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ORIGINAL ARTICLE

Antioxidant action and microflora modulation of Vitachelox® in subjects with acne-prone skin

Azione antiossidante di Vitachelox® e modulazione della microflora cutanea in soggetti con pelle a tendenza acneica

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Abstract - Riassunto

BACKGROUND: Vitachelox® is a multi-component powder containing three botanical standardized extracts rich in polyphenols, which exerts a protective action on the skin thanks to its antioxidative, anti-inflammatory and antimicrobial properties.
METHODS: A placebo-controlled trial has been conducted to compare the changes induced by a Vitachelox®-containing product and a placebo cream in the skin resident flora of 20 acne-prone subjects. We also investigated the antioxidant properties of Vitachelox®, by assessing its antioxidant power *in vitro* compared with butylated hydroxytoluene (BHT), a standard antioxidant.
RESULTS: Vitachelox® reported a superior and statistically significant modulation in the hyperproliferation of *Propionibacterium acnes* compared with placebo, without interfering with the concentration of other commensal bacterial species such as *Staphylococcus epidermidis* and *S. capitis*. The antioxidant properties analysis showed that Vitachelox® has a considerably higher antioxidant power compared with butylated hydroxytoluene and that this effect is stable over a prolonged period of time.
CONCLUSIONS: Vitachelox® may be effective in modulating the skin microflora and reducing the *P. acnes* population in patients with acne-prone skin and may provide a beneficial contribution to skin health thanks to its potent antioxidant activity.

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KEY WORDS: Microbiota; Acne vulgaris; Antioxidants; Polyphenols.

OBIETTIVO: Vitachelox® è una polvere multicomponente che contiene tre estratti naturali standardizzati ricchi in polifenoli e che esercita un'azione protettiva sulla pelle grazie alle sue proprietà antiossidanti, antinfiammatorie e antimicrobiche.
METODI: È stato condotto uno studio controllato con placebo per confrontare le alterazioni indotte nella microflora cutanea da un prodotto contenente Vitacheox® rispetto al corrispondente prodotto placebo in 20 soggetti con pelle a tendenza acneica. Abbiamo inoltre studiato le proprietà antiossidanti (AP) di Vitachelox® valutando il suo potere antiossidante in vitro rispetto all'idrossitoluene butilato (BHT), un agente antiossidante standard.
RISULTATI: Vitachelox® ha portato a una modulazione maggiore e statisticamente significativa nella iperproliferazione di P. acnes rispetto al placebo, senza interferire con la concentrazione di altre specie batteriche commensali come S. epidermidis e S. capitis. L'analisi delle proprietà antiossidanti ha dimostrato che Vitachelox® ha un potere antiossidante notevolmente superiore rispetto a BHT e che questo effetto è stabile per un periodo di tempo prolungato.
CONCLUSIONI: Vitachelox® sembra essere efficace nel modulare la microflora cutanea e ridurre la popolazione di P. acnes in pazienti con pelle a tendenza acneica, e può fornire un contributo positivo alla salute della pelle grazie alla sua potente attività antiossidante.

Acne is a chronic and relapsing inflammatory skin disease characterized by the formation of open (black) and closed (white) comedones, inflammatory papules, pustules, nodules and cysts, which may lead to scarring and pigmentary changes.[1] The disease is mostly common among adolescents, with over 80% of teenagers affected, but its prevalence seems to also be increasing among young adults.[2]

The exact pathogenesis of the disease is not entirely understood; however, four factors are recognized to play a role in its development: excessive sebum production, follicular hyper-keratinization, *Propionibacterium acnes* colonization and release of inflammatory mediators in the skin.[3] Interestingly, although *P. acnes* has been reported to be implicated in acne development since more than 100

years, its role in the disease pathogenesis is not entirely elucidated yet, and according to recent findings, acne may be linked not simply to *P. acnes* colonization, but to the presence of specific phylogenic groups with distinct genetic and phenotypic characteristics.[4, 5]

Additional factors, such as air pollution, UV exposure and oxidative damage may contribute to acne development or exacerbation, although these aspects are not officially recognized as causes of pathogenesis. Air pollution is known for its multiple negative effects on the skin, including the generation of free radicals, induction of the inflammatory cascade, and alteration of cutaneous microflora.[6] Exposure to air pollutants, such as particulate matter (PM2.5, PM10), nitrogen dioxide (NO_2) and sulfur dioxide (SO_2), seems to aggravate pre-existing skin diseases, such as eczema, urticaria and acne.[7]

