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A Topical Cream Containing Niacinamide 4% and Gluconolactone 14% Modifies the Local *C. Acnes Population* in Patients with almost no or Mild Facial Acne

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Abstract
Background: *C. acnes is involved in acne.*
Aim: *To assess the evolution of C. acnes phylotypes,the antimicrobial activity, the risk for inducing antibiotic resistance emergence and the clinical efficacy of a cream containing niacinamide and gluconolactone.*
Methods: *Prospective, open-label, non-comparative study performed in 21 adult patients with almost no or mild facial acne according to the GEA score.*
The main evaluation criterion was the genomic analysis of C. acnes phylotypes, using standard methods and clinical assessments made at baseline and Day56. Adverse events were reported throughout the study.
Results: *At baseline, the mean age was 23.5±5.5 years, 85.7% of the patients were female, main C. acnes phylotype was IA1 (71.4%). A C. acnes strain, resistant to clindamycin and erythromycin, potentially due to a mutation at position A2059G, was observed in two patients. The mean number of inflammatory lesions was 13.1±3.6 and the number of open or closed come dones was 20.2±5.4. At Day 56, a shift to a different C. acnes phylotype was observed in eight patients; in seven patients phylotypes remained unchanged. C. granulosum was still present in one patient, while no resistance or intermediate sensitivity was observed anymore. In one patient, RNA mutation 16S was absent, despite an intermediate susceptibility to tetracycline. The cream caused no antibiotic resistance emergence. Inflammatory and non-inflammatory lesion count and global severity had significantly improved from baseline; safety was excellent.*
Conclusion: *The tested cream improves acne by modifying C. acnes phylotypes, thus changing the C. acnes population, without causing antibiotic resistance emergence.*
Keywords: *Acne, C. acnes phylotypes, gluconolactone, niacinamide, antibiotic resistance, inflammatory lesions, microbiota.*

Introduction

In daily life, the skin microbiota equilibrium is challenged through intrinsic and extrinsic factors [1]. Disturbing this very fragile balance, also called dysbiosis, may lead to a disequilibrium among the cutaneous inhabitants or disturbed skin homeostasis, to an activation of the innate immunity and finally to inflammatory skin conditions such as acne[2].

C.acnes is a Gram-positive bacterium. It is a significant member of the human skin microbiota [3]. Multi-locus sequence typing (MLST) and single-locus sequence typing (SLST) schemes have allowed the *C. acnes* population to be subdivided into six main phylogenetic types: IA1, IA2, IB, IC, II, and III[4]. Of those, phylotype IA1 has been associated with acne[5]. In 2013, Fitz-Gibbon reported that other phylogenetic types, such as IB and II, were also associated with acne[6].

A certain number of studies assessing the ability of different *C. acnes* phylotypes to trigger specific immune responses focused on innate immunity[7]. Results showed that *C. acnes* phylotype II induced higher levels of interleukin- (IL)-8 in keratinocytes, and phylotype IA induced higher involucrin[8]. Both *C. acnes* IA and IB phylotypes induced a greater b-defensin response in sebocytes than phylotype II, while lysates from different *C. Acnes* phylotypes had various effects on human skin explants, and *C. acnes* phylotype I was more readily endocytosed than phylotype II [9], [10].

Dysbiosis is the result of an unbalanced microbiota. In acne, dysbiosis is associated with a loss of diversity of the skin microbiota and with the selection of specific phylotypes of the skin commensal *Cutibacterium acnes* (*C. acnes)* through antibiotic treatments [7],[11]. The use of topical antibiotics causes selective pressure on the skin microbiota, eliminating antibiotic-susceptible bacteria, resulting in the proliferation of mainly macrolide- and clindamycin-resistant bacteria, which both have been used to treat acne[12]. As a consequence, according to the selected phylotypes, the severity of inflammatory acne lesions may differ.

The present study aimed to assess the evolution of *C. acnes* phylotypes, the antimicrobial activity and the risk of inducing antibiotic resistance emergence after 56 days of topical daily use of a cosmetic product (Sebiaclear® active, Laboratoire SVR, France) containing niacinamide 4% and gluconolactone 14%. Niacinamide, or nicotinamide, is an amide form of vitamin B3, known for increasing cellular energy and regulating poly-ADP-ribose-polymerase 1, an enzyme involved in DNA repair and regulation of inflammatory cytokines[13]. Gluconolactone is a new-generation polyhydroxy acid has keratolytic properties but does not cause irritation or stinging[14].

