Br. J. clin. Pharmac. (1993), **35**, 199–203

The measurement of a new antimicrobial quinolone in hair as an index of drug exposure

T. UEMATSU, M. NAKANO, H. AKIYAMA[1] & M. NAKASHIMA
Department of Pharmacology, Hamamatsu University School of Medicine, Hamamatsu 431–31 and [1]Research Center, Otsuka Pharmaceutical Co., Ltd, Tokushima 771–01, Japan

1 Scalp hair samples were obtained at 1 month intervals up to 5 months from healthy male volunteers participating in a phase I study of a new antimicrobial quinolone, OPC-17116.
2 Hair was sectioned into 1 cm lengths from the scalp end. Corresponding portions from five pieces of hair were dissolved in 1 N NaOH and assayed for OPC-17116 by h.p.l.c.
3 In all subjects taking a single dose (400 mg, $n = 5$) or repeated doses (400 mg day^{-1}, twice daily, for 6.5 days, $n = 6$), the drug was detected in the portions of hair corresponding to the administration period, assuming a hair growth rate of 1 cm/month.
4 OPC-17116 (300 mg day^{-1}, three times daily, for 2 days) was given to four healthy male volunteers, from whom hair samples were obtained at 1 month and 3 months. The drug was detected in 1 to 4 consecutive 2.5-mm long portions of a single hair and there was no significant axial diffusion of the agent along the hair shaft with time.
5 These findings indicate the utility of measuring this quinolone derivative in hair as an index of exposure, and as a time-marker for the hair analysis of other drugs.

Keywords scalp hair quinolone derivative OPC-17116 index of exposure hair growth rate time-marker in hair

Introduction

Human scalp hair is a useful tissue for therapeutic drug monitoring as well as for forensic analysis (Airey, 1983; Balabanova *et al.*, 1987; Baumgartner *et al.*, 1981; Suzuki *et al.*, 1984). We have shown that haloperidol is excreted into hair in proportion to the dose, and that its distribution along a single hair length reflects the dosage history (Matsuno *et al.*, 1990; Sato *et al.*, 1989; Uematsu & Sato, 1990; Uematsu *et al.*, 1988, 1990, 1991).

The rate of hair growth varies both within and between subjects from about 0.5 to 1.5 cm/month (Montagna & Parakkal, 1974; Saitoh *et al.*, 1967). In addition, hair has a growth cycle of 2 to 8 years or more of anagen (growing stage), a few weeks of catagen (intermediate stage) and a few months of telogen (resting stage). When only a few strands of hair are used for drug analysis, the validity of the results must be considered in relation to whether resting phase hair might have been sampled. Therefore, if a marker drug could be found that was detectable in hair after a short exposure (e.g., a few or several days) and which moves outwards along the hair shaft without axial diffusion, it could be used to confirm that the sampled hair is in the growing stage, and to estimate the growth rate of the hair.

We have shown that ofloxacin, a widely used antimicrobial quinolone in Japan, can be detected in hair after a short period of administration (Miyazawa *et al.*, 1991) and that hair growth rate can be approximated from the distribution of the drug along the hair length (Uematsu *et al.*, 1991). Our studies on haloperidol and ofloxacin suggest that a drug with high affinity for melanin should accumulate in hair to a higher degree (Uematsu *et al.*, 1990, 1992a). Many quinolone derivatives possess a high affinity for melanin (Uematsu & Nakashima, 1992) including OPC-17116, (±)-1-cyclopropyl-6-fluoro-1,4-dihydro-5-methyl-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid, which is currently under development in Japan as an anti-bacterial agent. In the present study, hair samples were collected periodically from subjects who participated in a phase I study of OPC-17116. The concentrations of OPC-17116 in hair were compared with those in plasma and information on the discriminative power of hair analysis was

Correspondence: Dr Toshihiko Uematsu, Department of Pharmacology, Hamamatsu University School of Medicine, Hamamatsu 431–31, Japan

obtained with a view to using this quinolone derivative as a time-marker for the presence of drugs in the hair shaft.

Methods

Subjects

Several pieces of hair were collected at 1 month intervals for up to 4 or 5 months after the administration of OPC-17116 from each of 11 healthy male volunteers participating in a phase I study (single-dose study; 400 mg, $n = 5$ or repeat-dose study; 400 mg day^{-1}, twice daily for 6.5 days, total 2600 mg, $n = 6$). The ages and body weights of the subjects ranged from 21 to 43 years and from 55 to 68 kg, respectively. Hair samples were also obtained from four healthy male volunteers (age: 25–38 years; body weight: 56–80 kg), who had taken OPC-17116 (100 mg, three times daily for 2 days; total 600 mg), at 1 and 3 months after administration. Hair samples were collected by cutting close 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.

