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ORIGINAL RESEARCH ARTICLE

The Anti-Wrinkle Efficacy of Argireline, a Synthetic Hexapeptide, in Chinese Subjects

A Randomized, Placebo-Controlled Study

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Abstract

Background Argireline is a synthetic peptide that is patterned from the N-terminal end of the protein SNAP-25 and has been shown to reduce the degree of facial wrinkles. It is reported to inhibit vesicle docking by preventing formation of the ternary SNARE complex and by interfering in catecholamine release. The anti-wrinkle efficacy of argireline has not been studied in Chinese subjects.

Objective The objective of the study was to evaluate the safety and efficacy of argireline in the treatment of peri-orbital wrinkles in Chinese subjects.

Methods A total of 60 subjects received a randomized treatment of argireline or placebo in a ratio of 3:1. Argireline or placebo was applied to their peri-orbital wrinkles twice daily for 4 weeks, and then evaluations were made for the improvements in wrinkles. In the subjective evaluation, Daniell's classification and Seeman's standard were applied to make a global assessment of changes in the appearance of peri-orbital lines. In the objective evaluation, silicone replicas of the skin at the application area were made before and after the treatment, which were analyzed by a wrinkle-analysis apparatus.

Results In the subjective evaluation, the total anti-wrinkle efficacy in the argireline group was 48.9 %, compared with 0 % in the placebo group. In the objective evaluation, the parameters of roughness were all decreased in the argireline group ($p < 0.01$), while no decrease was obvious in the placebo group ($p > 0.05$).

Conclusions This study showed that argireline had a significant anti-wrinkle effect in Chinese subjects.

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1 Introduction

Nowadays, the desire to maintain a youthful appearance has driven the development of dermatologic cosmetics designed to rejuvenate the aging face. Argireline, a synthetic hexapeptide, is one of the new popular options to treat aging skin. It is a unique peptide that is used to reduce existing wrinkles, especially in the forehead and around the eyes.

The synthetic hexapeptide is acetyl hexapeptide-3 (AC-gly-glu-met-gln-arg-arg-NH_2), patterned from the N-terminal end of the protein SNAP-25. The identification of argireline is the result of efforts to find an effective but less toxic synthetic version of botulinum neurotoxin type A (BoNTA) [1, 2]. It has been found that this peptide can inhibit vesicle docking by preventing formation of the ternary soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex (a vesicular fusion complex required to drive Ca^{2+}-dependent exocytosis). It also interferes in catecholamine release, which is involved in synaptic vesicle exocytosis [3, 4]. These effects closely relate to the basic biochemical mechanisms of wrinkle formation. The hexapeptide is called argireline [5]. Argireline is currently marketed in China by McEit (Tianjin) International Trade Co. Ltd.

Argireline inhibits the repetitive contraction of the intrinsic muscles of facial expression and thereby reduces hyperkinetic facial lines [6]. One open-label trial in which ten women received twice-daily applications of 5 % argireline cream demonstrated a 27 % improvement in peri-orbital lines after 30 days, as measured by a silicone replica analysis [1]. In another study, with healthy American women volunteers, argireline solution reduced the depth of wrinkles up to 17 % after 15 days, and 30 % after 30 days [5]. Theoretically, argireline may mimic the effects of

BoNTA injection, by reducing hyperkinetic lines associated with muscles of facial expression. However, currently only BoNTA has been approved for subcutaneous, intradermal, and intramuscular injection for facial wrinkles by the US FDA [7].

The objective of this study is to test the efficacy and tolerability of argireline applied to peri-orbital wrinkles in Chinese subjects.

