Effects of Topical No Toner and Essence on Skin Changes in the Subjects with Acne Vulgaris

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Abstract

Background: Acne vulgaris (AV) is a very common condition with an estimated lifetime prevalence of 85% and occurs predominantly during adolescence. The aim of current study was to investigate how the NO-containing essences and toners change the skin condition of AV patients using nitric oxide (NO) with these various positive effects.

Method: The experiment proceded with 25 subjects in the acne skin experimental group with NO essence and toner (AE), 25 subjects in the normal skin experimental group with NO essence and toner (NE), and 20 subjects in the normal skin control group without any application of the products (NC). Skin analyzer (Mark-Vu, Korea) was used to examine the skin changes.

Results: The changes in pores, sebum, inflammation, wrinkles, flush, skin tone, radiance brightness, and radiance area before and after the experiment between three groups were statistically significant decreased and melanin increased in both AE and NE. A statistically significant change was only seen in AE ($p< 0.05$). There was a statistically significant decrease in NE and a statistically significant decrease in brown was only seen in NE ($p< 0.05$). After 8 weeks, Pore size and future wrinkles were significantly reduced in AE compared to the other groups ($p <0.05$). There was a statistically significant difference in pore sizes, future wrinkles in AE and NC in the post-test using the Sheffe test ($p <0.05$). After the experiment, sebum, inflammation, wrinkle decreased in AE and NE, but there was no statistically significant decrease between the three groups.

Conclusion: Essences and toners containing NO were found to be effective in improving AV and normal skin.

Keywords: *Acne vulgaris, Skin, Nitric oxide, Essences, Toner*

Introduction

Acne vulgaris (AV) is a disease affecting the pilosebaceous unit and forms a variety of scar tissues, including noninflammatory lesions such as open and closed comedones and inflammatory lesions such as papules, pustules, and nodules[1]. Acne causes residual scarring, which can lead to having a poor self-image, depression, and anxiety and can affect the quality of life and severe morbidity associated with psychological disturbances[2-4].

For the treatment of mild cases of AV, benzoyl peroxide (BP) or topical retinoids are used. More aggressive treatments, such as topical or oral combination therapy for BP, antibiotics and retinoids are used in moderate or more severe cases[5]. Since medical treatments with antibiotics have problems with developing resistance and there are different causes of acne, it is recommended that various method such as change of habits that worsen acne, proper guidance on the use of products such as cosmetics, education of

dietary habits, and stress management are carried out in combination with the treatment[5-7]

New therapies for treating AV patients are being continuously developed, and among them, products containing effective ingredients for acne in the form of ointments and creams have been actively tried[8]. The newest agents of this type include minocycline foam, topical nitric oxide (NO)-releasing agents, and cortexolone 17α-propionate. These latest method are undergoing various clinical stages of testing and there is a need for further studies[1]. Currently, there is a growing interest in products containing NO, including new patents on the development and delivery method of NO-containing substances for acne treatment[9]. However, few clinical studies have been conducted on topical NO-releasing agents.

NO can pass through the epidermal layers. Due to these properties, products containing NO have been studied to determine whether they improve dermatitis or acne with their antibacterial effects[8,10]; NO also has vasodilatory, melanogenesis, wound healing promotion and anti-inflammatory effects[11], and it has been used for aesthetic purposes and treatment of medical conditions. However, no research has been conducted on the development of a multi-purpose essence product containing NO, which is readily available for the general population without the need of prescription. Therefore, we tried to investigate how the NO-containing essences and toners change the skin condition of AV patients using NO with these various positive effects.

Method

Subjects: This study was conducted on adult females who volunteered to participate in the study through recruitment of women in their 20s to 60s.

Subjects were recruited by posting a recruitment notice for people with acne and healthy skin. Interviews were conducted to explain the detailed research plan, time required, and progress of the experiment. Informed written consent was received from all study participants. From the procedure, subjects with acne were graded from 1 to 3 on the Comprehensive Acne Severity Scale[12]. Subjects were selected with an inter-rater reliability of 0.75 or higher. Patients with cardiovascular diseases, immediately after surgery (6 months of a surgery), skin diseases, infectious diseases, cerebral infarction, severe depression, mental disorder, and pregnant women were excluded from the study for safety reasons.

Homogeneity was tested between the groups on the general age, body weight, and height. The general characteristics did not differ statistically between the two groups at baseline ($p>0.05$).

Experimental Procedure: The experiment proceeded with 25 subjects in the acne skin experimental group with NO essence and toner (AE), 25 subjects in the normal skin experimental group with NO essence and toner (NE), and 20 subjects in the normal skin control group without any application of the products (NC). In both AE and NE, 2 ml of natural NO toner was sprayed at least 3 times a day with a nebulizer across the face, and 2 ml of essence was applied 3 times per day (or 3 times or more) to the entire face and left for absorption. The NC received no treatment and all 3 groups continued with their daily lives.