Blood collection

Blood (5 ml) was collected into a heparinized tube just before and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48 and 72 h after the single administration of OPC-17116 (400 mg). Blood was collected in the same way before and at 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 12 h after the 1st administration, before and at 1, 2, 3, 4, 8 and 12 h after the 7th administration and before and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48, 72 and 96 h after the 13th (last) administration in the repeat-dose study. The plasma was separated immediately and stored at −20° C until assayed.

Plasma drug concentrations were measured in each subject and C_{max} and AUC values were calculated.

Preparation of hair samples

Five pieces of hair were sectioned together into 1 cm lengths, successively from the scalp end. In some cases, a single or five pieces of hair were sectioned into 2.5-mm lengths. These hair samples were washed with distilled water several times, blotted between two sheets of a paper towel and allowed to dry in room air. The hairs were then weighed, cut into pieces of about 2 mm or less and dissolved in 1 ml 1 N NaOH by heating at 80° C for 30 min. To this solution 0.5 ml 2 N HCl solution, 1 ml 0.5 M phosphate buffer (pH 7.0), 0.01 ml methanol containing 40 ng OPC-17203 (1-cyclo-propyl-6,8-difluoro-1,4-dihydro-5-ethyl-7-(4-methyl-1-piperazinyl)-4-oxoquinoline-3-carboxylic acid, 4 μg ml^{-1}) as internal standard and 5 ml chloroform were added successively and the mixture was agitated for 10 min in a shaker. The tube was centrifuged at 1700 *g* for 10 min and the organic layer was removed and evaporated under a stream of nitrogen at 40° C in a water bath. The residue was dissolved in 0.1 ml h.p.l.c. mobile phase and aliquots (0.025 ml) were injected onto the h.pl.c.

Analytical procedures

OPC-17116 in human hair and plasma was measured by h.p.l.c. using a system composed of a pump (Waters Model 510, Nihon Millipore, Tokyo, Japan), a fluorescence spectrophotometer (RF-535, Shimadzu, Kyoto, Japan; Excitation and emission wavelengths: 285 and 448 nm), an auto-sampler (WISP 710B, Nihon Millipore, Tokyo, Japan) and a recorder (Chromatopac C-R3A, Shimadzu, Kyoto, Japan). A TSKgel ODS-80TM analytical column (150X4.6 mm i.d., Tosoh, Tokyo, Japan) was used. The mobile phase was a mixture (27:73) of acetonitrile and 0.1% (v/v) H_3PO_4 in 20 mM solution of Na_2SO_4. The solution was filtered through a membrane filter (pore size: 0.45 μm) and degassed before use. The h.p.l.c. system was operated at ambient temperature and the flow rate was 0.8 ml min^{-1}.

The calibration curve was linear within the range 5-200 ng ml^{-1} and the coefficient of variation of the assay at OPC-17116 concentrations of 5-200 ng ml^{-1} was 0.8–5.4%. The detection limit was less than 1 ng ml^{-1}.

Results

In all five subjects who had taken a single dose (400 mg) of OPC-17116 the drug was detected in hair. The drug moved at a relatively constant rate along the hair in four subjects (Figure 1 upper panel) but moved rather slowly and irregularly in one subject (Figure 1 lower panel). However, the sum of the drug concentrations in hair, which was expressed as the largest sum of the concentrations found in any set of measurements, was similar in different subjects (Table 1). The rate of drug movement along the hair shaft varied from approximately 0.5 to 1.2 cm/month. In all six subjects who had taken repeated doses (total 2600 mg) of OPC-17116 over 6.5 days, the drug was also detected in hair. The month-by-month movement along 1 cm long segments is shown in Figure 2. The rate of movement, determined in hair collected 3 months after drug administration (3M in Figure 2), was less variable (0.8 to 1.3 cm/month) than that found in the single-dose study. The sum of the drug concentrations, expressed as the largest sum of the concentrations of OPC-17116 found in any set of measurements, was 81 ± 12 ng mg^{-1} (mean ± s.d.) and 295 ± 38 ng mg^{-1} after the administrations of single 400 mg and cumulative total 2600 mg doses, respectively. The amount of drug excreted into hair increased by a factor of about 3.7 relative to the dose ratio of 6.5. The AUC ratio for plasma drug concentration after single dose relative to a dosage interval on repeat dose was about 4.1. The maximum concentrations of drug in hair after single and repeated doses, estimated as the highest drug concentration in a 1 cm length of hair observed in any set of measurements, were 74 ± 8 ng mg^{-1} and 210 ± 38 μg mg^{-1}, respectively, and the ratio was 2.9 (Table 1).

