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Improvement of acne vulgaris by topical fullerene application: unique impact on skin care

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Abstract

Oxidative stress plays a major role in acne formation, suggesting that oxygen radical scavengers are potential therapeutic agents. Fullerene is a spherical carbon molecule with strong radical sponge activity; therefore, we studied the effectiveness of fullerene gel in treating acne vulgaris. We performed an open trial using a fullerene gel twice a day; at 4 and 8 weeks, the mean number of inflammatory lesions (erythematous papules and pustules) significantly ($P < 0.05$) decreased from 16.09 ± 9.08 to 12.36 ± 7.03 (reduction rate 23.2%) and 10.0 ± 5.62 (reduction rate 37.8%), respectively. The number of pustules, consisting of accumulation of neutrophils, was significantly ($P < 0.05$) decreased from 1.45 ± 1.13 to 0.18 ± 0.60 (reduction rate 87.6%), and further in vitro assays of sebum production in hamster sebocytes revealed that 75 μM polyvinylpyrrolidone-fullerene inhibits sebum production, suggesting that fullerene suppresses acne through decreasing neutrophil infiltration and sebum production. After treatment for 8 weeks, the water content of the skin significantly ($P < 0.05$) increased from 51.7 ± 7.9 to 60.4 ± 10.3 instrumental units. Therefore, the fullerene gel may help in controlling acne vulgaris with skin care benefit.

From the Clinical Editor: Fullerenes, spherical carbon cages with strong oxygen radical scavenging, with formulated into a gel and used to successfully treat acne vulgaris, an inflammatory disease associated oxidative stress.

Key words: Fullerene; Acne; Sebum; Oxidative stress

Acne vulgaris is an inflammatory skin disease involving the pilosebaceous follicles and has a multifactorial pathogenesis that includes sebaceous gland hyperplasia, increased sebum production, hyperkeratosis of hair follicle pores, and *Propionibacterium acnes* colonization. Oxidative stress also plays a major role in acne formation,[1,2] indicating that oxygen radical scavengers are potential therapeutic agents.

Fullerene is a spherical carbon molecule with strong radical sponge activity attributable to its unique cage structure. Its antioxidant activity is several hundred-fold higher than that of other antioxidants.[3] Because of this property, fullerene is useful in the treatment of neurodegenerative disorders[4] and arthritis,[5-7] and is expected to be effective against various oxidative diseases.[8] When applied to the skin, fullerene is reported to exhibit protective activity against keratinocyte apoptosis caused by reactive oxygen species formed because of ultraviolet exposure.[9] Fullerene penetrates deep into the epidermis[10] without causing skin irritation[11]; therefore, it is a potential candidate for topical treatment of acne vulgaris.[12] Hence, we decided to determine the effectiveness of fullerene gel in treating acne vulgaris.

Methods

Open trial for clinical efficacy of topical fullerene gel in treating acne vulgaris

The study population comprised 11 patients (5 men and 6 women) with acne vulgaris. Their ages ranged from 23 to 39 years, and the mean age was 30.7 years (standard deviation, 5.3 years). The number of facial papular and pustular lesions ranged from 6 to 39, corresponding to mild or moderate acne (1–20 lesions on half of the face) as defined by the Acne Study Group in Japan.[13] During the trial, the subjects applied 0.4 mL of 1% LipoFullerene [14,15] gel (the gel base: 1% squalene, 0.5% *phenoxyethanol, 49.25%* I.T.O. *gel base containing glycerin, carbomer,* sodium polyacrylate, *methylparaben, propylparaben,*

