

Direct Analysis in Real Time Mass Spectrometry (DART-MS) Analysis of Skin Metabolome Changes in the Ultraviolet B-Induced Mice

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Abstract

Ultraviolet (UV) radiation is a major environmental factor that leads to acute and chronic reactions in the human skin. UV exposure induces wrinkle formation, DNA damage, and generation of reactive oxygen species (ROS). Most mechanistic studies of skin physiology and pharmacology related with UV-irradiated skin have focused on proteins and their related gene expression or single-targeted small molecules. The present study identified and analyzed the alteration of skin metabolites following UVB irradiation and topical retinyl palmitate (RP, 5%) treatment in hairless mice using direct analysis in real time (DART) time-of-flight mass spectrometry (TOF-MS) with multivariate analysis. Under the negative ion mode, the DART ion source successfully ionized various fatty acids including palmitoleic and linolenic acid. From DART-TOF-MS fingerprints measured in positive mode, the prominent dehydrated ion peak (m/z : 369, M+H-H₂O) of cholesterol was characterized in all three groups. In positive mode, the discrimination among three groups was much clearer than that in negative mode by using multivariate analysis of orthogonal partial-least squares-discriminant analysis (OPLS-DA). DART-TOF-MS can ionize various small organic molecules in living tissues and is an efficient alternative analytical tool for acquiring full chemical fingerprints from living tissues without requiring sample preparation. DART-MS measurement of skin tissue with multivariate analysis proved to be a powerful method to discriminate between experimental groups and to find biomarkers for various experiment models in skin dermatological research.

Key Words: Cholesterol, Fatty acids, DART-TOF-MS, Retinyl palmitate, Skin photoaging, Ultraviolet B

INTRODUCTION

Ultraviolet (UV) irradiation of the skin leads to acute inflammatory reactions, such as erythema, sunburn, and chronic reactions, including premature skin aging and skin cancer (Chaquour *et al.*, 1995; Pacheco-Palencia *et al.*, 2008). UV light is composed of UVA (320-400 nm), UVB (280-320 nm) and UVC (200-280 nm). UVB radiation produces connective tissue damages more efficiently than does longer wavelength radiation in the hairless mouse model (Chaquour *et al.*, 1997). Acute exposure to UVB on human skin up regulates the expression of collagenase and stromelysin (Fisher *et al.*, 1996)

in UV-induced photoaging and reduces levels of type I and III collagen precursors, as well as increasing levels of matrix metalloproteinases (MMPs) and elastase. It was previously reported that collagenase was induced at 12-16 hours after UV irradiation and trails the initial loss of procollagen protein, which was observed 8 hours after UV irradiation. The reduction of procollagen expression and induction of collagen degradation maximizes collagen loss during the initial 24 hours following UV exposure (Fisher *et al.*, 2000). These factors serve as biomarkers in UV-irradiated skin. To date, there have been no studies on changes in intact UVB-irradiated skin. In this study, we attempted to identify parameters responding to

Open Access <http://dx.doi.org/10.4062/biomolther.2013.071>

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Received Sep 9, 2013 Revised Oct 21, 2013 Accepted Oct 24, 2013

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UVB irradiation using direct analysis in real time (DART) mass spectrometry (MS) as new analysis technique.

The DART ion source, a versatile new ion source that was introduced quite recently, is based on the reactions of metastable helium atoms generated from the plasma with ambient water, oxygen, or other atmospheric components to produce the reactive ionizing species (Cody *et al.*, 2005). Sample analysis by the DART technique is carried out at ambient temperature and ionization is achieved by exposing the sample to a stream of excited gas, typically helium (Williams *et al.*, 2006). Therefore, DART allows rapid, noncontact analysis of solid, liquid, and gaseous materials without sample preparation and directly detects on various surfaces including concrete, fruits, body fluids, and clothing (Morlock and Ueda, 2007; Pierce *et al.*, 2007). Although DART has low efficiency in ionization of hydrophilic large molecules such as peptide and protein, it has relatively low tendency toward matrix effect, compared with solid-liquid extraction-based ambient mass spectrometry such as DESI (desorption electrospray ionization) and LESA (liquid extraction surface analysis). Since time-consuming and labor-taking sample preparation steps can be omitted in DART-MS analysis, high throughput fingerprinting study is possible and this feature is one of the most advantageous characteristic of DART ion source in the files of metabolomics study. As DART ion source ionize metabolites directly from various surfaces with low matrix effect, mice skins can also be directly ionized for MS snapshots of various metabolomes in skin tissue. Since a highly variant and large volume of data are typically obtained from direct analysis in real time MS measurement, multivariate analysis tools must be used to achieve statistically significant discrimination between groups. The most frequently used tools for this purpose involve principal component analysis (PCA) (Jackson, 1991), partial least-squares (PLS) to latent structures (Wold *et al.*, 1984; Wold *et al.*, 2001), and orthogonal PLS (OPLS) (Trygg and Wold, 2002). The most common method is PCA and, but it is an untargeted reduction method which project data onto the principal subspace spanned by the leading eigenvectors of the covariance matrix (Trygg *et al.*, 2007). PCA also represents the pattern of similarity of the observations and the variables by displaying them as points in plots. Through PCA analysis of DART-MS data, a specific marker molecule was identified which could help distinguish between three groups.

