The anti-acne and anti-ageing activity of a new hexapeptide in complex with zinc and its comparison to retinol

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Abstract:

Background: In this study, we evaluated a new hexapeptide in complex with zinc (Zn-peptide) for its ability to inhibit key acne-related processes *in vitro* and to improve the appearance of the acne-prone skin *in vivo* and compared it with retinol.

Materials and methods: The hexapeptide was prepared by solid phase peptide synthesis and zinc sulfate was used for the preparation of the Zn-peptide complex. Expression of the selected genes was evaluated using quantitative RT-PCR in HaCaT or NIH-3T3 cells irradiated or not irradiated with UVB and treated with Zn-peptide. The antimicrobial activity was determined spectrophotometrically using *C. acnes* culture. We also performed a split-face, placebo-controlled in vivo study on 40 Caucasian volunteers with acne-prone skin treated with 13 μg/mL Zn-peptide or 0.2 % retinol for 6 weeks and evaluated various skin parameters.

Results and discussion: Zn-peptide inhibited all four key processes in acne pathogenesis *in vitro*: downregulated 5α-reductase involved in sebum production, suppressed keratinization and showed anti-inflammatory and antimicrobial effects. In the vivo study Zn-peptide significantly reduced number of inflammatory lesions, skin pores, skin redness, sebum level and *C. acnes* number. We also observed anti-ageing effect represented by wrinkle reduction, elasticity improvement and collagen increase. The effects of Zn-peptide were comparable or better than that of 0.2 % retinol. No negative adverse effects were observed in contrast to retinol which irritated the skin at the beginning of treatment and worsened skin barrier function.

Conclusion: Zn-peptide proved to be a new retinol alternative exerting anti-acne and anti-ageing properties with no negative side effects.

Keywords: Acne; Hexapeptide; Retinol; Skin ageing; Zinc

Introduction:

Acne vulgaris is a common chronic skin disease affecting individuals of all ages. The pathogenesis of acne is characterized by four core events: hyperseborrhoea, epithelial hyperkeratinization, *Cutibacterium acnes* colonization and inflammation [1]. Due to the multifactorial nature of the disease, a combination therapy or use of multifunctional compounds are the preferred approaches. Retinoids are among the most effective compounds targeting multiple acne-associated pathways [1]. However, they often cause negative adverse effects including skin dryness and irritation [1]. Therefore, there is still a need for new, more effective and safer alternatives. In this study, we evaluated a new hexapeptide in complex with zinc (Zn-peptide) for its ability to inhibit the key acne-related processes *in vitro* and to improve the appearance of the acne-prone skin *in vivo*. The effects were compared to its individual components and retinol as the most popular retinoid used in cosmetics.

Materials and methods:

Zinc hexapeptide complex preparation

The hexapeptide was synthesized by the standard solid phase peptide synthesis, purified by HPLC and lyophilized. Zinc sulfate heptahydrate ($ZnSO_4*7H_2O$) was used for the preparation of the zinc hexapeptide complex.

Evaluation of gene expression by qRT-PCR

HaCaT keratinocytes (CLS collection) or NIH-3T3 fibroblasts (ATCC collection) were treated with Zn-peptide, its individual components (hexapeptide and $ZnSO_4*7H_2O$) of corresponding concentrations; and 10 μM retinol for 72 h. Then, gene expression of 5α-reductase, type I (*SRD5A1*), proteins involved in epidermal keratinization (*FLG, OCLN, LCE2C, SPRRE2, KRT10*) and collagen I (*COL1A1*) was determined by standard quantitative, real-time RT-PCR (qRT-PCR).

For induction of the pro-inflammatory interleukins, HaCaT keratinocytes were firstly irradiated with 10 mJ/cm^2 UVB and then treated with the tested compounds as described above for 24 h. Gene expression of interleukins IL-6 and IL-8 was determined by standard qRT-PCR.

Antimicrobial activity towards *C. acnes* growing in biofilm

Cutibacterium acnes (strain DSM 1897, DSZM collection) growing in biofilms on spikes of microtiter plate lids (Nunc™ Immuno TSP Lids, Thermo Fisher Scientific) were treated with Zn-peptide complex or its individual components (hexapeptide and $ZnSO_4*7H_2O$) of

corresponding concentrations for 24 h. The lids with spikes covered with treated biofilms were then transferred to a new microtiter plate with fresh culture medium for 24 h and the optical density was measured at 590 nm (OD590).