2 Materials and Methods

The study design was a prospective, double-blind, randomized, placebo-controlled, parallel-group, comparative study in China to investigate the effects of argireline in subjects with moderate to severe peri-orbital lines as assessed at natural expression. A total of 60 subjects were randomized to receive a single treatment of argireline or placebo in a ratio of 3:1. A total sample size of 40 patients was sufficient to have an 85 % chance of detecting a 60-percentage point difference between the treatment groups in the proportion of patients reporting a global assessment score of 2 or greater, significant at the 0.05 level and with a randomization ratio of 3:1. We enlarged the number of patients to 60. Participants applied argireline or placebo to their peri-orbital wrinkles twice daily for 4 weeks, subjective and objective evaluations were made for the improvements in wrinkles. The study was approved by the Ethical Committee of the College of Medicine of Xi'an Jiaotong University, Xi'an, China.

The patient population of 60 Chinese volunteers with different degrees of peri-orbital wrinkles was randomly selected from outpatients in the Department of Dermatology from The Second Hospital of Xi'an Jiaotong University, Xi'an, China. All of them desired to reduce their wrinkles. Participants ranged from 25 to 60 years of age, whose natural expression showed peri-orbital lines of at least moderate severity. Exclusion criteria included known allergy or sensitivity to the medication or its components, infection, or other skin disease at the treatment region. The subjects were advised not to use any other facial cosmetic around the peri-orbital area or undergo any aesthetic medical treatment (e.g. face lift surgery, resurfacing, or filler treatment) during the study period. They were also requested to complete the entire course of the study and to comply with study instructions. All of the volunteers gave their consent before enrollment and they had the right to withdraw at any time during the study if they had any complaint.

Each vial contained 10 % argireline in an oil and water (O/W) emulsion without preservatives. The placebo solution was a non-active O/W emulsion alone, without argireline. Vials of argireline and placebo with identical investigational labels, which prevented identification of the contents, were all prepared by McEit (Tianjin) International Trade Co. Ltd.

For the subjective evaluation, investigators applied Daniell's [8] classification to evaluate the peri-orbital wrinkles at natural expression before use, and after the first, second, third, and fourth week (graded on a 4-point wrinkle severity scale: none, mild, and moderate to severe). After 4 weeks, investigators made a global assessment of changes in the appearance of peri-orbital lines by Seeman's [9] standard, graded on a 5-point scale ranging from 0 (no change) to 4 (100 % improved). The total anti-wrinkle efficacy was calculated as the percentage of subjects who were graded 3 or 4 on the global assessment of improvement scale after 4 weeks.

For the objective evaluation, silicone replicas of the skin at the application area were made before and after the treatment period. These were analyzed by a wrinkle-analysis apparatus (Skin-Visioline VL 650®; Courage+Khazaka Electronic GmbH, Germany). The silicone replicas were processed by confocal laser scanning microscopy to assess the evolution of the wrinkles and to record gray level images of the wrinkles. Confocal microscopy in reflection mode and three-dimensional analysis were used to assess the different parameters of roughness. Then, the relevant parameters of roughness obtained were analyzed by statistical analysis.

The statistical software used in this study was Windows SPSS 17.0.

3 Results

3.1 Subjective Evaluation

Patient demographics and baseline wrinkle evaluations are shown in Table 1. After 4 weeks, none of the subjects discontinued the study and no one experienced any adverse effect. After evaluation and classification of the wrinkles before and after treatment, the total anti-wrinkle efficacy in the argireline group was 48.9 % (22/45), compared with 0 % in the placebo group (Fig. 1). The improvement in the appearance of wrinkles in two patients is shown in Figs. 2 and 3.

3.2 Objective Evaluation

Two subjects' gray level images of peri-orbital wrinkles are shown in Figs. 4 and 5. It can be concluded from Fig. 4 that in this subject the wrinkles count is 14 before treatment, while it is 7 after treatment. In another subject, the wrinkles count is 13 before treatment but 9 after treatment

Table 1 Patient characteristics and baseline peri-orbital wrinkles at natural expression

Variable	Argireline ($n = 45$)	Placebo ($n = 15$)	p-Value
Patient characteristics			
Age, mean (y)	43.7	41.3	0.07
Sex, n (%)			
Female	38 (84.4)	12 (80)	0.08
Male	7 (15.6)	3 (20)	
Baseline severity of peri-orbital wrinkles at natural expression, n (%)			
None	0 (0)	0 (0)	0.13
Mild	14 (31.1)	4 (26.7)	
Moderate	19 (42.2)	8 (53.3)	
Severe	12 (26.7)	3 (20)	

in Fig. 5. Furthermore, the average depth of wrinkles, deepest wrinkle, total wrinkle volume, total wrinkle area, total form factor wrinkles, and total length of wrinkle are all decreased after the treatment in the two subjects.