Moreover, the study aimed to confirm its clinical efficacy and tolerance in the management of acne [15].

Methodology

This prospective, open-label, non-comparative study was conducted between November 2016 and December 2016 at one investigational site in France. The study received, prior to inclusion of any subject, approval from the local ethics committee (TLT/BB CPP N°564/2016), the ANSM health authorities and was conducted according to the principles of the declaration of Helsinki, Good Clinical Practices and to local legal requirements for the conduct of clinical studies

This study planned for the recruitment of 21 patients aged between 18 and 45 years with a facial acne score of 1 (almost no lesions) or 2 (mild acne) according to the GEA acne scale[16]. Suitable patients had to respect a washout period of up to 12 weeks if they received systemic and up to four weeks if they used topical acne treatments. A washout period of two weeks was to be respected if patients applied specific cosmetics to manage their acne.

The study lasted 56 days, during which patients received a cosmetic product containing niacinamide 4% (Sebiaclear® Active, Laboratoire SVR, France) to be applied twice daily, in the morning and evening; on the entire face. Prior to any application, patients were advised to gently wash their face using a provided foaming gel (Sebiaclear® foaming gel, Laboratoire SVR, France). Moreover, patients were provided with a sunscreen with a sun protecting factor of 50 (Sebiaclear SPF50®, Laboratoire SVR, France) which was to be applied if the subject planned to expose his/her face to sunlight.

The main evaluation criterion was the genomic analysis of *C. acnes* phylotypes at baseline and Day 56.

Bacteriological samples were made at baseline and Day 56. Intra-lesional sampling was performed on identified acne zones with a cotton swab, discharged in a Brain Heart broth and sent to the Bacteriology Department of Nantes University Hospital within 30 minutes, for standard testing of the resistance to antibiotics currently used to treat acne through counting of colony forming units (CFU) and for genomic analysis of *C. acnes*.

All *C. acnes* isolates were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry with a VitekMS® mass spectrometer (bio Mérieux SA, Marcy-l'Etoile, France). The Vitek MS IVD system was used for MALDI-TOF MS identification and based on a mass spectral fingerprint generated and identified automatically by the MYLA software database version 3.2.0-7. According to bio Mérieux recommendations, a calibration was performed using *Escherichia coli* ATCC3739. All strains were identified with an accuracy of >99.9%.

Total DNA from *C. acnes* isolates was extracted using the Insta Gene Matrix method (Bio-Rad Laboratories, Hercules, CA, USA). The procedure was performed according to the manufacturer's instructions. After centrifugation, the supernatant was used as a DNA template for PCR analysis, according to the method described by Furustr and-Tafin et al. [17].

The phylotype was determined according to the method recently developed by Barnard et al.[18] The Single Locus Sequence Typing (SLST) was carried out on all isolates as described by Scholz et al.[4]. Multi-Locus Sequence Typing (MLST) was conducted on all isolates, as described by Kilian et al[19].

Mutation of the 23S rRNA gene, the main macrolide resistance factor, was detected by PCR and DNA sequencing, according to methods reported by Oprica[20]. Moreover, mutations in the 16S rRNA gene (a tetracycline resistance factor) were made by DNA sequencing.

Susceptible control strains *C. acnes* ATCC 6919 and *C. acnes* ATCC 11828 were used as negative controls. Alignment analysis was made using the Clustal W interactive multiple sequence alignment at European Bioinformatics Institute (http://www.ebi.ac.uk). Sequences of all target genes were compared to those of *C. acnes* reference strain (Gen Bank accession number NC006085), using different free software available on the Internet (https://blast.ncbi.nlm.nih.gov/Blast.cgi, http://www.genome.jp/tools/clustalw/ and http://web.expasy.org/translate/).

Clinical assessments took place at baseline, Day 28 and Day 56. Clinical evaluations, including acne severity using the GEA scale, as well as inflammatory lesion and open or closed come done count, were performed at all three visits. The clinical efficacy, safety and local tolerance of the product were assessed by the investigator using a 4-point scale from none to excellent. Adverse events were reported by the subject throughout the study.

