ELSEVIER

Journal of Chromatography B, 695 (1997) 434–438

JOURNAL OF CHROMATOGRAPHY B

Short communication

High-performance liquid chromatographic measurement of urocanic acid isomers and their ratios in naturally light-exposed skin and naturally shielded skin

Katsumi Shibata[a,*], Yukari Nishioka[a], Teruo Kawada[b], Tohru Fushiki[b], Etsuro Sugimoto[c]

[a]*Department of Human Health Science, Faculty of Human Sciences, Osaka International University for Women, 6-21-57 Tohdacho, Moriguchi, Osaka 570, Japan*

[b]*Department of Food Science and Technology, Faculty of Agriculture, Kyoto University, Kyoto 606, Japan*

[c]*Course of Food Science and Nutrition, Department of Life Style Studies, School of Human Cultures, The University of Shiga Prefecture, Hikone 522, Japan*

Received 18 February 1997; revised 18 March 1997; accepted 18 March 1997

Abstract

We have developed methods for sampling and extraction of *trans*-urocanic acid and *cis*-urocanic acid from human skin, and subsequent high-performance liquid chromatographic measurement of these isomers. Sampling involves applying cellophane adhesive tape to the skin for 10 s. Urocanic acid isomers were completely extracted by immersing the tape in KOH solution. The HPLC column was a Tosoh ODS 80_{TS} (250×4.6 mm I.D., 7 μm average particle size) eluted with 20 m*M* potassium dihydrogenphosphate containing 1 g/l sodium heptanesulphonate (pH 3.7)–acetonitrile (93:7, v/v) at a flow-rate of 1.0 ml/min. The isomers were detected by UV absorbance at 264 nm. This technique was used to analyze the ratio of *trans*-urocanic acid/*cis*-urocanic acid on human skin at various sites on the body. It was found that the ratio was low in naturally light-exposed skin and high in naturally shielded skin. © 1997 Elsevier Science B.V.

Keywords: Urocanic acid

1. Introduction

Normal mammalian stratum corneum contains a high concentration of *trans*-urocanic acid (UCA) [1], which is synthesized from histidine by the catalytic action of histidine ammonia-lyase [2]. On exposure to UV radiation, *trans*-UCA is isomerized to *cis*-UCA [3]: *trans*-UCA is considered to act as a natural protective agent against sunburn and damage to DNA in skin [4]. *cis*-UCA which accumulates in skin after exposure to sunlight, has recently been postulated to have an adverse role in that it may be a chemical mediator of the transient immunosuppression that follows UV irradiation [5]. In fact, there is evidence to suggest that *cis*-UCA acts as a mediator of UV-induced immunosuppression [6–16]. Accordingly, the ratio of *trans*-UCA/*cis*-UCA on skin provides important information on the immune system.

Although methods for simultaneous determination of *trans*-UCA and *cis*-UCA by high-perfromance liquid chromatography (HPLC) have been reported [17–24], the procedures for sampling and extraction of UCA isomers from skin are more or less invasive

*Corresponding author.

PII S0378-4347(97)00195-3

[17–23], one of them is time-consuming [24], and none of them are readily applicable for experiments in the field. We, therefore, developed a simple procedure for sampling and extraction of the UCA isomers from human skin, followed by simultaneous HPLC analysis. Using this method, we investigated the ratio of UCA isomers in naturally light-exposed skin and naturally shielded skin.

2. Experimental

2.1. Chemicals

trans-UCA was purchased from Aldrich, (Milwaukee, WI, USA). All the other chemicals were of the highest purity obtainable from commercial sources.

2.2. Sampling and extraction method

Part of the stratum corneum was detached by applying a cellophane adhesive tape (20×18 mm wide; Nichiban, Tokyo, Japan) to skin for 10 s. UCA isomers were stable for at least 2 weeks on the sampling tape when it was stored in the dark at around 30°C. Therefore, this sampling method can be applied in the field. *trans*-UCA and *cis*-UCA on the tape were completely extracted by immersing the tape in 200 μl of 1 *M* KOH for 30 min at room temperature. The extract was neutralized with H_3PO_4 to minimize column damage. The neutralized solution was centrifuged for 3 min at 8000 *g* to remove large solid particles, the supernatant filtered through a 0.45-μm filter and injected onto the HPLC column. The procedure is summarized in Fig. 1.

