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ORIGINAL ARTICLE

Efficacy of the continuous use of a lotion with carbon dioxide on male subjects with mild acne

Aiko Kiyozuka MS[1] | Asami Kajiyama MS[1] | Rie Ootsuki PhD[1] | Masaru Hosokawa MS[1] | Masahiro Miyaki MS[1] | Yutaka Takagi PhD[1,2]

[1]Kao Corporation, Tokyo, Japan

[2]Josai University, Saitama, Japan

Correspondence
Yutaka Takagi, Department of Pharmaceutical Sciences, Josai University, 1-1 Keyakidai, Sakado, Saitama 350-0295, Japan.
Email: ytakagi@josai.ac.jp

Abstract

Objective: Acne vulgaris is caused by dyslipidemia, dyskeratosis and/or abnormal bacterial growth. The obstruction of skin pores due to hyperkeratosis of the infundibulum contributes to the formation of comedones. Thus, normalizing keratinization of epidermal cells in skin pores might be useful to improve acne. Recently, it has been found that the transcutaneous application of carbon dioxide (CO_2) regulates imbalances of the desquamatory process. In this study, we evaluated the efficacy of a skin lotion containing CO_2 on mild acne.

Methods: Twenty-four healthy Japanese males (20–29 years old) with mild acne attended this evaluation. The subjects were divided into 2 groups, one group used a skin lotion containing CO_2 and the other group used a skin lotion without CO_2. Following facial washing, each subject topically applied the skin lotion with or without CO_2 twice a day for 4 weeks. Prior to the start of the evaluation (week 0) and following 2 and 4 weeks of treatment, acne symptoms were assessed by a dermatologist and by instrumental measurements.

Results: Topical application of the skin lotion with CO_2 for 4 weeks significantly improved acne symptoms, which was recognized by the subjects. However, treatment with the skin lotion without CO_2 did not improve acne symptoms. This improvement of acne symptoms by CO_2 was not accompanied by changes in sebum levels, skin surface pH, skin capacitance, or porphyrin levels.

Conclusion: The transcutaneous application of a lotion with CO_2 improves acne symptoms by normalizing keratinization without affecting skin surface conditions.

KEYWORDS
acne, CO_2, skin care

1 | INTRODUCTION

Acne is a common skin disease of teenagers but it can occur in most age groups[1] and many adults are bothered with acne. Acne involves seborrheic areas of the face and results from the obstruction of hair follicles followed by inflammation. The key pathogenic factors that play important roles in the development of acne are the following four major factors: androgen-dependent sebogenesis, hyperkeratinization of the infundibulum, *Cutibacterium acnes* (*C. acnes*) colonization, and inflammation.[2]

Inflammation precedes hyperkeratinization in early acne lesions, which occurs in the follicular infundibulum.[3,4] Thus, improving the hyperkeratinization may be effective for improving acne. There are many substances, including retinoids, their derivatives and benzoyl peroxide, that regulate or improve abnormal keratinization in the follicular infundibulum.[5–9] Medications, including systemic and topical antimicrobials and retinoids, have been used as prescribed medicines.[2,10,11] In addition to those medications, appropriate daily skin care is very important to prevent new acne or the aggravation of mild acne.

Hot springs containing CO_2 have been believed to have good effects on some skin diseases[12] and the effects of CO_2 on the skin, mainly for wound healing, have been reported.[13–17] Those effects of CO_2 are suggested to be caused by increased blood flow due to vasodilation,[18] microcirculation improvement[19] and changes in the hemoglobin–oxygen affinity in the blood (Bohr effect).[20] Recently, it was reported that a 8-week application of transcutaneous CO_2 decreased the scaling of cheek skin and concomitantly improved the epidermal barrier function.[21] The transcutaneous application of CO_2 ameliorated the reduced desquamatory process in xerotic skin, with concomitant mild acidification of the stratum corneum.[22] Thus, the transcutaneous application of CO_2 is expected to normalize keratinization of the follicular infundibulum, resulting in the improvement of acne symptoms.

In this study, we evaluated the efficacy of the topical application of a skin lotion containing CO_2 on mild acne symptoms for 4 weeks.

2 | METHODS

2.1 | Subjects

Post-adolescent Japanese male volunteers with mild acne were prescreened by a well-trained dermatologist. Subjects with nodules, more than 30 acne on their whole face and/or progressive diseases were excluded from this study. Prior to and during the trial period, the use of topical or oral anti-acne therapies was prohibited. Of the 39 Japanese males who were prescreened, 28 male subjects aged 20–37 years old (25.7 ± 4.7 mean ± standard deviation) who had mild acne, which was judged based on Hayashi's grade[23] were selected and enrolled in the present study. Based on their acne conditions during the screening, the subjects were divided into two groups with similar levels of acne symptoms, one group (Gr CO_2) used a skin lotion with CO_2 and the other group (Gr Control) used a skin lotion without CO_2.

