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A new antimicrobial quinolone (AM-1155) analysed in hair as an index of drug exposure and as a time-marker

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Abstract—Scalp hair samples were obtained at one-month intervals for up to four months after the administration from each of twelve healthy male volunteers participating in a phase I study of a new antimicrobial quinolone, AM-1155, (±)-1-cyclopropyl-6-fluoro-1, 4-dihydro-8-methoxy-7-(*cis*-3,5-dimethyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid. After hair was sectioned into 1 cm lengths from the scalp end, corresponding portions from five pieces of hair were dissolved in 1 M NaOH and assessed for AM-1155 by HPLC. In all subjects who had taken a single dose (600 mg, n = 6) or repeated doses (300 mg twice daily for 6·5 days, n = 6), the drug was detected in hair. The hair portions containing the drug were shown in most subjects to move outwards month by month at the rate of about 1 cm month^{-1}. A single hair, which was obtained from each subject of the repeated-dose study 3 months after the completion of administration, was cut into 2·5-mm lengths from the scalp side and analysed for AM-1155. The drug was shown to be contained in 4 to 6 consecutive 2·5-mm lengths, showing that there was no large axial diffusion of the drug along the hair shaft even after 3 months. These findings indicate the utility of measuring this quinolone derivative in hair as an index of drug exposure and, furthermore, as a time marker for analysing other drugs in hair.

Human scalp hair is a useful tissue for therapeutic drug monitoring as well as for forensic and toxicological analysis (Baumgartner et al 1981; Airey 1983; Suzuki et al 1984; Balbanova et al 1987). We have shown that haloperidol is excreted into hair in proportion to the given dose, and that its axial distribution along a single hair shaft reflects the dosage history over several months up to a year, depending on the hair length (Sato et al 1989; Uematsu et al 1989, 1990; Uematsu & Sato 1990; Matsuno et al 1990).

The rate of hair growth is variable both within and between subjects from about 0·5 to 2 cm month^{-1}. Moreover, hair has its own growth cycle of 2 to 8 years or more of growing stage, a few weeks of intermediate stage and a few months of resting stage. When only a few strands of hair are used for the analysis, the validity of the obtained results must be considered cautiously in relation to whether resting stage hair might have been sampled. Therefore, if there would be such a drug that could be detected in hair after a short exposure over a few or several days, and that would move outwards along the hair shaft with no large axial diffusion as the hair grows, it should serve as a time marker within hair to confirm whether the sampled hair is in the growing stage, and to estimate the growth rate of that hair itself.

We have shown that ofloxacin, a widely used antimicrobial quinolone derivative in Japan, could be detected in hair after a short exposure to it (Miyazawa et al 1991), and that the hair growth rate could be roughly evaluated from its axial distribution along the hair shaft (Uematsu et al 1991). Our studies on haloperidol and ofloxacin suggest that a drug with high affinity for melanin accumulates in hair to a high degree (Uematsu et al 1990, 1992b; Uematsu & Nakashima 1992). AM-1155, (±)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(*cis*-3,5-dimethyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid, is a new quinolone derivative currently under development in Japan. In the present study, hair samples were collected periodically from subjects who participated in a phase I study of AM-1155 and the drug in hair was analysed as an index of exposure to it. In addition, the information on the discriminative power of hair analysis was obtained with a view to using this quinolone derivative as a time marker for analysing other drugs in the hair and as a measure of hair growth rate.

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Materials and methods

Subjects. Several pieces of hair were collected at 1 month intervals for up to 4 months after the administration of AM-1155 from each of twelve healthy male volunteers who had participated in a phase I study (single-dose study; 600 mg, n = 6 or repeat-dose study; 300 mg twice daily for 6·5 days, total 3900 mg, n = 6). The ages and body weights of the subjects ranged from 20 to 44 (mean 30·6) years and from 53·4 to 78·3 (mean 63·8) kg, respectively. Hair samples were collected by cutting closest to the scalp. All subjects gave their informed consent to taking the drug and to hair sampling. The protocol was approved by a local ethics committee.

Preparation of hair samples. Five pieces of hair were sectioned together into 1 cm lengths successively from the scalp end. In a separate measurement, a single piece of hair was sectioned into 2·5 mm lengths. These hair samples were successively washed with 0·1% sodium dodecyl sulphate and distilled water, blotted between two sheets of a paper towel and allowed to dry in room air. The hairs were then weighed and dissolved in 0·5 mL 1 M NaOH by heating at 80 °C for 30 min. To this solution 0·5 mL of 1 M HCl and 0·1 mL of a solution containing AM-1587 *cis*-((±)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(*cis*-3,5-dimethyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid, 0·2 μg mL^{-1}) as internal standard were successively added. The mixture was filtered through a membrane filter (pore size 0·45 μm) and an aliquot (50 μL) of the filtrate was injected onto the HPLC.