When hair was sectioned successively into 2.5 mm lengths and OPC-17116 was measured in corresponding lengths collected from 5 pieces of hair per subject, the drug was found to distribute widely along the hair shafts (Figure 3 upper panel). However, when only a single hair was used, the drug was detected in only 1 to 4

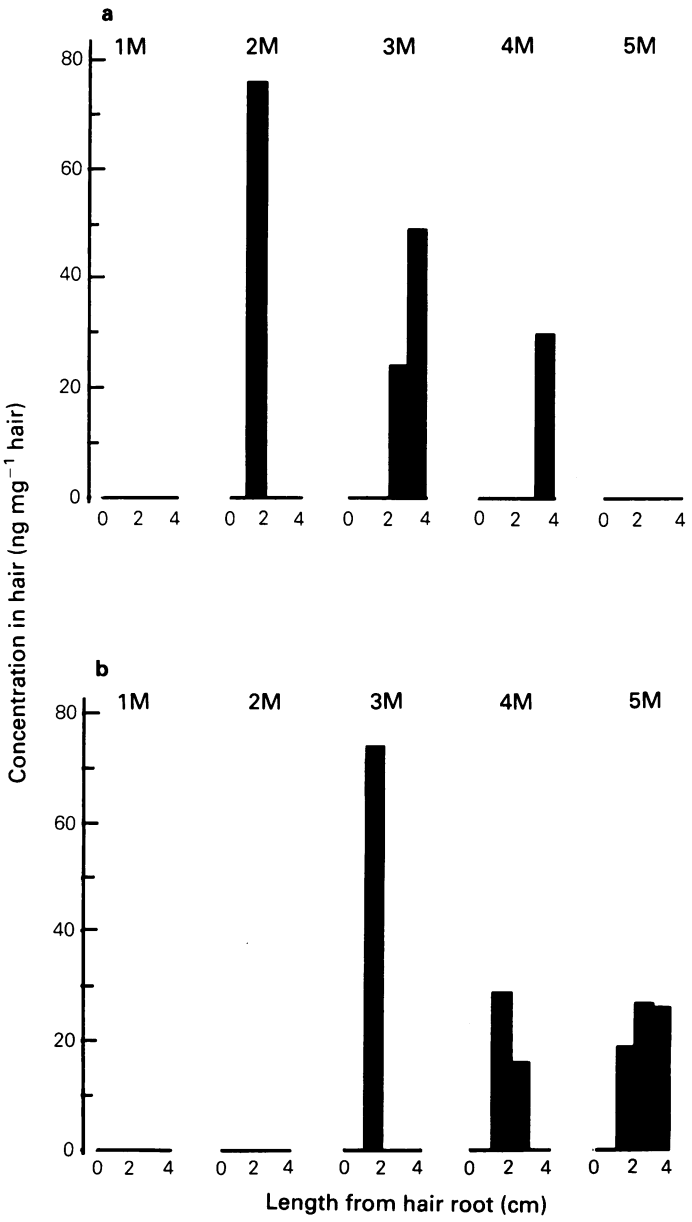

Figure 1 Distribution of OPC-17116 along the hair shaft at 1, 2, 3, 4 and 5 months (1M–5M) after a single oral dose of 400 mg OPC-17116.
Five pieces of hair were sectioned together into 1 cm lengths successively from the scalp ends and the concentration of OPC-17116 in each corresponding 1 cm length was measured. The distribution of OPC-17116 was examined cm by cm over the entire 4 cm length of hair.
Data from two representative subjects (upper and lower panels) are shown.