This study adhered to the tenets of the Declaration of Helsinki. This controlled usage trial was reviewed and approved by the Review Board of the Kao Corporation, (#T235-190715). A formal written informed consent was obtained from each subject before the study.

2.2 | Test materials

All test aqueous lotions were provided by the Kao Corporation. The ingredients of the test foaming lotion (pH 6.0) were water, dipropylene glycol, PEG-32, isotridecyl isononanoate, lauryl methacrylate/sodium methacrylate crosspolymer, acrylates/C10-30 alkyl acrylate crosspolymer, sodium hydroxide, isostearyl glyceryl ether, sodium laureth-4 phosphate, succinic acid, xanthan gum, disodium ethylenediamine tetraacetic acid, phenoxyethanol, methyl paraben, and CO_2 gas (1700 mg L^{-1}). The placebo lotion contained the same ingredients except for CO_2. Changing the current facial skin care products and cosmetics except for the test materials was prohibited during the study. Following facial washing, each subject topically applied the test lotion or the placebo lotion on their face twice a day for 4 weeks.

2.3 | Observation and trial period

Baseline assessment of acne condition was done (week 0) and the efficacy was assessed at the end of weeks 2 and 4. The investigation was performed in Tokyo, Japan, from October to November 2019. All instrumental measurements and visual assessments were performed following acclimation in a room at 22 ± 2°C, 50 ± 5% relative humidity for 20 min.

2.4 | Evaluation

Following acclimation, the sebum level on the forehead of each subject was measured using a Sebumeter (Courage + Khazaka Electronic, Cologne, Germany) and the skin surface pH was measured using a PH 905 (Courage + Khazaka Electronic). Following those measurements, each subject washed his face with the mild facial skin cleanser and acclimated for 20 min. Cutaneous capacitance was analyzed as the moisture-retention ability (MRA) on the cheek using a Corneometer (Courage + Khazaka Electronic).

A well-trained dermatologist performed visual assessments of the acne on the facial skin throughout this study. The numbers of papules and pustules were counted by the dermatologist on the entire face. Microcomedones were not included in this evaluation by the dermatologist. To count the number of acne lesions, the facial area was roughly divided into 4 areas, the forehead, the cheek, the nose, and the face-line (around the mouth and jaw).

Images of the face of each subject were obtained with a VISIA-CR (Canfield Scientific, Inc. NJ, USA). Right and left oblique and frontal views were captured using a standard procedure. Right and left oblique views were used to analyze the cheek and the side of the jaw, and frontal views were used to analyze the forehead, the nose, and around the mouth. The number of microcomedones was counted from each VISIA image by a well-trained specialist. Porphyrin levels on the cheek and forehead skin were evaluated by UV fluorescence imaging.[24] The total luminance of porphyrin per scanned area was analyzed with Microsoft Visual Studio 2017.