After silicone replicas were processed by Skin-Visioline VL 650®, various parameters of roughness were obtained and analyzed by SPSS 17.0 statistical software. The parameter Sa represents the average wrinkle height in one place, the parameter Smax is the difference from a peak to the lowest point of all the wrinkles in the region, and the parameter St represents the average wrinkle height over all the wrinkles in the region. These parameters were all decreased in the argireline group ($p < 0.01$), while they were not obviously decreased in the placebo group ($p > 0.05$) [Table 2].

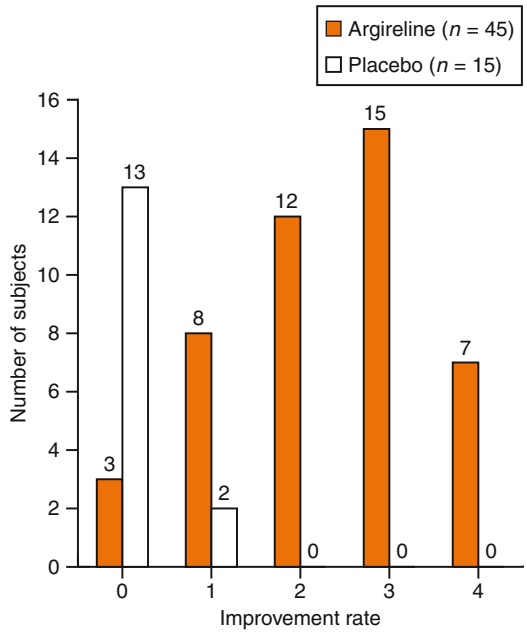

Fig. 1 The number of subjects with different improvement rates. 0 = no improvement; 4 = 100 % improvement

4 Discussion

BoNT brought about a revolution in cosmetic science because of its remarkable and long-lasting anti-wrinkle activity. BoNT rejuvenates the aging face by reducing hyperkinetic lines associated with muscles of facial expression and is the most popular aesthetic product. In 1992, Carruthers and Carruthers [10] investigated selectively injecting BoNTA to treat glabellar wrinkles. Since then, other studies have continuously corroborated their results [11–16]. In 2006, injection of BoNTA was the most frequently performed cosmetic procedure in the USA, with over 3 million patients receiving injections [17].

Physiologically, the formation of wrinkles appears to be due, at least partly, to the excessive stimulation of the muscle fibers in the face, which pull the skin inwards giving rise to the well known wrinkle [18, 19]. Thus, a useful strategy to reduce the intensity of wrinkles is to downregulate muscle action either directly or by attenuating the activity of the innervating neuron [20, 21]. In support of this tenet, treatment with BoNTA significantly reduces the intensity of wrinkles. BoNTA strongly inhibits the Ca^{2+}-dependent neurotransmitter release in neurons. These proteins are metalloproteases that specifically cleave synaptic proteins essential for regulated neuronal exocytosis, specifically the vesicular protein VAMP (a vesicle-associated membrane protein, which is essential for the docking and fusion of the synaptic vesicle to the presynaptic membrane for the release of acetylcholine) and the membrane proteins syntaxin and SNAP-25. As a result, the critical protein fusion complex assembled by these proteins, known as the SNARE complex, is destabilized preventing vesicle fusion with plasma membrane, and consequently abrogating Ca^{2+}-triggered exocytosis [22].