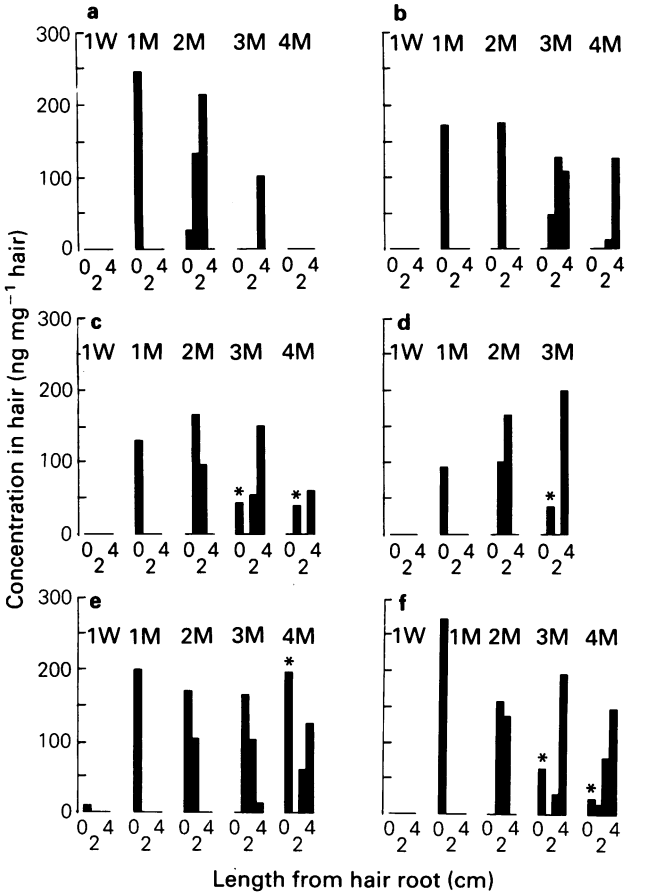

Figure 2 Distribution of OPC-17116 along the hair shaft at 1 week (1W), 1, 2, 3 and 4 months (1M–4M) after administration of repeated oral doses of OPC-17116 over a 6.5-day period (total 2600 mg, $n = 6$ subjects).
Four of the subjects took OPC-17116 on other occasions. Thus, the drug was also detected at the hair lengths denoted by *, corresponding to single oral administration of 200 mg at 2 months and 11 days (Subjects c, d and f) or to repeated doses (300 mg day^{-1} for 6.5 days) at 2 months and 5 days after the repeat-dose study (Subject e).

consecutive portions at both 1 and 3 months after administration (Figure 3 lower panel).

Discussion

The results show that OPC-17116 can be measured in hair after a single oral dose of 400 mg or a total oral dose of 600 mg over a 2-day period, that it moves along the hair shaft month by month at different rates in different subjects, and that there is little axial diffusion of the drug along the hair shaft with time. Thus the discriminating hair length for analyzing OPC-17116 administered over a 1- to 2-day period is 2.5 to 10 mm.

Concentrations of haloperidol found in human hair are in proportion to the daily dose and plasma AUC (Matsuno *et al.*, 1990; Uematsu *et al.*, 1988, 1992b). In contrast, the concentration of ofloxacin in rat hair was correlated more closely with plasma C_{max} than dose or AUC partly because ofloxacin exhibits non-linear pharmocokinetics in rats (Uematsu *et al.*, 1992a). From this finding, we suggest that ofloxacin which has a high affinity for melanin, might be incorporated into hair bulb cells in proportion to maximum blood drug concentration. In the present study the amount of OPC-17116 excreted into hair was not proportional to the total dose nor to plasma C_{max} but to plasma AUC when comparing the results from single- and repeat-dose studies (Table 1). The administration period was different between both treatment groups of the present study and this might be an additional variable influencing the amount of drug incorporated in hair bulb cells. The difference in elimination half-life between OPC-17116 and ofloxacin

Table 1 Concentrations of OPC-17116 in hair and plasma after the oral administration of a single 400 mg dose and repeated 200 mg (b.i.d.) doses over 6.5 days. In the repeat-dose study the C_{max} and AUC values refer to each of the 1st, 7th and 13th (last) administrations

I. *Single-dose study (400 mg)*

	Hair		Plasma			
		C_{max}++	C_{max}	AUC (μg ml^{-1} h)		
Subject	*Sum*+	(ng mg^{-1})	(μg ml^{-1})	*0–12 h*	*0–24 h*	*0–48 h*
1	79	79	1.79	13.0	19.8	25.2
2	71	58	1.76	10.4	14.6	18.1
3	76	76	1.86	14.0	20.8	23.7
4	74	74	2.02	13.3	19.5	24.3
5	103	81	2.14	15.4	23.1	30.2
Mean	81	74	1.91	13.1	19.6	24.3
± s.d.	12	8	0.14	1.6	2.8	3.9