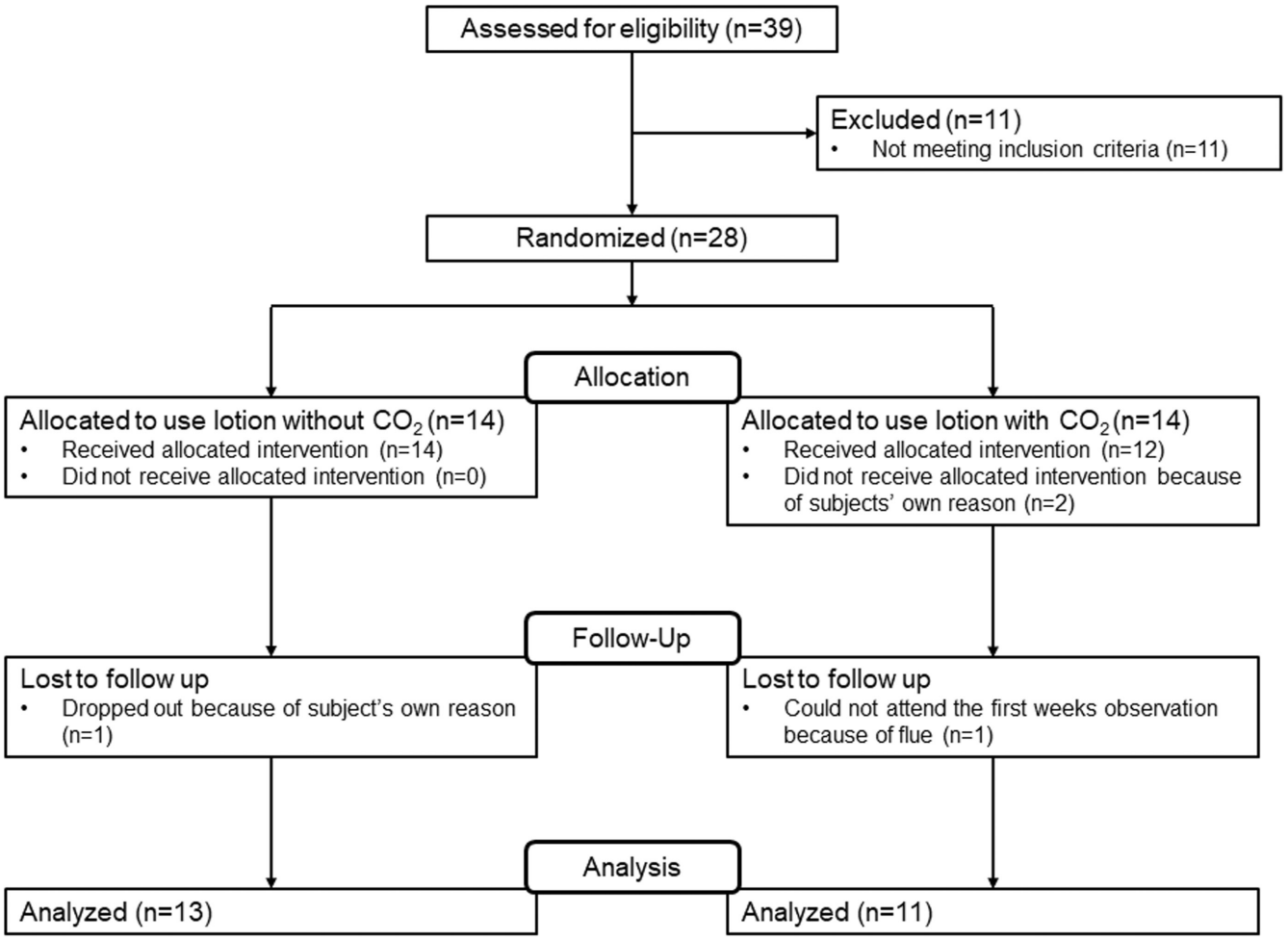

FIGURE 1 Research flow diagram of this trial

2.5 | Judgment of overall improvement by a dermatologist

At the evaluation at the end of week 4, a dermatologist judged the overall improvement rate with the test materials based on the acne condition of whole face. Overall improvement rates were comprehensively judged by a dermatologist using a 4-step grading system (improved, slightly improved, no change, or aggravated).

2.6 | Subjective evaluation by the test subjects

Subjective scores of changes in skin conditions for symptoms including acne conditions, total number of acne lesions, number of non-inflammatory acne lesions, and number of inflammatory acne lesions were graded as follows: improved, no change, or aggravated.

2.7 | Statistical analysis

Changes in acne number over the time course of the study were determined using the Wilcoxon signed rank test, and changes in data of instrumental analysis over the time course of the study were determined using the paired Student's *t*-test.

3 | RESULTS

3.1 | Visual assessments of acne conditions

Twenty-eight Japanese males participated in the present study. Based on their acne conditions during screening, they were divided into two groups with similar levels of acne symptoms; one group used a skin lotion with CO_2 (Gr CO_2, $n = 14$) and the other group used a skin lotion without CO_2 (Gr Control, $n = 14$). Prior to the start of the study, 2 subjects in Gr CO_2 group refused to attend this test because of their own reasons. Within 4 weeks of use, 1 subject dropped out because of his own reason and 1 subject could not attend the first week of observation because of the flu. Thus, a total of 24 subjects were involved in the analysis (Gr CO_2: $n = 11$, Gr Control: $n = 13$, Figure 1).

At the beginning of the study (week 0), the subjects in Gr CO_2 had 8.5 ± 6.4 (3–23) inflammatory acne lesions and those in Gr Control had 8.4 ± 6.6 (1–23) inflammatory acne lesions, with no significant difference between both groups (Table 1). There were no acne

	Papules	Pustules	Nodules	Total
Gr CO_2 ($n = 11$)	6.1 ± 4.2 (2–17)	2.4 ± 3.0 (0–8)	0.0 ± 0.0 (0–0)	8.5 ± 6.4 (3–23)
Gr Control ($n = 13$)	5.9 ± 5.3 (1–20)	2.5 ± 2.4 (0–8)	0.0 ± 0.0 (0–0)	8.4 ± 6.6 (1–23)

Note: The value in brackets indicate the minimum number to maximum number within each group. There are no significant differences between these 2 groups on either acne lesions.

