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Report

Antimelanogenic activity of a novel adamantyl benzylbenzamide derivative, AP736: a randomized, double-blind, vehicle-controlled comparative clinical trial performed in patients with hyperpigmentation during the summer

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Conflicts of interests: None.

Abstract

Background/Purpose AP736 is a novel compound with an adamantyl benzylbenzamide moiety that has shown antimelanogenic activity in melanocytes *in vitro* and in artificial skin equivalent through the inhibition of key melanogenic enzymes and suppression of the cAMP-phosphokinase A-cAMP response element-binding protein signaling pathway. To estimate the clinical effectiveness of AP736 for the treatment of facial hyperpigmentation, we examined the efficacy and safety of a topical formulation containing AP736 compared with a vehicle formulation in human facial skin. To evaluate the degree of whitening when used in a real-life situation, subjects with hyperpigmentation conditions were selected and the trial was performed from mid-May to the end of June, when there are strong UV rays in Korea.

Materials and Methods Forty-eight healthy Korean women aged 20–60 years were enrolled in this study for 6 weeks. Women who were pregnant or undergoing any concurrent therapy were excluded. Subjects were instructed to apply a randomly assigned formulation containing 0.5% AP736 (test formulation; $n = 24$) or vehicle (vehicle control; $n = 24$) in addition to an assigned sunscreen with a twice-daily application protocol. The degree of facial pigmentation was measured objectively using a Mexameter MX18 and Chromameter CM700, in addition to assessment by physicians using clinical photographs.

Results The AP736 formulation was significantly ($P < 0.05$) more effective than the vehicle control formation in reducing the appearance of pigmentation at 3- and 6-week follow-up visits.

Conclusion A formulation containing a novel skin whitening ingredient, AP736, effectively reduced pigmentation and was well tolerated by study subjects in summer season.

Introduction

As the standard of beauty is considered fair skin and a flawless complexion, an increasing number of medical procedures are being performed for skin whitening, and the need for safe and active ingredients is growing. Agents such as HQ, arbutin, kojic acid, rucinol, ascorbic acid, licorice, resveratrol, niacinamide, soy, and tranexamic acid are being used as depigmentation agents.[1–3] Most of these compounds reduce melanin production through the inhibition of tyrosinase, a key enzyme of melanogenesis. Niacinamide and soy inhibit melanosome transfer from melanocytes to keratinocytes.[1–4] Tranexamic acid has antiplasmin activity that prevents hyperpigmentation through inflammatory mediators.[5,6]

In our previous study, we synthesized various derivatives of *N*-benzylbenzamides as novel skin-lightening agents and found that AP736 downregulates microphthalmia-associated transcription factor (MITF) and tyrosinase by inhibiting the elevation in cAMP and activation of cAMP response element binding protein (CREB) and protein kinase A (PKA).[7–9]

In this study, we conducted a clinical trial to compare the whitening effects of a topical formulation containing

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AP736 with that of a vehicle control formulation to verify the findings obtained using the *in vitro* model.

Materials and methods

Product and subjects

A novel adamantyl benzylbenzamide derivative, AP736, was synthesized and formulations with or without AP736 were prepared at the AMOREPACIFIC R&D Center (Yongin-si, South Korea). These formulations were water-in-oil emulsions and had an identical composition except that the vehicle lacked AP736. The formulations were confirmed to have no safety issues in humans with respect to irritation and hypersensitivity through a repeat insult patch test (data not shown).

Forty-eight Korean women aged 20–60 with moderate hyperpigmentation (Fitzpatrick skin type II–IV) were enrolled. Subjects were not pregnant, nursing, or receiving any concurrent therapy. Subjects who had used any other topical whitening agents or chemical peels within the last 3 months and who had used any prescription topical products for more than 1 month were excluded from the study. Subjects were randomly assigned to receive the AP736 ($n = 24$) or vehicle control ($n = 24$) regimen. The study was approved by the relevant institutional review boards. All subjects provided informed consent, and the study protocol followed the Declaration of Helsinki and Korean Good Clinical Practice guidelines.