In vivo study

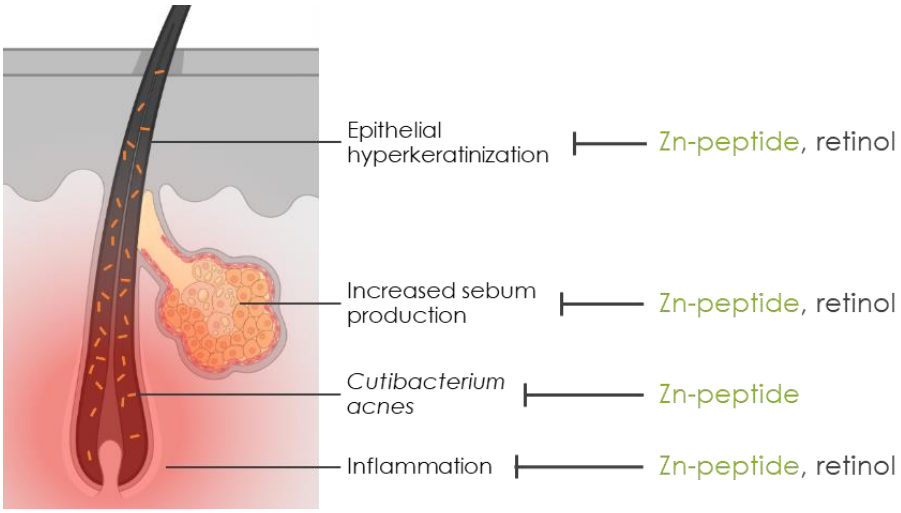
We performed a double-blind, placebo-controlled, split-face *in vivo* study on 40 Caucasian subjects with acne-prone skin. The study was approved by the Contipro´s ethical committee and informed consent was obtained from all participants. The volunteers applied two emulsions with 1 % Zinnerine (solution of Zn-peptide complex, its final concentration in the emulsion was 13.5 µg/mL) and placebo (30 subjects, 27 women/3 men, 18-48 years) or 0.2 % retinol and placebo (10 subjects, 9 women/1 man, 24-49 years) on the two respective halves of the face once daily in the evening for 6 weeks. Compositions of the tested emulsions are described in **Table 1**. The number of inflammatory acne lesions, number of skin pores, overall skin redness and the amount of protoporphyrin IX was determined by image analysis of whole-face pictures obtained by a VisiaCR camera (Canfield). Sebum was determined by a sebumeter SM815, transepidermal water loss (TEWL) by a tewameter TM300, elasticity by a cutometer dual MPA580 (all Courage-Khazaka electronics), wrinkle depth by a Primos 3D camera (Canfield), and collagen level by reflectance confocal microscopy (Vivascope 3000, Mavig).

Table 1. Composition of the tested emulsions used in the *in vivo* study:

INCI/name	**Supplier**	**% w/w**		
		Placebo	**Zn-peptide**	**Retinol**
Aqua	-	96.62	95.62	96.42
Capric/Caprylic Triglyceride	ACE Trade	0.90	0.90	0.90
Phenoxyethanol	Lachner	1.00	1.00	1.00
Hydroxyethylcellulose	Making Cosmetics	0.70	0.70	0.70
Tocopheryl Acetate (vitamin E)	Míča a Harašta	0.50	0.50	0.50
Carbomer	Lubrizol	0.25	0.25	0.25
Sodium Hydroxide	Lachner	0.03	0.03	0.03
Retinol	Sigma-Aldrich	-	-	**0.20**
Zinnerine (solution of 1.35 mg/mL Zn-peptide complex; INCI: Aqua, Hexapeptide-2, Zinc Sulfate, Phenoxyethanol)	Contipro	-	**1.00**	-

Statistical analysis

T test was used for the statistical evaluation of the results. *p< 0.05, **p<0.01, ***p<0.001 in comparison to the respective (UVB) controls or placebo if not stated otherwise.



Schema 1. Anti-acne mechanism of action of the zinc hexapeptide complex targeting all key events in acne pathogenesis and its comparison to retinol

Results and discussion:

Inhibition of sebum production (5α-reductase)

An increased production of sebum is considered one of the key events in the pathogenesis of acne. Among the most potent stimulators of sebum production are androgens whose upregulation is often associated with acne development [2]. Within the skin cells, testosterone, the most common androgen, is quickly reduced to 5α-dihydrotestosterone (DHT) by enzyme 5α-reductase [3]. Both testosterone and DHT bind to androgen receptors (ARs) promoting sebum production. However, DHT is 5-10 times more potent AR activator [4]. In the skin of acne patients, 5α-reductase is commonly upregulated leading to the higher rate of conversion of testosterone to DHT and increased sebum production [5].

Zn-peptide complex significantly inhibited gene expression of 5α-reductase. It was more effective than its individual components of corresponding concentrations and retinol (**Figure 1A**). These results show that both the hexapeptide and zinc components contribute to the observed effect of the Zn-peptide complex.

Inhibition of hyperkeratinization

Another typical event in acne pathogenesis is abnormal differentiation and hyperkeratinization of follicular keratinocytes leading to the increased cohesiveness of corneocytes and their impaired desquamation within hair follicles. The accumulated mass of corneocytes together with sebum obstruct the follicles resulting in their extension and creating favorable conditions for the colonization of *C. acnes* [6].