Furthermore, we investigated metabolite changes in hairless mouse skin treated with vitamin A derivative, retinyl palmitate (RP), using the MS technique as mentioned above. Vitamin A (retinol) is an essential human nutrient that plays important roles in immunity and critical physiological functions including vision, reproduction and developmental morphogenesis (Fu *et al.*, 2007; Tsai *et al.*, 2008). RP is the major esterified form of retinol and accounts for >90% of the retinyl esters endogenously formed in skin. As retinyl esters are more chemically and thermally stable than retinol, retinyl esters are preferred retinoid used in skin care products, and are commercially used as an ingredients in cosmetic products (Yan *et al.*, 2006a; Yan *et al.*, 2006b).

The present study aims to analyze and identify changes in the composition of skin following acute exposure to UVB irradiation and topical RP treatment, using DART- time-of-flight (TOF) MS analysis in hairless male mice. Furthermore, we investigated the possibility of using this technique as a new targeted profiling method for searching skin photodamage-

related diagnostic markers.

MATERIALS AND METHODS

Reagents

Retinyl palmitate (RP), propylene glycol and ethanol were purchased from Sigma (St. Louis, USA).

Animal

Six-week-old male SKH-1 hairless mice (25 g each) were purchased from OrientBio/Charles River (Seongnam, Korea). After 3 days of quarantine, they were housed in individual cages with free access to food pellets and drinking water throughout the experiment. The animals were kept in an air-conditioned room at $22 \pm 1^\circ\text{C}$ and $60 \pm 5\%$ humidity under a 12 hr light/12 hr dark cycle. The experimental protocol [KHUASP-08-008] was approved by Institutional Animal Care and Use Committee of Kyung Hee University.

Animal UV irradiation schedule and treatment

The hairless mice were divided into 3 groups, with 4 or 5 mice in each group: normal (UV-) group, UVB-irradiated control (UV+) group and UVB-irradiated and RP-treated (UV+RP) group. UVB was supplied by an array of 5 Sankyo Denki G5T5E lamps (Sankyo Denki Co., Japan) without filters. The peak emission was near 312 nm; the amount of irradiation delivered was between 290 and 320 nm, representing 97% of the total amount of UVB. The irradiation intensity was measured at the bottom of the cage, and the animals could move around freely in the cage during the period of exposure to UVB light. The distance between the lamp and the each animal's back was approximately 15 cm. The energy output of the lamps was measured with an UV radiometer (Waldmann radiometer, Waldmann, Medizintechnik, Germany).

UVB radiation was applied to the backs of mice 3 times for 1 week. The amount of irradiation was 850 mJ/cm² per exposure (1 minimal erythema dose (MED)=100 mJ/cm²). Immediately following UVB irradiation (850 mJ/cm² per exposure), hairless mice were treated with either a vehicle (7:3 [v/v] propylene glycol:ethanol) or 5% RP in UV+ and UV+RP group, respectively. And 2 week, UVB exposure was discontinued, and only the sample was topically administrated daily.

Sacrifice and skin sample collection

To perform DART-MS analysis, mice were sacrificed by cervical dislocation 24 hours after the last sample treatment, and skin samples (4 mm diameter) were obtained from the central dorsum of the mice and collected. Non-irradiated mice were used as negative controls.

DART-MS analysis conditions

The operating conditions of the DART ion source (Ion Sense, Tokyo, Japan) coupled to a AccuTOF-TLC (JEOL, Tokyo, Japan) in the positive and negative ion modes were a discharge needle voltage of 3,000 V, electrode 1 at ± 100 V, electrode 2 at ± 100 V, helium gas flow at 3 L/min, at 200°C. The first orifice lens was set to ± 10 V and the ring lens voltage was set to ± 5 V. The TOF-MS was set with an Ion-guide RF voltage of 500 V and a detector voltage of 2,200 V.

DART-MS measurement of skin tissue sample

Direct analysis was carried out with intact tissue, with each sample placed between the inlet of MS analyzer and the DART ion source. Mass spectral data were obtained by averaging the mass spectra recorded during the exposure of the skin tissue to the DART gas stream. Four specimens were used for the normal, and UVB-irradiated groups and five specimens were used for the UV+RP group.