TABLE 1 Acne conditions of subjects at baseline

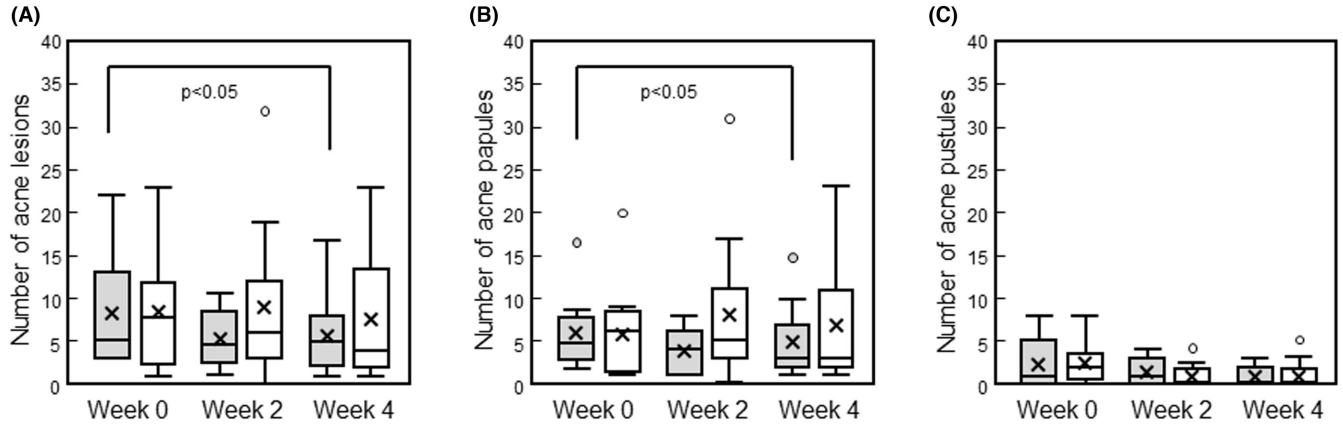

FIGURE 2 Changes in the numbers of inflammatory acne lesions (A), papules (B), and pustules (C) on the entire face within the 4 weeks of the study. Horizontal lines inside the boxes indicate medians, and the lower and upper ends of the boxes are the first and third quartiles. The whiskers indicate values within 1.5× the interquartile range from the upper or lower quartile (or the minimum and maximum if within 1.5× the interquartile range of the quartiles), and data more extreme than the whiskers are plotted individually as outliers (open circles). The crosses in the boxes indicate mean values. Gray boxes indicate subjects in Gr CO_2 (with CO_2, $n = 11$) and open boxes indicate subjects in Gr Control (without CO_2, $n = 13$)

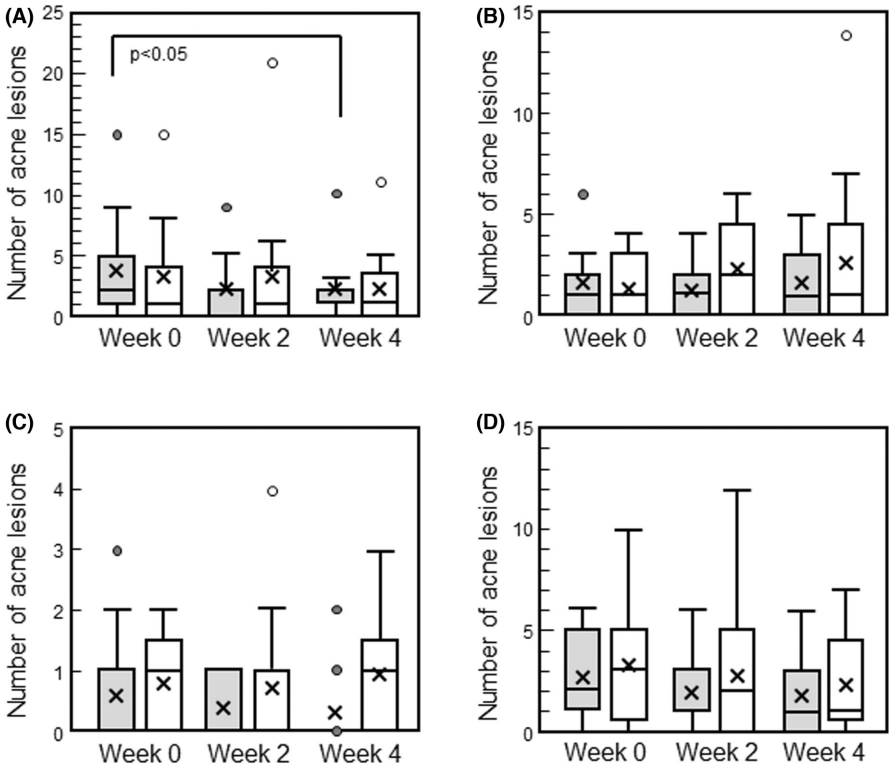

FIGURE 3 Changes in the numbers of inflammatory acne lesions on the forehead (A), the cheek (B), the nose (C), and the face-line (around the mouth and jaw, D) within the 4 weeks of the study. Horizontal lines inside the boxes indicate medians, and the lower and upper ends of the boxes are the first and third quartiles. The whiskers indicate values within 1.5× the interquartile range from the upper or lower quartile (or the minimum and maximum if within 1.5× the interquartile range of the quartiles), and data more extreme than the whiskers are plotted individually as outliers (circles). The crosses in the boxes indicate mean values. Gray boxes indicate subjects in Gr CO_2 (with CO_2, $n = 11$) and open boxes indicate subjects in Gr Control (without CO_2, $n = 13$)

nodules in any of the subjects. There were no significant changes in the number of total inflammatory acne lesions or inflammatory papules in the Gr Control within the 4 weeks of treatment; however, in the Gr CO_2, the total numbers of inflammatory acne lesions and inflammatory papules were significantly decreased. The numbers of pustules were decreased in both groups within the 4 weeks but