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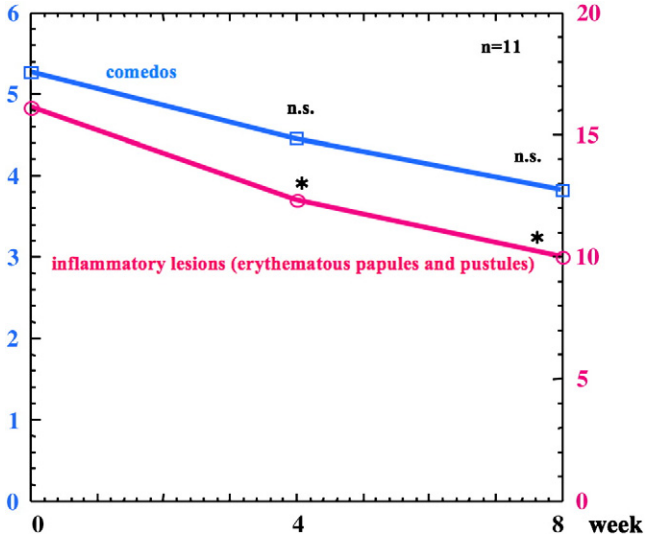

Figure 1. Effect of fullerene gel on the number of acne lesions. The open squares and circles indicate the mean number of comedos and inflammatory lesions (erythematous papules and pustules) (n = 11), respectively. Left y-axis: Number of comedos, Right y-axis: Number of inflammatory lesions. ns, not significant. *$P < 0.05$.

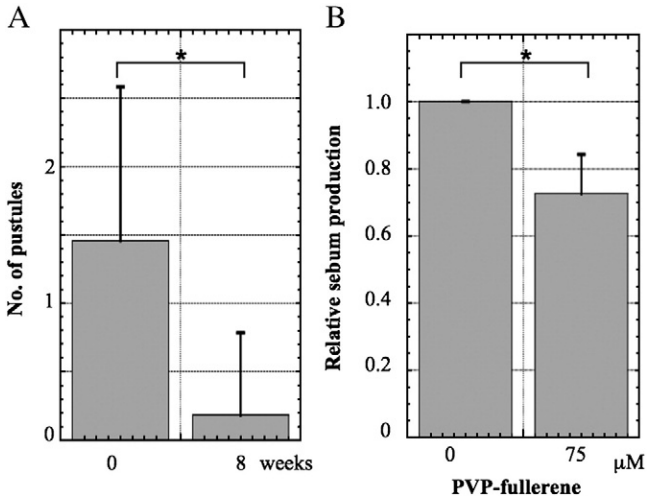

Figure 2. Evaluation of fullerene in suppressing pustules (neutrophil infiltration) in vivo and sebum production in vitro. **(A)** Effect of the fullerene gel on the number of pustules in the open trial. The number of pustules at 0 and 8 weeks after starting application of the fullerene gel is shown. *$P < 0.05$. **(B)** Effect of PVP-fullerene on in vitro sebum production in hamster sebocytes. Hamster sebocytes were cultured in HuMedia-BB (Kurabo Co.) containing 8% fetal bovine serum, 2% human serum, and 10 μg/mL insulin (differentiation medium) supplemented with 75 μM PVP-fullerene or a mock control (water) for 14 days. Subsequently, the lipid levels in the cell lysates were measured using an SE-3001 kit. *$P < 0.05$. Sebum production was determined relative to that in the mock control (water) in each experiment.

water) (I.T.O. Co. Ltd., Tokyo, Japan) *and 49.25% water)* (Vitamin C60 BioResearch Corporation, Tokyo, Japan) on their entire face twice a day (immediately after washing the face in the morning and night); they were instructed to not apply it on erosions. At 0, 4, and 8 weeks after starting the treatment, facial photographs were taken; the acneiform comedos, erythematous papules, pustules, and nodules were counted. The water and sebum content of the skin were measured using the Corneometer CM 825 and Sebumeter SM815 (Courage + Khazaka electronic GmbH, Köln, Germany), respectively. The porphyrin concentration and the conspicuous pores on the skin were measured using the VISIA complexion analysis system (Canfield Scientific, Inc., Fairfield, New Jersey). The number of acne lesions was confirmed by careful and repeated observations of the photographs of the patients' faces and necks. During the trial the patients were prohibited from using any drugs or other skin care treatments for acne. This study was conducted in accordance with the tenets of the Declaration of Helsinki and was reviewed and approved by the institutional review board of Vitamin C60 BioResearch Corporation. All the patients provided written informed consent before enrolling in this study.