Zn-peptide complex as well as the hexapeptide alone were shown to downregulate genes associated with keratinocyte differentiation in the same manner as retinol whereas zinc of corresponding concentrations had no such effect (**Figure 1B**). The results show that the hexapeptide component of Zn-peptide complex is responsible for this effect.

Antimicrobial activity towards *C. acnes*

C. acnes is a bacterium living in and on the human skin as part of the normal human skin microbiome. It predominantly resides deep within the sebaceous hair follicles. It is well-established that *C. acnes* overgrowth plays a significant role in the pathogenesis of acne [7]. In the human skin, *C. acnes* does not live in a planktonic form but creates a biofilm, a community of bacteria embedded in self-produced matrix protecting it against the human immune system and also against antimicrobial therapies [8].

Zn-peptide complex showed enhanced antimicrobial activity towards *C. acnes* growing in biofilm in comparison to its separate components: hexapeptide and zinc (**Figure 1C**). Because retinol does not possess any direct antimicrobial properties, it was not evaluated.

Anti-inflammatory activity

Inflammation is regarded as a key part in the pathogenesis of acne. In the past, inflammation was thought to be a secondary event induced by *C. acnes* overgrowth. Recently, inflammatory processes are believed to be involved in all stages of acne development and acne is nowadays considered as a genuinely inflammatory disease [9].

UVB irradiation of keratinocytes strongly induced gene expression of the pro-inflammatory interleukins IL-6 and IL-8 as expected (**Figure 1D**). Subsequent treatment with Zn-peptide complex or the hexapeptide alone significantly inhibited UVB-induced gene expression of both interleukins similarly to retinol whereas zinc alone had no effect. The results suggest that the observed effect of Zn-peptide can be attributed to the hexapeptide part of the complex.