Analytical procedures. AM-1155 was measured by a Hitachi HPLC system (L-6200, Tokyo, Japan) together with a fluorescence spectrophotometer (F-1050; excitation and emission wavelengths: 295 and 485 nm) and a system analyser (D-2000). An analytical column (TSKgel ODS-80TM; particle size 5 μm; 4·6 mm i.d., 150 mm, Tosoh, Tokyo, Japan) and guard column (TSKgel ODS-80TM; particle size 10 μm; 4·0 mm i.d. × 10 mm, Tosoh, Tokyo, Japan) were used. The mobile phase was a mixture (18:82, v/v) of acetonitrile and 0·01 M phosphoric acid. The solution was filtered through a membrane filter (pore size: 0·45 μm) and degassed before use. The temperature of the column was maintained at 40 °C by a column oven (555, GL Science, Tokyo, Japan) and the flow rate was 1·0 mL min^{-1}.

The calibration curve was linear within the range 0·25–100 ng/tube (r > 0·999) and the detection limit was less than 0·25 ng/tube. Within- and between-run precisions are described in Table 1. The recovery of AM-1155 added to blank hair samples ranged from 94–95% within the concentration range of 4·8 48·1 ng/tube.

Table 1. Within- and between-run precisions of AM-1155 in hair measured by HPLC.

Added (ng/tube)	Within-run (n = 5) Found (ng/tube)	CV (%)	Between-run (n = 4) Found (ng/tube)	CV (%)
4·8	4·5 ± 0·08	1·8	4·9 ± 0·29	5·9
14·4	13·6 ± 0·23	1·7	13·7 ± 0·10	0·7
48·1	45·7 ± 0·47	1·0	45·2 ± 1·01	2·2

Data are expressed as means ± s.d.

Results

In all 12 subjects, who had taken either a single dose (600 mg) or repeated doses (300 mg twice daily, total 3900 mg) of AM-1155, the drug was detected in all hair samples collected 1, 2, 3 and 4 months after the drug administration. Representative chromatograms are shown in Fig. 1. In most cases the drug was shown to move along the hair shaft month by month at a relatively constant rate after the administration of single (Fig. 2) and repeated doses of AM-1155 (Fig. 3). However, in some cases the distribution tended to be broader or even biphasic in the later samples, suggesting that the hairs of different stages or those with different growth rates might have been sampled together in such cases.

In six subjects of the repeated-dose study a single hair, which was obtained three months after the study, was analysed in detail. Since the hair portion containing the drug had been roughly specified from the above results, a 2- to 4-cm-long hair portion was obtained from the other single hair strand and analysed by cutting into 2·5 mm lengths successively from the scalp side. The results are shown in Fig. 4. Only 4 to 6 consecutive 2·5-mm lengths contained the drug and the hair growth rate of each single hair was calculated from the length in which the drug peaked. This was about 1·20, 1·17, 1·33, 1·00, 1·08 and 0·67 cm month^{-1} in subjects A, B, C, D, E and F of Fig. 4, respectively.

Discussion

The present study clearly showed the following. A newly developed quinolone derivative, AM-1155, was excreted into hair and could be quantitatively analysed in the hair even after a single dose of 600 mg. The hair portion containing the excreted drug moved outwards along the hair shaft month by month at different rates among different subjects, which corresponded to their own hair growth rates. There was no large axial diffusion of the drug along the hair shaft with time, showing that the discriminative power for analysing AM-1155 along the hair length was 10–15 mm after its administration over a 7-day period.

AM-1155 incorporated in hair was shown to move along the hair shaft month by month for up to 4 months after the drug administration. The speed of movement was variable among subjects in relation to hair growth rate. When five pieces of hair were analysed together for AM-1155, the distribution was shown to be broader or to have two separate peaks, especially in the later samples of some cases. Our earlier studies (Uematsu et al 1991; Uematsu & Nakashima 1992) suggest that this phenomenon might be due to the difference of growth rate or that of hair cycle stage among the pieces of hair used, and these

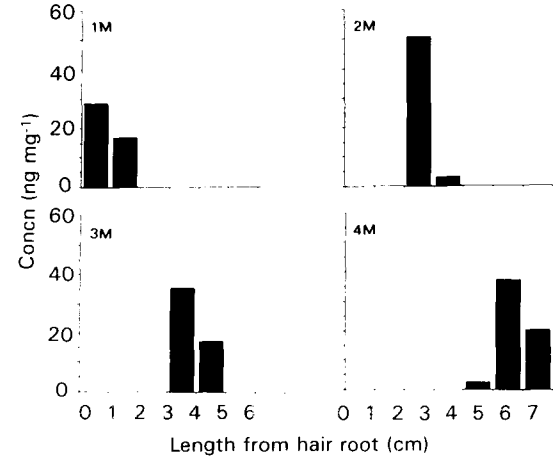

FIG. 2. Distribution of AM-1155 along the hair shafts at 1, 2, 3 and 4 months (1M–4M) after a single oral dose of 600 mg. The distribution of AM-1155 was examined cm by cm over the entire 6–7 cm length of five pieces of hair. Data from a representative subject are shown. Each column shows the concentration of AM-1155.