II. *Multiple-dose study (200 mg, twice daily for 6.5 days; total 2600 mg)*

	Hair		Plasma								
			1st dose		7th dose		13th dose				
		C_{max}++	C_{max}	AUC	C_{max}	AUC	C_{max}	AUC (μg ml^{-1} h)			
Subject	*Sum*+	(ng mg^{-1})	(μg ml^{-1})	*0–12 h*	(μg ml^{-1})	*0–12 h*	(μg ml^{-1})	*0–12 h*	*0–24 h*	*0–48 h*	*Total*+++
6	283	200	0.82	4.61	0.91	8.04	1.11	9.94	15.05	19.42	95.32
7	285	176	0.50	3.18	0.85	7.34	0.98	9.06	13.58	16.98	80.10
8	267	200	0.66	3.66	1.91	8.35	1.32	8.79	12.59	15.47	87.53
9	297	273	0.80	5.59	1.49	12.20	1.81	13.39	20.17	26.21	132.95
10	262	166	0.42	2.93	0.86	6.27	0.88	6.74	9.47	11.42	66.62
11	375	243	0.62	5.03	1.30	11.85	1.70	14.42	21.59	28.25	129.53
Mean	295	210	0.64	4.17	1.22	9.01	1.30	10.39	15.41	19.63	98.68
± s.d.	38	38	0.14	0.98	0.39	2.23	0.35	2.68	4.23	5.91	24.61

+ Sum: the largest sum of the concentrations found in any set of hair measurements
++ C_{max} the highest concentration in a 1-cm long portion in any set of hair measurements
+++ AUC total = AUC (0,12 h) (1st dose) × 6 + AUC (0,12 h) (7th dose) × 6 + AUC (0,48 h) (13th dose).

(about 11 h *vs* 5 h; Uematsu & Nakashima, 1992) may have some influence on the ability of hair bulb cells to concentrate the drug.

OPC-17116 incorporated in hair was shown to move along the hair shaft month by month up to 5 months after drug administration. The speed of movement was variable among subjects, ranging from about 0.5 to 1.3 cm/month, in relation to hair growth rate. In each subject of the repeat-dose study the drug moved at a relatively constant rate along the hair. However, in one subject in the single-dose study (Figure 1 lower panel) the earlier samples might have been of hair in the resting stage, and the growth rate calculated at 5 months after drug administration was relatively low. In any event, the hair growth rate could be deduced from the distribution of OPC-17116 along the hair shaft given the interval between drug ingestion and hair sampling. This has also been pointed out using ofloxacin (Uematsu *et al.*, 1991). The measurement of a quinolone derivative along the hair length could serve as a time-marker for analyzing other drug(s) in hair. It could also be used to estimate hair growth rate. Reliable and quantitative measurement of hair growth rate is essential for assessing treatments intended to increase hair growth (Barth, 1986: Price & Menefee, 1990).

When a single hair was sectioned into smaller portions (2.5 mm lengths), OPC-17116 was found to be restricted to 1 to 4 consecutive segments, i.e., 2.5–10.0 mm lengths, even at 3 months after drug administration over a 2-day period. In addition, the drug peaked only in one to two 2.5 mm lengths and, therefore, the discriminative power of hair analysis as a time-marker of dosage is considered to be good. When five pieces of hair were sectioned together into 2.5 mm lengths, OPC-17116 was shown to distribute more broadly along the hair length. This probably reflects varied growth rates among hair samples.

In the case of haloperidol, which is usually taken by psychiatric patients for a long period of time, an abrupt change of daily dose was shown to cause a corresponding change in drug concentration in hair. The hair length reflecting such a change of dose was shown to move outwards along the hair shaft month by month (Matsuno *et al.*, 1990; Sato *et al.*, 1989; Uematsu *et al.*, 1990). Therefore, hair growth rate is expected to be estimated by the distribution of haloperidol concentration along the hair shaft only if dosage is changed. Haloperidol, however, is only used in a limited population with mental disorders. In contrast, antimicrobial quinolones are administered intermittently and repeatedly for short periods of time for the treatment of infectious diseases independent of the original disease or in otherwise healthy people. Therefore, hair analysis of a quinolone derivative seems superior to that of haloperidol for estimating hair growth rate.

In conclusion, the growth rate of individual hairs could be estimated by analyzing the distribution of OPC-17116 along the hair shaft. Because there was little axial diffusion of the drug along the hair shaft, a precise measurement of hair growth seems possible several months after drug ingestion. Also, the distribution of the drug along the hair shaft may serve as a time-marker for therapeutic monitoring of other drugs.

This work was supported by a Grant-in-Aid for Scientific Research 04671410 from the Ministry of Education, Science and Culture in Japan.