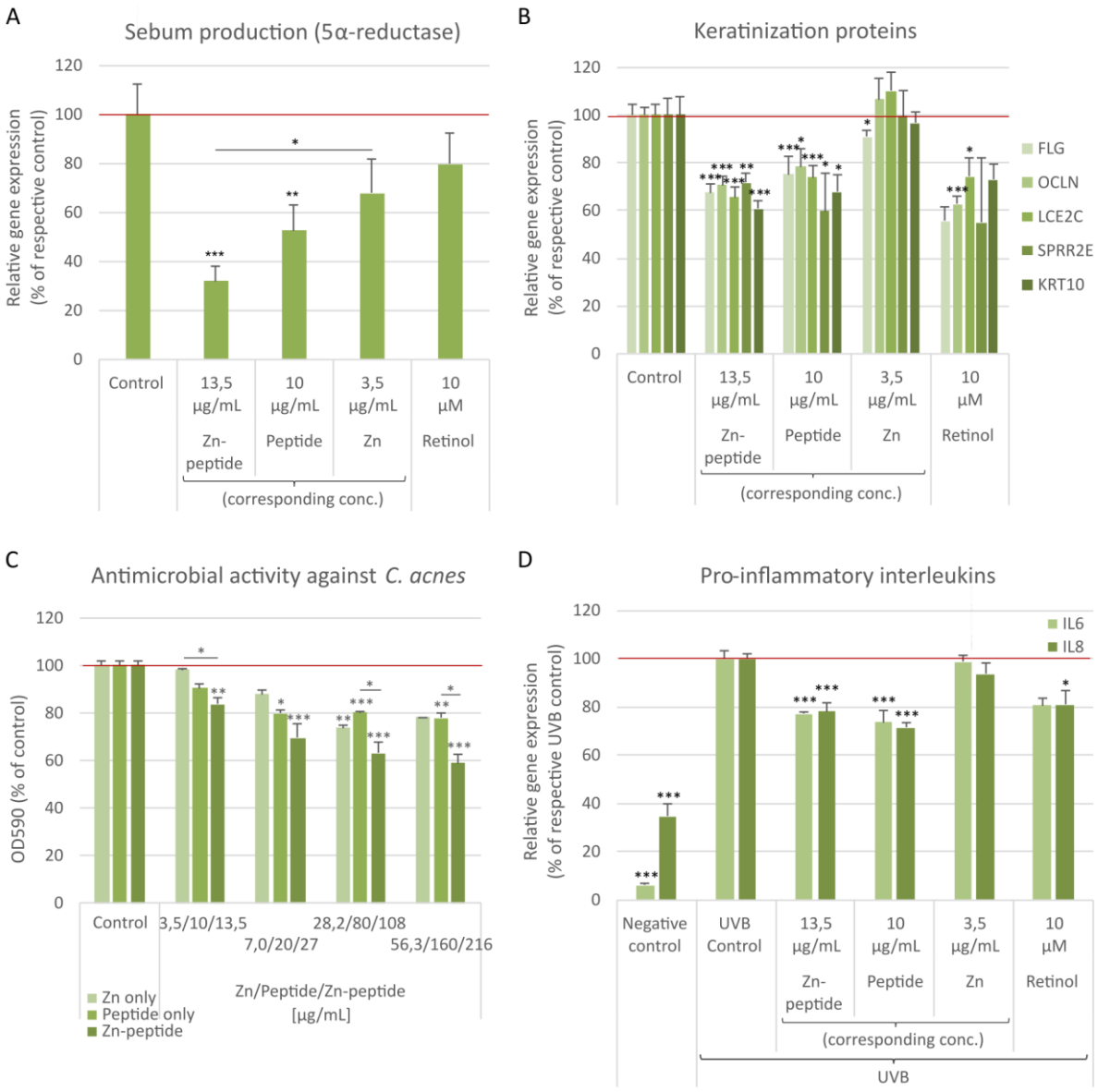


Figure 1. Zn-peptide inhibited all four key events in acne pathogenesis: sebum production (5α-reductase), hyperkeratinization, *C. acnes* overgrowth and inflammation. When compared to retinol, its effect was similar or even better. Gene expression of (A) 5α-reductase (*SRD5A1*) and (B) various proteins involved in keratinocyte differentiation: filaggrin (*FLG*), occludin (*OCLN*), late cornified envelope 2C protein (*LCE2C*), small proline rich protein 2E (*SPRR2E*) and keratin 10 (*KRT10*) was determined by qRT-PCR in HaCaT keratinocytes treated with Zn-peptide, its individual components (hexapeptide and $ZnSO_4*7H_2O$) of corresponding concentrations and retinol for 72 h. (C) Antimicrobial activity towards *C. acnes* growing in biofilm and treated with the tested compounds for 48 h. (D) Gene expression of pro-inflammatory interleukins IL-6 and IL-8 was determined by qRT-PCR in HaCaT keratinocytes irradiated with UVB and treated with the tested compounds for 24 h.

Stimulation of collagen

Retinoids have been shown to stimulate collagen production leading to the effective anti-ageing effect such as wrinkle reduction and elasticity improvement [10]. However, in *in vitro* studies using 3T3 fibroblasts, retinoids have been shown to inhibit cell proliferation and collagen production [11] as we also observed in our study (**Figure 2**). On the other hand, Zn-peptide

significantly increased collagen production. The hexapeptide part of the complex was shown to be responsible for this effect as zinc did not affect collagen gene expression.

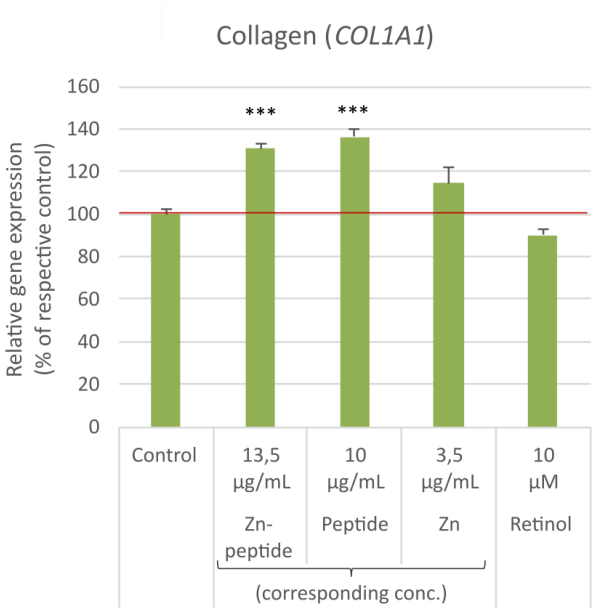


Figure 2. Zn-peptide stimulated collagen 1 gene expression in contrast to retinol. The hexapeptide part of the complex was responsible for this effect. Gene expression of collagen I (*COL1A1*) was determined by qRT-PCR in NIH-3T3 fibroblasts treated with Zn-peptide, its individual components (hexapeptide and $ZnSO_4*7H_2O$) of corresponding concentrations and retinol for 72 h.

Anti-acne and anti-ageing effect of Zn-peptide *in vivo*

In vivo study on human subjects with acne-prone skin showed that Zn-peptide significantly improved overall acne skin conditions, reduced number of the inflammatory acne lesions (**Figure 3**), sebum level (**Figure 4A**), number of skin pores (**Figure 4B, 4C**), it also slightly decreased the amount of protoporphyrin IX corresponding to the *C. acnes* number (**Figure 5**) although this effect did not reach statistical significance. Overall skin redness was also reduced (**Figure 6A, 6B**). Zn-peptide also stimulated collagen production leading to the effective reduction of wrinkles and elasticity improvement (**Figure 7**) representing the anti-ageing activity. In most cases, both the anti-acne and anti-ageing effects of Zn-peptide were comparable or even better than that of 0.2 % retinol.