Moreover, oxidative stress, as that induced by exposure to UV radiation, may be partially involved in the pathogenesis and progression of acne vulgaris.[8] In fact, the formation of reactive oxygen species (ROS) and lipid peroxide may play a role in the initiation and progression of epithelial inflammation in the pilosebaceous unit,[9, 10] and ROS have been reported to alter the viscosity and composition of the sebum.[10, 11]

At present, multiple topical and systemic therapies are available to treat acne, including retinoids, corticosteroids and hormones; however, they cause common side effects, such as erythema, irritation, xerosis and menstrual irregularities.[12] Other treatment options include non-pharmacological interventions, such as optical therapy, comedone extraction, cryoslush therapy, cryotherapy and electrocauterization.[13] An increasing interest has also emerged towards complementary and alternative medicines, regarded as natural and safer options.[13]

Vitachelox® is a multi-component powder including three botanical standardized extracts rich in polyphenols [*Vitis vinifera* (grape) seed extract, *Camellia sinensis* leaf (green tea) extract, and *Quercus robur* (oak) wood/bark extract].[13] These active components present multiple important properties, such as antioxidative, anti-inflammatory and antimicrobial activities, which make Vitachelox® an efficient topical treatment for the protection of the skin from the effect of multiple environmental stressors, including UV exposure, air pollution and oxidative stress.[14, 15]

Recent studies have shown that Vitachelox® is able to reduce different markers of oxidative stress, including lipid peroxidation (-66% in malondialdehyde [MDA]), DNA oxidation (-59% in 8-hydroxy-deoxyguanosine) and proteins carbonylation (-87%) (data on file).

Vitachelox® has been also extensively tested in the protection of the skin from air pollutants, showing remarkable results.[14, 15] Notably, a study conducted in vivo on 30 healthy subjects showed that treatment with Vitachelox® completely prevents the permeation of heavy metals — namely chromium, nickel, iron and zinc — in the stratum corneum of the skin after exposure to a polluted environment for 6 hours.[15]

Vitachelox® has been shown to efficiently complex metal ions such as Cu(II) (data not shown).

Considering the multiple beneficial properties of Vitachelox® on the skin, we evaluated the effects of this natural product in subjects with acne-prone skin, measuring the changes in the skin resident flora after treatment with Vitachelox® compared with placebo. Moreover, we further investigated the antioxidant properties of Vitachelox® by assessing its antioxidant power *in vitro*.

Materials and methods

Placebo-controlled clinical trial

We conducted a placebo-controlled trial on 20 healthy adult male and female subjects showing clinical signs of acne-prone skin. Inclusion criteria were: 1) age between 18 and 65 years old; 2) Caucasian ethnicity; 3) mild-to-moderate acne lesion on the face (presence of 10-25 inflammatory lesions on each side of the face); 4) adequate washout period before enrollment; and 5) willingness not to vary the normal daily routine, to use the test products and to avoid intensive UV exposure during the whole study period. Exclusion criteria included use of topical or systemic treatment known to interfere with skin metabolism/physiology; use of food supplements that could interfere with the functionality of the test product; presence of skin alteration in the monitored area; intense UV exposure in the 4 weeks prior to enrolment; positive history for atopy or hypersensitive skin; past history of allergy or sensitivity to cosmetics, toiletries, solar and/or topical medications; any skin condition deemed inappropriate for participation; diagnosis of immunosuppressive diseases, such as AIDS and HIV, or use of immunosuppressive medications; and severe concurrent diseases.

Patients enrolled in the study used a neutral detergent and a neutral cream for a week before the initiation of the study (baseline phase). After this conditioning phase, the subjects used the Vitachelox® containing product daily for 30 days, applying the product on the left or right side of the face, on clean skin. Subjects were randomly assigned to receive the test product on the left side of the face and the placebo product in the right side of the face or vice versa.

