Determination of Uric Acid in Scalp Hair for Non-Invasive Evaluation of Uricemic Controls in Hyperuricemia

Kunio Kobayashi,∗,a Yasuhide Morokoka, Yoshitaka Isakab, and Tatsuo Tozawac

Diagnostic Science Division, Shionogi & Co., Ltd.,a 2–5–1 Mishima, Settsu 666–0022, Japan, First Department of Internal Medicine, School of Medicine, Osaka University,b 2–2 Yamada-oka, Suita 565–0871, Japan, and Department of Clinical Laboratories, Hyogo College of Medicine,c 1–1 Mukogawa-cho, Nishinomiya 663–8131, Japan.

Received September 19, 1997; accepted January 7, 1998

The uric acid concentration in blood has been widely accepted as a diagnostic indicator of hyperuricemia and gout, and its assay method is well established. In the present study, we developed a simple and rapid method for the determination of uric acid in hair, which can be obtained non-invasively. The concentration (nmol/mg hair) of uric acid extracted from 10–20 mg hair with 0.1 N potassium hydroxide was determined by an enzymatic method using uricase. The concentration of uric acid (nmol/mg hair, mean±S.D.: 0.49±0.157, n=16) in hair from hyperuricemic patients was significantly higher than that (0.26±0.107, n=8) in healthy volunteers (p<0.01). The within-run and between-day precision (CVs) of the assay was 9.6–10.3% (n=10 each) and 11.6–16.3% (n=7 each), respectively. The concentration (nmol/mg hair, y) of uric acid in hair correlated well with that in serum (mg/l, x): y=0.09x−0.12 (r=0.75, Sxy=0.122, n=23). Changes in the concentration of uric acid in the hair of antihyperuricemic drug-treated patient paralleled that in serum, suggesting that the concentration of uric acid in hair is a reliable indicator of the metabolic control in hyperuricemia.

Key words scalp hair; uric acid; uricemic control; hyperuricemia; gout

Hyperuricemia can be detected and diagnosed by measuring the concentration of uric acid in blood.3) However, a non-invasive method with easy sample collection would be more useful. It has been reported that uric acid distributes in hair and its concentration is related to the severity of gout with renal insufficiency.2) If the uric acid content of hair can be correlated with that in serum, this would offer a stable measure for the long-term control of hyperuricemia and gout. Hair samples could be collected at clinical examination instead of blood. We reported that the glycation- and cholesterol-index of hair can be applied to the clinical examination.3)–5) Moreover, hair has many advantages as a clinical sample, i.e., it can be quickly obtained at non-medical facilities and stored for a long period because of its physicochemical stability.

Conventionally, prolonged extraction with a large amount of boiling water and concentration of the extract have been required for the assay of uric acid in hair.6,7) In the present study, we developed a simple and rapid alternative. We examined the optimal conditions for the extraction of uric acid from hair and established an assay procedure. We also evaluated the correlation between the concentration of uric acid in hair and serum.

MATERIALS AND METHODS

Chemicals Uric acid, potassium hydroxide, hydrochloric acid, ethyl alcohol and other chemicals were of analytical reagent grade from Wako Pure Chemical Industries, Ltd., Osaka, Japan. Uric acid B-testwako (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and Clinimate UA reagent (Daichichi Chemical Co., Ltd., Tokyo, Japan) were used for the assay of uric acid in hair and serum, respectively.

Apparatus Absorbance was measured using a spectrophotometer (Hitachi SPECTROPHOTOMETER, model U-3210, Hitachi, Ltd., Tokyo, Japan).

Subjects Scalp hairs and/or blood were collected from healthy volunteers (age 25–51 years, n=23) and hyperuricemic patients (age 38–59 years, n=16). The blood samples were used for measurement of uric acid. Temporal hair, 10 mm from the scalp, was taken within 3 d of blood sampling for the assay of uric acid.

Measurement of Uric Acid in Hair A hair sample (10–20 mg) less than 1 mm in length was rinsed with 1 ml ethyl alcohol at 37 °C for 0.5 h. After removing the ethyl alcohol using a capillary pipet, the sample was incubated in 0.1 M potassium hydroxide (0.1 ml) at 37 °C for 2 h. The reaction mixture was neutralized with 0.2 M hydrochloric acid (50 μl). For the reagent blank, 0.1 M potassium hydroxide (0.1 ml) was incubated under the same conditions.

A standard solution of uric acid in the Uric acid B-testwako (298 μg, 50 μl) was incubated with 0.2 M potassium hydroxide (50 μl) in the same manner. After the reaction mixture was neutralized with 0.2 M hydrochloric acid (50 μl), reagent A (Uric acid B-testwako, 0.5 ml) was added, and the mixture was left standing for 5 min at room temperature (25–27 °C). Then, reagent B (Uric acid B-testwako, 1 ml) was added, and the mixture was left standing for 10 min at room temperature. The absorbance of this reaction mixture was measured at 550 nm, and the concentration (nmol/mg hair) of uric acid was calculated from the net absorbances of sample and standard solution, corrected for absorbance of the reagent blank and sample weight (mg).