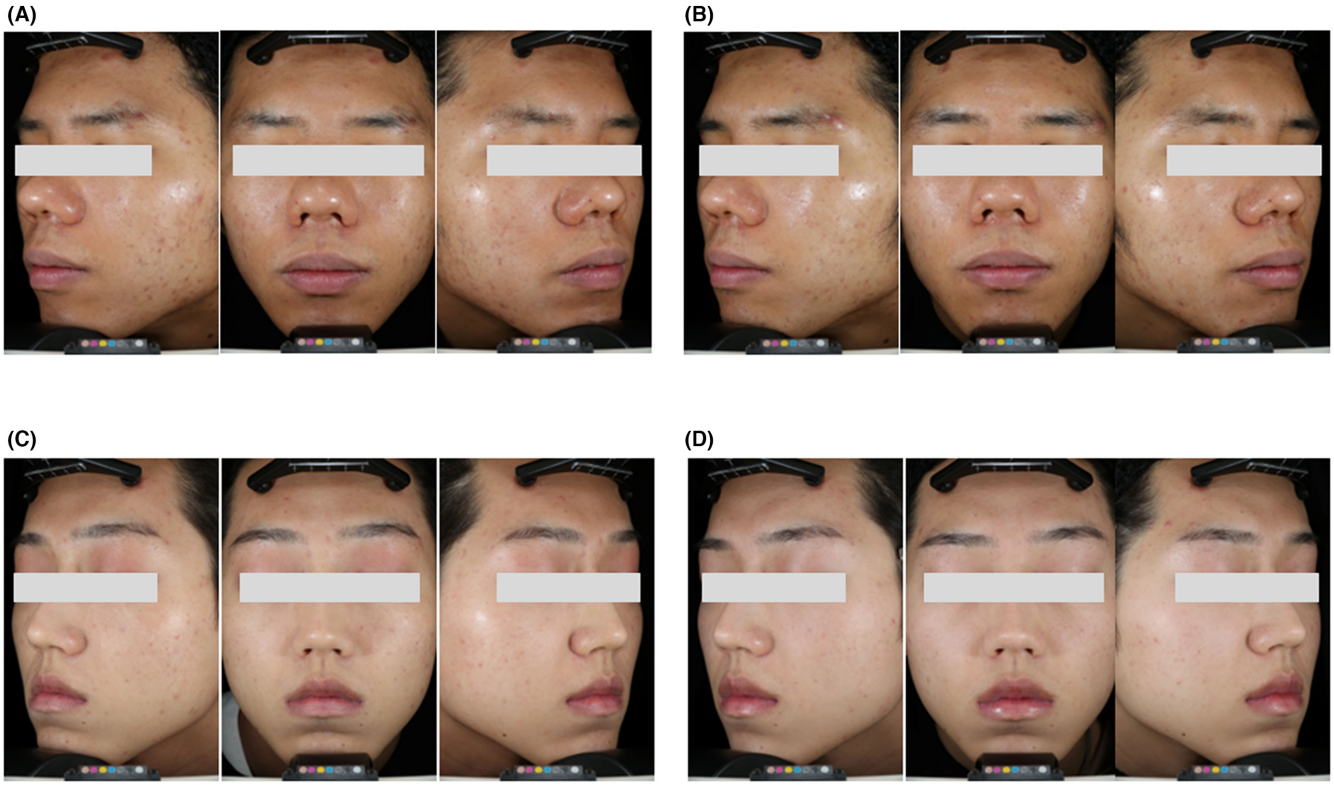

FIGURE 4 Typical clinical features with frontal view, left and right oblique view at week 0 (A and C) and week 4 (B and D). A and B are 23-year-old Japanese male and C and D are 22-year-old Japanese male

those numbers were small and their average number at week 0 was only around 2.5 on the entire face (Figure 2).

The analysis of changes in the number of acne lesions on each facial area indicated that there was a significant decrease in inflammatory acne lesions on the forehead in Gr CO_2 but not in Gr Control. There were no significant changes in the cheek over the 4 weeks. The results revealed that the number of subjects with more than 4 acne lesions on the mouth and jaw decreased, but that was not statistically significant (Figure 3).

Figure 4 shows a representative photograph of clinical improvement of acne on the face through the present study; a 23-year-old Japanese male at week 0 and at week 4 had an improved acne (total acne number 22 to 17), and a 22-year-old Japanese male at week 0 and at week 4 had an improved acne (total acne number 17 to 13).

