Cutaneous microdialysis is a new tool for direct sampling and real-time monitoring in dermatological research. However, its use in general laboratory practice poses several problems, one of which is determination of the relative recovery of the target material. Uric acid, one of the major antioxidants in the skin, was analysed using cutaneous microdialysis in 11 healthy subjects. Two methods for in vitro recovery were adopted, one in which a standard solution of uric acid was used and another in which serum was used. Although differences between the two methods were found, it is suggested that establishing the in vitro recovery using serum might be a simple approach for microdialysis.

Key words: antioxidants; in vitro recovery; microdialysis; uric acid.

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MATERIALS AND METHODS

Subjects

Eleven healthy male volunteers were enrolled in this study. They were divided into two groups according to age. Six subjects were in their 3rd decade (the young group), the others in their 8th (the aged group). None showed evidence of cardiac problems or a history of gout. All subjects provided informed consent. The Institutional Review Board, Seoul National University Hospital, approved the study conducted by the Department of Dermatology (Seoul, Korea).

Relative recovery

Relative recovery (RR) was determined in two ways. One was from a dialysate collection from a vial containing a standard uric acid solution. The other was from serum and was compared with a direct measurement of uric acid.

In vitro procedure for relative recovery: standard solution

Uric acid (2,4,6-trihydroxypurine; Sigma Chemical Co., St. Louis, USA) was used for the standard solutions in concentrations ranging from 2.5 to 20.0 μg/ml in diluted water in order to determine relative recovery. A microdialysis probe was placed in a vial containing uric acid solution with a known concentration. The standard solution was pre-warmed at 37°C on a heating block (Barnstead/Thermolyne, Iowa, USA). The microdialysis procedure was done in identical conditions (see below for details of the human study). All
procedures were performed in a light-shielded state in duplicate.

**In vitro procedure for relative recovery: human serum**

Four healthy male medical students, aged 24–27 years, were investigated. None had a history of cardiac disease or metabolic disorders, including gout. All had been checked for febrile conditions or intake of anti-inflammatory medication. Fasting venous blood samples were collected in two evacuated plain collection tubes (Beckton-Dickinson, USA) and stored in a light-shielded state for 1 h at room temperature. The total volume of blood was approximately 10–12 ml. The serum was collected from the coagulated blood by centrifugation (2,500 rpm at 4°C). Each serum sample was divided into six microcentrifuge tubes and immediately stored at −70°C. The microdialysis probe was placed in a vial containing serum. The microdialysis procedure and uric acid analysis were done under identical conditions as used for *in vitro* recovery with the standard solution and human experiments. All were performed in triplicate.

**Microdialysis procedure**

All procedures were performed in an environmentally controlled room at 26°C with 60% humidity. Three 2.5-cm-diameter circles were marked on the ventral side of the left forearm and separated at intervals of 5.0 cm. Topical anaesthetic cream, EMLA (Astra, Stockholm, Sweden), 5.0 g, was applied to the marked regions. Tegaderm (3M, Germany) was attached to the applied site for 2 h. The CMA/20 microdialysis probe, with a molecular weight cutoff of 20 Kd (CMA/Microdialysis AB, Stockholm, Sweden), was inserted into the forearm skin. The microdialysis system was perfused with isotonic normal saline at a rate of 5.0 ml/min. The tube was connected to the CMA100 microdialysis pump (CMA/Microdialysis AB) and the procedure was performed essentially as described previously (12). Dialysate samples were collected at 30-min intervals in a light-shield state and immediately stored at −70°C until analysed by high performance liquid chromatography (HPLC) analysis.

The depth of the inserted probe was evaluated by an ultrasound device (Dermascan C, Cortex Technology, Hadsund, Denmark). None experienced a febrile sensation during the examination.

**Analysis of uric acid**

The uric acid concentration in the dialysate was assayed using HPLC. Chromatographic separation was obtained with a Higgins Clipeus C18 5-μm column. A mobile phase was a mixture of monochloroacetic acid, sodium hydroxide and Na₂EDTA. Analysis was done at a flow rate of 1 ml/min. The peak detection was performed using a TOA amphorometric detector (TOA Electronics, USA).