Skin flora collection was performed at baseline and after 30 days of treatment, by swabbing different areas of the skin (cheeks, forehead or chin, depending on the location of acne lesions), 8 hours after face washing with water. Collected samples were subjected to quantitative PCR analysis to determine the relative abundance of selected bacterial species represented in the skin flora (*P. acnes*, *Staphylococcus epidermis*, *S. capitis*, and *S. aureus*). In addition to this, we conducted a more extensive metabarcoding analysis on 20 different volunteers (40 samples, one swab sample per cheek) including global skin flora DNA sequencing and bioinformatics data analysis.

The study was approved by the local ethics committee and all the participants signed an informed consent form prior to enrollment.

Data are presented as mean, percentage and standard error. Data were also submitted to paired t-test. Statistical analysis was carried out using a Microsoft® Excel 2013 v. 15.0 (Redmond, WA, USA) worksheet running on Microsoft® Windows 8.1 Professional.

In-vitro determination of antioxidant power

We conducted an *in-vitro* test to measure the antioxidant power (AP) of Vitachelox® in comparison with a known antioxidant (butylated hydroxytoluene [BHT]), and to assess the stability of the two molecules over time.

The AP test is an innovative method that determines the antioxidant capacity and the antioxidant activity of a tested substance by measuring the reduction of a stable radical (diphenyl-picryl-hydrazyl) through the electron spin resonance spectroscopy. Different concentrations of the tested substances are usually evaluated, and the test is repeated over time to evaluate the long-term stability. The AP is calculated by taking into account both the reaction time (t_r) and the reduction capacity (w_c) (AP = N. free radicals / [$w_c \times t_r$]), and the results are expressed as antioxidant units (AU), where 1 AU corresponds to the activity of a 1 ppm solution of pure vitamin C (ascorbic acid).[16, 17]

Vitachelox® and BHT were incorporated each in a different placebo formulation (Physiogel) at a final concentration of 0.5%; samples were stored at room temperature protected from light. The AP test was performed at baseline, and repeated at subsequent time point (48 hours, 4, 7, 10, and 15 days) to assess the long-term stability of each substance.

Results

Placebo-controlled clinical trial

The study flow chart is represented in Figure 1. As summarized in Figure 2, the areas of the skin treated with Vitachelox® reported a considerable decrease in the concentration of *P. acnes*, which was reduced from 4.75±0.09 at baseline to 4.05±0.15 after 30 days. No difference in *P. acnes* was observed after treatment with placebo (4.76±0.15 at 30 days). The concentration of other bacterial species, namely *S. epidermidis* and *S. capitis* (2.43±0.16 and 1.76±0.10, respectively, at baseline) were unchanged after 30 days of treatment both with Vitachelox® (2.49±0.10 and 1.77±0.21, respectively) and with placebo (2.56±0.14 and 1.64±0.25, respectively). The concentration of *S. aureus* was not significantly represented in the collected skin flora and therefore was not analyzed. Moreover, the metabarcoding analysis shown that, in the semi-quantitative approach used, Vitechelox® did not alter the natural skin microbiota (data on file).

In-vitro determination of antioxidant power

The results of the AP analysis are summarized in Table I. At T0 Vitachelox® showed an over 17-fold increase in AP value compared to BHT at the same concentration (1.563 *vs.* 88 AU, respectively). The antioxidant capacity (w_c) of the two molecules was comparable (0.548 *vs.* 0.492 mg), while a considerable difference was measured in terms of t_r (6.85 *vs.* 0.43 min).

The analysis of the long-term stability showed that BHT maintained a low but stable antioxidant activity over 15 days. On the other hand, the Vitachelox® containing gel reported a decreased in its antioxidant power, with a reduction of 63% of the initial value after 15 days of storage. This decrease, however, was only attributed to a slight decrease in reactivity (t_r value increased from 0.43 to 0.62 min), while the capacity remained almost stable (from 0.492 to 0.576 mg). At the end of the study period the AP of Vitachelox® was approximately 14 times superior to that of BHT (976 *vs.* 76 AU) (Table I).