Data analysis

The DART-TOF-MS spectra were automatically converted to ASCII files using the MassCenter software (JEOL, Tokyo, Japan). PCA studies were performed with SIMCA-P software (v. 12.0, Umetrics, Umea°, Sweden).

Statistical analysis

One-way analysis of variance (ANOVA) followed by least significant difference (LSD) test was used for statistical analysis. Statistical significance was set at $p < 0.05$.

RESULTS

In this study, UVB irradiation for 1 week (total amounts of UVB irradiation, 2,550 mJ/cm²) induced serious wound and erythema in dorsal skin of hairless mice, while topical RP treatment had no effect on a cure for skin (Fig. 1A). Furthermore, the skin from only vehicle-treated mice without UVB irradiation showed the suppression of UVB-induced scars on their dorsum and topical RP treatment for 1 week accelerated these conditions (Fig. 1B). And also, we investigated skin metabolite profiles following the exposure to UVB radiation and topical

RP treatment in hairless mice skin using DART-TOF-MS. Skin tissue was directly analyzed by placing the biopsied samples under a stream of metastable helium gas. This avoided the necessity of additional sample preparation, including organic solvent extraction and lyophilization, securing real time analysis of small organic compounds directly from intact tissue. Under negative mode, the DART ion source ionized various fatty acids in the skin tissue as expected. Among the characterized ion peaks were palmitoleic acid (m/z : M-H, 253.8), linolenic acid (m/z : M-H, 277.4), linoleic acid (m/z : M-H, 279.7), and oleic acid (m/z : M-H, 281.7). These molecular ions of common physiologic fatty acids were easily detected in all three groups (Fig. 2). Although slight differences in ion peaks from DART-MS fingerprints among the three groups were observed in the region of m/z 130 and between m/z 160 and 180, it was difficult to detect significant differences among the three groups. PCA of the DART-MS spectral data was useful in differentiating the intact normal mouse skin group (UV-) from the UVB irradiated (UV+) and RP treated (UV+RP) groups (Fig. 3A). However, the UV+RP group was not easily distinguished

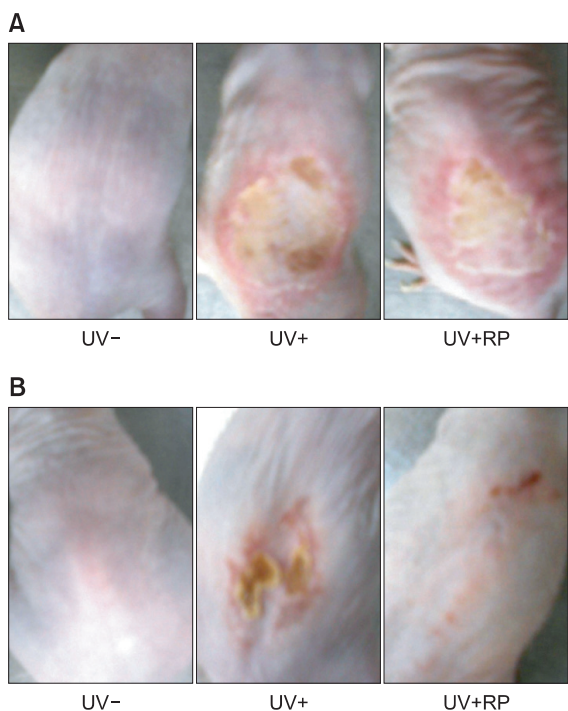


Fig. 1. Phenotypic features of a dorsal skin of mice. (A) at 1 week, UVB-irradiation and topical treatment with RP in dorsal skin of mice. (B) at 2 week, only topical treatment with RP in dorsal skin of mice.

