J. Soc. Cosmet. Scientists Korea
Vol. 33, No. 1, March 2007, 23-28

Flexible Thin Layer Battery가 부착된 Iontophoretic Gel Patch를 이용한 Vitamin C 유도체의 경피 흡수 증진

조 완 구† · 랑 문 정* · 송 영 숙** · 임 영 호** · 박 현 우**

전주대학교 건강자원학부, *배재대학교 분자과학부, **엘지 생활건강 연구소
(2007년 2월 22일 접수, 2007년 3월 4일 채택)

Enhanced Transdermal Delivery of Vitamin C Derivative using Iontophoretic Gel Patch with Flexible Thin Layer Battery

Wan Goo Cho†, Mun Jeong Rang*, Young Sook Song**, Young Ho Lim**, and Hyeon Woo Park**

Health Resources Department, Jeonju University, Hyoja-dong, Wansan-gu, Jeonju-si, Jeonranam-do 560-759, Korea
*Molecular Chemical Division, Baejae University, Daejon
**R & D Institute, LG Household & Health Care, Daejon
(Received February 22, 2007; Accepted March 4, 2007)

요 약: 비타민 C는 강력한 환원제로서 멜라닌 색소의 합성을 저해하는 것으로 알려져 있다. 그러나 일반적인 화장품 제형에서는 낮은 안정성과 경피 흡수의 문제점으로 만족할 만한 효과를 나타내지 못한다. 본 연구에서는 안정성이 개선된 비타민 C의 유도체인 ascorbyl glucoside (AsAG)을 유효성분으로 하여 경피흡수를 증가시키고자 하였다. 본 실험에서는 유연하면서도 박막 형태의 배터리를 장착한 패치 화장품을 제조하고 안정성과 경피흡수성을 평가하였다. 피부에 낮은 전류를 증가하는 이온토포레시스를 활용하여 피부에 적용하는 전류의 세기를 증가시키면 물질의 경피흡수는 증가한다. 그러나 전류의 세기를 증가시키면 피부 부작용이 증가하기 때문에 본 연구에서는 한국인의 피부에 맞는 적절한 전류를 선택하여 피부 부작용을 최소화 하였다. 이런 결과들을 바탕으로 유연하면서도 가벼운 박막 배터리를 개발하였으며, 2%의 AsAG을 함유한 이온토포레시스 패치의 안전성, 경피흡수정도, 미백효과 등을 검토한 결과 피부에 가하는 최적의 전류는 1.5 V의 배터리를 사용하여 피부 부작용과 경피흡수를 고려하여 평균 0.1 mA이었다. 또한 패치의 임상실험 결과 유의한 수준의 미백효과를 보였으며 피부 자극도도 통상의 화장품 수준을 나타냈다.

Abstract: Ascorbic acid (vitamin C, AsA) has been known as a strong reducing agent and is supposed to retard the synthesis of melanin pigment. A main problem that arose in using vitamin C in cosmetic formulation was its poor stability and low skin permeability, which result in low lightening efficacy in clinical trials. In this study, iontophoretic gel patch with flexible thin layer battery was employed in order to enhance skin permeation of vitamin c derivative (ascorbyl glucoside, AsAG) and to increase its lightening efficacy. *In vitro* iontophoretic skin permeation and stability of AsAG, safety and clinical lightening efficacy of iontophoretic patch containing 2% AsAG solution were examined. A optimun current of ionthophoretic patch for korean women was 0.1 mA, considering the skin permeability and skin irritation of consumers. We suggest that iontophoretic gel patch could be a safe system for enhancing the skin permeation of AsAG and lightning efficacy.

Keywords: *vitamin C derivative, iontophoresis, skin whitening, flexible thin layer battery, tropical delivery*

1. Introduction

In both the cosmetic and pharmaceutical areas, significant efforts have been put forth on how to overcome the skin barrier to deliver functional ingredients into the skin topically. Recent progress in skin drug delivery has been reviewed in several articles[1-3]. Three primary routes across the stratum corneum are

† 주 저자 (e-mail: wgcho@jj.ac.kr)

available for molecular transport[1]. (1) Small molecules diffusing through skin usually follow an intercellular path within the stratum corneum lipids. (2) Transcellular pathways, which cross both the cells and intercellular lipids of the stratum corneum, can be accessible following skin permeabilization by electrophoresis. (3) Flux through the hair follicles and sweat ducts may be utilized during iontophoresis, pressure-mediated delivery, and liposomal transport. Electroporation is believed to involve the creation of new transient aqueous pathways in lipid bilayers by the application of the shorts electric current and to drive molecules through the pores by electrophoresis[4,7].