Discussion

The results of this study show that Vitachelox®, a natural product known for its protective properties against multiple

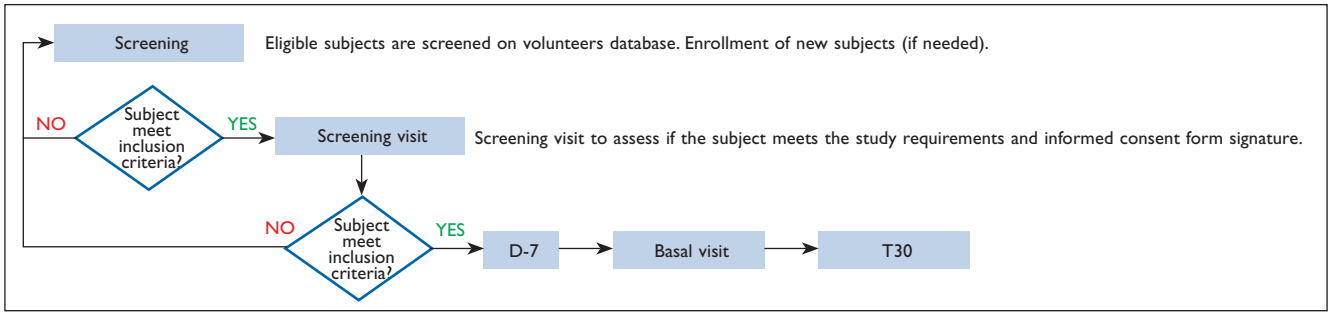

Figure 1.—Flow chart of the study and scheduled assessment. Skin flora collection was performed at basal visit and at 30 days, 8 hours after last washing with water.
D-7: 1-week conditioning period during which subjects use a neutral detergent and a neutral cream supplied by Complife.

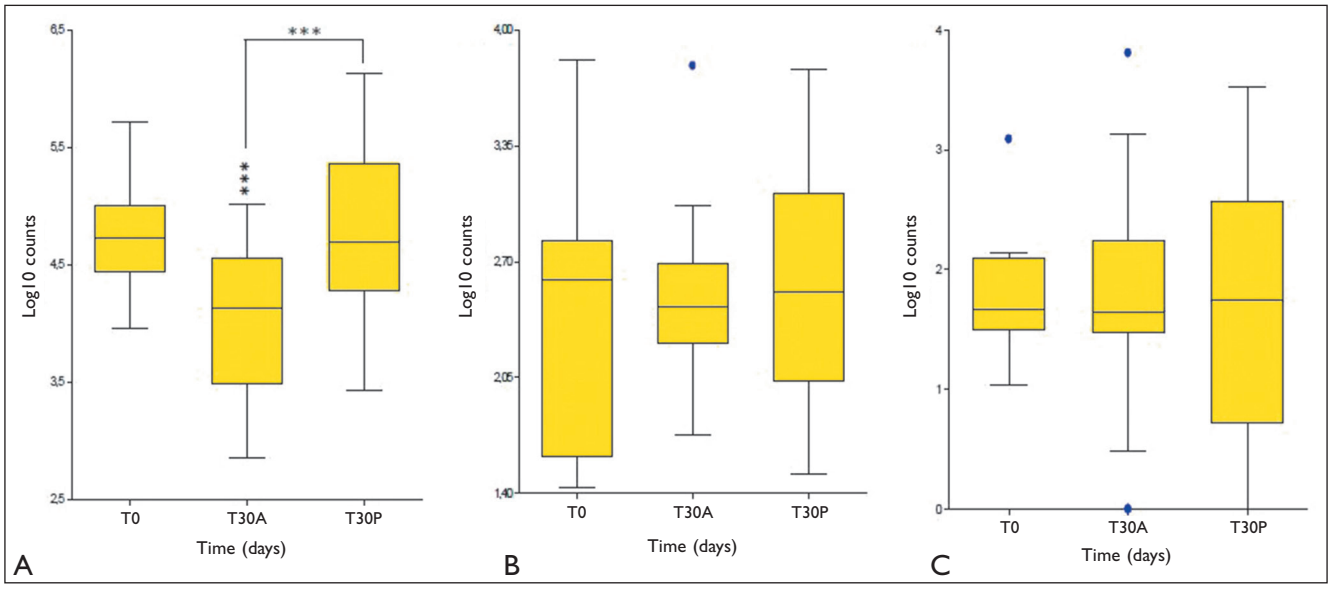

Figure 2.—A) *P. acne* counts; B) *S. epidermis* counts; C) *S. capitis* counts. The intragroup statistical analysis (*versus* T0) is reported between two boxes. The intergroup statistical analysis (T30A *vs.* T30P) is reported above each box. The box portion of the box plot is defined by two lines at the 25th percentile and 75th percentile.
*P<0.05; **P<0.001; ***P<0.001; ● mild outlier.

