water resistance capacity of the anti-acne pack was established by using a very sensitive instrument called Mexameter useful to measure the skin erythema value. 15 minutes of contact time with the anti-acne pack was sufficient to have a greater residency over the skin.

After 15 minutes when the cream was washed and the skin was exposed to sun activity, the recovered erythema was extremely low in skin regions treated with higher concentration of anti-acne pack. This suggests that the cream not only has a greater affinity and residency over the skin structure but also it is resistant to water washing.

This study based on volunteers reaffirms our laboratory finding about the effect of our anti-acne pack in inhibiting the bacterial growth. Although, the anti-acne pack could not affect the life of bacteria, certainly it seems to inhibit their growth. Once the microbial growth is inhibited, the acne eruptions can be significantly reduced.

The above finding clearly establishes a new strategy for the treatment of acne even with static preparations.

References

1) **Fitz-Gibbon S, *et al* (2013)** *Propionibacterium acnes* Strain Populations in the Human Skin Microbiome Associated with Acne. *J. Invest. Dermatol.,* **133:**2152-2160.
2) **Titus S, Hodge J. (2012)** Diagnosis and treatment of Acne. *American Family Physician,* **86(8):**734-740.
3) **Janani S, Gayathri R. (2015)** 'Quick kill effect' of a herbal shampoo versus contact time. *J. Appl. Cosmetol.,* **33:**1-7.
4) **Aruna V, Janani S, Gayathri R, Abbas A. (2014)** Adhesion and Arrest of Skin Microbes as Reason for Efficacy of a Pimple Cure Cream Paste (PCCP) – A Siddha Drug. *J. Appl. Cosmetol.,* **32:**153-163.
5) **Nakatsuji T, Kao MC, et al (2009)** Antimicrobial Property of Lauric Acid against *Propionibacterium Acnes:* Its Therapeutic Potential for Inflammatory Acne Vulgaris. *Journal of Investigative Dermatology,* **129:**2480–2488.
6) **Zhai H. and Maibach H.I. (2002)** Skin occlusion and irritant and allergic contact dermatitis: an overview. *Contact Dermatitis,* **44 (4):**201-206.
7) **Giese AC. and Wells J.M. (2006)** Sweat and water resistant sunburn preparations. *J. Amer. Pharma. Assn.,* **35 (7):**208-212.

Author Address:

Janani S.
Dr. JRK's Siddha Research and Pharmaceuticals Pvt Ltd.
19, Perumal Koil Street
Kunrathur, Chennai - 600 069 - India
E-mail: 89jananis@gmail.com
E-mail: labs@jrksiddha.com