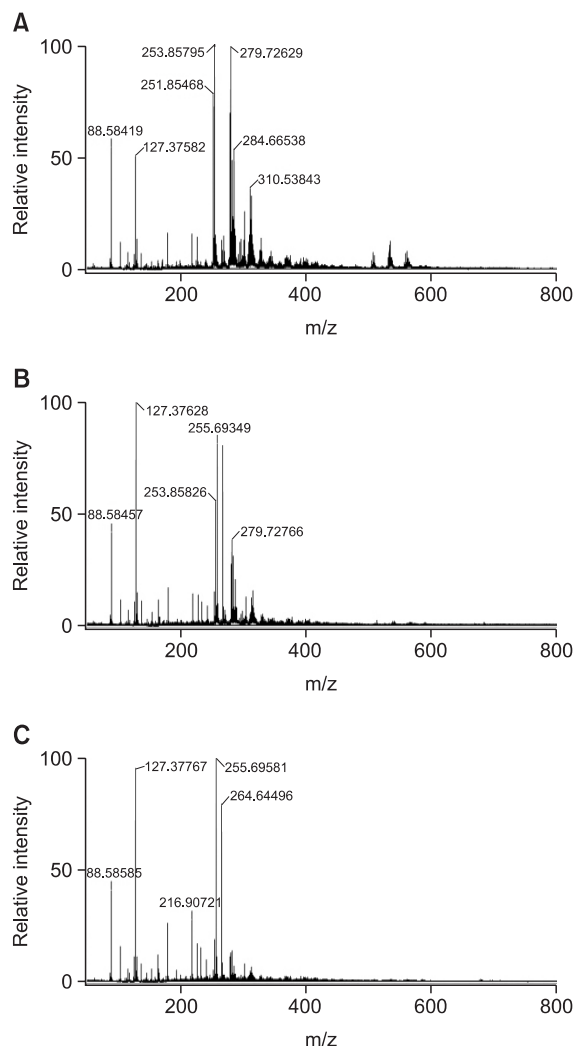


Fig. 2. Representative DART-MS spectra in the negative ion mode. (A) UV- group, (B) UV+ group, (C) UV+RP group.

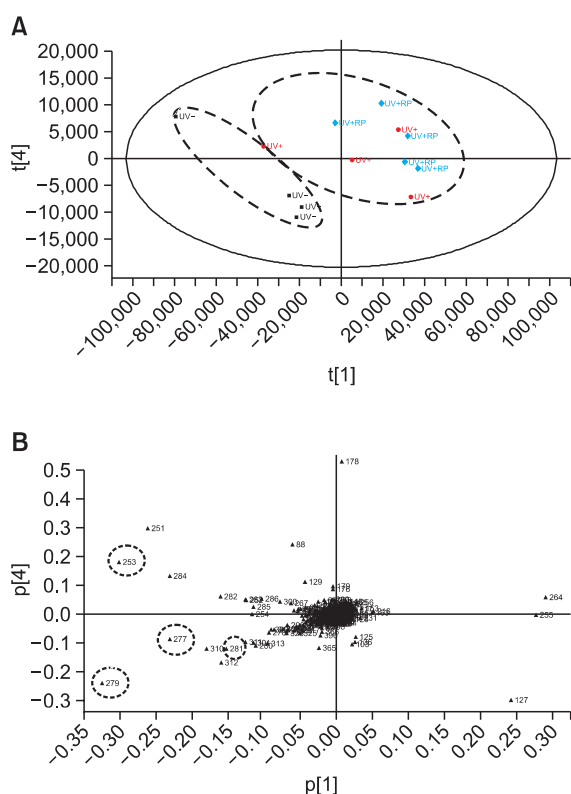


Fig. 3. PCA score plot (A) and loading plot (B) of DART-MS spectral data in the negative ion mode. Symbols represent: UV-, squares; UV+, circles; UV+RP, diamonds.

from only UV+ group. In MS-based metabolomic analysis, the large amount of x variables (usually m/z values) compared to the limited sample sizes obscured the borders between the groups. In the PCA loading plot, a few of the molecular ion peaks were dramatically separated from each other, and could be useful candidate molecules for discriminating between these groups. Palmitoleic acid, linolenic acid, linoleic acid, and oleic acid were the most prominent ion peaks from normal tissue samples. The intensities of m/z 127, 255, and 264 peaks were significantly higher in the UV+ group than in the normal tissues, but the identities of these peaks were not apparent (Fig. 3B).

Prominent significant differences between the groups were not found from the DART-MS fingerprints measured in positive mode, but the dehydrated ion peak (m/z :369, $M+H-H_2O$) of cholesterol was characterized in all three groups (Fig. 4). This demonstrated significant differences between the UV- and UVB-irradiated (UV+ and UV+RP) groups by LSD statistical analysis when the cholesterol m/z 369 peak was used as a variable. PCA demonstrated clear discrimination between UV-, UV+, and UV+RP groups (Fig. 5A). The discrimination between these three tissues was much clearer in the positive mode than in the negative mode. From loading plot of the positive mode DART-TOF-MS analysis data, an unidentified peak of m/z 329 was found to be a molecular marker peak in normal tissue samples and the dehydrated cholesterol ion peak (m/z 369) was found to be a marker ion peak in the UV+ group (Fig. 5B).