2.3. Sweat

Sweat taken from a cheek was directly filtered through a 0.45-μm filter and injected onto the HPLC column.

2.4. Liquid chromatography

The HPLC system consisted of an LC-9A liquid chromatograph (Shimadzu, Kyoto, Japan), a Model 7125 syringe-loading sample injector (Rheodyne,

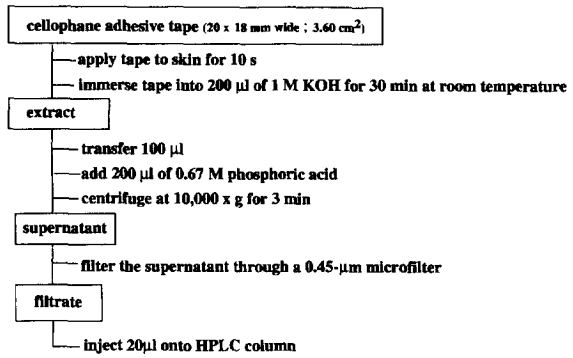

Fig. 1. Sampling and extraction procedure for urocanic acid isomers.

Cotati, CA, USA), a Tosoh ODS 80_{TS} column (250×4.6 mm I.D., average particle size 7 μm) (Tosoh, Tokyo, Japan), and an SPD-6A spectrophotometer (Shimadzu) and Chromatopac C-R6A for data processing (Shimadzu). The mobile phase was a mixture of 20 m*M* potassium dihydrogenphosphate containing 1 g/l sodium heptanesulphonate (pH was adjusted to 3.7 by addition of 14.7 *M* phosphoric acid)–acetonitrile (93:7, v/v), the flow-rate was 1.0 ml/min, the detection wavelength was 264 nm. The column temperature was maintained at 40°C with a CTO-6A column oven (Shimadzu). Under these conditions, *cis*-UCA and *trans*-UCA were eluted at about 6.5 and 8.5 min, respectively.

2.5. Subjects

Subjects were Japanese females aged between 19 and 22 years. The number of subjects was dependent on the sampling ($n=17$ to 48).

3. Results and discussion

3.1. Calibration curve and limit of detection

The concentration of standard *trans*-UCA was determined by using the molar absorptivity value of 19 000 at 264 nm in H_2O. To obtain *cis*-UCA, which is unavailable commercially, we irradiated a solution of $1.54 \cdot 10^{-4}$ *M* *trans*-UCA in H_2O with a 100 W Hanovia Xenon lamp filtered at 280 nm for 30 min. The amount of *cis*-UCA formed by this treatment

was determined as follows. A known amount of the starting solution of *trans*-UCA was injected onto the HPLC column, yielding a single peak at around 8.5 min. From this, we calculated the integrated peak area of 1 nmol of *trans*-UCA to be 560 618±5216 (mean±S.D., $n=10$). An irradiated sample was then injected onto the column, yielding the chromatogram shown in Fig. 2A. A new peak at 6.5 min, which was taken to represent *cis*-UCA, increased with irradiation time until about 30 min and remained constant thereafter. The amount of *cis*-UCA isomerized from *trans*-UCA was calculated from the difference between the amounts of *trans*-UCA before and after UV-irradiation. The integrated peak area of 1 nmol of *cis*-UCA corresponded to 504 284±4025.

Calibration curves for *cis*-UCA and *trans*-UCA were obtained by using the solution irradiated for 30 min. They were linear in the range of 2 pmol (276 pg) to 1.5 nmol per injection, with correlation coefficients of 0.999. The amount of UCA isomers

