Efficacy and safety of liposome-encapsulated 4-n-butylresorcinol 0.1% cream for the treatment of melasma: A randomized controlled split-face trial

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ABSTRACT
Melasma is an acquired pigmentary disorder that most commonly occurs in women of child-bearing age. Melasma is therapeutically challenging, and most commercially available hypopigmenting agents include tyrosinase inhibitors, which regulate the rate-limiting step of melanogenesis. 4-n-Butylresorcinol has received considerable attention as a novel hypopigmenting agent in the last 15 years because it has an inhibitory effect against tyrosinase and tyrosinase-related protein-1. However, the hypopigmenting effect of 4-n-butylresorcinol in human subjects has only been shown in a few studies. Liposome encapsulation is known to improve stabilization and enhance penetration of the product. Therefore, this study was conducted to evaluate the hypopigmenting efficacy and safety of liposome-encapsulated 4-n-butylresorcinol 0.1% cream in patients with melasma. This was a randomized, double-blind, vehicle-controlled and split-face comparison study. Twenty-three patients with a clinical diagnosis of melasma were included. 4-n-Butylresorcinol 0.1% cream or vehicle was applied to each side of the face twice daily for 8 weeks. Clinical and photographic evaluations, Mexameter measurements and assessment of patient satisfaction and side-effects were performed at baseline, 4 and 8 weeks. All subjects completed the study. Mexameter measurements demonstrated that the melanin index of the 4-n-butylresorcinol-treated side showed a significant decrease when compared with the vehicle-treated side after 8 weeks (P = 0.043). No adverse reactions were observed throughout the study. Subjectively, 4-n-butylresorcinol was considered to be efficacious in more than 60% of the patients after 8 weeks of treatment. In conclusion, liposome-encapsulated 4-n-butylresorcinol 0.1% cream was well tolerated and showed significant higher efficacy than vehicle alone for the treatment of melasma.

Key words: liposome-encapsulated 4-n-butylresorcinol, melasma, randomized controlled trial, split-face design, treatment.

INTRODUCTION
Melasma is a common acquired hypermelanosis that primarily occurs in women in their 30s or 40s. It is more common in people with darker skin types, especially Asians and Hispanics. The precise cause of melasma remains unknown; however, there are many possible contributing factors, such as exposure to sunlight, the use of oral contraceptive pills, pregnancy, cosmetics, endocrine dysfunction and genetic predisposition. Among these, exposure to ultraviolet radiation is thought to be the most important factor involved in the development of melasma.

It is well known that tyrosinase plays a key role in melanogenesis because it regulates the rate-limiting step of melanogenesis.1,2 Accordingly, most
commercially available hypopigmenting agents include tyrosinase inhibitors as the major active ingredients. Hydroquinone, kojic acid and arbutin are the most common compounds used to treat melasma or hyperpigmentary conditions, and these products are often used in combination with other therapies such as retinoic acid, topical corticosteroids or laser treatment. However, the therapeutic results of such treatments are not satisfactory.

In 1995, Okubo et al. reported that 4-n-butylresorcinol had an inhibitory effect on melanogenesis in cultured B16 melanoma cells through its direct inhibition of tyrosinase activity as well as the suppression of tyrosinase synthesis without inducing any cytotoxicity. Since then, further in vitro studies have demonstrated that 4-n-butylresorcinol inhibits melanin production as well as the activity of both tyrosinase and tyrosinase-related protein-1 (TRP-1). Furthermore, resorcinol has been reported to induce skin irritation. Skin irritation can also be induced by 4-n-butylresorcinol, which is a derivative of resorcinol derivative, particularly when it is applied in high concentrations. Therefore, we attempted to investigate the hypopigmenting efficacy of 4-n-butylresorcinol at lower concentrations than have been evaluated in previous studies. 4-n-Butylresorcinol was encapsulated in liposomes produced using γ-linolenic acid and an attached amino acid (arginine). Liposome encapsulation is known to enhance penetration and improve stabilization of the product. Moreover, liposome encapsulation may help reduce skin irritation through hydration of the epidermis. γ-Linolenic acid also has an anti-inflammatory property and has been shown to support the barrier function of the skin.

This study was conducted to evaluate the hypopigmenting efficacy and safety of liposome-encapsulated 4-n-butylresorcinol 0.1% cream for the treatment of melasma.