The number of microcomedones on the entire face determined by VISIA imaging was significantly decreased both in Gr CO_2 and in Gr Control (Figure 5). The numbers of subjects with more microcomedones was obviously decreased, particularly in Gr CO_2.

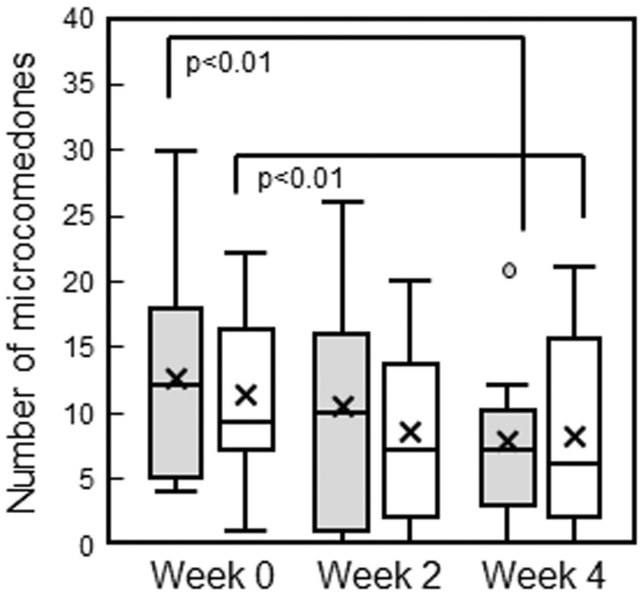

FIGURE 5 Changes in the numbers of microcomedones in the entire face within the 4 weeks of the study. Horizontal lines inside the boxes indicate medians, and the lower and upper ends of the boxes are the first and third quartiles. The whiskers indicate values within 1.5× the interquartile range from the upper or lower quartile (or the minimum and maximum if within 1.5× the interquartile range of the quartiles), and data more extreme than the whiskers are plotted individually as outliers (circles). The crosses in the boxes indicate mean values. Gray boxes indicate subjects in Gr CO_2 (with CO_2, $n = 11$) and open boxes indicate subjects in Gr Control (without CO_2, $n = 13$)

3.2 | Sebum level, skin surface pH, MRA and porphyrin level

The sebum level on the forehead (Figure 6A) and the skin surface pH on the forehead and cheek (Figure 6B,C, respectively), which were analyzed prior to facial washing did not change during the 4 weeks of evaluation. Further, the MRA, which was analyzed following facial