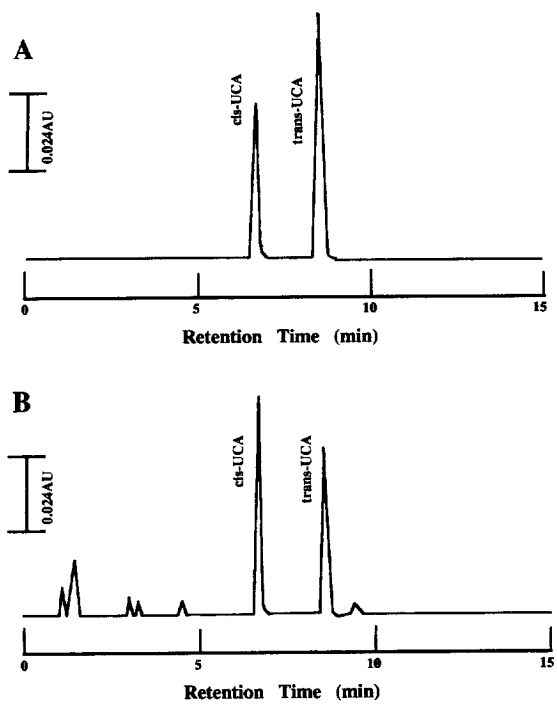

Fig. 2. Chromatograms of urocanic acid (UCA) isomers. (A) Standard *cis*-UCA (632 pmol) and *trans*-UCA (818 pmol). (B) Extract from skin on the back of the hand; sample size, 20 μl; *cis*-UCA, 985 pmol; *trans*-UCA, 625 pmol. See Section 2.4 for conditions.

were calculated from the following equations: *cis*-UCA (nmol)=(integrated peak area eluted at around 6.5 min)/504 000 and *trans*-UCA=(integrated peak area eluted around 8.5 min)/560 000.

The detection limit for each isomer was 2 pmol at a signal-to-noise ratio of 5:1. The coefficients of variation in the analyses of *cis*-UCA (41 pmol injection, $n=10$) and *trans*-UCA (82 pmol injection, $n=10$) were each within 1%. We periodically measured the standard sample contained in a polyethylene tube to determine the stability of *cis*-UCA and *trans*-UCA. It was found that the standard sample solution was stable for at least four months when it was kept in a refrigerator (4°C) or freezer (−25°C)

3.2. Extraction of cis-UCA and trans-UCA from the tape

As shown in Fig. 1, the sampling and extraction procedure is straightforward, non-invasive and takes less time than other methods [17–24]. Cellophane adhesive tape is readily available at local stationery stores, and sampling in the field is simple. The complete removal of both isomers from the tape during the 30-min extraction was confirmed by their absence in the second extraction.

A typical chromatogram of an extract from skin on the back of the hand is shown in Fig. 2B. The total analysis time required was around 15 min.

3.3. Ratio of trans-UCA/cis-UCA in naturally light-exposed skin and naturally shielded skin

The roles of *trans*-UCA as a sunscreen [4] and of *cis*-UCA as an immunodepressive mediator [5–16] have been postulated, but only one paper [25] reported on the relationship between *cis*-UCA and *trans*-UCA content at different sites on the body, presumably because a practical method for sampling of UCA isomers on the human body was not available. The method described here allows the ready determination of the *trans*-UCA/*cis*-UCA ratio on any site on the body. Thus, we compared *trans*- UCA/*cis*-UCA ratios in naturally light-exposed skin and naturally shielded skin.

Fig. 3 shows histograms of the *trans*-UCA/*cis*-UCA ratios at various sites on the human body. The