Briefly, the topical delivery of vitamin C is as follows: the main factors causing skin aging are natural processes, lifestyle, and environmental stressors (UV and chemical pollutants). Vitamin C reacts with oxidative radicals to prevent cellular damage. In spite of these important activities of vitamin C, the drawback is instability. In order to improve the instability, several types of vitamin C derivatives which are modified by chemical synthesis were developed. The typical derivative is AsAG. The stability of AsAG is better than that of vitamin C. Topical delivery of the vitamin C and its derivatives for skin improvement and lightening appears comfortable than chemical peels, laser skin resurfacing or large doses of pills. There are several types of vitamin C derivatives which are modified by chemical synthesis. Topical delivery of the vitamin C and vitamin C derivatives for skin improvement appears comfortable than chemical peels, laser skin resurfacing or large doses of pills.

Many asian women want to have lighter skin and want to avoid hyperpigmentation, such as melasma or freckling. In order to meet this desire, many cosmetic companies have been researching the pigmentation process to develop whitening products. While skin thickness, hemoglobin and minor pigment like carotenoids affect skin color, the amount of melanin produced by the melanocytes primarily determines skin color[8]. For this reason, research for the development of whitening products has focused on reducing melanin production in the melanocyte. Melanin formation is induced mainly by UV radiation and other stimuli such as toxic chemical agent[8]. UV radiation has been shown to generate active oxygen species (AOS) such as superoxide anion radical, hydrogen peroxide, hydroxyl radical, and singlet oxygen or free radicals in living organisms. AOS accelerate tyrosinase activity to increase dopa and dopaquinone generation from tyrosine in melanocyte[9] resulting in excess melanin formation[10]. Whitening products usually contain kojic acid, arbutin or ascorbic acid derivatives to decrease skin pigmentation. It is known that ascorbic acid (vitamin C) scavenges AOS and free radicals as a chain-breaking antioxidant and inhibits melanin formation by reducing the intermediate dopaquinone and resulting eumelanin in melanogenesis. These characteristic biological activities of vitamin C are derived from the enediol structure, which has a strong electron-donating ability.

A main problem that arose in using vitamin C in cosmetic formulation was its stability and permeability. A substantial effort has been made through the cosmetic industry to improve vitamin C stability, effectiveness, and delivery. The required duration of treatment is relatively long and skin irritation will occur with acidic pH formulations. Can more vitamin C be delivered quickly? It is possible the permeability of skin substantially? Combining electroporation-mediated tropical delivery with formulation appears to be promising choice. People go to the hospital to treat the skin damage, however, it is very inconvenient and rather expensive.

We believe that electrophoresis may enhance the penetration of cosmeceuticals into the skin over conventional methods, thus, shortening the duration of treatment. The most important issues to be addressed are efficacy, convenience, and safety.

The objectives of this study were to investigate the feasibility of iontophoretic gel patch with flexible thin layer battery of vitamin C derivative by measuring the penetration of the skin and the irritation of the skin. We also considered the correlation between the electric field strength and skin layers. Ultimately, we aim to develop an electrically assisted, safe, effective, and user-friendly delivery patch system for cosmetic applications to assist making the skin younger and white.

2. Materials and Methods

2.1. Materials

The chemical used, and their commercial sources, were as follows: AsAG (Hayashibara, Tokyo, Japan). Flexible battery ($MnO_2/ZnCl_2/NH_4Cl/Zn$) was developed by us and the specification was as follows: voltage (1.5 V); thickness (500 mm) diameter (1.4 mm). The Ag wire (99.9%, 1 mm diameter) was purchased from Sigma.

2.2. Methods

2.2.1. Skin Permeation Studies

Vertically assembled Franz type diffusion cells (Hanson Research Corporation, USA) were used for *in vitro* skin permeation experiments. The system consisted of Franz type diffusion cells with an effective diffusion area of 1.776 cm^2 and receptor volume of 7.0 mL and cell drive system with rpm controller. The fundamental experiments were performed according to the method given in our previous report[10]. Briefly, the excised skin of female hairless mouse was obtained from 8 ~ 9 weeks old, 27 ~ 33 g animals. The skin was mounted on diffusion cell, and the receiver compartment was filled up with 7 mL of 50 mM phosphate buffer saline pH 7.4 (PBS) and maintained at 32℃ by circulating water within a jacket around the lower chamber.

3% AsAG aqueous was applied at donor compartment on the skin surface (500 μL). Inlets in the Franz cell permitted the positioning of electrodes on either side of the skin. AsAG was delivered cathodically (cathode placed in the donor side) using a continuous constant current. Silver-silver chloride electrode and power supply (Type 2553, Yokogawa, Japan) were used for current delivery.

