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SHORT REPORTS

A Non-invasive Chamber Sampling Technique for HPLC Analysis of Human Epidermal Urocanic Acid Isomers

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A new, non-invasive chamber sampling technique for HPLC analysis of human epidermal urocanic acid isomers is described. Easily processed samples are obtained in a rapid and non-invasive manner, without discomfort to the patient. HPLC data from chamber samples are reproducible, and recorded urocanic acid isomer levels are comparable to those measured in samples taken with tape stripping technique. ***Key words: Finn chambers, Extraction, Urocanic acid.***

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Considerable interest has recently been focused on epidermal urocanic acid (UCA) isomers, particularly in relation to the possible immunoregulatory function of its *cis*-isomer (1). To obtain epidermal samples of human skin for UCA isomer analysis, several sampling techniques have been used, none of which are optimal for large scale clinical use. Previously used epidermal sampling techniques are more or less invasive, and include rising of suction blisters (2, 3), skin surface clipping (4, 5, 6) or tape stripping (7, 8, 9). In this paper we describe a simple, new chamber sampling technique which is suitable for repeated sampling or in the investigation of large patient populations.

MATERIAL AND METHODS

To obtain samples with varying proportions of the UCA isomers 64 atopic dermatitis (AD) patients who were starting ultraviolet (UV) phototherapies or had recently been treated with UV light, and 12 non-atopic control subjects were recruited into the study, after having obtained their informed consent.

Samples were taken on one or two occasions from each person. In the chamber sampling techniques, 6 Finn Chambers (Epikon, Helsinki, Finland) were attached with Leucoplast (Norgesplaster, Oslo, Norway) onto the left upper back. In the majority of cases the application time was 30 min. In selected cases 1, 2.5, 4, 8, 12 and 24 h samples were used. Standard filter papers were placed in each chamber, moistened with 9.0 µl of 0.100 M KOH immediately before application. The total area covered with the 6 chambers was 3 cm^2. Triplicate samples were taken and the mean (±SD) values of the 3 samples were calculated. The filter papers from the removed chambers were soaked for 24 h in a total volume of 1.0 ml of 0.100 M KOH, including also the 9.0 µl filter paper moistening volume. In preliminary experiments (data not shown), we determined that *cis*-UCA is stable in water and in buffer solutions at room temperature for over several months, whereas *trans*-UCA is more susceptible to decomposition. Therefore temperatures of −20°C or less are recommended in sample storage, and were used in this study.

In the tape stripping sample technique, an area of 6 cm^2 on the right upper back was demarcated. Five subsequent strippings were taken from that area with Tesafilm tape and the strips were soaked in 2.0 ml of 0.100 M KOH for 24 h with occasional vortexing. Three parallel tape stripping samples were always obtained and the mean UCA isomer levels (± SD) of the triplicates were calculated. For an unbiased comparison of results obtained with the two different techniques, a *cis*/*trans*-isomer ratio was calculated, to obviate the many obstacles of a direct comparison, including different size of sampling areas, differences in dilution schemes during extraction procedures, etc.

The sample liquids were kept in dark and frozen at −20°C until analysis. For HPLC analysis, samples were adjusted to pH 5 with 0.5 M H_3PO_4, filtered through 0.2 µm Gelman Acro LC filters, and 100 µl aliquots were injected. HPLC equipment consisted of a Perkin Elmer 400 solvent delivery system with a Rheodyne 7125 sample injection valve, a Perkin Elmer variable wavelength 550 UV controller fitted with a continuous flow cell, and a Perkin Elmer LCI-100 electronic integrator. We employed a 250 x 4.0 mm Lichrosorb NH_2 Hibar RT 250–4, 5 µm, separation column (Merck AG), protected by a 30 x 4.0 precolumn. The eluent was a 50/50 mixture (v/v) of acetonitrile and 0.05 M KH_2PO_4 in water (pH 5) operated at a flow rate of 1 ml/min. This method successfully resolves the *cis*- and *trans*-isomers; retention volumes were 4.2 and 6.0 ml, respectively. The UCA isomers were detected at 264 nm and peak areas were used for quantitation. The response of the UV detector was linear over the essential range (0.2 – 25 µg/ml) for both isomers (correlation coefficient 0,99973 for *trans*- and 0.99993 for *cis*-UCA). The precision of repeated

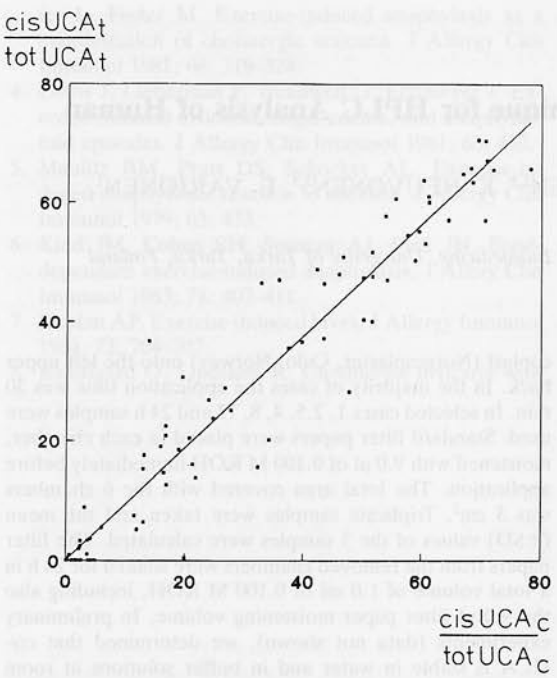

Fig. 1. Comparison of data for urocanic acid isomers in samples obtained with either the chamber method (UCA_c) or the tape strip ping method (UCA_t). Ratio of *cis*-UCA and *trans*-UCA is given as percentage.

injections (at least 5) was better than 0.1 μg/ml and the detection limit lower than 0.1 μg/ml. Linear regression analysis was employed when comparing the values obtained with the two different methods.