Mean values of clinically assessed parameters were compared to baseline and quantitative values were tested at all visits using the Student t-test or the Wilcoxon test in the intent-to-treat (ITT) and per protocol (PP population). Percentages were compared using the Chi2 test or the Fisher exact

test, if the number was inferior to 5. Qualitative data were tested using the Mc Nemar test for binary and the Stuart-Maxwell test for more than two modalities. A bilateral risk α of 5% was applied. Statistical analyses were performed using SAS® version 9.4.

Results

Subject and baseline data

Overall, 21 acne patients were recruited. Due to major protocol violations, one patient was not considered for the PP statistical analyses of clinical parameters. No patient withdrew from the study prematurely. The patient mean age was 23.5±5.5 years, 85.7% of the patients were female and 57.1% had phototype III according the Fitzpatrick scale. Mean acne duration prior to inclusion was 9.4±5.1 years, 95.2% of the patients had mild facial acne (grade 2) and 4.8% had almost no acne (grade 1). The mean number of inflammatory lesions was 13.1±3.6 and that of open or closed come dones was 20.2±5.4.Detailed demographic data are provided in Table 1. Twelve patients had previously used an anti-acne preparation and respected specific wash-out periods.

Microbiological testing and genomic analysis

Microbiological testing and genomic analyses were performed in all 21 patients.

At baseline, the main *C. acnes* phylotype was IA1 (71.4%, 15 patients). One *C. acnes* strain resistant to clindamycin and erythromycin, potentially due to a mutation at position A2059G was observed in two patients.

Detailed information about microbiological investigations and genomic analyses at baseline is provided in Table 2.

After 56 days, the number of CFU had changed in 18 patients; in nine patients the number had increased, while in nine other patients the number had decreased. In three patients the number of CFU remained unchanged.

Moreover, a shift to a different *C. acnes* phylotype was observed in eight patients, while in seven patients phylotypes remained unchanged.

C. granulosum was still present in the investigated zone of subject n° 7,while no resistance or intermediate sensitivity was observed anymore for *C. acnes* strains present inpatientn°14 and n°17.In patient n° 14, RNA mutation 16S was absent, despite an intermediate susceptibility to tetracycline.

Niacinamide 4% caused no antibiotic resistance emergence to any of the tested antibiotics.

Detailed results for microbiological investigations including changes of the single locus and multi-locus sequence typing by patient after 56 days are provided in Table 3.

Clinical efficacy

The mean number of inflammatory lesions had significantly decreased after 28 days (9.3±4.2 vs 13.1±3.6 lesions at baseline, $p<0.001$) and 56 days (5.7±2.4 lesions,) compared to baseline, corresponding to a mean reduction of 55.3±17.5% ($p<0.0001$). A statistically significant decrease of open and closed come dones was observed after 56 days (15.4±5.3 vs 20.2±5.4 come dones at baseline), corresponding to a mean reduction of 22.7±20.2%, $p<0.0001$).

At Day 28, a total of 33.3% ($p=.0.0143$) of all patients had almost no acne lesions (grade 1) and 66.7% had a grade 3 acne observed on their face. After 56 days of use, 57.1% vs 4.8% at baseline of all patients had almost no acne lesions anymore (grade 1), 42.9% vs 95.2% at baseline still had mild facial acne (grade 2). Improvement from baseline was statistically significant ($p=0.0009$). None of the patients had grade 0 acne observed. The investigator considered the cosmetic product efficacious in 61.9% of all patients and very well tolerated in 81.0% of the patients.

A total of five patients reported five local mild adverse events, mainly dryness, considered by the investigator as being potentially related to the product.

Complementary analysis

A complementary statistical analysis was conducted to assess the correlation between clinically assessed parameters and microbiological and genomic analysis. As a result, in one patient presenting with a *C. granulosum* strain, the percent decrease of inflammatory lesions was less important compared to all other patients, while there was no difference for the open or closed come done count. In another patient presenting with a macrolide-resistant strain with a point mutation at position A2059G, the number of inflammatory lesions had slightly increased between Day 28 and Day 56, while a slight linear decrease was observed for patient n° 14. The percent change in the number of open or closed comedones for these two did not differ from those for other patients.