**Statistics**

The statistical significance of the *in vitro* relative recovery of uric acid was tested with simple linear regression analysis. Student’s *t*-test and the Mann-Whitney U-test were used to analyse the uric acid concentration *in vivo*. All statistical analyses were performed using a Microsoft Office Package and SPSS.

**RESULTS**

**Relative recovery of uric acid**

Fig. 1 shows the dialysate concentrations from the standard uric acid solutions (2.5, 5.0, 10.0, 20.0 μg/ml). Linear regression demonstrated a significant correlation between the standard concentration level and the dialysate. *In vitro* recovery of uric acid using the standard solution at a perfusion rate of 5.0 μl/min was approximately 12.3 ± 2.9% for the CMA/20 catheter. Table I presents the concentration of uric acid from the sera and dialysate of four subjects. The normal uric acid level in the serum is known to be in this range (men 25–80 μg/ml, women 15–60 μg/ml) (13). *In vitro* recovery of uric acid from serum was approximately 10.1 ± 1.2%. Linear regression analysis also showed a similar linearity compared to that of the standard uric acid solution, but it was not statistically significant (Y = 0.06X + 1.81, R² = 0.867, p > 0.05).

**In vivo studies for uric acid**

Table II indicates the dialysate and estimated uric acid levels using cutaneous microdialysis for the two methods for determining the relative recovery. In the

<table>
<thead>
<tr>
<th>Table I. In vitro recovery of uric acid using sera</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subject</strong></td>
</tr>
<tr>
<td><strong>(age)</strong></td>
</tr>
<tr>
<td>1 (24)</td>
</tr>
<tr>
<td>2 (24)</td>
</tr>
<tr>
<td>3 (24)</td>
</tr>
<tr>
<td>4 (27)</td>
</tr>
<tr>
<td>Mean±SD</td>
</tr>
</tbody>
</table>

SD = standard deviation.
Methods were used for the direct testing of measuring the relative recovery (1–4, 19). Two expanding into dermatological research, one of which is adaptations have been used in dermatology (12, 14). Since Shindo and other factors, reports on the antioxidants them- selves in the skin are uncommon (14). Since Shindo et al. (9) reported the level of enzymatic and non-enzymatic antioxidants in the epidermis and dermis of human and murine skin using biopsy specimens, some data relating ageing and antioxidants have been reported (15). However, they did not present the results including ageing process supports this hypothesis. The anti-ageing process has been a major interest to many researchers, including dermatologists. Although many studies have reported an interrelation among antioxidants, UV light, chronological ageing, vitamins and other factors, reports on the antioxidants themselves in the skin are uncommon (14). Since Shindo et al. (9) reported the level of enzymatic and non-enzymatic antioxidants in the epidermis and dermis of human and murine skin using biopsy specimens, some data relating ageing and antioxidants have been reported (15). However, they did not present the results from a direct or real-time sampling.

Microdialysis is regarded as a new sampling method for overcoming some limitations (1, 2) and several adaptations have been used in dermatology (12, 16–19). However, it has certain limits for further expanding into dermatological research, one of which is measuring the relative recovery (1–4, 19). Two methods were used for the direct testing of in vitro recovery modified from the method reported by Wennberg et al. (17). We found dissimilarity in the relative recovery measured by the two methods. It is believed that such results reflect the limitation of a direct application of in vitro relative recovery. Factors considered to have an influence on the experimental results include over time sampling, the metabolic state of the volunteer and the skin temperature. For a correct relative recovery determination using human serum, the

DISCUSSION

The anti-ageing process has been a major interest to many researchers, including dermatologists. Although many studies have reported an interrelation among antioxidants, UV light, chronological ageing, vitamins and other factors, reports on the antioxidants themselves in the skin are uncommon (14). Since Shindo et al. (9) reported the level of enzymatic and non-enzymatic antioxidants in the epidermis and dermis of human and murine skin using biopsy specimens, some data relating ageing and antioxidants have been reported (15). However, they did not present the results from a direct or real-time sampling.