Moreover, retinol worsened many skin parameters after two weeks of treatment when it increased sebum production (**Figure 4A**), *C. acnes* number (**Figure 5**) and the overall skin redness suggesting skin irritation (**Figure 6A, 6B**). Retinol also gradually increased TEWL values reflecting skin barrier impairment (**Figure 6C**). Zn-peptide, on the other hand, significantly reduced TEWL representing skin barrier reinforcement.

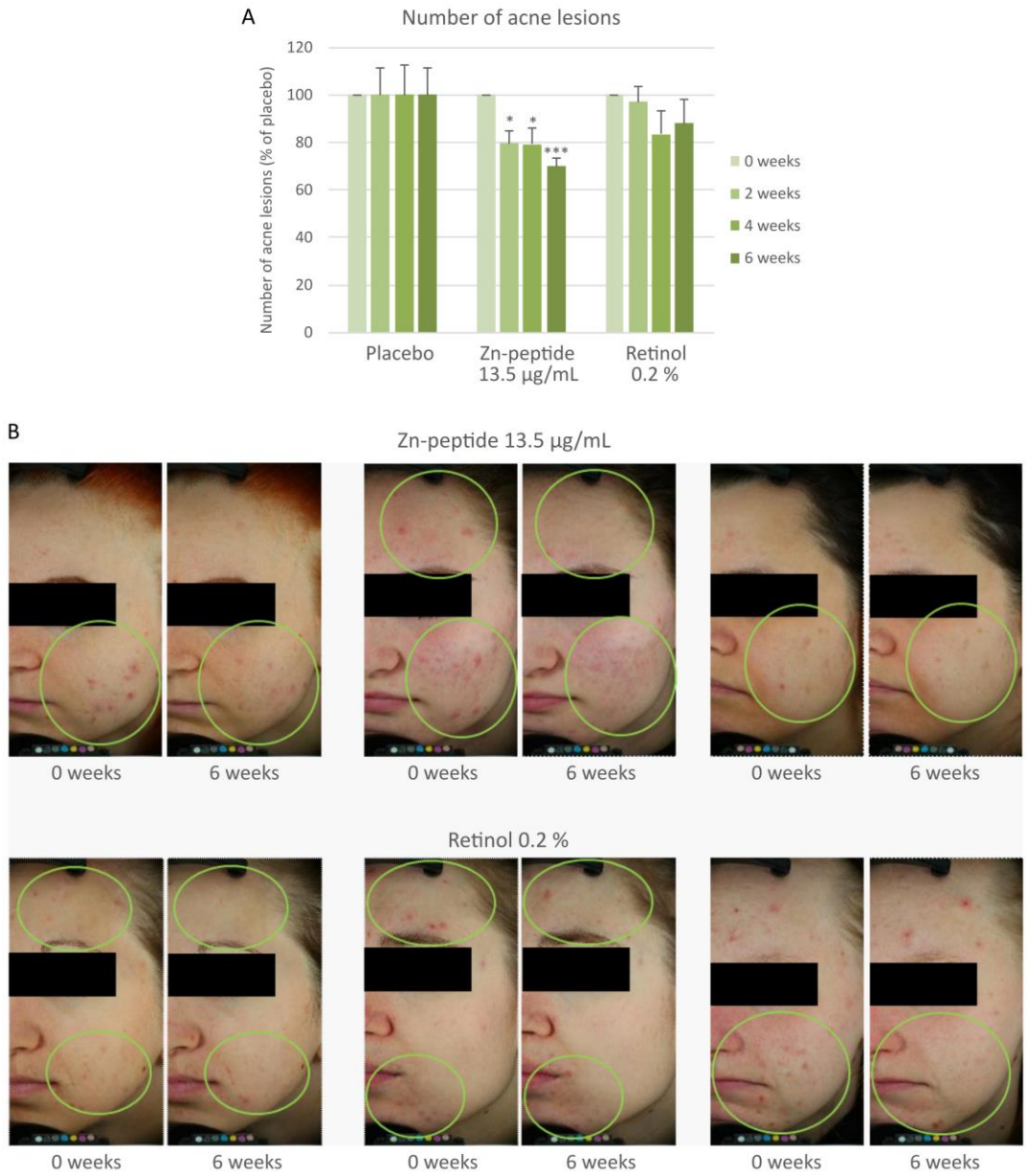


Figure 3. Zn-peptide decreased the number of acne lesions and improved the overall appearance of acne-prone skin better than retinol. The number of acne lesions was determined by an image analysis of the whole-face images: (A) quantification, (B) representative images.