Study design

This was a 6-week, prospective, randomized, double-blind, vehicle-controlled clinical study. Subjects in the treated group received a topical emulsion formulation containing 0.5% AP736 and sun protection factor 50 sunscreen agents. Subjects in the control group received a vehicle emulsion with the same sunscreen agents. The samples were provided in identical 40 g blind-coded pump jars labeled with the subject number. Volunteers applied approximately 0.5 g of the test product to their entire face twice daily in the morning and evening.

Efficacy and safety assessments

Subjects were assessed at baseline and after 3 and 6 weeks by photography and using objective instrumental measurement systems. The primary efficacy endpoint was the difference in skin lightness (L value) between pre- and post-treatment and was assessed with the Chromameter CM700 (Konica Minolta, Osaka, Japan). Degree of facial pigmentation was determined using the Mexameter MX18 (Courage-Khazaka electronic GmbH, Cologne, Germany). Clinical appearance was assessed by two blinded expert graders using high-quality clinical photographs and a 1–10 scoring system (from bright and clear to dark and dull) and by patient satisfaction (Visia-CR; Canfield, NJ, USA). At each visit, adverse effects were assessed by the subjects and researchers.

Statistical analysis

All data were expressed as means and standard deviations. Skin lightening was analyzed by repeated measures ANOVA and independent *t*-test. Melanin index and pigmentation intensity scoring were analyzed using Friedman and Mann–Whitney *U*-tests. In all cases, differences were considered statistically significant when $P < 0.05$. Statistical analyses were performed using SPSS Statistics 19.0 version (Chicago, IL, USA).

Results

All 48 subjects successfully completed the trial with no dropouts; none of the subjects discontinued participation because of a lack of effectiveness or adverse events.

Skin lightening effects were significantly greater in the treated group than in the control group. The L values and average changes in L value are shown in Fig. 1. In the treated group, L values increased significantly from baseline at both 3 and 6 weeks: 61.86 ± 2.41 vs. 62.81 ± 2.32 ($P < 0.001$) vs. 63.34 ± 2.41 ($P < 0.001$), respectively. In contrast, in the control group, L values decreased with marginal significance from baseline to 3 weeks (61.93 ± 2.64 vs. 61.56 ± 2.52; $P = 0.040$) and were not significantly altered at 6 weeks (61.64 ± 2.14; $P > 0.05$).

Comparison of the average change in L value at 3 and 6 weeks from baseline revealed significant differences between the treated group and the control group (0.94 vs. −0.37 at week 3; $P < 0.001$ and 1.48 vs. −0.29 at week 6; $P < 0.001$).

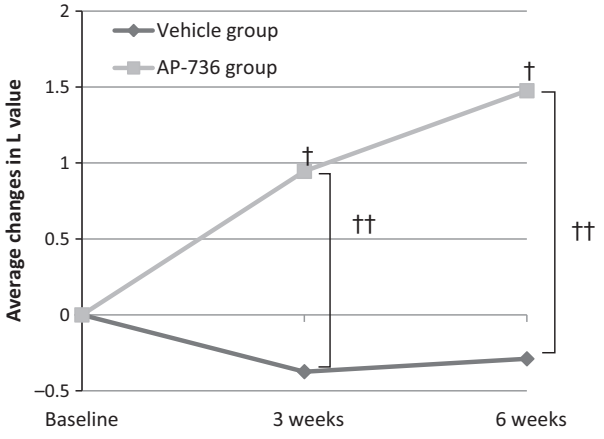

Figure 1 Average changes in skin lightness (L value) compared to the baseline for each group. The L value of the treated group increased significantly, whereas the L value of the control group showed a slight but insignificant decrease. †$P < 0.05$ by repeated measures ANOVA; ††$P < 0.05$ by independent *t*-test