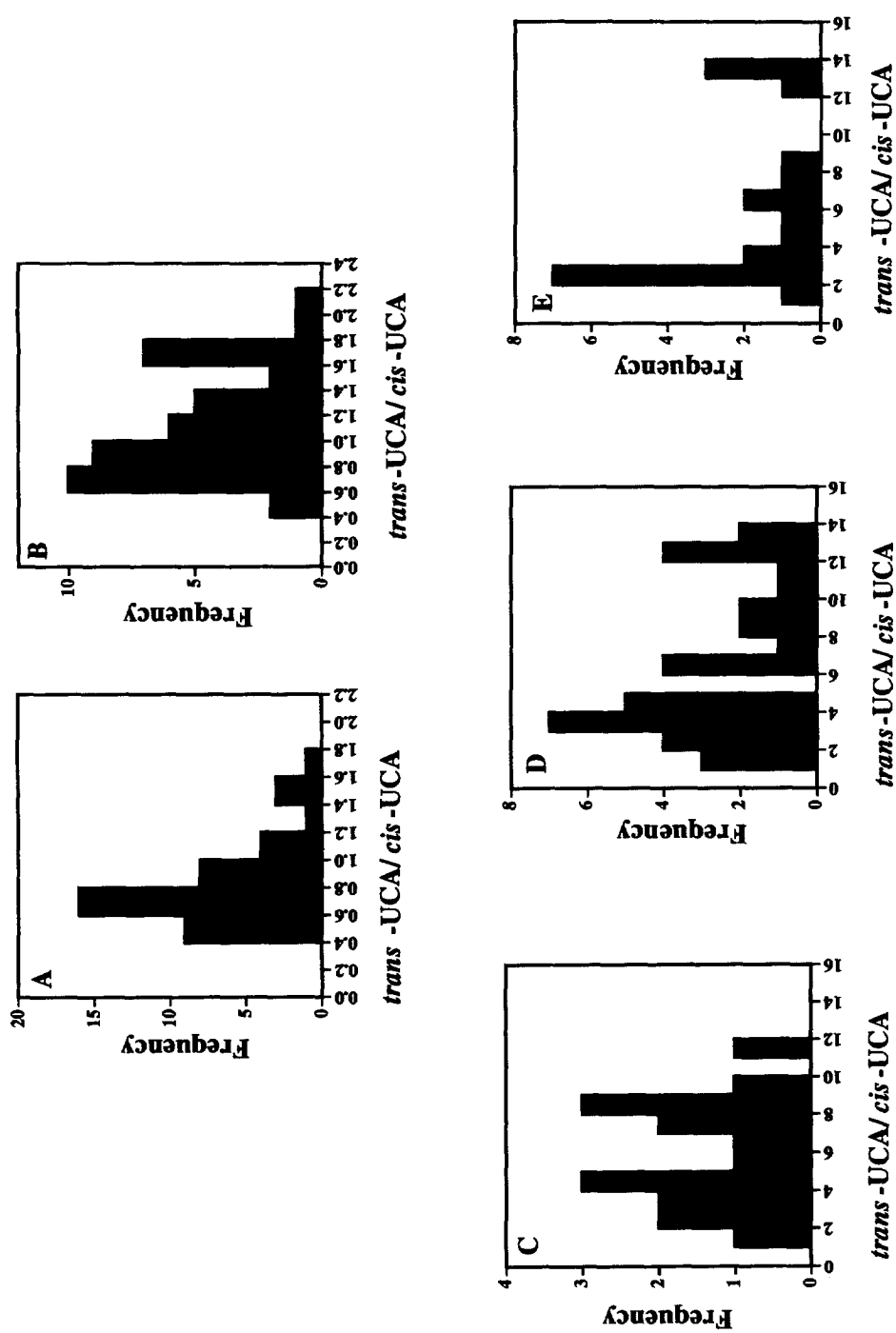

Fig. 3. Ratio of *trans*-urocanic acid (UCA)/*cis*-UCA on skin on the back of the hand ($n=42$) (A), cheek ($n=43$) (B), lower abdomen ($n=17$) (C), inner thigh ($n=36$) (D) and armpit ($n=20$) (E).

average ratios for skin on the back of the hand and the cheek, which are naturally light-exposed sites, were 0.83 and 1.14, respectively, while those on the lower abdomen, inner thigh and armpit, which are naturally shielded sites, were 5.86, 7.88 and 8.28, respectively. These results demonstrate that *cis*-UCA is mainly detected on naturally light-exposed sites. A similar result was reported by Kavanagh et al. [25], who found, however, a higher ratio for naturally light-exposed skin. This difference may be due to a racial difference between the Japanese and the British subjects who participated in these studies. Stäb et al. [26] reported that the concentration of total UCA was higher in black and Asian skin than in European Caucasian skin.

When the skin is exposed to strong sunlight, *trans*-UCA is isomerized to *cis*-UCA and the resulting *cis*-UCA is thought to cause a systemic immunosuppression [5–16]. Thus *cis*-UCA must be quickly metabolized or removed in order to maintain the normal function of the immune system. However, further metabolism of *cis*-UCA is unknown. To examine the possibility that *cis*-UCA may be removed specifically by perspiration, we measured the ratio of *trans*-UCA/*cis*-UCA in sweat on the cheek. The result is depicted in Fig. 4. The mean±S.D. of the ratio was 1.57±0.58, not statistically significant different from that of skin on the cheek, 1.14±0.46.