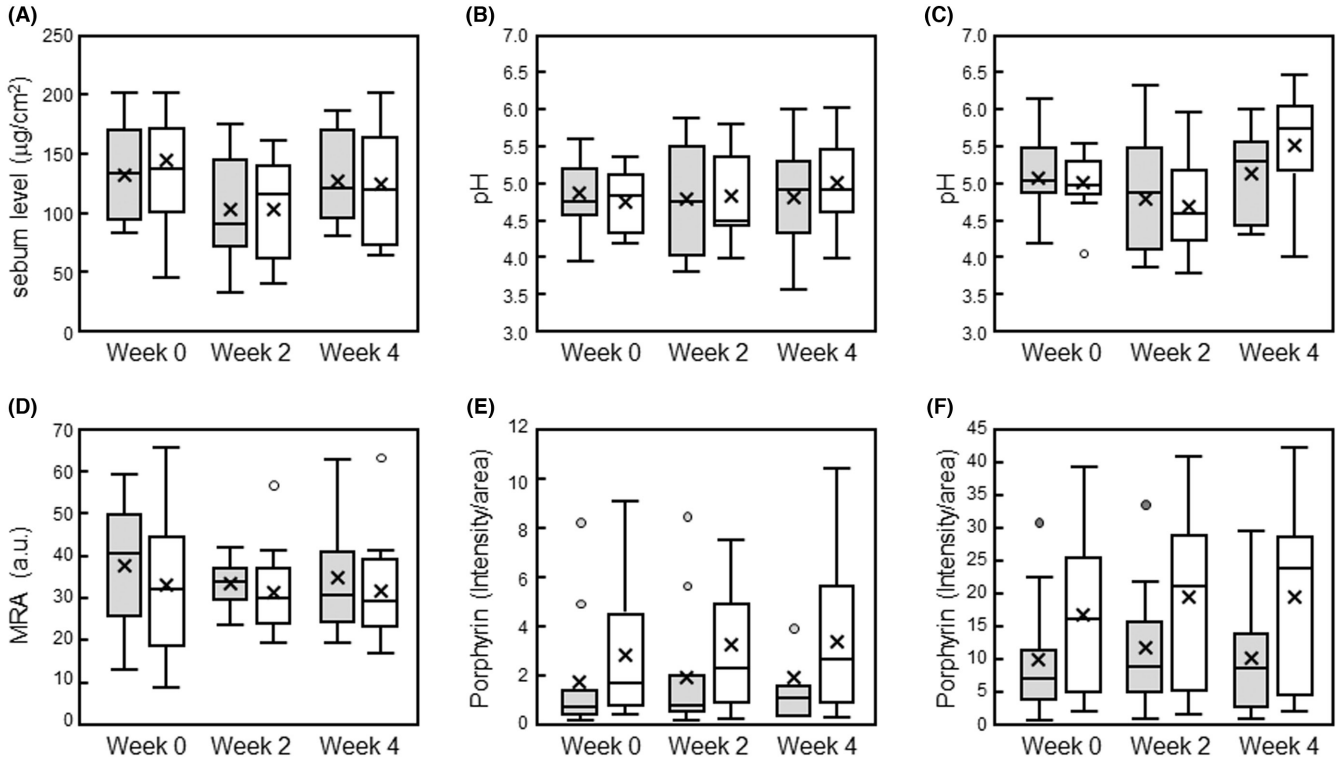

FIGURE 6 Changes in sebum levels on the forehead (A), pH on the forehead (B), pH on the cheek (C), MRA on the cheek (D), porphyrin levels on the forehead (E), and porphyrin levels on the cheek (F) within the 4 weeks of the study. Sebum levels and skin surface pH were evaluated prior to face washing and MRA and porphyrin levels were evaluated following face washing. Horizontal lines inside the boxes indicate medians, and the lower and upper ends of the boxes are the first and third quartiles. The whiskers indicate values within 1.5× the interquartile range from the upper or lower quartile (or the minimum and maximum if within 1.5× the interquartile range of the quartiles), and data more extreme than the whiskers are plotted individually as outliers (circles). The crosses in the boxes indicate mean values. Gray boxes indicate subjects in Gr CO_2 (with CO_2, $n = 11$) and open boxes indicate subjects in Gr Control (without CO_2, $n = 13$)

TABLE 2 Judgment of improvements by dermatologists

	Improved	Slightly improved	No changes	Aggravated	*p* value
Gr CO_2 ($n = 11$)	4 (36%)	3 (27%)	4 (36%)	0 (0%)	0.078
Gr control ($n = 13$)	1 (8%)	4 (31%)	8 (61%)	0 (0%)	–

Note: The value in brackets indicate the ratio of subjects within each group.

washing and acclimation, also indicate no changes on the cheek within this 4-week evaluation period (Figure 6D). There were slight increases in porphyrin levels in both groups but there were no significant changes in either group (Figure 6E,F).

3.3 | Judgment of efficacy by a dermatologist

No adverse events due to either test material were identified in the present study. The evaluation of improvement classified as slightly improved or better was 7 (64%) and improved was 4 (36%) in Gr CO_2. Compared with this group improved or better was 5 (38%) and improved was only 1 (8%) in Gr Control, which approached significance ($p = 0.078$, Table 2).

3.4 | Subjective score

Within the 11 subjects in Gr CO_2, 8 (73%) recognized that their acne symptoms were improved and 8 (73%) recognized that their number of acne lesions decreased. Seven subjects (64%) recognized that the number of inflammatory papules decreased and 5 (45%) recognized that the number of pustules/nodules decreased and none of them recognized that their acne worsened within the 4 weeks test period. On the contrary, 7 subjects (54%) in Gr Control recognized that their acne symptoms improved and 5 (38%) recognized that the number of acne lesions decreased but one subject (8%) recognized that his acne increased within the test period. Furthermore, 4 subjects (45%) recognized that the number of inflammatory papules decreased and 2 recognized that the number of pustules/nodules decreased while

TABLE 3 Recognition of improvement of skin conditions by subjective evaluation

	Improved[a]	No changes	Aggravated	*p* value
Acne symptoms				
Gr CO_2 ($n = 11$)	8 (73%)	3 (27%)	0 (0%)	0.35
Gr control ($n = 13$)	7 (54%)	6 (46%)	0 (0%)	–
Acne number				
Gr CO_2 ($n = 11$)	8 (73%)	3 (27%)	0 (0%)	0.086
Gr control ($n = 13$)	5 (38%)	7 (54%)	1 (8%)	–
Inflammatory papules				
Gr CO_2 ($n = 11$)	7 (64%)	4 (36%)	0 (0%)	0.076
Gr control ($n = 13$)	4 (31%)	7 (54%)	2 (15%)	–
Pustules/nodule				
Gr CO_2 ($n = 11$)	5 (45%)	6 (55%)	0 (0%)	0.37
Gr control ($n = 13$)	4 (31%)	8 (61%)	1 (8%)	–

Note: The value in brackets indicate the ratio of subjects within each group.

[a]Slightly improved and improved patients.

2 subjects recognized that the number of inflammatory papules increased, and 1 recognized that the number of pustules/nodules increased (Table 3).