Tool: Skin analyzer (Mark-Vu, Korea) was used to examine the skin changes. Four different measurement positions were used: the center of the lower third of the forehead, the nose wing, nose bridge, and the middle of both cheeks. These permits analysis of the whole face, and quantitative comparative analysis was performed by presenting numerical data. For the measurement method, the total mean value from three measurements was used.

Statistical: Data are expressed as mean ± SD values. All variables were tested for normality using the one-sample Kolmogorov-Smirnov test. The paired t-test was performed to compare differences within groups before and after interventions. One-way ANOVA was used to compare the differences in the changes after intervention in each group, and Sheffe was used for the post-hoc test. The collected data were analyzed using a statistical package program (SPSS ver. 21.0). A two-tailed probability of $p<.05$ was considered statistically significant.

Results

The changes in pores, sebum, and inflammation before and after the experiment between three groups were statistically significant decreased, in both AE and NE. After 8 weeks, pores, sebum volume, and inflammation were compared (Table 1). Pore size was significantly reduced in AE compared to the other groups ($p <0.05$). There was a statistically significant difference in pore sizes in AE and NC in the pos t-test using the Sheffe test ($p <0.05$). The changes in wrinkles and future wrinkles before and after the experiment between three groups showed statistically significant decreases in both

AE and NE. After 8 weeks, when wrinkles and future wrinkles were compared (Table 2), future wrinkles were significantly reduced in AE compared to the other groups ($p < 0.05$). There was a statistically significant difference in future wrinkles in AE and NC in the post-test using the Sheffe test ($p < 0.05$). The changes in melanin before and after the experiment between three groups showed an increase in melanin in AE and NE but a statistically significant change was only seen in AE ($p < 0.05$) and for pigmentation (Table 3). There was a statistically significant decrease in NE before and after the experiment ($p < 0.05$). Flush was statistically significantly decreased in AE and NE ($p < 0.05$) and a statistically significant decrease in brown was only seen in NE ($p < 0.05$). The changes in skin tone, radiance brightness, and radiance area before and after the experiment between three groups showed statistically significant decrease of all of them in AE and NE ($p < 0.05$)., however, after 8 weeks there was no significant difference between the 3 groups (Table 4).

Table 1: Changes of pore size, sebum and porphyrin after application of essence with nitrogen oxide (unit: %).

	AE (n=25)		NE (n=25)		NC (n=20)		F	p
	Pre	Post	Pre	Post	Pre	Post		
Pore size (%)	53.80±5.01	47.08±5.72	52.88±4.57	48.84±4.27	52.80±5.55	52.05±5.55	5.14	0.00†
t	8.19		7.01		1.22			
p	0.00*		0.00		0.24			
Sebum (%)	20.99± 9.82	16.75±7 .77	19.46± 6.00	17.40±5 .61	19.78± 9.68	20.51± 10.34	1.38	0.25
t	4.84		3.72		-0.91			
p	0.00*		0.00*		0.38			
Porphyrin (%)	14.02±9.11	8.51±5.86	6.86±2.86	6.16±2.61	6.37±3.42	6.05±2.60	1.67	0.19
t	5.24		2.80		1.19			
p	0.00*		0.01*		0.24			

Value are mean±SD.*p<0.05 †Significantly different for post-intervention compared 3 groups (p<0.05) AE: Acne experimental group with NO essence and spray, NE: normal skin experimental group with NO essence and spray, NC: normal skin control group

Table 2. Changes of wrinkle and future wrinkles after application of essence with nitrogen oxides (unit: %)

	AE (n=25)		NE (n=25)		NC (n=20)		F	p
	Pre	Post	Pre	Post	Pre	Post		
Winkle	27.08±2.81	23.76±2.22	26.08±2.46	23.92±2.44	26.20±2.56	25.05±2.39	1.91	0.15
t	10.21		6.35		1.92			
p	0.00*		0.00*		0.69			
Future wrinkle	12.41± 4.34	9.77±3.42	12.92± 3.21	10.48±2.83	13.22±3.13	12.09±3.12	3.12	0.05†
t	7.78		7.73		1.83			
p	0.00*		0.00*		0.08			

Value are mean±SD. *p<0.05 †Significantly different for post-intervention compared 3 groups (p<0.05) AE: Acne experimental group with NO essence and spray, NE: normal skin experimental group with NO essence and spray, NC: normal skin control group

Table 3. Changes of skin color after application of essence with nitrogen oxides (unit:%).