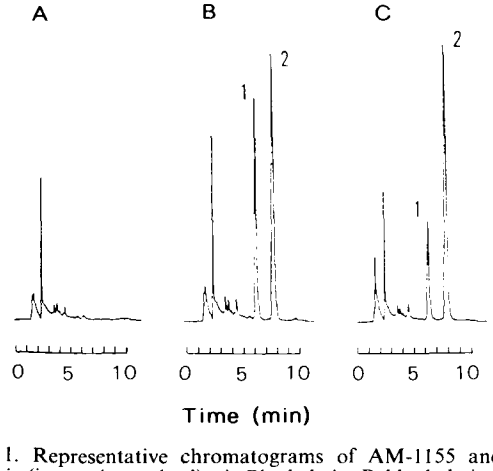

FIG. 1. Representative chromatograms of AM-1155 and AM-1587*cis* (internal standard). A Blank hair, B blank hair supplemented with AM-1155 (28·9 ng mL^{-1}, peak 1) and AM-1587*cis* (20 ng mL^{-1}, peak 2) and C hair collected at 1 month after the administration to a subject who had taken a single dose (600 mg) of AM-1155.

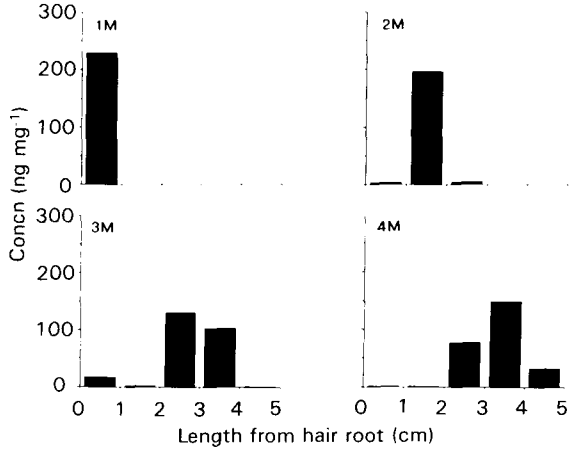

FIG. 3. Distribution of AM-1155 along the hair shafts at 1, 2, 3 and 4 months after repeated oral doses of 600 mg day^{-1}, b.i.d. for 6·5 days. The distribution of AM-1155 was examined cm by cm over the entire 5 cm length of five pieces of hair. Data from a representative subject are shown. Each column shows the concentration of AM-1155.

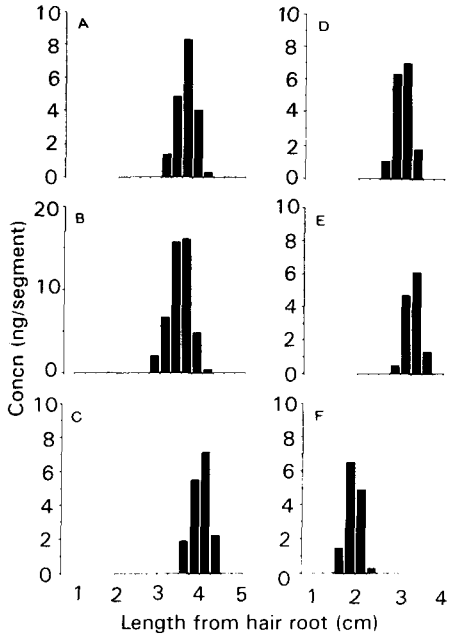

FIG. 4. Distribution of AM-1155 along a single piece of hair collected from each of six subjects (Subject A-F) 3 months after the repeated-doses of AM-1155.

differences should have become more evident in the later samples as a broader or even biphasic distribution of AM-1155 along the hair shafts.

To clarify this phenomenon, a single hair was cut into smaller portions, i.e. 2·5-mm lengths, and the distribution of AM-1155 along a single hair shaft was analysed. AM-1155 was found to be contained only in 4 to 6 consecutive sections and the distribution was always monophasic even 3 months after the drug administration over a 7-day period. The length of hair from the scalp end, in which the drug peaked, was obtained and the growth rate of a single hair could be calculated, ranging from 0·67 to 1·33 cm month^{-1}.