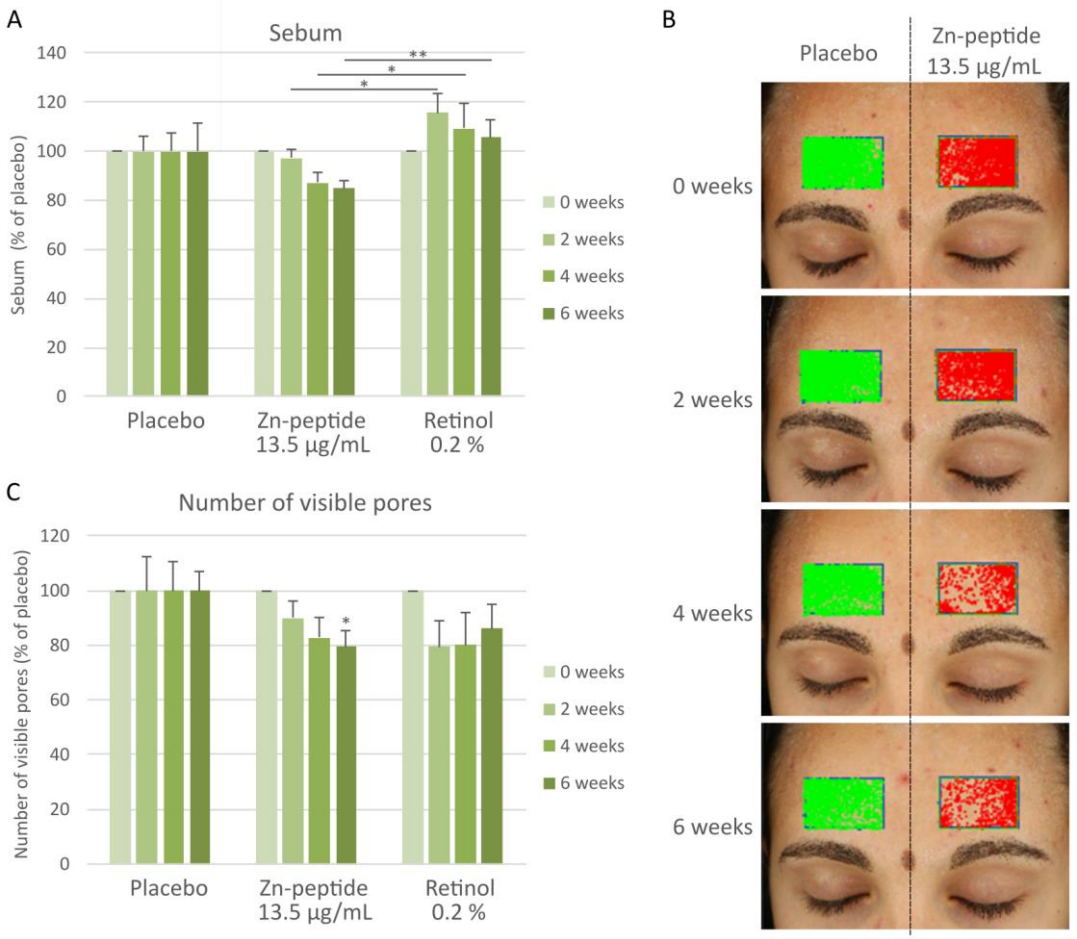


Figure 4. Zn-peptide inhibited sebum production and reduced the number of visible skin pores. Retinol, on the other hand, increased skin oiliness after two weeks of treatment which normalized later although it did not fall below the placebo level. Sebum level was determined by a sebumeter (A). The number of visible skin pores was determined by an image analysis of whole-face images: (B) representative pictures of the image analysis, (C) quantification.

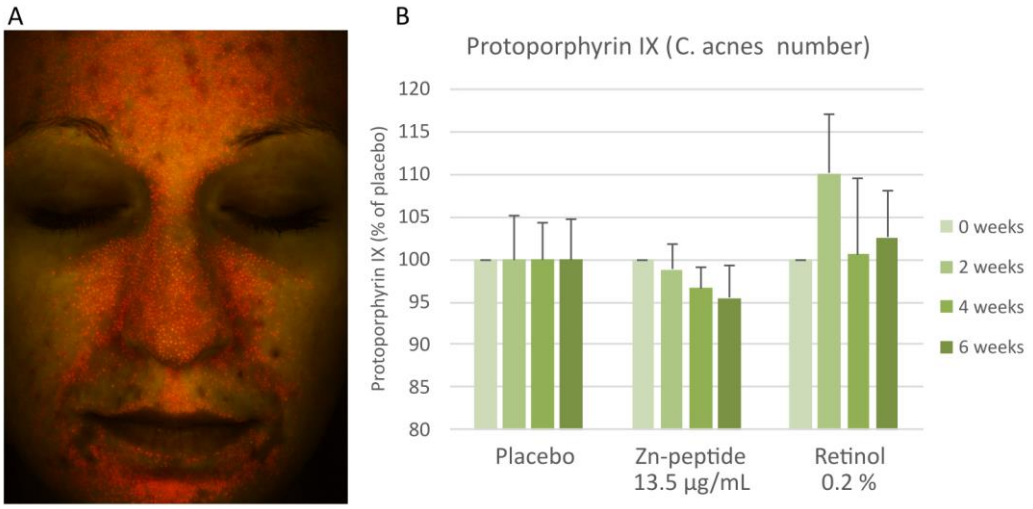


Figure 5. Zn-peptide slightly reduced the number of *C. acnes*. Retinol, on the other hand, increased *C. acnes* number after two weeks of treatment which normalized later although it did not fell below the placebo level. These changes are probably associated with the similar trend in the sebum level observed after retinol treatment. The level of protoporphyrin IX was determined by an image analysis of the whole-face images: (A) representative image of orange fluorescence of porphyrins, products of *C. acnes* corresponding to the bacteria number [12], (B) quantification.