	AE (n=25)		NE (n=25)		NC (n=20)		F	p
	Pre	Post	Pre	Post	Pre	Post		
Melanin	34.87±7.74	37.98±6.74	34.14±35.42	35.42±6.05	34.47±6.82	34.44±6.85	1.81	0.17
t	-4.82		-1.75		0.08			
p	0.00*		0.09		0.93			
Pigmentation	31.31±32.45	32.45±10.37	32.66±6.93	29.96±7.25	30.12±30	30.09±6.49	0.68	0.50

	AE (n=25)		NE (n=25)		NC (n=20)		F	p
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**		
t p	-0.93 0.36		4.20 0.00*		0.08 0.93			
Redness	16.956± 14.70	14.70±5.47	15.11±5.13	14.02±3.66	13.89±5.37	13.80±4.68	0.23	0.79
t p	4.26 0.00*		2.37 0.03*		0.33 0.74			
Brown spot	19.23± 18.83	18.83±4.10	19.58±4.72	17.45±4.35	18.08±4.21	18.58±4.22	0.73	0.48
t p	0.97 0.34		3.82 0.00*		-1.86 0.07			

Value are mean±SD. *p<0.05, AE: Acne experimental group with NO essence and spray, NE: normal skin experimental group with NO essence and spray, NC: normal skin control group

Table 4.:Changes of skin brightness and tone after application of essence with nitrogen oxides (unit:%).

	AE (n=25)		NE (n=25)		NC (n=20)		F	p
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**		
Tone	52.72±53.80	53.80±3.74	53.56±3.58	55.40±2.94	53.40±3.50	54.10±3.33	1.57	0.21
t p	-2.61 0.01*		-4.47 0.00*		-0.95 0.35			
Brightness	59.74± 61.73	61.73±3.35	60.46± 3.89	62.82±3.26	61.16±3.88	61.77±10.65	0.79	0.45
t p	-4.10 0.00*		-3.89 0.00*		-0.73 0.47			

Value are mean±SD.*p<0.05, AE: Acne experimental group with NO essence and spray, NE: normal skin experimental group with NO essence and spray, NC: normal skin control group

Discussion

This experiment was conducted to investigate how the essence and toner containing a small amount of NO changed the AV condition and affected normal skin.

When NO essence and toner were applied for 8 weeks, the pore size, sebum and inflammation in the AE and NE groups were significantly reduced before and after the experiment, indicating that NO essence and toner were effective for both acne and normal skin. In particular, the pore size showed a statistically significant decrease in the AE group compared to the NC group when compared between the three groups, demonstrating a positive effect in reducing the pore size of acne patients. Previous studies have shown that NO-containing products have potent antibacterial effects that not only eliminate bacteria of atopic dermatitis but also modulate inflammatory components[10]. In this study, the reduction of sebum and porphyrin in AE with NO-containing essence and toner is thought to be due to the NO component inhibiting NLRP3 inflammasome assembly and downstream production of cytokines, suggesting that these main cytokines in AV development were inhibited from acting as a driving force blocking the acne pores[8].

In AE, the AV group with NO-containing essence and toner, the pore size was reduced statistically significantly before and after the application. When acne develops, the pore size increases due to inflammation, and it is thought that due to the use of the NO essence and toner, the inflammation reduced and the continuous use of the toner induced astringent effect, reducing the pore size in AE. NO upregulates collagen synthesis of dermal fibroblasts without affecting collagen breakdown activity. These results indicate that NO is metabolized in wounds and synthesized by wound-derived fibroblasts[13], and such action of NO is thought to have led to the decrease in pore sizes.

Wrinkles and future wrinkles showed statistically

significant decrease before and after the experiment in the AE and NE groups, indicating that the products were effective in improving wrinkles. This action is known to play an important role in reducing the adverse effects of skin aging and carcinogenesis[14,15]. In addition, it is thought to reduce the formation of wrinkles by causing the increase of type I collagen synthesis in fibroblasts[16]. The continuous use of toners and essences leads to increased moisturization and enhancement of skin tone, which in turn reduces wrinkles.[17,18]

The results of this study showed an increase in melanin in AE and NE with NO essence and toner, and especially AE showed statistically significant increase. This is because NO-releasing materials act as non-UV-based tanning agents to stimulate melanogenesis, which leads to an increase in endogenous pigmentation, thereby reducing the UV-induced photo damage and skin cancer risk at the same time, and this results in attaining the positive effect of melanin increase[19,20]. Flush also showed significant decrease in AE and NE. Inflammation is characterized by vasodilation, swelling, redness and fever, and AV is accompanied by inflammation and this in turn in most times causes the redness, a representative symptom of inflammation[21]. These symptoms disappear naturally as inflammation improves or heals. In this study, flushing was reduced when NO essence and spray were applied in AE and NE, suggesting an anti-inflammatory effect on AV.

This study had several limitations. First, no pre-clinical experiments were conducted on essences and toners containing NO. If pre clinical experiments were conducted to investigate the extent of skin penetration according to various NO concentrations and the resulting skin changes, more clinical effects could be obtained based on more effective concentrations and skin penetration method in this study on acne patients. Second, there was a limit in the reliability and validity of the measurement method for skin changes. The reliability of the measuring equipment was very high, but there was a lack of practical research on its validity.

Conclusion

Essences and toners containing NO were found to be effective in improving AV and normal skin. Further studies are required to study the optimum amount of NO and the effects of the penetration method.

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Conflict of Interest: Nil

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