Measurement of Uric Acid in Serum The concentration of uric acid in serum was measured by the method used for routine clinical examination (an automated clinical analyzer, Hitachi-736; Clinimate UA reagent).

RESULTS AND DISCUSSION

Since uric acid is an acidic compound (pK a, 5.57), alkaline solvent should be effective in extracting it from hair. However, it is unstable in a medium containing a high concentration of alkali. Thus, effective extraction of uric acid requires
careful consideration of the concentration of the alkali, pH, temperature and time of extraction. We investigated the optimal conditions for the stable extraction of uric acid from hair, and minimal assay time. Uric acid was stable (% control, 95.4—101.5%) in alkaline medium (0.001—0.1 M KOH) at room temperature and 37 °C for 2 h, and at 60 °C for 2 h (0.001—0.01 M KOH). On the other hand, at room temperature to 60 °C (0.5 h), a higher concentration of potassium hydroxide (1 M KOH) reduced uric acid by 12.4—83.9% (Fig. 1). Based on these results, medium containing 0.1 M potassium hydroxide and an incubation temperature of 37 °C were selected for the extraction of uric acid from hair.

As shown in Fig. 2, when scalp hair (ca. 10 mg) was incubated in medium containing 0.1 M potassium hydroxide at 37 °C for 1—2 h, the amount of uric acid extracted reached a maximum, then remained constant (0.5 nmol/mg hair). In medium containing 0.001 or 0.01 M potassium hydroxide, only 0.33 nmol uric acid/mg hair was extracted even after 2 h (Fig. 2A). When uric acid was repeatedly extracted from scalp hair under various conditions (1 h/extraction), the cumulative extraction of uric acid was 81% (first time), 95% (second time) and 100% (third time) with medium containing 0.1 M potassium hydroxide at 37 °C. At room temperature, the extraction rate was 59% (second time). Under other conditions, the cumulative extraction rate was less than 30%. Since uric acid usually binds to proteins such as albumin in circulating blood, a medium (0.07 M phosphate buffer, pH 7.4) containing 3% human serum albumin (HSA) was used to extract uric acid from hair. However, the extraction was not effective (less than 20%) (Fig. 2B). Consequently, it seemed optimal (95%<) to incubate the hair sample in medium containing 0.1 M potassium hydroxide at 37 °C for 2 h based on the results in Fig. 2A, B and the stability of uric acid. The method for measuring uric acid in hair was as described in the section Materials and Methods.

The concentration [0.49±0.157 (range, 0.28—0.82) nmol/
mg hair, mean ± S.D.) of uric acid in the hair from well controlled (uric acid < 6.0 mg/dl serum) or uncontrolled (uric acid > 8.0 mg/dl serum) hyperuricemic patients was significantly higher (p < 0.01) than that [0.26 ± 0.107 (range, 0.11—0.37) nmol/mg hair] in normal subjects (Fig. 3A). The present results agreed well with those reported by Bolliger and Gross [normal subjects, 5 mg/100 g (0.30 nmol/mg hair); patients with mild-severe gout, 10—14 mg/100 g (0.60—0.63 nmol/mg hair)]. The concentration (nmol/mg hair, y) of uric acid in hair correlated with that (mg/dl, x) in blood (y = 0.09x — 0.12, r = 0.75, S_y = 0.122, n = 23) (Fig. 3B).

When the hair sample weighed from 10 to 50 mg, the relationship between the amount of uric acid (nmoles) measured and the hair weight (mg) was linear and the concentration of uric acid (nmol/mg hair) was constant. When a hair sample less than 10 mg was used, there was not only a large variation, but the concentration of uric acid was low (Fig. 4). Thus, hair samples weighing more than 10 mg should be used for the assay. The within-run and between-day reproducibility (CVs) for the assay was 9.6—10.3% (n = 10 each) and 11.6—16.3% (n = 7 each), respectively (Table 1).

As shown in Fig. 5, when an antihyperuricemic drug (allopurinol, 100 mg/d) was administered every day after breakfast to a mild hyperuricemic patient (uric acid; 8 mg/dl serum; 0.61 nmol/mg hair), the concentrations of uric acid in serum and hair fell to 6.6 mg/dl, 0.52 nmol/mg hair (month 1) and 6.0 mg/dl, 0.38 nmol/mg hair (month 2), showing a good therapeutic effect. Thus, the changes in the concentrations of uric acid in hair paralleled those in serum, showing that uric acid in hair closely reflects that in blood. As human hair grows at a rate of about 10 mm per month, the concentration of uric acid in hair in the portion 10 mm from the scalp may reflect the average degree of uricemic control over the previous month.

This study shows that by measuring the concentration of uric acid in hair (10—20 mm from the scalp), taken every few months, the degree of uricemic control may be followed non-invasively. Furthermore, our method may be extended to the medical examination of other adult diseases including diabetes mellitus, hypercholesterolemia and hyperuricemia by combining it with assays developed by Kobayashi and Igimi for the glycine index and cholesterol index using the same hair sample.

REFERENCES