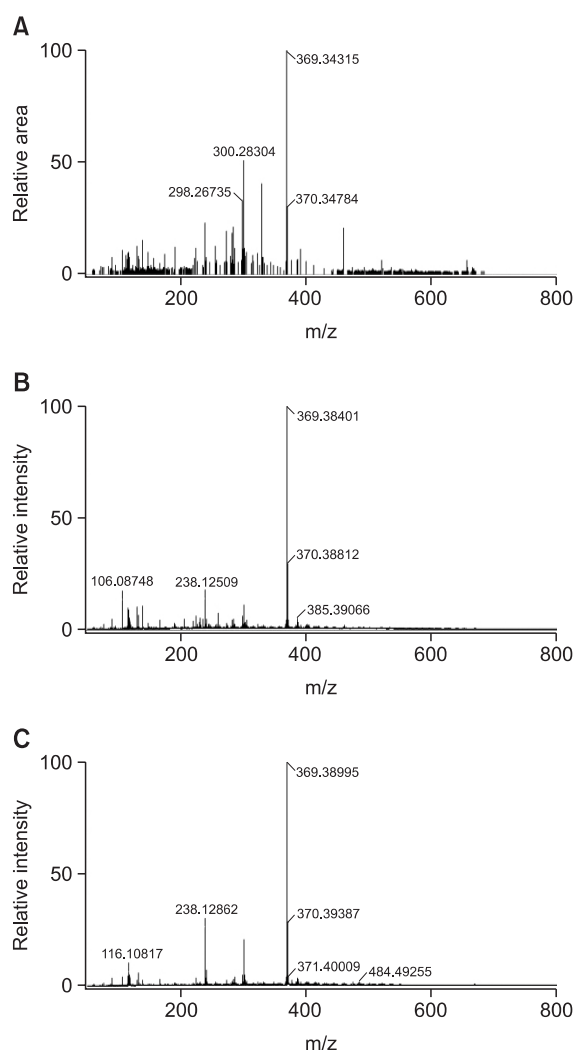


Fig. 4. Representative DART-MS spectra in the positive ion mode. (A) UV- group, (B) UV+ group, (C) UV+RP group.

DISCUSSION

UV radiation leads to several biochemical events including DNA damage, generation of oxygen radicals, and photoisomerization of urocanic acid (Picardo *et al.*, 1991; Eckhart *et al.*, 2008) that subsequently induce skin inflammation (Guahk *et al.*, 2010), photoaging (Park *et al.*, 2010) and contribute to skin cancers (Lejeune, 1986). We observed serious skin wound and eryderma in acute UVB-irradiated mice skin and further confirmed natural healing over time and the effect of RP on UVB-induced skin with the naked eye (Fig. 1). Our novel experiments demonstrated that cholesterol was the most prominent compound characterized in all three groups following UVB exposure and topical RP treatment in hairless mice skin. Moreover, characteristic ion peaks of fatty acids were successfully detected under the negative mode. The roles and functions of cholesterol and fatty acids in skin are well known. Several studies have already been carried out on acute and chronic UV-induced morphological and cellular changes, such as epidermal lipids and the free fatty acid composition of the

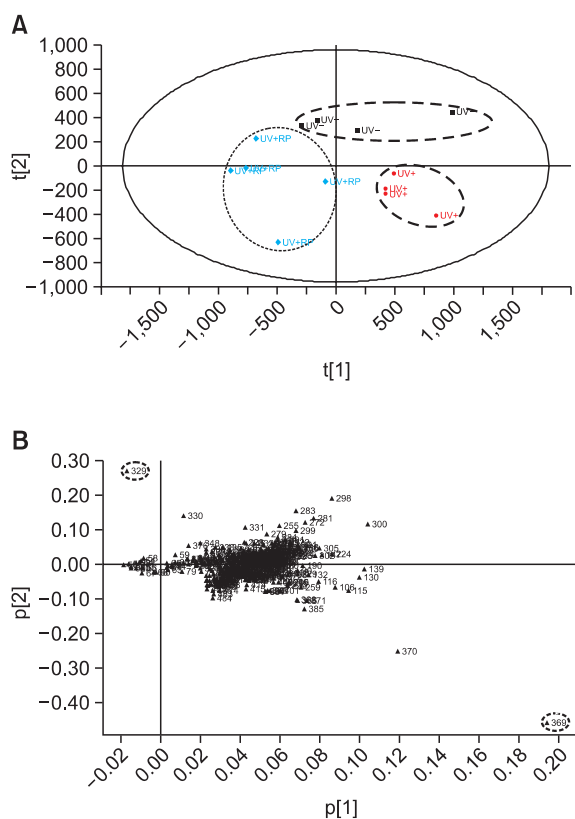


Fig. 5. PCA score plot (A) and loading plot (B) by DART-MS spectral data in the positive ion mode. Symbols represent: UV-, squares; UV+, circles; UV+RP, diamonds.

skin, especially the stratum corneum (SC) which consists of ceramides cholesterol and fatty acids (Lampe *et al.*, 1983, Kim *et al.*, 2010a).