Table 1 Demographic and baseline data (ITT population)

	N = 21
Gender	
Female	18 (85.7%)
Male	2 (14.3%)
AGE (year)	
Mean±SD	23.5±5.5
Median	22.5
Min / Max	18.2 / 41.1
PHOTOTYPE	
I	1 (4.8%)
II	4 (23.8%)
III	12 (57.1%)
IV	2 (9.5%)
V	1 (4.8%)
ACNE HISTORY (Years)	
Mean±SD	9.4±5.1
Median	7.9
Min / Max	2.9 / 26.9
ACNE SEVERITY ACCORDING TO GEA SCALE	
Grade 1	1 (4.8%)
Grade 2	19 (95.2%)
NUMBER OF INFLAMMATORY LESIONS	
Mean±SD	13.1±3.6
Median	12.0
Min / Max	7.0 / 20.0
NUMBER OF OPEN OR CLOSED COMEDONES	
Mean±SD	20.2±5.4
Median	18.0
Min / Max	13.0 / 30.0

Table 2 Microbiological investigations at Day 0 (Intent to treat population, N=21)

Patient	Zone	Count (CFU/cm²)	Phylotype	SLST	MLST	E	CLI	TET	Other
1	T-zone	$1.43x10^3$	IA2	**D4***	CC28	S	S	S	
2	Chin	$2.86x10^3$	IA1	D1	CC28	S	S	S	
3	T-zone	$>6.67 \times 10^3$	II	K1	CC53	S	S	S	
4	right cheek	1.17×10^3	IA1	A1	CC18	S	S	S	
5	left cheek	1.47×10^3	IB	**H6***	CC36	S	S	S	
6	Chin	$>6.67 \times 10^3$	IA1	A1	CC18	S	S	S	
7	below mouth	4×10^2				S	S	S	*C. granulosum*
8	left cheek	$>6.67 \times 10^3$	II	K16	CC53	S	S	S	
9	right cheek	0.93×10^3	II	K1	CC53	S	S	S	
10	right cheek	1.2×10^3	IA1	A1	CC18	S	S	S	
11	left cheek	1.43×10^3	IA1	D1	CC28	S	S	S	
12	Chin	3.3×10^1	IA1	A1	CC18	S	S	S	
13	Chin	$>6.67 \times 10^3$	IA1	C1	CC3	S	S	S	
14	Chin	6.6×10^1	IA1	A1	CC18	**R**	**I**	S	A2059G
15	right cheek	2.2×10^3	IA1	A1	CC18	S	S	S	
16	left cheek	2.4×10^3	IA1	A1	CC18	S	S	S	
17	T-zone	1.17×10^3	IA1	A1	CC18	**R**	**R**	S	A2059G
18	left cheek	5.3×10^3	IA1	A1	CC18	S	S	S	
19	T-zone	$>6.67 \times 10^3$	IA1	A1	CC18	S	S	S	
20	T-zone	$>6.67 \times 10^3$	IA1	**A30***	CC18	S	S	S	
21	left cheek	$>6.67 \times 10^3$	IA1	D1	CC28	S	S	S	

SLST: Single Locus Sequence Typing, MLST: Multi-locus Sequence Typing, E: erythromycin, CLI: clindamycin, TET: tetracycline
S: sensitive, R: resistant, I: intermediary, * SLST described for the first time and confirmed by ChristanScholz, SLST data base curator, Department of Biomedicine, Aarhus University, Aarhus, Denmark

Table 3 Microbiological investigations at Day 56 (Intent to treat population, N=21)