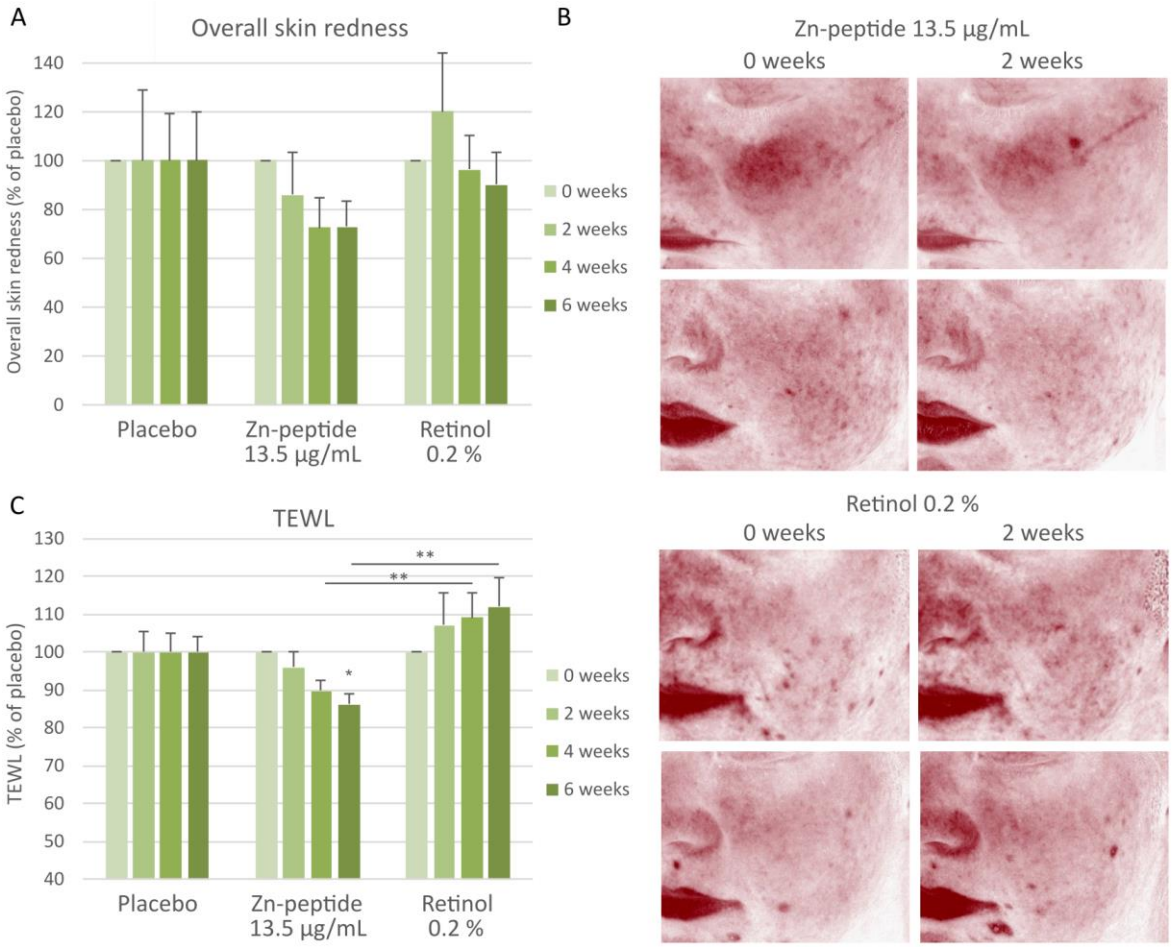


Figure 6. Zn-peptide calmed the skin, reduced the overall skin redness and reinforced skin barrier function by reducing TEWL. Retinol, on the other hand, irritated the skin after two weeks of treatment as shown by the increased skin redness. Retinol also gradually increased TEWL values reflecting skin barrier impairment. These results confirm a well-documented skin irritation potential of retinol which hinders its use by many people especially those with the sensitive skin. The overall skin redness was determined by an image analysis of the whole-face images: (A) quantification, (B) representative images after two weeks of treatment. TEWL was determined by a tewameter (C).