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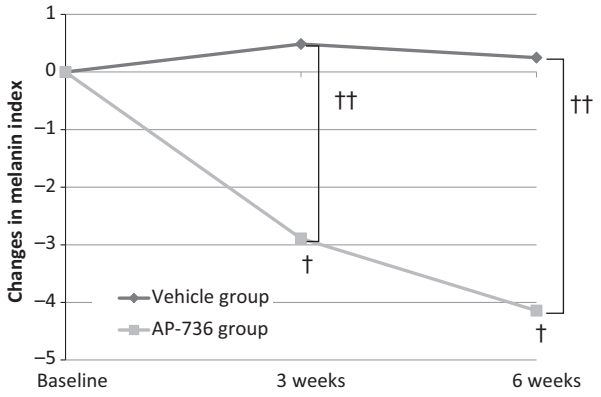

Figure 2 Average changes in the melanin index compared to the baseline for each group. The melanin index of the treated group decreased significantly, whereas the melanin index of the control group showed a slight but insignificant increase. †$P < 0.05$ by Friedman test; ††$P < 0.05$ by the Mann–Whitney *U*-test

Table 1 Pigmentation intensity scoring (1–10 scale) by two blinded expert graders

	Treated group (mean ± SD)	Control group (mean ± SD)	*P* value
Baseline	5.29 ± 0.62	5.25 ± 0.68	0.863
3 weeks	4.92 ± 0.78	5.21 ± 0.72	0.017*
6 weeks	4.67 ± 0.76	4.92 ± 0.65	0.038*

*$P < 0.05$, by Mann–Whitney *U*-test.

Average changes in the melanin index compared to baseline for each group are shown in Figure 2. In the treated group, the pre- and post-treatment levels indicated a significant decrease from 153.1 at baseline to 150.2 at 3 weeks ($P < 0.001$) and 149.0 at 6 weeks ($P < 0.001$). In the control group, the level of melanin increased slightly but non-significantly from 159.6 at baseline to 160.1 ($P = 0.215$) and 159.8 ($P = 1.000$), respectively. Comparison of the average change in melanin index at 3 and 6 weeks from baseline showed a significant difference between the treated group and the control group (−2.9 vs. 0.5 at week 3; $P < 0.001$ and −4.1 vs. 0.2 at week 6; $P < 0.001$).

Regarding clinical appearance, the mean scores of two blinded expert graders at 3 and 6 weeks after treatment showed significantly greater improvement in the treated group compared with the control group (Table 1).

Clinical photographs of facial hyperpigmentation in the treated group before and 6 weeks after treatment are presented in Figure 3. The facial tone and hyperpigmentation of the representative subjects showed an improvement in spot size and intensity.

Both regimens were well tolerated by subjects, and none of the participants experienced adverse events.

Discussion

Numerous botanical and other ingredients have skin-lightening effects, and an increasing number of these agents have been studied in small controlled clinical trials.[1,3,5] Many skin care products containing these ingredients are commercially available and can serve as adjunct or alternative treatments in the management of hyperpigmentation.[2]

Most depigmenting agents act specifically to reduce the function of tyrosinase through several mechanisms, including interference with its transcription and/or glycosylation, enzyme inhibition, reduction of by-products, and post-transcriptional control. Some agents inhibit melanosome transfer from melanocytes to keratinocytes and accelerate skin turnover.[4,10]

Because tyrosinase is the enzyme involved in the most important rate-determining step in melanin biosynthesis, the degree of tyrosinase inhibition is widely used to screen depigmenting agents.[11] However, most known tyrosinase inhibitors have unsatisfactory efficacy in humans compared with their *in vitro* efficiency, in addition to low stability and skin irritation.[3]

Hydroquinone (HQ) is considered one of the most effective inhibitors of melanogenesis *in vitro* and *in vivo* and is widely used in the treatment of melasma and other hyperpigmentary disorders. However, HQ has a number of reported side effects that restrict its use.[1]