Assay for sebum production in hamster sebocytes

Hamster sebocytes established from the sebaceous glands of the auricles of 5-week-old male golden hamsters were purchased from Kurabo Co., Osaka, Japan. The cells were grown in HuMedia-BB (Kurabo Co.) supplemented with 8% fetal bovine serum, 2% human serum, and 10 ng/mL human epidermal growth factor. After reaching confluence, the culture was continued for another 7 days. Following this, the cells were fed with HuMedia-BB (Kurabo Co.) supplemented with 8% fetal bovine serum, 2% human serum, and 10 μg/mL insulin for induction of differentiation and subsequent sebum production by sebocytes. The medium was changed every second day, and fresh solution of polyvinylpyrrolidone-fullerene (PVP-fullerene), as a reference compound for the pristine fullerene, or water was added to it. After incubation for 14 days, the cells were harvested and subjected to an assay for lipid production (SE-3001; Kurabo Co.) based on Oil Red O staining, according to the manufacturer's instructions. Using this assay we measured the sebum production per cell.

Statistical analyses

Alterations in skin-related parameters, including the number of acne lesions, during the open trial were statistically determined by a Wilcoxon signed-rank test, and the in vitro sebum production in hamster sebocytes was statistically examined using Student's t-test. Differences were considered to be significant at $P < 0.05$.

Results

Effect of fullerene gel on number of acne lesions

After the 8-week trial, the comedos decreased in number in only 3 (27.3%) of the 11 patients. At 4 and 8 weeks after starting the treatment, the mean number of comedos decreased from 5.27 ± 5.57 to 4.45 ± 3.80 (reduction rate 15.6%) and 3.82 ± 3.52 (reduction rate 27.5%), respectively, but this reduction was not statistically significant. In contrast, after the 8-week trial, the number of inflammatory lesions (erythematous papules and

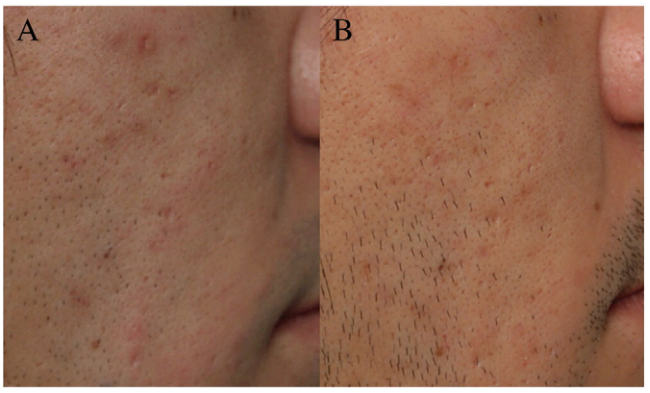

Figure 3. Clinical findings in a representative case (29-year-old man) with good improvement in acne after treatment with topical fullerene gel. **(A)** Before treatment. **(B)** After topical application of fullerene gel for 8 weeks. In active acne lesions a large amount of sebum is produced and neutrophils infiltrate into pilosebaceous units. However, after fullerene is applied onto the skin, superoxides are scavenged and apparently neutrophil infiltration and sebum production is reduced, resulting in suppression of inflammation.

pustules) decreased in 9 (81.8%) of the 11 patients. Further, at 4 and 8 weeks, the mean number of inflammatory lesions significantly ($P < 0.05$) decreased from 16.09 ± 9.08 to 12.36 ± 7.03 (reduction rate 23.2%) and 10.0 ± 5.62 (reduction rate 37.8%), respectively (Figure 1). We could not statistically analyze the number of nodules, because there were too few (mean 0.091). The number of pustules, consisting of accumulation of neutrophils, significantly decreased from 1.45 ± 1.13 to 0.18 ± 0.60 (reduction rate 87.6%, $P < 0.05$) (Figure 2, *A*), indicating that fullerene strongly suppresses neutrophil infiltration, possibly through its potential antioxidant effect on skin cells.[16] Additionally, our in vitro assays of sebum production using hamster sebocytes showed that 75 μM PVP-fullerene, as a reference compound for the pristine fullerene, significantly ($P < 0.05$) decreases sebum production from hamster sebocytes by 27.4% (Figure 2, *B*), suggesting suppression of sebum as another possible pathway of fullerene's effect on acne, although here we utilized hamster cells instead of human cells, because it is difficult to isolate and culture human sebocytes. We report a representative case of a 29-year-old male patient, whose acne lesions remarkably improved after topical application of fullerene gel for 8 weeks (Figure 3, *A*, *B*). In this case, the number of inflammatory lesions decreased from 39 to 13. During this trial no apparent side effects were experienced by any of the patients, and all the patients completed the treatment protocol.