The route of drug disposition to hair needs to be clarified; a drug may adsorb onto the hair from the outside through sweat or sebaceous material, although it is generally accepted that drug molecules are trapped in the core of hair strands from blood (Holden 1990). However, when the concentration of ofloxacin in white hair is compared with that in black hair sampled from the same subject with grizzled hair, the former is always very much lower than the latter or even undetectable (Uematsu & Nakashima 1992; Uematsu et al 1992a, b). This phenomenon has been ascertained by an animal experiment using both albino and pigmented rats. These findings indicate that almost all of the ofloxacin measured in black hair was due to the drug trapped in the core of hair from blood because the absolute amount of drug adsorbed from the outside might be, if any, identical between both types of hair. Because AM-1155 was also undetectable in white hair (data not shown) and almost all Japanese have black hair, we analysed only black hair in the present study. Therefore, we consider, at the moment, that the still rather wide distribution of AM-1155 along a single hair shaft (Fig. 4) should be due to some uneven transport of drug from each hair bulb cell into hair and to the slower dissociation of drug tightly bound to melanin in the cell.

The possibility of using a drug as a time marker within hair has been suggested by using ofloxacin (Uematsu et al 1991). The measurement of another quinolone derivative, AM-1155, along the hair length has been shown to serve as a time marker for analysing other drugs in hair. This method could also be used to estimate the growth rate itself of a single hair because reliable and quantitative measurement of hair growth rate is essential for assessing treatments intended to increase hair growth (Barth 1986; Price & Menefee 1990).

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References

Airey, D. (1983) Total mercury concentrations in human hair from 13 countries in relation to fish consumption and location. Sci. Total Environ. 31: 157–180

Balbanova, S., Brunner, H., Nowak, R. (1987) Radioimmunological determination of cocaine in human hair. Zeitschrift Rechtmed. 98: 229–234

Barth, J. H. (1986) Measurement of hair growth. Clin. Exp. Dermatol. 11: 127–138

Baumgartner, A.M., Jones, P.F., Black, C.T. (1981) Detection of phencyclidine in hair. J. Forensic Sci. 26: 576–581

Holden, C. (1990) Hairy problems for new drug testing method. Science 249: 1099–1100

Matsuno, H., Uematsu, T., Nakashima, M. (1990) The measurement of haloperidol and reduced haloperidol in hair as an index of dosage history. Br. J. Clin. Pharmacol. 29: 187–194

Miyazawa, N., Uematsu, T., Mizuno, A., Nagashima, S., Nakashima, M. (1991) Ofloxacin in human hair determined by high-performance liquid chromatography. Forensic Sci. Int. 51: 65–77

Price, V.H., Menefee, E. (1990) Quantitative estimation of hair growth I. Androgenetic alopecia in woman: effect of minoxidil. J. Invest. Dermatol. 95: 683–687

Sato, R., Uematsu, T., Sato, R., Yamaguchi, S., Nakashima, M. (1989) Human scalp hair as evidence of individual dosage history of haloperidol: prospective study. Ther. Drug Monit. 11: 686–691

Suzuki, O., Hattori, H., Asano, M. (1984) Detection of methamphetamine and amphetamine in a single human hair by gas chromatography/chemical ionization mass spectrometry. J. Forensic Sci. 29: 611–617

Uematsu, T., Nakashima, M. (1992) Pharmacokinetic aspects of newer quinolones. In: Mitsuhashi, S. (ed.) Progress in Drug Research 38. Birkhaeuser Verlag, Basel pp 39–56

Uematsu, T., Sato, R. (1990) Human scalp hair as evidence of individual dosage history of haloperidol: longer-term follow-up study. Ther. Drug Monit. 12: 582–583

Uematsu, T., Sato, R., Suzuki, K., Yamaguchi, S., Nakashima, M. (1989) Human scalp hair as evidence of individual dosage history of haloperidol: method and retrospective study. Eur. J. Clin. Pharmacol. 37: 239–244

Uematsu, T., Sato, R., Fujimori, O., Nakashima, M. (1990) Human scalp hair as evidence of individual dosage history of haloperidol: a possible linkage of haloperidol excretion into hair with hair pigment. Arch. Dermatol. Res. 282: 120–125

Uematsu, T., Miyazawa, N., Nakashima, M. (1991) The measurement of ofloxacin in hair as an index of exposure. Eur. J. Clin. Pharmacol. 40: 581–584

Uematsu, T., Matsuno, H., Hirayama, H., Matsumoto, K., Nakashima, M. (1992a) Steady-state pharmacokinetics of haloperidol and reduced haloperidol in schizophrenic patient and analysis of factors determining their concentrations in hair. J. Pharm. Sci. 81: 1008–1011

Uematsu, T., Miyazawa, N., Okazaki, O., Nakashima, M. (1992b) Possible effect of pigment on the pharmacokinetics of ofloxacin and its excretion in hair. J. Pharm. Sci. 81: 45–48