Arbutin, a naturally occurring β-D-glucopyranoside derivative of HQ, was found to inhibit melanosomal tyrosinase and 5,6-dihydroxyindole-2-carboxylic acid polymerase activity.[5] In a human study, treated subjects underwent ultraviolet (UV) irradiation (210 mJ) on the inner forearm and were then administered 10% arbutin four times a day for 15 days. Arbutin treatment significantly suppressed pigmentation by 43.5% compared with the control ($n = 15$).[12] In another human study, a significant decrease in levels of melanin was determined in all 10 patients who used a formulation containing 1% arbutin twice daily for 6 months.[13]

An arbutin derivative, deoxyarbutin, demonstrated effective inhibition of mushroom tyrosinase *in vitro* with a Ki 10-fold lower than that of HQ and 350-fold lower than that of arbutin. A 12-week paired forearm test comparing 3% deoxyarbutin in a moisturizing cream with a vehicle control was conducted from mid-November to mid-February in the United Kingdom. The formulation containing 3% deoxyarbutin significantly reduced skin color compared to the placebo in the total population and in the Caucasian subset but not in the subset of

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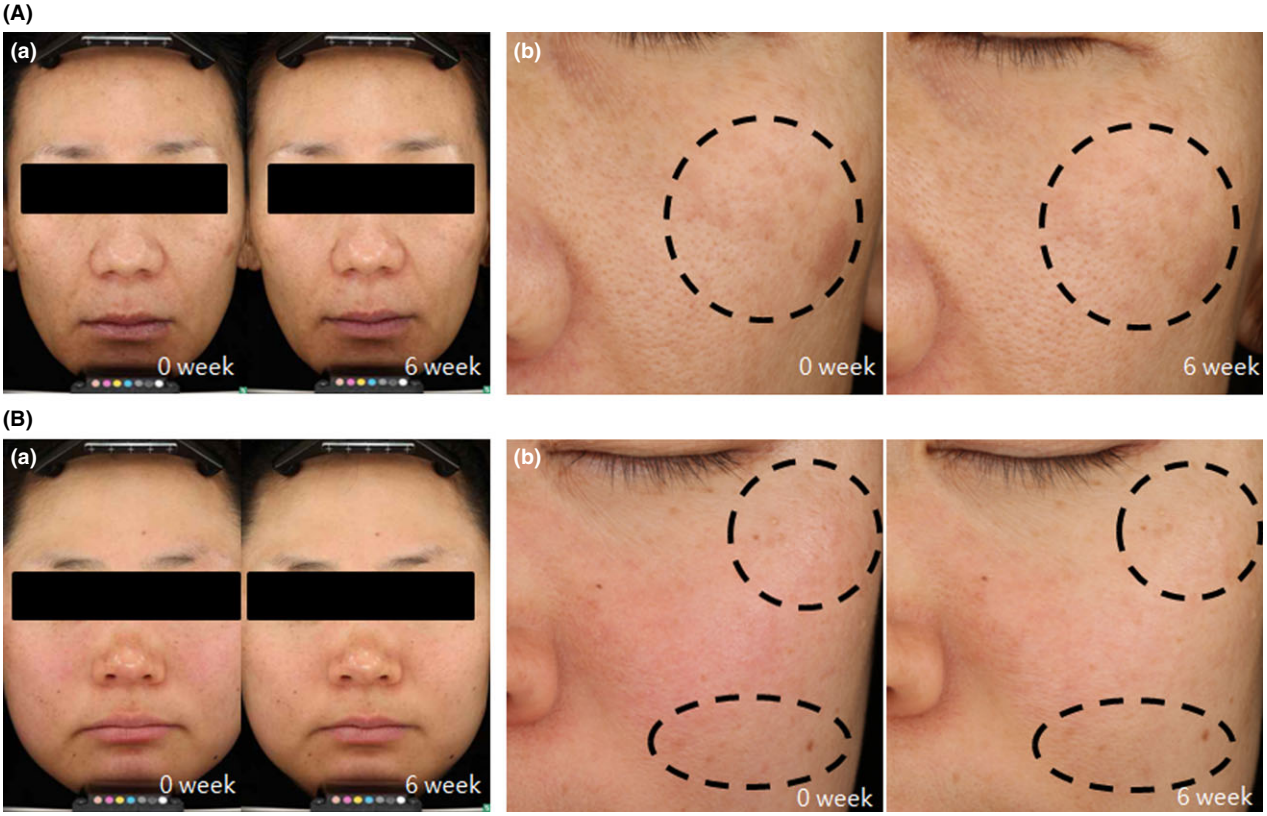