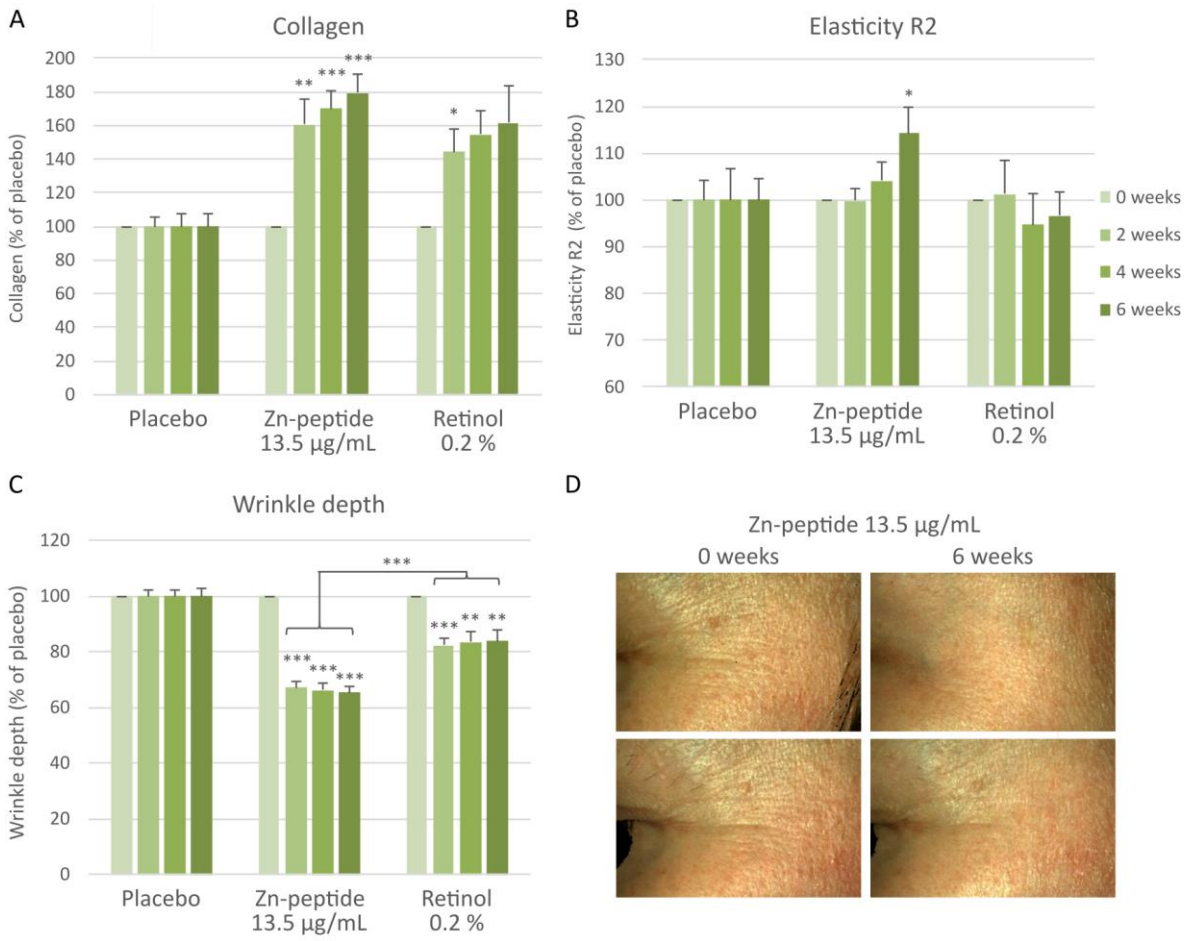


Figure 7. Zn-peptide stimulated collagen production, reduced wrinkles and increased skin elasticity better than retinol. Collagen level was determined by an image analysis of pictures obtained by reflectance confocal microscopy (A). Skin elasticity represented by a parameter R2 was determined by a cutometer (B). Wrinkle depth was determined by a 3D camera: (C) quantification, (D) representative images.

Conclusion

Taken together, zinc hexapeptide complex is a new cosmetic active ingredient with anti-acne and anti-ageing activity comparable or even superior to retinol. Its major benefit is the complex mechanism of action and inhibition of all key events in acne pathogenesis: sebum production, follicular hyperkeratinization, *C. acnes* overgrowth and inflammation (**Schema 1**). It also stimulates collagen production leading to the effective wrinkle reduction and elasticity improvement representing the anti-ageing activity. The hexapeptide component is highly active itself while the presence of zinc in the complex further enhances mainly the effect on the sebum production and the antimicrobial activity.

We also showed that retinol worsened many skin parameters and irritated the skin at the beginning of the treatment. It also caused skin barrier disruption after long-term use. The well-known irritation potential of retinol hinders its use by many people especially those with the

sensitive skin. On the other hand, Zn-peptide did not have any negative adverse effects and even reinforced the skin barrier function making it an effective, safer retinol alternative.

Conflict of interest statement

None

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