The receptor fluid was mixed by a magnetic stirrer throughout the experiment. The receptor fluid was collected from the receiver compartment at predetermined time and replaced by fresh fluid.

After filtration of the receptor solution on Millex filter FG (pore size: 0.2 μm, Millipore, USA), solutions were assessed by a high performance liquid chromatography system.

2.2.2. Stability Studies

To measure the temperature stabilities, aqueous solution containing 2% AsAG were prepared at various pH (pH 2, 4, 5, 6, 7). Samples were stored at 25℃ and 40 ℃ up to 2 months. Periodically, 1 mL aliquots of each sample were pipetted out and diluted with distilled water. The amount of residual AsAG was measured by HPLC.

2.2.3. High Performance Liquid Chromatography

The HPLC consisted of solvent delivery pump (Hewlett Packard, Germany), C_{18} column (HP ODS Hypersil 5 μm, 4.6 × 200 mm, Hewlett Packard, Germany). AsAG was analyzed with the mobile phase of distilled water in 0.05% phosphoric acid and at the flow rate of 1 mL/min. The absorbance at 245 nm was measured and the retention time was 5.5 min. Temperature of the column was kept at 30℃.

2.2.4. Toxicity

In order to examine the effect of current density on the skin irritation, the various current was applied on the face of the 30 volunteers. Irritation such as itching, prickling, burning sensation, edema, and erythema was evaluated by both subjects and well-trained professionals. Evaluation was performed by CTFA/ICDRG guideline.

2.2.5. Preparation of Iontophoretic Patch

Iontophoretic patch containing anode and cathode (Ag-AgCl electrode, anode area: 6 cm^2, cathode area: 10 cm^2) was prepared using flexible thin layer battery (1.5 V) for iontophoretic skin permeation of AsAG.

2.2.6. Treatment Clinical Test and Colorimeter Measurement

For estimation of lightening effectiveness, 20 participants with facial melasma were treated with iontophoretic patch with battery on one side of their face and patch without battery containing 2% AsAG solution on the other side as a control for 4 weeks. All of the volunteers were allowed to use a sunscreen which was applied a day to both sides of the face. The L value (lightness) of colorimeter (CR 300, Minolta, Japan) was used to measure the change in lightening of skin color and the postauricular areas

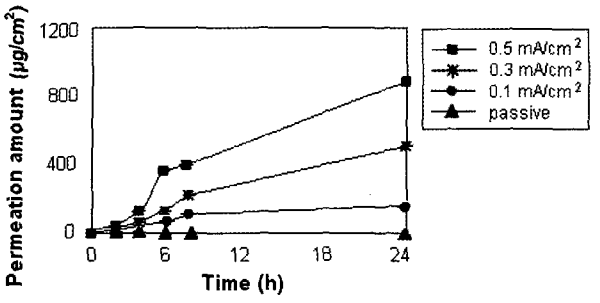

Figure 1. Iontophoretic and passive skin permeated amount of AsAG and effect of current density on the permeability of AsAG.

were selected as the control site. As for the spot localization, the measured site was marked with circle and was photographed as a follow-up reference. On each visit, the measurement was conducted on the same site by reference to the baseline marking photographs. The L, a, b system, recommended by the Commission International de l'Eclairage, was used to measure the skin color. It is expressed in a three-dimensional scale: the L value (luminance) gives the relative lightness ranging from total black (L = 0) to total white (L = 100). Three consecutive readings were taken at the treated sites, and the controlled sites of the subjects and their mean values were calculated. A calibration was performed before each measurement.

2.3. Volunteers

The subjects included in this study were all females. The mean age was 35.8 years (27 ~ 44 years). All subjects were free of systemic or skin diseases and had not used any tropical preparations on the test areas during the study. Written informed consent was obtained from all the subjects.

2.4. Statistical Analysis

Statistical analysis was conducted using SPSS for windows computer software (SPSS Science, USA). The values of sensory irritation were statistically analyzed using ANOVA test. $p < 0.05$ was considered significant.

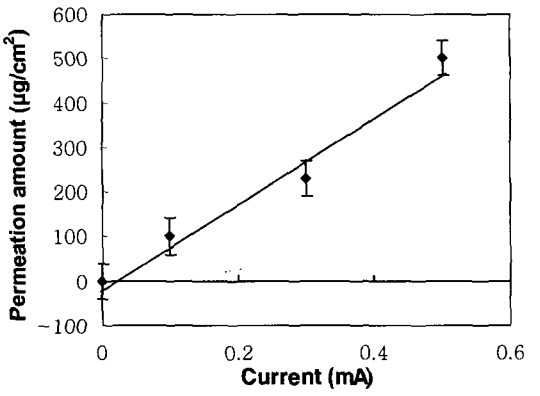

Figure 2. Effect of applied current on AsAG iontophoresis (after 12 min).