METHODS

Study design and patients
This double-blind, randomized, vehicle-controlled and split-face study was conducted between July and October 2006. This study was approved by the Institutional Review Board. Prior to enrollment, all patients were informed of the possible risks and complications of the treatment and signed consent was obtained from each patient. All patients were healthy female subjects aged 20 years or older who had been diagnosed with melasma. The exclusion criteria included pregnancy, breastfeeding, infectious skin disease, serious medical disorders, and recent hormone or corticosteroid therapy. At baseline visits, patients were provided with two products packaged similarly, one containing vehicle plus 4-n-butylresorcinol 0.1% cream and the other containing vehicle alone. They were instructed to apply the two formulations to each half of their face, twice daily, for 8 weeks. Randomization was carried out by patients drawing lots in the form of sealed envelopes containing cards with "left" and "right", indicating the side of face to be treated with 4-n-butylresorcinol cream or vehicle alone. Investigators were completely blinded to the assigned treatment throughout the study.

Patients were warned to avoid the use of any bleaching agents during the study. They were also instructed to avoid sun exposure and wear a broad-spectrum sunscreen in case of exposure to sunlight.

Efficacy and safety assessments
Patients were evaluated three times (baseline, weeks 4 and 8). Objective skin color measurements were performed using Mexameter (MX-18; Courage & Khazaka Electronic GmbH, Cologne, Germany). Three successive measurements of the melanin index (MI) were conducted on the same (darkest) portion of the 4-n-butylresorcinol-treated skin and vehicle-treated skin during each visit. The mean value of the data collected from each half of the face was then calculated and compared. Additionally, clinical photographs of the patients were taken at baseline, weeks 4 and 8.

After 4 and 8 weeks, patients were asked to complete a questionnaire concerning their overall improvement in response to treatment according to the following scale: excellent, good, fair, poor and very poor. Adverse events were recorded throughout the study. The investigator graded the degree of erythema, scaling, itching and burning at each visit, using a 0–3 scale (0, none; 1, mild; 2, moderate; 3, severe).
Statistical analysis
For statistical analysis, an independent samples Student’s t-test was used to compare the change in the mean MI in response to treatment between the 4-n-butylresorcinol-treated and vehicle-treated hemi-faces. Data were analyzed using SPSS software ver. 12.0K. $P < 0.05$ was considered statistically significant.

RESULTS
Twenty-three melasma patients were enrolled in the study. All patients were Korean women, and their mean age was 40.7 ± 5.4 years (age range 32–50 years). All patients completed the study.

The results of the Mexameter measurements demonstrated that the mean MI of the 4-n-butylresorcinol-treated skin showed a significant decrease (−7.51%) at week 8 when compared with the vehicle-treated skin (−3.26%) ($P = 0.043$) (Table 1). At week 4, the MI of the 4-n-butylresorcinol-treated skin and the vehicle-treated skin showed a 4.31% decrease and 2.50% decrease, respectively, although these values did not differ significantly ($P = 0.210$). Changes in the mean MI during the 8-week study period are shown in Figure 1. Representative photographs showing improvement observed on a patient treated with 4-n-butylresorcinol 0.1% cream are shown in Figure 2.

All patients completed the questionnaire and assessed their improvement subjectively. After 4 weeks, 4.3% and 43.5% of patients graded their improvement on the 4-n-butylresorcinol-treated skin as excellent or good, respectively. After 8 weeks, 65.2% of patients rated their response as excellent (13.0%) or good (52.2%). No new adverse events related to the study product were experienced by the patients or observed by the investigators during the study period.

DISCUSSION
Melasma is a chronic distressing condition that is often recalcitrant to various treatment modalities. 4-n-Butylresorcinol is a newly developed hypopigmenting agent that has an inhibitory effect against both tyrosinase and TRP-1. The hypopigmenting efficacy of 4-n-butylresorcinol has been demonstrated through several in vitro and in vivo studies since it was first reported in 1995.3–10 In an 18-week, placebo-controlled clinical study, 4-n-butylresorcinol 0.3% serum induced significant improvement in patients with post-laser pigmented lesions when compared with a placebo group.8 For melasma patients, 4-n-butylresorcinol 0.3% serum was found to be effective in 84% of patients with melasma in a 24-week open-treatment study.9 In another recent