4 | DISCUSSION

There are four major pathogenic factors that play important roles in the development of acne: androgen-dependent sebogenesis, hyperkeratinization of the infundibulum, *C. acnes* colonization and inflammation.[2] Excess sebum and sebum metabolites on the skin surface induce skin irritation, and that might be a major factor to increase *C. acnes*, which was reported to have significant correlations between sebum levels and the number, proportion and location of acne lesions.[2,25–27] In this study, the topical application of a skin lotion containing CO_2 decreased acne symptoms. However, there were no changes in sebum or porphyrin levels, which may be an indicator of *C. acnes*. Thus, this improvement of acne symptoms caused by CO_2 might be independent of sebum levels and *C. acnes* levels. Hyperkeratinization of follicular ducts is also one of the main factors that play a role in acne development.[2,28] It was reported that the transcutaneous application of CO_2 improves skin conditions including skin dryness[29] and reduces the desquamatory process in xerotic skin.[22] This reduction of the desquamatory process by CO_2 was concomitant with mild acidification of the stratum corneum, resulting in the activation of proteinase in the stratum corneum.[22] Thus, it is presumed that the enhancement of desquamation by transcutaneous applied CO_2 might improve acne symptoms. The increase in cutaneous water contents also improves hyperkeratosis.[30] However, in this test, most subjects did not have dry skin or low MRA values, and there were no significant changes in MRA values in subjects with or without CO_2 application. Thus, it is presumed that this improvement of acne did not correlate with the cutaneous water content.

Microcomedones, which are decreased by improvements in keratinization, were decreased significantly by treatment with both lotions but only the lotion with CO_2 decreased microcomedones to almost less than ten in the entire face. Thus, these results may indicate that improvements in keratinization is crucial factor for decrease the acne with mild acne symptoms. Topical application of retinoids or their derivatives are also used to improve acne by normalizing abnormal desquamation; however, these compounds also induce skin dryness.[31] In contract, transcutaneous application of CO_2 decreased acne without changing sebum secretion and did not cause skin dryness. This analysis about changes in keratinization caused by transcutaneous application of CO_2 on the skin with acne symptoms might be needed for the confirmation.

It is known that skin pH affects protease activity in the stratum corneum, which controls skin desquamation. In this test, the transcutaneous application of CO_2 did not affect the skin surface pH during the measurement period. Topically applied CO_2 might easily penetrate the skin, which is indicated by microcirculation improvement. Thus, it is speculated that the penetrated CO_2 might affect the skin pH and enhance cutaneous protease activity during this period as Fukagawa et al. reported;[22] however, because of the high buffering function of skin, the skin pH might be normalized. As transcutaneous application of CO_2 affects the blood flow promotion by vasodilation,[18] microcirculation improvement,[19] the induction of irritation or inflammation was a concern but there were no aggravated subjects judged by the dermatologist, which suggests that these formulations of lotions did not enhance the formation of comedones or acne symptoms. In this evaluation, one-third of the subjects who applied the lotion without CO_2 also improved their acne. They did not change their skincare habits except for the use of the skin lotion. We presume that this improvement might be caused because these subjects might wash their face more carefully than before due to attending the acne skin test.

Acne in adolescents is mainly caused by androgen-dependent sebogenesis,[32] and there are ethnic variations in androgen productivity. Asians produce less androgen than Europeans[33,34] and have less oily skin with less sebum production, and the severity of acne in Japanese patients is milder than in European patients.[35] In this evaluation, the subjects who indicated the efficacy of the CO_2 lotion were only post-adolescent Japanese males. Thus, the evaluation of CO_2 for Europeans might be necessary to determine whether CO_2 is effective for treating mild acne globally.

Medical treatments such as systemic and topical antimicrobials and retinoids are recommended for acne symptoms.[2,10,11] However, many subjects do not medicate when their acne symptoms are not severe. Even microcomedones have subclinical inflammation, which

may cause scars,[36] and thus, in addition to medical treatments, appropriate daily skin care to help reduce new acne and prevent aggravation of mild acne at an early stage might be necessary. Proper facial washing is effective for preventing acne.[37-42] Skin care that normalizes the desquamation process might also be useful to reduce acne. Retinoids or their derivatives are also known for effecting cellular proliferation; however, the topical application of these materials may cause skin dryness. In contrast, applies CO_2 normalize the desquamation process and also improve skin dryness. There were no adverse effects observed on this trial. Thus, this daily skin care material which applies CO_2 transcutaneously might be effective for decreasing mild acne by normalizing the desquamation process.

AUTHOR CONTRIBUTIONS

A.K., A.K., D.W., and Y.T. performed the research. A.K., A.K., M.H., M.M., and Y.T. designed the research study. A.K., A.K. R.O., and Y.T. analyzed the data. Y.Y. wrote the paper.

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CONFLICT OF INTEREST

This study was funded in full by the Kao Corporation. The authors have no other conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

ETHICAL APPROVAL

This study adhered to the tenets of the Declaration of Helsinki. This controlled usage trial was reviewed and approved by the Review Board of the Kao Corporation (Tokyo, Japan, #T235-190715). A formal written informed consent was obtained from each subject before the study.

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