Effect of fullerene gel on various skin-related parameters

After applying fullerene gel for 8 weeks, the mean water content of the skin, measured by Corneometer CM 825 (Courage + Khazaka electronic GmbH), significantly ($P < 0.05$) increased from 51.7 ± 7.9 to 60.4 ± 10.3 instrumental units, indicating improved skin barrier function, but the other parameters such as porphyrin content and number of conspicuous pores were not affected. Moreover, sebum production, measured using the Sebumeter SM815 (Courage + Khazaka electronic GmbH), was not altered despite the suppressive effect of fullerene on sebum production as shown above (Figure 2, *B*). This discrepancy is probably due to limited suppression by fullerene (only 27.4% suppression in vitro, Figure 2, *B*), precision of the device, and/or improved barrier function as mentioned above.

Discussion

Our open study revealed that fullerene exhibits a preferential efficacy for acne vulgaris. However, after application for 8 weeks, the inflammatory lesions were reduced by only 37.8%; this value is lower than that observed for adapalene gel in a previous study (median 63.7%),[17] suggesting that the fullerene has a mild effect on acne vulgaris. However, 9 of the 11 patients (81.8%) experienced a decrease in the number of inflammatory lesions, suggesting that fullerene can be used as a skin care product for acne patients. In addition, there might be possibility that the gel base itself exerts an antiacne effect, and so a placebo-controlled study using the gel base only is necessary to rule it out. However, among the gel base ingredients, it is unlikely that squalene shows a therapeutic effect on acne, because it is increased in acne patients.[18] On the other hand, the combination of octenidine dihydrochloride and phenoxyethanol, the other component in the gel base used in this study, has been reported to be effective in treating inflammatory acne lesions,[19] but because octenidine dihydrochloride has microbicidal activity,[20] phenoxyethanol is likely to be contained as a vehicle. The water content of skin increased during the treatment. The 1% squalene in the gel may have moisturized the skin, so a controlled study should be performed using the gel base to determine whether this hydrating effect was caused by fullerene. In addition, we have previously reported that the decrease in the conspicuous pores reflects the attenuation of perifollicular pigmentation.[21] Initially, the results of a previous study revealing the suppressive antimelanogenic effect of fullerene in vitro[9] led us to expect that fullerene might decrease the conspicuous pores, but such an effect was not observed in our study. Therefore, a large-scale study is required to determine the effect of fullerene on skin pigmentation.

Porphyrins, which are metabolic products of *P. acnes*, are reported to stimulate the expression of keratinocyte-derived interleukin-8 in pilosebaceous units, resulting in perifollicular inflammation[22]; the porphyrin concentration significantly decreases during isotretinoin treatment.[23] However, in our study no significant difference in porphyrin concentration was observed after fullerene treatment, as determined using the VISIA system (Canfield Scientific, Inc.). This finding may be ascribed to differences in the mechanism of action of fullerene and isotretinoin or antibiotics against *P. acnes*. The above speculation is based on the recent findings that fullerene exhibits no antibacterial activity against *P acnes*, *Staphylococcus epidermidis, Candida albicans*, and *Malassezia furfur*.[24] Here, our clinical and experimental data suggest that fullerene can suppress acne by inhibition of neutrophil infiltration and sebum production, possibly through antioxidative function.

In conclusion, we consider that fullerene gel is a useful and safety skin care product for controlling acne vulgaris.

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