Table 1. Melanin index (MI) changes for the 4-n-butylresorcinol 0.1%-treated side and vehicle-treated side during the 8-week study period

<table>
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<th>4-n-Butylresorcinol 0.1%</th>
<th>Vehicle</th>
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<tbody>
<tr>
<td></td>
<td>Mean ± SD MI</td>
<td>Reduction rate</td>
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<tr>
<td>Baseline visit</td>
<td>200.68 ± 38.24</td>
<td>4.31%</td>
</tr>
<tr>
<td>Week 4</td>
<td>191.84 ± 38.15</td>
<td>7.51%</td>
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<td>Week 8</td>
<td>185.42 ± 38.81</td>
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*Statistically significant difference between the 4-n-butylresorcinol-treated side and vehicle-treated side (independent samples Student’s t-test), $P < 0.05$. SD, standard deviation.
randomized, double-blind, vehicle-controlled, split-face study of 28 patients with melasma, 4-n-butylresorcinol 0.3% serum was found to have significantly greater efficacy than a vehicle control after 12 weeks application based on clinical assessments and colorimetric measurements. In that double-blind study for melasma, relatively long-term application of 4-n-butylresorcinol was necessary to achieve the hypopigmenting effect. Additionally, 12 adverse events including mild erythema, dryness, peeling and desquamation were experienced in eight of 28 patients (28.6%). Hence, the clinical trial conducted for the present study used lower concentrations of 4-n-butylresorcinol. To increase the efficacy, we used liposomes as carriers because liposome encapsulation may provide a safer and more effective treatment of melasma.

4-n-Butylresorcinol was encapsulated in liposomes constructed using γ-linolenic acid and an attached amino acid (arginine). Liposomes are microscopic spheres composed of amphipathic phospholipid bi-layers, which were initially used as a model for membrane system studies. It is well known that topical applications of liposomal preparations provide great advantages with respect to drug delivery and restoration of the skin barrier because of the similarity in the lipid composition of liposomes and membranes in the epidermis. Furthermore, liposome encapsulation may also improve stabilization of the product and help reduce skin irritation through hydration of the epidermis.

Liposomes can be designed using a variety of methods by changing the phospholipid composition or surface characteristics. Liposomes containing γ-linolenic acid may help alleviate skin irritation due to its anti-inflammatory property. The attachment of arginine to the outer surface of liposomes can lead to increased hydrophilicity of liposomes. To the best of our knowledge, this study is the first to evaluate treatment of melasma with liposome-encapsulated 4-n-butylresorcinol 0.1% cream.

The MI of the 4-n-butylresorcinol-treated skin determined using the Mexameter decreased gradually from 200.68 at baseline to 185.42 after 8 weeks. In addition, the mean MI of the vehicle-treated skin decreased from 201.13 to 194.43 throughout the study. This efficacy of the vehicle could be explained by increased use of sunscreen and avoidance of sun exposure.

Figure 2. Clinical photographs of a patient treated with 4-n-butylresorcinol 0.1% cream showing decrease of pigmentation over time. (a) At baseline visit; (b) after 4 weeks; (c) after 8 weeks.
exposure by the patients, or due to a beneficial effect of local permeation of 4-n-butylresorcinol through the skin that can occur during a split-face study. Overall, the mean reductions in MI at the end of the study were 7.51% and 3.26% for the 4-n-butylresorcinol-treated skin and the vehicle-treated skin, respectively, with a statistically significant difference (P = 0.043).

According to the patient self-assessment, 4-n-butylresorcinol was considered to be efficacious in more than 60% of patients after 8 weeks of treatment. Unexpectedly, no adverse reactions were observed, and the liposome-encapsulated 4-n-butylresorcinol 0.1% cream was well tolerated by the patients.

The results of this study showed that safer and better tolerated treatments may be achieved using reduced concentrations of 4-n-butylresorcinol. The fact that all 23 patients included in this study tolerated the study product without any adverse reactions emphasizes this suggestion. Moreover, liposome encapsulation of 4-n-butylresorcinol enabled effective treatment in a shorter time when compared with previous studies.

In conclusion, liposome-encapsulated 4-n-butylresorcinol 0.1% cream showed rapid efficacy and was well tolerated when used for the treatment of melasma.

REFERENCES

9 Researching Committee of Rucinol 
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