Although botulinum toxins, especially BoNTA, have been extensively used to attenuate facial signs of aging, their use is seriously limited because of their high toxicity

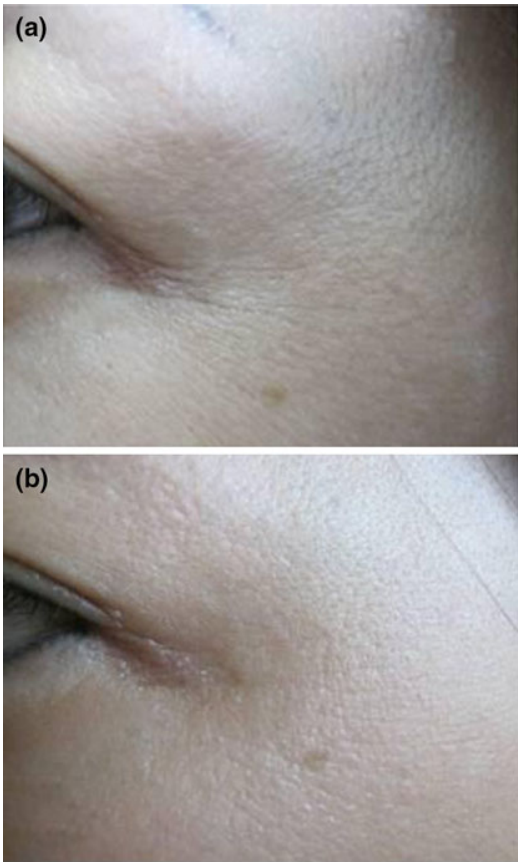

Fig. 2 Changes seen in a subject (female, aged 40 years), (**a**) before and (**b**) after treatment with argireline

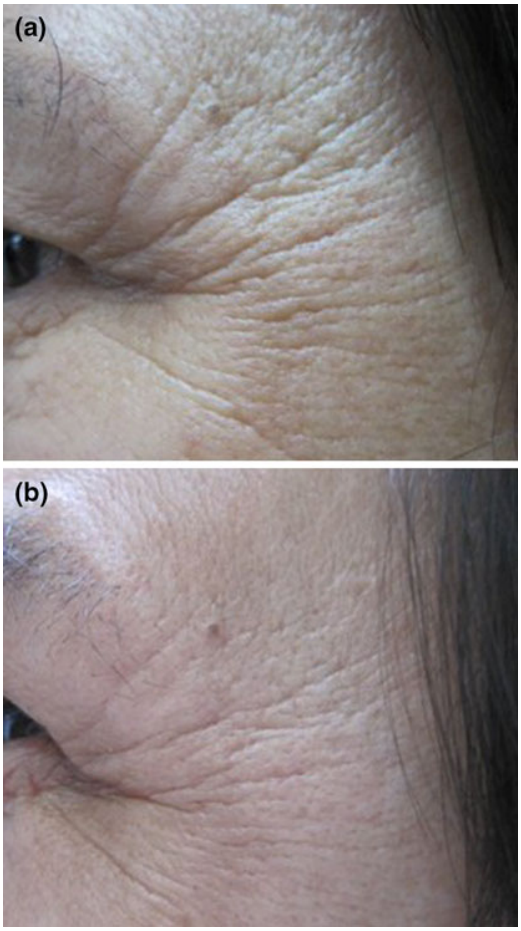

Fig. 3 Changes seen in a subject (male, aged 49 years), (**a**) before and (**b**) after treatment with argireline

(human lethal dose, 50 % [LD_{50}] $\approx$ 2,500 biologic mouse units) [20]. Thus, there is a need to design and validate non-toxic molecules that mimic the action of BoNTA [1, 2]. In this regard, a 6-mer peptide (Ac-EEMQRR-NH_2) that emulates the amino acid sequence of the synaptic protein SNAP-25 is shown to be a specific inhibitor of neurosecretion at micromolar concentrations. It is patterned after the N-terminal domain of SNAP-25 (aa 12–17) and exhibits a significant capacity to permeate through the skin. Toxicologic and primary irritation data indicate that it is well tolerated. This hexapeptide is called argireline [5].