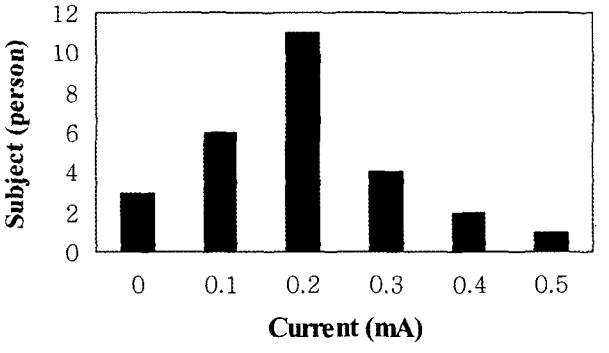

Figure 3. Measurement of current among the subjects (applied voltage = 3.7 V, n = 27).

3. Results and Discussion

The rate of membrane penetration of active ingredients may be increased by means of an external energy source. *In vitro* skin permeation experiment (Figure 1) showed skin permeability of iontophoresis was higher than that of passive delivery of AsAG from solutions of 2% concentration. Iontophoretic permeated amount of AsAG increased to about 8 ~ 40 fold that obtained by passive delivery, depending on the magnitude of current density and pH. As the magnitude of current density increased and pH decreased, the skin permeability increased.

According to Figure 1, the flux of AsAG was proportional to the current density, provided transport number is constant. A linear relationship has been observed between flux and applied current density. The steady state flux of AsAG increased (Figure 2). A linear increase in steady state flux with current has

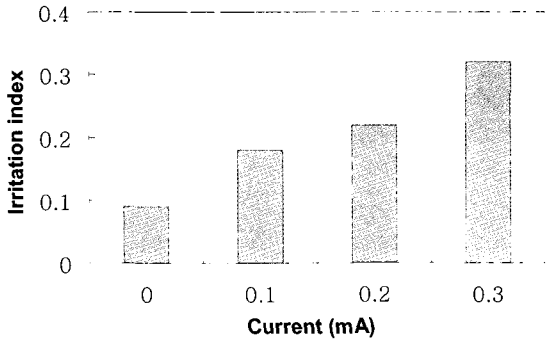

Figure 4. Irritation index against current.

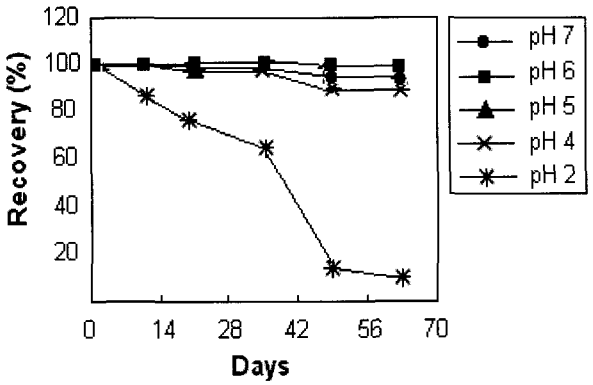

Figure 5. Recovery percent of AsAG in aqueous solution stored at 40℃ and effect of pH on the stability of AsAG.

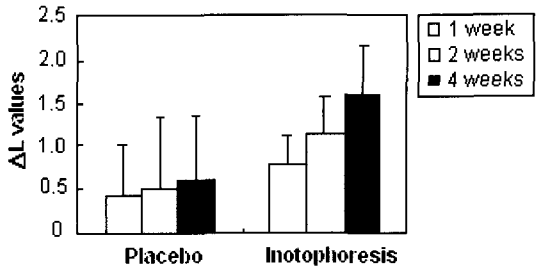

Figure 6. The ΔL value by colorimeter measurement before and after (1, 2, 4 weeks). * $p < 0.05$.

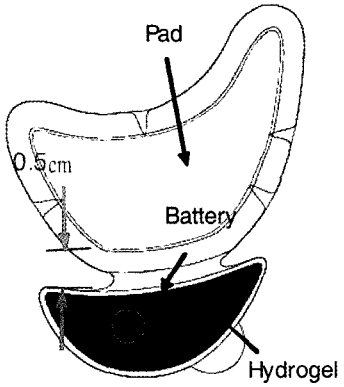

Figure 7. The picture of iontophoretic gel patch with flexible thin layer battery.

also been reported for number of other compounds[11].