Microdialysis is regarded as a new sampling method for overcoming some limitations (1, 2) and several adaptations have been used in dermatology (12, 16–19). However, it has certain limits for further expanding into dermatological research, one of which is measuring the relative recovery (1–4, 19). Two methods were used for the direct testing of in vitro recovery modified from the method reported by Wennberg et al. (17). We found dissimilarity in the relative recovery measured by the two methods. It is believed that such results reflect the limitation of a direct application of in vitro relative recovery. Factors considered to have an influence on the experimental results include over time sampling, the metabolic state of the volunteer and the skin temperature. For a correct relative recovery determination using human serum, the

Table II. Microdialysis data of uric acid from 11 subjects

<table>
<thead>
<tr>
<th>Subject (age)</th>
<th>Dialysate (µg/ml)</th>
<th>Tissue conc. 1a (µg/ml)</th>
<th>Tissue conc. 2b (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (73)</td>
<td>2.1</td>
<td>16.6</td>
<td>20.3</td>
</tr>
<tr>
<td>B (70)</td>
<td>1.3</td>
<td>10.7</td>
<td>13.1</td>
</tr>
<tr>
<td>C (74)</td>
<td>1.2</td>
<td>10.0</td>
<td>12.3</td>
</tr>
<tr>
<td>D (75)</td>
<td>1.3</td>
<td>10.5</td>
<td>12.8</td>
</tr>
<tr>
<td>E (78)</td>
<td>2.4</td>
<td>19.4</td>
<td>23.7</td>
</tr>
<tr>
<td>F (29)</td>
<td>8.6</td>
<td>69.4</td>
<td>84.8</td>
</tr>
<tr>
<td>G (28)</td>
<td>3.6</td>
<td>29.4</td>
<td>36.0</td>
</tr>
<tr>
<td>H (25)</td>
<td>1.4</td>
<td>11.2</td>
<td>13.7</td>
</tr>
<tr>
<td>I (25)</td>
<td>2.8</td>
<td>22.9</td>
<td>27.9</td>
</tr>
<tr>
<td>J (30)</td>
<td>2.2</td>
<td>17.7</td>
<td>21.7</td>
</tr>
<tr>
<td>K (22)</td>
<td>2.3</td>
<td>18.9</td>
<td>23.1</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>2.7 ± 2.1</td>
<td>21.5 ± 17.0</td>
<td>26.3 ± 20.8</td>
</tr>
</tbody>
</table>

a = Estimated tissue concentration by relative recovery using standard solution.
b = Estimated tissue concentration by relative recovery using sera.

Aged group, the mean level of uric acid was lower than that in the young group (13.4 ± 4.3 vs. 28.3 ± 21.0 with RR in standard solution, or 16.4 ± 5.2 vs. 34.5 ± 25.7 with RR in sera, µg/ml). However, no statistically significant difference in the uric acid concentration was found between the two groups.

An attempt to investigate the association between uric acid and ageing was also made in this study. Although the differences in uric acid level between the young group and the aged group did not show statistical significance, the data indicated that the uric acid level was higher in the young group than in the aged group, as has been reported elsewhere (21). It is suggested that this difference should be interpreted by two mechanisms. One is the change in the constitution of the dermis with the ageing process. According to Gniadecka’s explanation for dermal echogenicity (22), the chronological or photo-ageing process might result in a build-up in dermal water content, leading to structural alterations. These changes could induce the uric acid level to decrease with age. However, Rhie et al. (21) reported that the uric acid level was similar during both the photo-ageing and the natural ageing process. Therefore, more factors that differ with a constitutional change need to be investigated if the change in uric acid concentration is to be explained. The other explanation is the balance of uric acid during restoration of the antioxidant chain reaction. Recent hypotheses and reports have proposed that a new evaluation of the chain reaction of the antioxidants mechanism above the concentration itself be done (23, 24). Uric acid is considered to fill the role of an electron sink in the antioxidant redox cycle (11). We hypothesize that the levels of uric acid, a-tocopherol and ascorbic acid interact with one another for homeostasis. It is proposed that the uniform decrease in the level of these three antioxidants during photo-ageing and the natural ageing process supports this hypothesis.

Although more studies evaluating the relative recovery determination using serum and reducing the
inter- and intra-individual variations are needed. This approach may be a short way of making cutaneous microdialysis more useful in human studies.

ACKNOWLEDGEMENTS

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REFERENCES