Analysis of the mechanism of action showed that argireline significantly inhibited neurotransmitter release with a potency similar to that of BoNTA. Inhibition of neurotransmitter release was due to the interference of the hexapeptide with the formation and/or stability of the SNARE ternary complex that is required to drive Ca^{2+}-dependent exocytosis. Notably, this peptide did not exhibit in vivo oral toxicity or primary irritation at high doses [5]. Therefore, this hexapeptide represents a biosafe alternative to BoNTA in cosmetics to attenuate facial wrinkles.

In this study, after 4 weeks of application of argireline on peri-orbital wrinkles, none of the human subjects experienced any adverse effect, the total anti-wrinkle efficacy was 48.9 %, and the wrinkle parameters were all decreased in the argireline group. All the findings demonstrated that the anti-wrinkle activity of argireline was significant, in agreement with its cellular activities.

Although our study identified that argireline had a significant anti-wrinkle effect, there were some limitations. Most importantly, the best way to demonstrate changes in the skin is to do pre- and post-biopsies and then to compare the histologic changes, which is the gold standard to determine effectiveness in improving quality of the skin. Unfortunately, we were unable to persuade the volunteers to do biopsies because of concerns they had regarding postoperative scarring. Potential errors with silicone impressions include that tissue edema caused by the study medication or even mild rubbing can produce better than deserved results, although these errors can also impact on