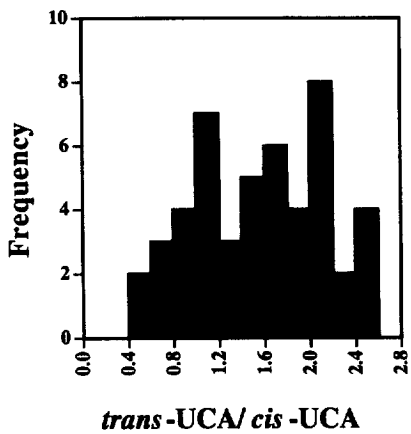

Fig. 4. Ratio of *trans*-urocanic acid (UCA)/*cis*-UCA in sweat from the cheek (n=48).

Thus, sweat dissolved not only *cis*-UCA but also *trans*-UCA.

References

[1] J. Tabachnick, Arch. Biochem. Biophys. 70 (1957) 295.
[2] I.R. Scott, Biochem. J. 194 (1981) 829.
[3] H.P. Baden, M.A. Pathak, D. Butter, Nature 210 (1966) 732.
[4] H. Morrison, Photodermatology 2 (1985) 158.
[5] E.C. De Fabo, F.P. Noonan, J. Exp. Med. 157 (1983) 84.
[6] J.A. Ross, S.E.M. Howie, M. Norval, J. Maingay, J. Invest. Dermatol. 89 (1987) 230.
[7] L. Rasanen, C.T. Jansen, T. Reunala, H. Morrison, Photodermatology 4 (1987) 182.
[8] F.P. Noonan, E.C. De Fabo, H. Morrison, J. Invest. Dermatol. 90 (1988) 92.
[9] J.A. Ross, S.E.M. Howie, M. Norval, J. Maingay, Photodermatology 5 (1988) 9.
[10] M. Norval, T.J. Simpson, E. Bardshiri, S.E.M. Howie, Photochem. Photobiol. 49 (1989) 633.
[11] E.W. Palaszynski, F.P. Noonan, E.C. De Fabo, Photochem. Photobiol. 55 (1992) 165.
[12] I. Kurimoto, J.W. Streilein, J. Immunol. 148 (1992) 3072.
[13] D.B. Yarosh, L. Alas, J.T. Kibitel, S. Ullrich, Photodermatol. Photoimmunol. Photomed. 9 (1992) 127.
[14] P.H. Hart, C.A. Jones, K.L. Jones, C.J. Watson, I. Santucci, L.K. Spencer, J.J. Finlay-Jones, J. Immunol. 150 (1993) 4514.
[15] J.W. Gilmour, J.P. Vestey, S. George, M. Norval, J. Invest. Dermatol. 101 (1993) 169.
[16] V.E. Reeve, M. Bosnic, C. Boehm-Wilcox, R.B. Cope, Am. J. Clin. Nutr. 61 (1995) 571.
[17] J.C. Caron, B. Martin, B. Shroot, J. Chromatogr. 230 (1982) 125.
[18] W. Schwarz, K. Langer, A. Haag, J. Chromatogr. 310 (1984) 188.
[19] W. Schwarz, J. Chromatogr. 342 (1985) 247.
[20] M. Norval, C.R. McIntyre, T.J. Simpson, S.E.M. Howie, E. Bardshiri, Photodermatology 5 (1988) 179.
[21] L. Vasantha, Ind. J. Med. Res. 58 (1970) 1079.
[22] S. Yokoya, E. Tokuhiro, S. Suwa, H. Maesaka, Eur. J. Pediatr. 140 (1983) 330.
[23] M. Norval, T.J. Simpson, E. Bardshiri, J. Crosby, Photodermatology 6 (1989) 142.
[24] C.T. Jansén, K. Lammintausta, P. Pasanen, K. Neuvonen, E. Varjonen, K. Kalino, P. Ayras, Acta Derm. Venereol. (Stockholm) 71 (1991) 143.
[25] G. Kavanagh, J. Grosby, M. Norval, Br. J. Dermatol. 133 (1995) 728.
[26] F. Stäb, U. Hoppe, G. Sauerman, J. Invest. Dermatol. 102 (1994) 666A.