Table I.—*Antioxidant power of Vitachelox® and BHT over time.*

Tested substance	Timepoints					
	Baseline	48 hours	4 days	7 days	10 days	15 days
BHT						
AP, AU	88	85	75	67	85	76
t_r, min	6.85	7.72	8.03	9.98	6.59	6.21
w_c, mg	0.548	0.505	0.552	0.495	0.589	0.595
Vitachelox®						
AP, AU	1.563	1.185	1.110	1.037	957	976
t_r, min	0.43	0.56	0.61	0.67	0.69	0.62
w_c, mg	0.492	0.498	0.488	0.475	0.501	0.576

AP: antioxidant power; AU: antioxidative units; BHT: butylated hydroxytoluene; t_r: reaction time; w_c: reaction capacity.

skin stressors, might be effective in modulating the skin microflora in patients with acne-prone skin. The broad-spectrum metabarcoding analysis of the totality of skin microbiome also reveals that the product does not alter the natural variability of cutaneous microbiome, suggesting specific selectivity to determined hyper-proliferating populations. Moreover, the results of the *in-vitro* AP test provide additional evidence on the antioxidant power of Vitachelox®, which may represent a beneficial contribution to skin health.

Several studies have shown that the unbalance in the skin microbiota is associated with the development of different skin diseases.[3, 4, 18-21] In particular, in the case of acne, the overgrowth of bacteria *P. acnes* seems to play a role in the disease pathogenesis, although a clear picture of acne etiology is still lacking.[22, 23] On the other hand, *P. acnes* is believed to contribute to skin health, preventing the colonization of opportunistic pathogens via its ability to convert sebum to free fatty acids and to maintain an acidic skin pH.[24, 25] Recently, Wang *et al.* have reported that human hosts may take advantage of *S. epidermidis* fermentation within an anaerobic acne lesion to combat the overgrowth of *P. acnes*.[26]

The results of this clinical trial show that Vitachelox® led to a considerable decrease (-0.7 logarithmic units) in the concentration of *P. acnes*, with superior and statistically significant results compared with placebo, and without interfering with the concentration *of S. epidermidis* and *S. capitis*. These preliminary results indicate an effect

of Vitachelox® in influencing the skin resident flora with a selective effect on *P. acnes* population, which might be beneficial in acne treatment. Such selectivity is additionally confirmed by the maintenance of cutaneous microbiome integrity that has been demonstrated through the metabarcoding DNA analysis.

Another interesting aspect explored in this study is oxidative damage, a potential stressor involved in the development and exacerbation of acne. In fact, the presence of excessive ROS has been implicated in all the major alterations observed in acne,[8] and a study comparing subjects with acne vulgaris and healthy volunteers showed significantly higher signs of oxidative stress among patients with acne.[26]

The results of the *in-vitro* AP test further confirm the potent antioxidant activity of Vitachelox®, which showed an extremely higher AP (>17-fold) compared with a standard antioxidant (BHT). Notably, the difference in AP was mainly attributed to the higher reactivity of Vitachelox®, which — although slightly decreasing over time — remained in the range of polyphenols at a low oxidation state even after 15 days of storage. The potent AP showed by Vitachelox® and the stability over a prolonged period of time may play an important role in reducing oxidative stress in the skin, with possible beneficial effects on skin health.

Of note, the protective effect of Vitachelox® against oxidative stress has been previously reported in an *in-vitro* study, in which Vitachelox® significantly reduced protein oxidative damage (carbonylation) on human keratinocytes exposed to blue light (460 nm) (Indena, data on file).

Finally, although not directly investigated in this study, we can speculate that the protective action of Vitachelox® against skin damage induced by air pollution[14] might have an additional beneficial effect on acne-prone skin, considering the multiple negative effects that polluted air has on different skin diseases.[7] However, this aspect requires further investigation.

Conclusions

The results of this study, although preliminary and limited in number, suggest that Vitachelox® may contribute to the treatment of acne thanks to its known antioxidant properties combined with a positive modulation of the cutaneous microflora, which leads to a selective reduction in *P. acnes* colonization without affecting the other beneficial commensal bacteria.

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