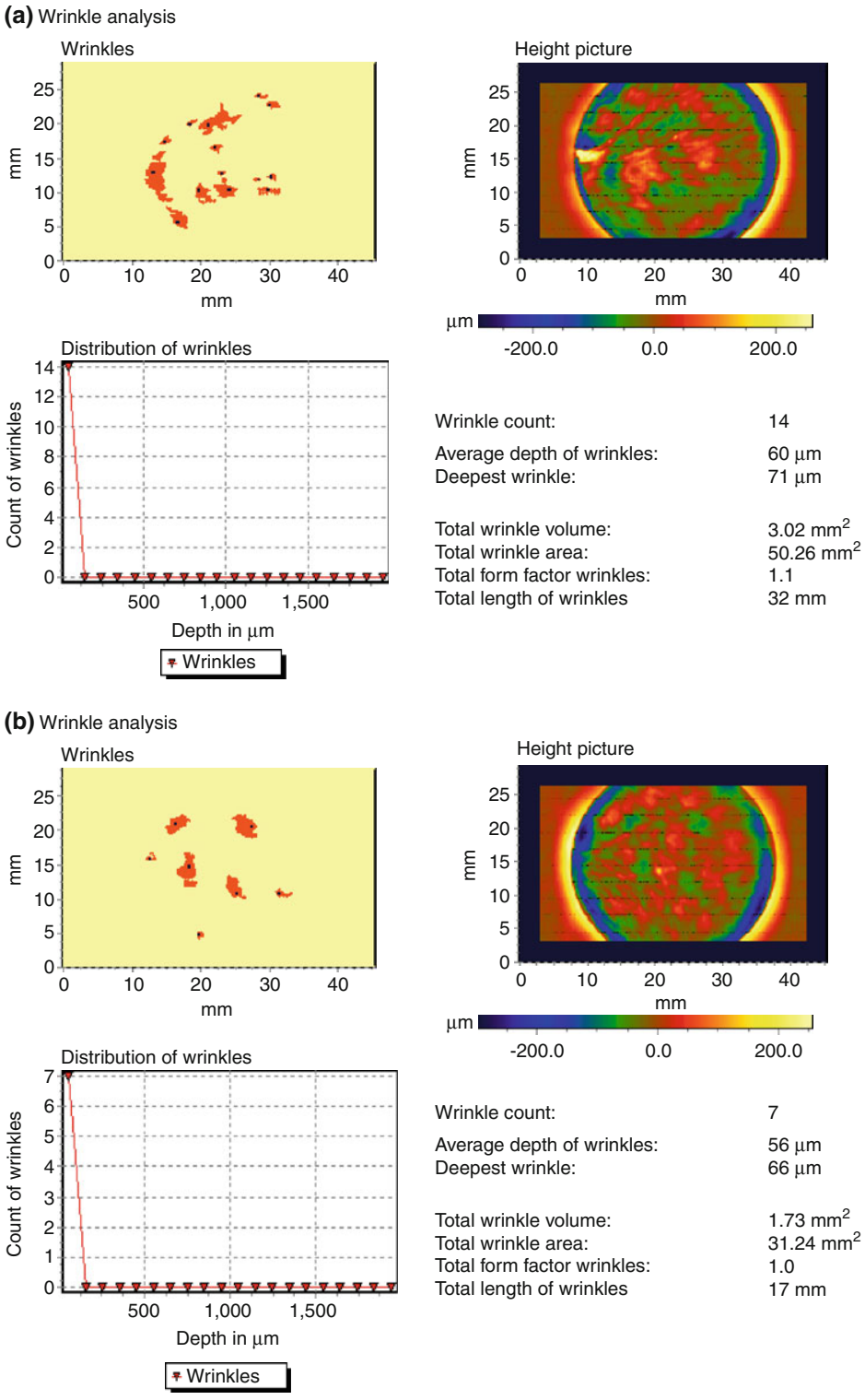

Fig. 4 A subject's (female, aged 45 years) gray level images of peri-orbital wrinkles processed by Skin-Visioline VL 650®, (**a**) before and (**b**) after treatment

placebo results. However, silicone impressions provide non-invasive results that are far better than photography, and this method is relatively mature having been used in many previous studies [1, 5]. For these reasons, it was selected for the objective measurement in this study, and our best efforts made to avoid potential errors.