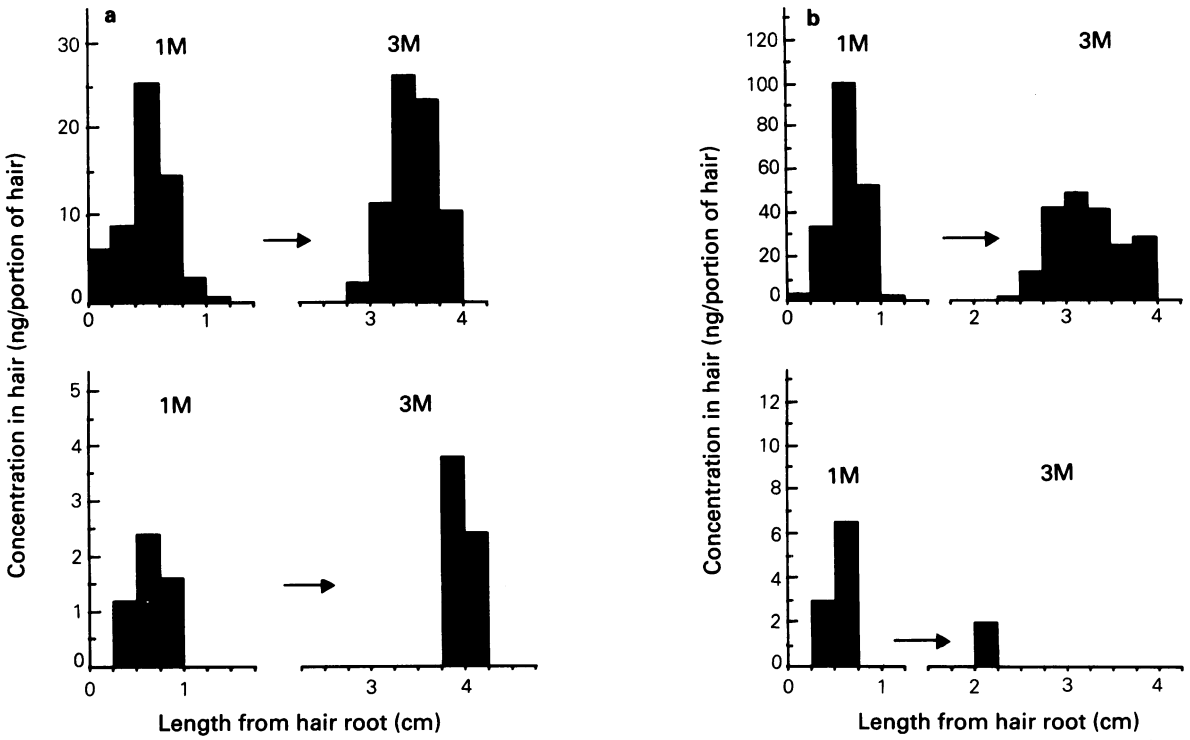

Figure 3 Change in the distribution of OPC-17116 along the hair shaft between 1 (1M) and 3 months (3M) after administration of 600 mg OPC-17113 over a 2-day period.
a) Five pieces of hair were cut into 2.5-m lengths successively from the scalp ends. OPC-17116 are measured in each hair portion.
b) A single piece of hair from the same subject was cut into 2.5 mm lengths successively from the scalp end.
Data from two representative subjects a) and b) are shown.

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. *Z. 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.

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. Pharmac.*, **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.

Montagna, W. & Parakkal, P. F. (1974). *The structure and function of skin*, 3rd edition, pp. 83–105. New York: Academic Press.

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.

Saitoh, M., Uzuka, M., Sakamoto, M. & Kobori, T. (1967). Rate of hair growth. In *Advances in biology of skin* (vol. 5), eds Montagna, W. & Dobson, R. L. New York: Pergamon.

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., Matsuno, H., Hirayama, H., Matsumoto, K. & Nakashima, M. (1992b). Steady-state pharmacokinetics of haloperidol and reduced haloperidol in schizophrenic patient and analysis of factors determining their concentrations in hair. *J. pharm. Sci.*, (in press).

Uematsu, T., Miyazawa, N. & Nakashima, M. (1991). The measurement of ofloxacin in hair as an index of exposure. *Eur. J. clin. Pharmac.*, **40**, 581–584.

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

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

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., 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., 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. Pharmac.*, **37**, 239–244.

(Received 5 February 1992, accepted 14 July 1992)