Figure 3 Clinical photographs of facial hyperpigmentation in the treated group before and after 6 weeks of treatment (A)44-year old subject, (B) 36-year old subject, (a) a full face, (b) focus on the hyperpigmentation area, respectively

non-Caucasian individuals with darker skin.[14] These results indicate that the efficacy of *in vitro* tyrosinase inhibition and *in vivo* human studies are not completely matched.

The resorcinol derivative rucinol (4-n-butylresorcinol) was the first substance shown to inhibit the activity of both tyrosinase and tyrosinase-related protein 1 (TRP-1). *In vitro* data show that rucinol has a strong and dose-dependent inhibitory effect on B16 mouse melanoma TRP-1 and tyrosinase (IC_{50} = 0.93 and 44 μM, respectively). The inhibitory potency of rucinol was reported to be 5.6, 100, and 380 times greater than that of kojic acid, HQ, and arbutin, respectively.[15] In a randomized, double-blind, vehicle-controlled, split-face study of 28 patients with melasma conducted from January to October, serum containing 0.3% rucinol was found to have significantly greater efficacy than a vehicle control after application for 12 weeks, based on clinical assessments and colorimetric measurements. The L values at 12 weeks varied significantly between the rucinol-treated group and the vehicle-treated group (61.6 vs. 61.1; P = 0.013).[15] The difference in L value at 12 weeks between the treated group and the control group was approximately 0.5. In comparison, that of AP-736 at 6 weeks was approximately 1.77. This difference may reflect the difference in the season performed.

Niacinamide (also called nicotinamide) is the physiologically active amide of niacin (vitamin B3). Niacinamide has been used to treat cutaneous lesions of dermatitis and is a main ingredient of several treatments for hyperpigmentation because it has known lightening effects. Clinical trials included 18 Japanese subjects with hyperpigmentation who used a 5% niacinamide moisturizer and vehicle moisturizer in a split-face paired design, and 120 subjects with facial tanning who were assigned to two of three treatments: vehicle, sunscreen, and 2% niacinamide + sunscreen. Niacinamide significantly decreased hyperpigmentation and increased skin lightness compared with vehicle alone after 4 weeks of use.[16] This study also showed that the magnitude of the difference between niacinamide + sunscreen and sunscreen alone is similar to the difference between sunscreen and vehicle, thus use of only sunscreen in summer can result in skin lightening compared to use of no agent at all.

In our previous study, in which we sought to develop a new material with depigmenting effects, we investigated

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various polyhydroxybenzamide derivatives based on the structure of kazinol or broussonin, which are present in the extract of *Broussonetia kazinoki* and have strong depigmenting activity.[7,11] Among these agents, we found that AP736, a polyhydroxylated *N*-benzylbenzamide derivative containing an adamantyl moiety, inhibits tyrosinase and has an antimelanogenic effect approximately 30 times more potent than that of kojic acid.[2,4,5] In subsequent research, we reported that AP736 attenuated melanin production induced by diverse melanogenic stimuli in murine and human melanocytes. It also suppressed the expression of key melanogenic enzymes tyrosinase, TRP-1 and -2, and decreased the expression of a major promoter of melanogenesis, MITF. AP736 inhibited the elevation in cAMP and activation of CREB and PKA, indicating suppression of the cAMP–PKA–CREB signaling axis resulting in downregulation of the MITF and tyrosinase.[8]

In addition to these depigmentation studies, it was recently reported that AP736 has potential anti-inflammatory effects through suppression of NF-κB-IKK/IκBα and AP-1-IRAK1/TAK1 signaling in macrophages. AP736 suppressed multiple macrophage-mediated inflammatory responses, including nitric oxide/prostaglandin E_2 production and inflammatory gene (inducible nitric oxide synthase, cyclooxygenase-2, and interleukin-1β) expression, phagocytic uptake, and morphological changes in activated macrophages.[17] These results suggest that AP736 might be useful for the treatment of inflammatory and/or pigmentation diseases of the skin.