However, the maximum strength of current that can be used is limited by consumer safety considerations. The irritation of the skin was directly related to the iontophoretic current density (0.05 ~ 0.3 mA/cm^2). The voltage of iontophoretic gel patch was determined at 1.5 V in order to supply safe current to the Korean women (0.05 ~ 0.2 mA/cm^2). The upper limiting value of current has been suggested 0.2 mA/cm^2 (Figure 3 and 4).

The stability of AsAG was examined in aqueous solution at various pH during 2.5 months storage at 2 5℃ and 40℃. AsAG was stable above pH 5 and the recovery percent was above 95%. Therefore, we selected optimal pH 5 in AsAG solution contained in the iontophoretic patch, considering both iontophoretic permeability and stability (Figure 5).

In the clinical test, iontophoretic patch containing 2% AsAG solution treated area showed the significant improvement of melasma in both of clinical and self assessment compared to control. ΔL values by colorimeter measurements (Figure 6) showed that increase of skin lightness was significantly higher in iontophoretic patch containing AsAG treated area than control ($p < 0.05$). The clinical test was performed using a iontophoretic gel patch with flexible thin layer battery (Figure 7).

4. Conclusion

1) Iontophoretic skin permeability of AsAG was enhanced about 8 ~ 40 fold compared to passive delivery. As the magnitude of current density increased and pH decreased, the skin permeability of AsAG increased.

2) Skin irritation was directly related to the iontophoretic current density. The safe voltage of iontophoretic patch for a skin was 1.5 V.

3) AsAG was stable above pH 5 at 40℃. The optimum pH was 5 in AsAG solution for the ionto-

phoretic patch, considering both iontophoretic permeability and stability.

4) Patch containing anode and cathode (anode area: 6 cm^2, cathode area: 10 cm^2) was prepared using thin flexible battery (1.5 V) for iontophoretic skin permeation of AsAG.

5) Clinical test conducted with 20 females with facial melasma showed that the iontophoretic patch containing 2% AsAG had a significantly higher skin lightning effect than passive patch.

6) This research suggested that iontophoretic gel patch could be a safe system for enhancing the skin permeation of AsAG and lightning efficacy.

References

1. S. Y. Oh, S. Y. Jeong, T. G. Park, and J. H. Lee, Enhanced transdermal delivery of AZT (zidovudine) using iontophoresis and penetration enhancer, *J. Controlled Release*, **51**, 161 (1998).
2. L. Zhang, S. Lerner, W. Rustrum, and G. Hofmann, Electroporation-mediated topical delivery of vitamin C for cosmetic application, *Bioelectrochemistry and Bioenergetics*, **48**, 453 (1999).
3. M. R. Prausnitz, The effects of electric current applied to skin: A review for transdermal drug delivery, *Advanced Drug Dilivery Reviews*, **18**, 395 (1996).
4. M. Ebihara, M. Akiyama, Y. Ohnishi, S. Tajima, K. Komata, and Y. Mitsui, Iontophoresis promotes percutaneous absorption of *L*-ascorbic acid in rat skin, *J. Dermatological Sci.*, 1 (2003).
5. C. H. Huh, K. I. Seo, J. Y. Park, J. G. Lim, H. C. Eun, and K. C. Park, A randomized, double-blind, placebo-controlled trial of vitamin C iontophoresis in melasma, *Dermatology*, **206**, 316 (2003).
6. M. R. Prausnitz, Do high-voltage pulses cause changes in skin structure, *J. Controlled Release*, **40**, 321 (1996).
7. M. R. Prausnitz, C. S. Lee, C. H. Liu, J. C. Pang, T. P. Singh, R. Langer, and J. C. Weaver, Transdermal transport efficiency during skin electroporation and iontophoresis, *Biotechnology*, **20**, 1205 (1996).
8. M. Kubo and H. Matsuda, Development studies of cuticle and medicinal drugs from natural sources on melanin biosynthesis, *Fragrance J.*, **8**, 48 (1995).
9. G. Imokawa and Y. Mishima, Loss of melanogenic, properties in tyrosinases induced by glycosylation, inhibitors within malignant melanoma cell, *Cancer Res.*, **42**, 248 (1994).
10. Y. S. Song, B. Y. Chung, S. G. Park, M. E. Park, S. J. Lee, W. G. Cho, and S. H. Kang, Polyethoxylated retinamide as an anti-wrinkle agent, *Cosmetics & Toiletries*, **114**(6), 53 (1999).
11. J. B. Phipps, R. V. Padmanabhan, and G. A. Lattin, Iontophoretic delivery of model inorganic and drug ions, *J. Pharm. Sci.*, **78**, 365 (1989).