RESULTS

Fig. 1 compares the HPLC data for the UCA isomers in samples obtained with the chamber method (30 min occlusion) or the tape stripping method. An excellent correlation was found between the *cis*/*trans*-isomer ratios obtained with the two different methods, with a correlation coefficient of $r^2 = 0.91$ ($p < 0.001$).

In a direct comparison of results from the 30 min occlusion chamber samples, the UCA isomer levels in the chamber series were of a similar magnitude as in the samples obtained by the tape sampling method. From the 99 parallel samples of Fig. 1, a mean *trans*-UCA value of 5.20 nmol/cm^2 was calculated for the chamber samples and 5.14 nmol/cm^2 for the tape samples.

Analysis of chamber samples obtained after different occlusion times disclosed a clear-cut increase in UCA yield with increasing occlusion time, and the *trans*-UCA yield was more than tripled when the occlusion was increased from 30 min to 24 h (Fig. 2). The mean relative amount of *cis*-UCA (out of total UCA) was 25.5–25.8% in the 30-min to 2.5-h samples, 21.1–23.2% in the 4- to 12-h samples, and 16.0% in the 24-h samples. Even with 24 h occlusion, no skin irritation was caused at the chamber sites.

DISCUSSION

Of previously described sampling techniques, the tape stripping method has probably been the most employed one. An inherent advantage of the tape method is that samples from different depths of the epidermis can be analysed separately (9, 10). When used as a standard method, however, a certain amount of tape strips are taken and pooled, and the information from different layers is lost. Our study shows that a similar yield of UCA isomers is obtained with either the five-strip tape method or the 30 min chamber occlusion method. Interestingly, the total UCA amount obtained in 24 h occlusion chambers was of similar order of magnitude as the reported UCA yield from tape stripping to the full depth of epidermis (9, 10).

Although superficial, nevertheless tape stripping

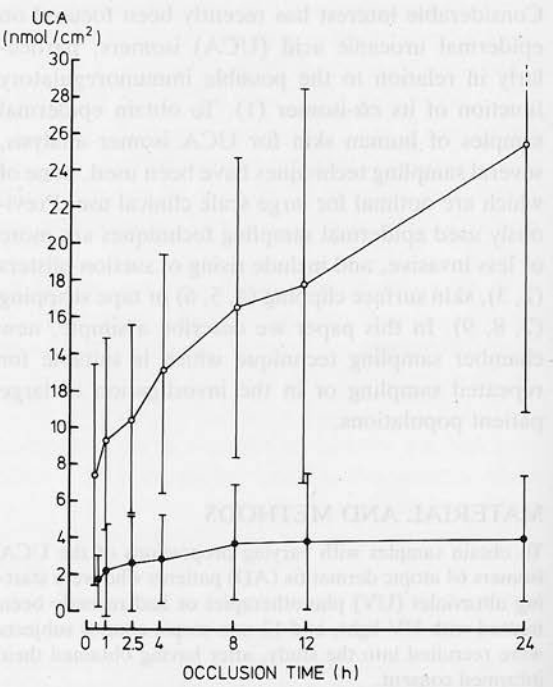

Fig. 2. Concentration of *trans*-(○) and *cis*-(●) isomer of urocanic acid in chamber samples obtained after different occlusion times.

represents an invasive procedure, and in part of our patients it elicited an unpleasant sensation, while chamber sampling caused no subjective sensations. Furthermore, the handling and processing of the tape strips was technically more difficult, and less amenable to standardisation than the handling of the chamber filter discs. In particular, the standardisation of the skin area from which samples are obtained is inherent in the chamber technique, while the area of tape application most often is subject to some strip-to-strip variation. We also found that tape stripping samples sometimes suffered from opaque contaminants, obscuring the HPLC columns after prolonged use, whereas the samples taken with the chamber technique were clear and did not cause any disturbances in the chromatograms.

Other epidermal samples used for UCA isomer analysis include suction blister roofs and clipping material (2–6). The suction blister technique is much too time consuming and discomfortable to be used e.g. on patients in an ambulatory setting. Some residual scarring and hyperpigmentation are also often unavoidable after suction blisters. The clipping method is only suitable for thick skin areas and the size of the sampling area is difficult to define. Furthermore, suction blister roofs, clipped material, and biopsy specimens all represent solid pieces of tissue material requiring some sort of grinding and further extraction before HPLC chromatography, making the procedure more tedious and open for several sources of assay error.

We conclude that the chamber sampling technique is a rapid, noninvasive, and reliable method of sampling epidermal isomers for HPLC UCA analysis. The method can easily be applied in clinical settings to investigate e.g. the levels of epidermal UCA isomers in different skin diseases and after UV exposures.

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