Cholesterol is an important component in the manufacture of bile acids, steroid hormones, and vitamin D. UV radiation affects the cholesterol contents of the skin *in vivo* depending on experimental conditions, such as the dosage of UV radiation and the exposure time (Knudson *et al.*, 1939; Picardo *et al.*, 1991). And the increase of cholesterol level caused by UVB irradiation leads to aggregation and activation of apoptotic signaling pathways in human keratinocytes (George *et al.*, 2012). Moreover, epidermal synthesis of the major barrier lipid species such as cholesterol, fatty acids, and ceramides, is unchanged or reduced at 24 and 48 h, it increases significantly 72 h after exposure to a high dose of UVB (7.5 MED) indicating the delayed disruption of the permeability barrier following acute UVB exposure and the rapid recovery of the barrier (Holleran *et al.*, 1997). Given these literatures, it is not surprising that cholesterol was detected as skin metabolites in significantly different amounts among experimental groups.

Together with cholesterol, various fatty acids including palmitoleic, linolenic, linoleic and oleic acid were present in each experimental tissues. Fatty acids play an important role in the control of inflammation and are mainly composed of saturated fatty acids such as palmitic (C16:0) and stearic (C18:0) acids, monounsaturated acids such as palmitoleic (C16:1) and oleic (C18:1) acids, and polyunsaturated acids such as linoleic (C18:2) and linolenic (C18:3) acids (Lampe *et*

al., 1983; Kawamoto *et al.*, 2001; Calder, 2003; Schallreuter *et al.*, 2009). Acute and chronic UV irradiation in human skin induces significant differences of the amounts of triglycerides and total free fatty acids by decreasing lipid metabolism and lipid synthesis (Kim *et al.*, 2010b). This study showed alteration of some fatty acids suggesting that these changes of free fatty acids in UVB-exposed skin are associated with fatty acid synthesis.

Our study also focused on the possibility for the practical use of DART-MS as new analysis technique in skin metabolome. Numerous methods to qualitatively and quantitatively analyze skin tissue have been reported based on HPLC-MS, GC-MS, FTIR and NMR (Norlén *et al.*, 1998; Merle *et al.*, 2008; Robosky *et al.*, 2008; Abaffy *et al.*, 2010). The above methods usually require sample preprocessing, such as extraction, lyophilization and derivatization, and often require large amounts of sample. In contrast, DART-TOF-MS can directly analyze material surfaces without requiring preparation with wipes or solvent extraction (Kim *et al.*, 2010c) and determine rapid qualitative and/or quantitative analysis of metabolites with a very small quantity of analyst in raw, unprocessed body fluids and skin (Zhou *et al.*, 2010). Plus, considering the increasing international pressure to reduce animal numbers used in *in vivo* experiments, the successful grouping between UV- and UV+ group and the observation of significant quantitative differences in cholesterol as one of skin biomarkers in acute UVB-induced skin using DART-MS measurement are very encouraging result of the current study; this may reduce the number of animals that are needed to obtain significant results. The small size of biopsied skin tissue was sufficient to obtain full chemical fingerprints for meaningful data analysis, including multivariate statistics. By adopting multivariate analysis for high resolution data of DART-TOF-MS fingerprints, statistically significant groupings between specified experimental groups and characterizing compounds and/or signals were successfully identified. Since DART analysis mostly shows metabolome profiles on the surface of sample, the analyzed data can be significantly different from the data acquired by conventional method that require extraction of whole tissues. However, it also can be a very specific characteristic of DART-MS analysis in metabolomics studies. These methods and results may be useful in various fields, such as the cosmetic research and developmental industries, as powerful tools for the rapid identification of relevant skin metabolome changes and for determining their physiologic significance.

Concerning the metabolite changes assessed by DART-MS analytic technique in mouse skin following acute UVB irradiation, we suggest not only cholesterol and some fatty acids are potential biomarkers for the skin caused by UVB irradiation but also DART-MS has the capacity to ionize small organic molecules in living skin tissues. And DART maybe a suitable alternative analytical tool for acquiring full chemical fingerprints from living tissues in real time, without tedious sample preparation.

ACKNOWLEDGMENTS

This work was supported by a grant from the Next-Generation BioGreen 21 Program (No. PJ009511), Rural Development Administration, Republic of Korea.