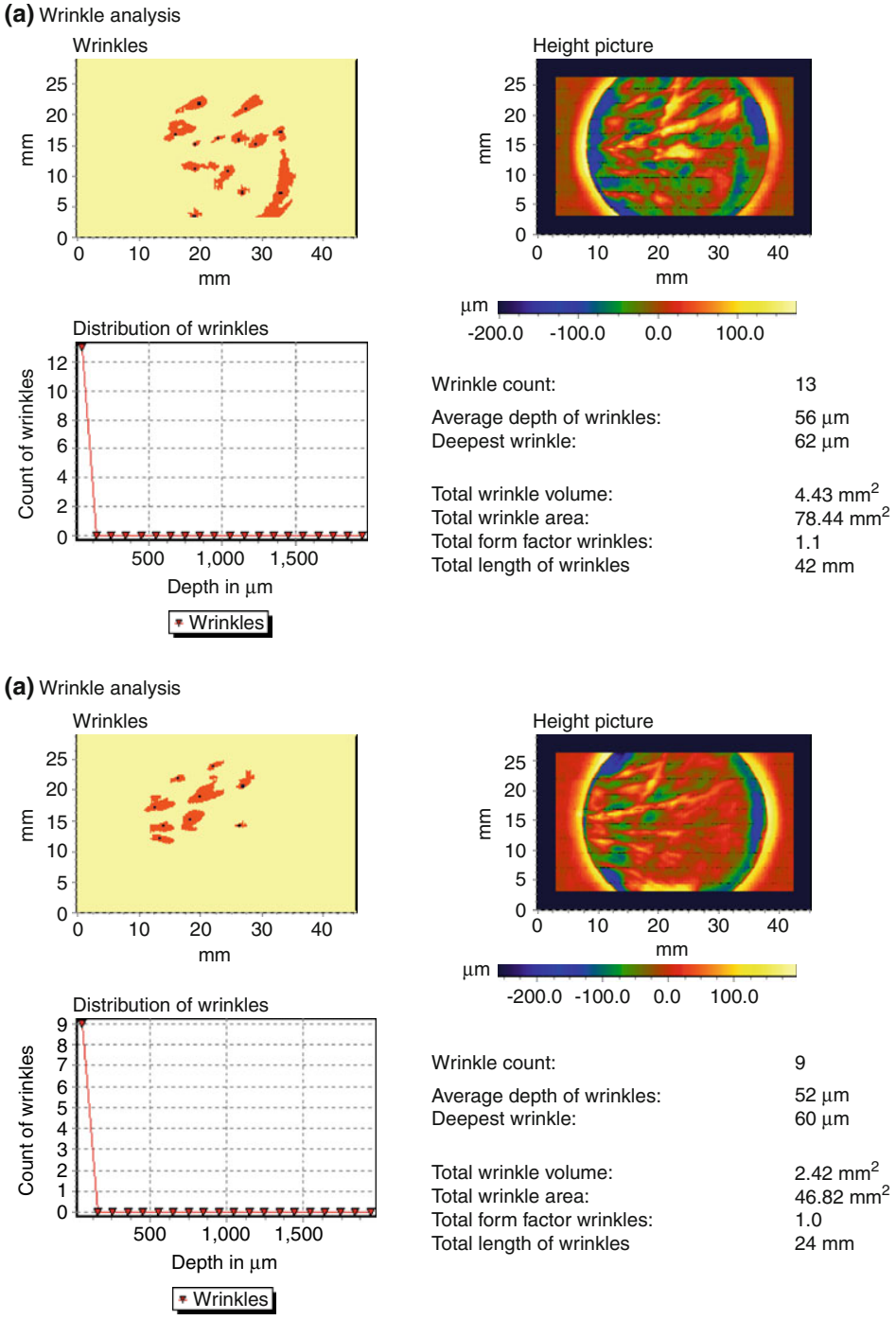

Fig. 5 A subject's (male, aged 55 years) gray level images of peri-orbital wrinkles processed by Skin-Visioline VL 650®, (**a**) before and (**b**) after treatment

5 Conclusions and Prospects

Applying argireline around the eyes for up to 4 weeks was an effective treatment for reducing the severity of peri-orbital wrinkles in Chinese people with moderate and severe peri-orbital lines, and argireline was safe and well tolerated in this study group.

Argireline, one of the cosmeceutical peptides, is a new popular option to treat aging skin. It is not considered as a drug and is therefore not regulated by the FDA [1, 2]. Although much less potent than BoNTA (12 vs. 0.003 assigned amount units), this small peptide exhibits the great advantage of insignificant acute toxicity (≥2000 mg/kg) as compared with BoNTA (20 ng/kg). Furthermore, the

Table 2 The parameters of the different groups ($\bar{x} \pm$ sd)

Parameter	The argireline group	The placebo group
Sa	9.2000 ± 2.28576*	5.3000 ± 2.22919
Smax	138.000 ± 30.69133*	99.3000 ± 14.05754
St	131.5000 ± 41.66569*	108.0000 ± 17.01732

Sa the average wrinkle height in one place, *Smax* the difference from a peak to the lowest point of all the wrinkles in the region, *St* the average wrinkle height over all the wrinkles in the region

* $p < 0.01$ for differences between before and after the treatment

hexapeptide does not show primary skin irritation in an intracutaneous test or genotoxicity as determined by the Ames test, thus making its use safe and physician independent [5]. Therefore, peptides that mimic the action of BoNTA, such as argireline, represent the next generation of biosafe products with anti-wrinkle activity that could be extensively used in cosmetic preparations.

Most studies used to assess the incorporation of these ingredients into skin care products are in vitro. In this study, clinical data are presented to suggest that argireline may have a place in a comprehensive skin care protocol for aging skin. A study with larger samples is planned that will provide more comprehensive data to support the present study.

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