Patient	Zone	Count (CFU/cm²)	Phylotype	SLST	MLST	E	CLI	TET	Other
1	T-zone	>6.67 x 10^3	II	**K21***	CC53	S	S	S	
2	chin	2.86 x 10^3	IA1	**D5***	CC28	S	S	S	
3	T-zone	>6.67 x 10^3	II	K1	CC53	S	S	S	
4	right cheek	4 x 10^2	IA1	D1	CC28	S	S	S	
5	left cheek	4.2 x 10^3	IB	**H6***	CC36	S	S	S	
6	chin	5.1 x 10^3	II	K1	CC53	S	S	S	
7	above lips left side	3.23 x 10^3	IA1	D1	CC28	S	S	S	
	above lips left side	6.6 x 10^1				S	S	S	*C. granulosum*
8	left cheek	8.7 x 10^2	IA1	E3	CC31	S	S	S	
9	right cheek	1.07 x 10^3	IA1	A1	CC18	S	S	S	
10	right cheek	3.33 x 10^2	IA1	A1	CC18	S	S	S	
11	left cheek	>6.67 x 10^3	III	L5	CC43	S	S	S	
12	chin	8.6 x 10^2	IA1	A1	CC18	S	S	S	
13	chin	>6.67 x 10^3	IA1	C1	CC3	S	S	S	
14	chin	6 x 10^2	IA1	A1	CC18	S	S	**I**	Mutation 16S missing
15	right cheek	6,67 x 10^3	IA1	A1	CC18	S	S	S	
16	right cheek	3.47 x 10^3	IB	H4	CC36	S	S	S	
17	T-zone	7 x 10^2	IA1	C1	CC3	S	S	S	
18	left cheek	3.33 x 10^2	IA1	D1	CC28	S	S	S	
19	T-zone	>6.67 x 10^3	II	K2	CC53	S	S	S	
20	left temple	6.67 x 10^1	IB	H1	CC36	S	S	S	
21	right cheek	4.3 x 10^3	IA1	D1	CC28	S	S	S	

SLST: Single Locus Sequence Typing, MLST: Multi-locus Sequence Typing, E: erythromycin, CLI: clindamycin, TET: tetracycline
S: sensitive, R: resistant, I: intermediary, * SLST described for the first time and confirmed by ChristanScholz, SLST data base curator, Department of Biomedicine, Aarhus University, Aarhus, Denmark

Discussion and conclusion

The present study confirmed through genomic analyses that *C. acnes* phylotype IA1 is frequently found in patients with acne [21].

Genomic analyses after 56 days of twice daily use of a cosmetic product containing Niacinamide 4% showed a qualitative difference among the predominant *C. acnes* strains in culture. In two thirds of the patients, this shift was due to a genetic evolution, while in one third a shift of the predominant cluster in cultureoccurred within the same phylogenetic group with a slight modification in the recovered SLST types. These results indicate that the tested product might have led to a modification of *C. acnes* phylotypes and thus to an impact on the dynamic *C. acnes* population with a potential modification of the composition of the skin microbiota.

Based on our results, one may hypothesize that when correlating clinical results with results from genomic analysis, changing the composition of the *C. acnes* population on the skin may reduce the number of pro-inflammatory *C. acnes* strains. As a result, the number of less- or non-inflammatory strains of *C. acnes* may increase, leading ultimately to an improvement of the acne severity. However, this hypothesis still has to be confirmed through a larger controlled clinical study.

The study confirmed that a point mutation at position A2059G, observed in two strains, is an indicator of resistance to macrolides, as shown by the resistance to erythromycin and clindamycin in two lesion samples[22].The absence of the typical 16S RNA mutation at position G1058C in one *C. acnes* sample suggested the potential involvement of other mechanisms[23].

After 56 days of daily application, no antibiotic resistance emergence to erythromycin, clindamycin or tetracycline was observed; there was no significant evolution of the overall number of CFU. However, we observed a change in the qualitative evolution of *C. acnes* clones, a modified balance of the *C. acnes* population, no selection of resistance to any of the tested antibiotics and an increase in sensitivity to macrolides in one patient.

Even though this study was neither comparative nor randomised, results showed that after 56 days of daily applications on skin of patients with grade 1 and 2 acne, the tested cosmetic significantly reduced the number of superficial inflammatory lesions, as well as of open and closed comedones,

and improved the severity of the condition. The product was considered efficacious and well tolerated, with only a few, mild, local adverse events.

In conclusion, our study demonstrated that the tested cosmetic product containing niacinamide at 4% and gluconolactone at 14% improves acne by modifying *C. acnes* phylotypes, thus changing the *C. acnes* population present on the patients' skin without causing antibiotic resistance emergence to any of the tested antibiotics.

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Conflict of interest

The study was funded by Laboratoire SVR, France.

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