This study was designed to evaluate the clinical efficacy and safety of a topical formulation containing AP736 with that of a vehicle control formulation in humans. AP736 has an *in vitro* antimelanogenic effect on B16 mouse melanoma cells that is approximately 14 times more potent than that of rucinol by molar concentration. The tyrosinase inhibitory potency of AP736 was found to be 1.3, 34, and 770 times greater than that of rucinol, kojic acid, and arbutin, respectively, in our cell-free mushroom tyrosinase assay system. The molecular weight of AP736 is approximately three to four times greater than that of rucinol, kojic acid, and arbutin, and the published range of inhibitory activity is extremely broad and divergent according to data from the *in vitro* assay system.[18–20] Because the significant depigmenting efficacy of rucinol was observed after application of a 0.3% dose for 12 weeks in a clinical trial, the selected dose of AP736 for the first clinical study was 0.5%.

In previous clinical studies, the upper back or upper arm of the subject was pigmented artificially by UV irradiation to evaluate the depigmenting effect of the test compounds. The protocol of irradiation with UV rays has the advantages of relative ease of recruitment of subjects and regulation of other influencing factors, but to evaluate the degree of the whitening effect in real life situations, we used study subjects with hyperpigmentation conditions who applied the treatment to the entire face twice a day, similar to the typical use of cosmetics. Furthermore, similar to the model of UV-induced hyperpigmentation, this trial was performed from mid-May to the end of June when there are strong UV rays in Korea, unlike most clinical studies performed in autumn to winter, and all subjects used sunscreen. In our control group, L values decreased from baseline to 3 weeks, and the melanin level increased slightly but not significantly. This is presumed to be because of induction of melanin synthesis by strong UV rays during the experimental period. In contrast, in the AP736-treated group, the L values increased and the melanin index was reduced significantly from baseline at 3 weeks.

This study was not designed as a split-face study because of ethical issues, and thus we were unable to assess intra-individual variation. Even though basic skin lightness and pigmentation were evaluated through this trial, we did not include clinical photographic analysis with a UV lamp. In addition, the decrease of the melanin index induced by AP736 was weak compared to the *in vitro* melanogenesis inhibitory effect seen in B16 melanoma cells. This difference between *in vitro* and *in vivo* results is probably due to species difference and skin penetration. In spite of this limitation, the AP736 formulation was significantly ($P < 0.05$) more effective than the vehicle control formation in reducing the appearance of pigmentation. In addition, regarding safety, the enrolled participants experienced no adverse events. This study demonstrates that cosmeceuticals containing AP736 could improve facial hyperpigmentation in Asians.

This was the first study conducted to evaluate the depigmenting effect of AP736 alone in a human clinical trial. Tyrosinase inhibitors are the active ingredients in most commercially available skin-lightening cosmetics and, at present, there are no multi-action inhibitors of melanogenesis. Rucinol is known to inhibit the enzyme activity of tyrosinase and TRP-1 simultaneously. AP736 inhibits transcription and expression of melanogenesis enzymes. These actions may allow prompt inhibition of melanin production and may prevent later melanin synthesis through continued use. Because AP736 has various action mechanisms, as discussed above, further studies are required to determine whether these effects are beneficial or unrelated to the skin-lightening effect and what the main depigmenting pathway is. Furthermore, to confirm the feasibility of AP736 for cosmetic use, it will be necessary to perform additional safety evaluations and further studies on the formulation type and its stability when mixed with other ingredients, its efficacy in comparison

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with other agents, and the effect in combination with other depigmenting agents with a different mechanism of action. Based on this study, we plan to extend the scope of studies to figure out these questions in the near future.

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