REFERENCES

- Abaffy, T., Duncan, R., Riemer, D. D., Tietje, O., Elgart, G., Milikowski, C. and DeFazio, R. A. (2010) Differential volatile signatures from skin, naevi and melanoma: a novel approach to detect a pathological process. *PLoS One* **5**, e13813.
- Calder, P. C. (2003) *N*-3 polyunsaturated fatty acids and inflammation: from molecular biology to the clinic. *Lipids* **38**, 343-352.
- Chaquour, B., Bellon, G., Seite, S., Borel, J. P. and Fourtanier, A. (1997) All-trans-retinoic acid enhances collagen gene expression in irradiated and non-irradiated hairless mouse skin. *J. Photochem. Photobiol. B* **37**, 52-59.
- Chaquour, B., Seit e, S., Coutant, K., Fourtanier, A., Borel, J. P. and Bellon, G. (1995) Chronic UVB- and all-trans retinoic-acid-induced qualitative and quantitative changes in hairless mouse skin. *J. Photochem. Photobiol. B* **28**, 125-135.
- Cody, R. B., Laramee, J. A. and Durst, H. D. (2005) Versatile new ion source for the analysis of materials in open air under ambient conditions. *Anal. Chem.* **77**, 2297-2302.
- Eckhart, L., Schmidt, M., Mildner, M., Miltz, V., Abtin, A., Ballaun, C., Fischer, H., Mrass, P. and Tschachler, E. (2008) Histidase expression in human epidermal keratinocytes: regulation by differentiation status and all-trans retinoic acid. *J. Dermatol. Sci.* **50**, 209-215.
- Fisher, G. J., Datta, S., Talwar, H. S., Wang, Z., Varani, J., Kang, S. and Voorhees, J. J. (1996) Molecular basis of sun-induced premature skin ageing and retinoid antagonism. *Nature* **379**, 335-339.
- Fisher, G. J., Datta, S., Wang, Z., Li, X. Y., Quan, T., Chung, J. H., Kang, S. and Voorhees, J. J. (2000) c-Jun-dependent inhibition of cutaneous procollagen transcription following ultraviolet irradiation is reversed by all-trans retinoic acid. *J. Clin. Invest.* **106**, 663-670.
- Fu, P. P., Xia, Q., Yin, J. J., Cheng, S. H., Yan, J., Mei, N., Chen, T., Boudreau, M. D., Howard, P. C. and Wamer, W. G. (2007) Photodecomposition of vitamin A and photobiological implications for the skin. *Photochem. Photobiol.* **83**, 409-424.
- George, K. S., Elyassaki, W., Wu, Q. and Wu, S. (2012) The role of cholesterol in UV light B-induced apoptosis. *Photochem. Photobiol.* **88**, 1191-1197.
- Guahk, G. H., Ha, S. K., Jung, H. S., Kang, C., Kim, C. H., Kim, Y. B. and Kim, S. Y. (2010) *Zingiber officinale* protects HaCaT cells and C57BL/6 mice from ultraviolet B-induced inflammation. *J. Med. Food* **13**, 673-680.
- Holleran, W. M., Uchida, Y., Halkier-Sorensen, L., Haratake, A., Hara, M., Epstein, J. H. and Elias, P. M. (1997) Structural and biochemical basis for the UVB-induced alterations in epidermal barrier function. *Photodermatol. Photoimmunol. Photomed.* **13**, 117-128.
- Jackson, J. E. (1991) *A User's Guide to Principal Components*. John Wiley, New York.
- Kawamoto, S., Kita, M., Hamada, M., Aki, T., Shigeta, S., Suzuki, O. and Ono, K. (2001) Lack of effect of the abnormal fatty acid metabolism in NC/Nga mice on their atopic dermatitis. *Biosci. Biotechnol. Biochem.* **65**, 431-434.
- Kim, E. J., Kim, M. K., Jin, X. J., Oh, J. H., Kim, J. E. and Chung, J. H. (2010a) Skin aging and photoaging alter fatty acids composition, including 11,14,17-eicosatrienoic acid, in the epidermis of human skin. *J. Korean Med. Sci.* **25**, 980-983.
- Kim, E. J., Jin, X. J., Kim, Y. K., Oh, I. K., Kim, J. E., Park, C. H. and Chung, J. H. (2010b) UV decreases the synthesis of free fatty acids and triglycerides in the epidermis of human skin in vivo, contributing to development of skin photoaging. *J. Dermatol. Sci.* **57**, 19-26.
- Kim, H. J., Jee, E. H., Ahn, K. S., Choi, H. S. and Jang, Y. P. (2010c) Identification of marker compounds in herbal drugs on TLC with DART-MS. *Arch. Pharm. Res.* **33**, 1355-1359.
- Knudson, A., Sturges, S. and Bryan, W. R. (1939) Cholesterol content of skin, blood, and tumor tissue in rats irradiated with ultraviolet light. *J. Biol. Chem.* **128**, 721-727.
- Lampe, M. A., Burlingame, A. L., Whitney, J., Williams, M. L., Brown, B. E., Roitman, E. and Elias, P. M. (1983) Human stratum corneum lipids: characterization and regional variations. *J. Lipid Res.* **24**, 120-130.
- Lejeune, F. J. (1986) Epidemiology and etiology of malignant melanoma. *Biomed. Pharmacother.* **40**, 91-99.
- Merle, C., Laugel, C. and Baillet-Guffroy, A. (2008) Spectral monitoring of photoirradiated skin lipids: MS and IR approaches. *Chem. Phys. Lipids* **154**, 56-63.
- Morlock, G. and Ueda, Y. (2007) New coupling of planar chromatography with direct analysis in real time mass spectrometry. *J. Chromatogr. A* **1143**, 243-251.
- Norl en, L., Nicander, I., Lundsjo, A., Cronholm, T. and Forslind, B. (1998) A new HPLC-based method for the quantitative analysis of inner stratum corneum lipids with special reference to the free fatty acid fraction. *Arch. Dermatol. Res.* **290**, 508-516.
- Pacheco-Palencia, L. A., Noratto, G., Hingorani, L., Talcott, S. T. and Mertens-Talcott, S. U. (2008) Protective effects of standardized pomegranate (*Punicagranatum L.*) polyphenolic extract in ultraviolet-irradiated human skin fibroblasts. *J. Agric. Food Chem.* **56**, 8434-8441.
- Park, H. M., Moon, E., Kim, A. J., Kim, M. H., Lee, S., Lee, J. B., Park, Y. K., Jung, H. S., Kim, Y. B. and Kim, S. Y. (2010) Extract of *Punica granatum* inhibits skin photoaging induced by UVB irradiation. *Int. J. Dermatol.* **49**, 276-282.
- Picardo, M., Zompetta, C., De Luca, C., Cirone, M., Faggioni, A., Nazaro-Porro, M., Passi, S. and Protta, G. (1991) Role of skin surface lipids in UV-induced epidermal cell changes. *Arch. Dermatol. Res.* **283**, 191-197.
- Pierce, C. Y., Barr, J. R., Cody, R. B., Massung, R. F., Woolfitt, A. R., Moura, H., Thompson, H. A. and Fernandez, F. M. (2007) Ambient generation of fatty acid methyl ester ions from bacterial whole cells by direct analysis in real time (DART) mass spectrometry. *Chem. Commun.* 807-809.
- Robosky, L. C., Wade, K., Woolson, D., Baker, J. D., Manning, M. L., Gage, D. A. and Reily, M. D. (2008) Quantitative evaluation of sebum lipid components with nuclear magnetic resonance. *J. Lipid Res.* **49**, 686-692.
- Schallreuter, K. U., Hasse, S., Rokos, H., Chavan, B., Shalhaf, M., Spencer, J. D. and Wood, J. M. (2009) Cholesterol regulates melanogenesis in human epidermal melanocytes and melanoma cells. *Exp. Dermatol.* **18**, 680-688.
- Trygg, J., Holmes, E. and Lundstedt, T. (2007) Chemometrics in metabolomics. *J. Proteome Res.* **6**, 469-479.
- Trygg, J. and Wold, S. (2002) Orthogonal projections to latent structures (O-PLS). *J. Chemom.* **16**, 119-128.
- Tsai, Y. C., Chang, H. W., Chang, T. T., Lee, M. S., Chu, Y. T. and Hung, C. H. (2008) Effects of all-trans retinoic acid on Th1- and Th2-related chemokines production in monocytes. *Inflammation* **31**, 428-433.
- Williams, J. P., Patel, V. J., Holland, R. and Scrivens, J. H. (2006) The use of recently described ionisation techniques for the rapid analysis of some common drugs and samples of biological origin. *Rapid Commun. Mass Spectrom.* **20**, 1447-1456.
- Wold, S., Ruhe, A., Wold, H. and Dunn, W. J. III (1984) The collinearity problem in linear regression. The partial least squares (PLS) approach to generalized inverse. *SIAM J. Sci. Stat. Comput.* **5**, 735-743.
- Wold, S., Trygg, J., Berglund, A. and Antti, H. (2001) Some recent developments in PLS modeling. *Chemom. Intell. Lab. Syst.* **58**, 131-150.
- Yan, J., Wamer, W. G., Howard, P. C., Boudreau, M. D. and Fu, P. P. (2006a) Levels of retinyl palmitate and retinol in the stratum corneum, epidermis, and dermis of female SKH-1 mice topically treated with retinyl palmitate. *Toxicol. Ind. Health* **22**, 181-191.
- Yan, J., Xia, Q., Webb, P., Warbritton, A. R., Wamer, W. G., Howard, P. C., Boudreau, M. and Fu, P. P. (2006b) Levels of retinyl palmitate and retinol in stratum corneum, epidermis and dermis of SKH-1 mice. *Toxicol. Ind. Health* **22**, 103-112.
- Zhou, M., Guan, W., Walker, L. D., Mezencev, R., Benigno, B. B., Gray, A., Fern andez, F. M. and McDonald, J. F. (2010) Rapid mass spectrometric metabolic profiling of blood sera detects ovarian cancer with high accuracy. *Cancer Epidemiol. Biomarkers Prev.* **19**, 2262-2271.