

Sequential Concentration of Chloroquine in Human Hair Correlates with Ingested Dose and Duration of Therapy*

U. RUNNE¹, F. R. OCHSENDORF¹, K. SCHMIDT² and H.-W. RAUDONAT²

¹Center of Dermatology and Venereology and ²Center of Forensic Medicine University Hospital of the J. W. Goethe-Universitaet, Frankfurt/Main, Germany

Human scalp hair was analyzed for chloroquine using gaschromatography. In 5 patients it was demonstrated that the amount of uptake of chloroquine into the hair varied proportionally with the dosage (from 500 mg/week to 10 g single dose) and with the time of administration. The chloroquine concentrations ranged from 8 to 1100 µg/g hair. Chloroquine could be determined quantitatively after a single toxic dosage used in a suicidal attempt and also after low therapeutic doses. The sequential examination of the hair shaft allows an assessment of the chloroquine amount taken over time, the individual dosage, the initiation and termination of therapy. As hairs can be collected easily, they are a unique specimen for investigation, and it is suggested that they can virtually be used as a "tachogram" of chloroquine drug-therapy or intoxication. **Key word:** Gaschromatography.

(Accepted March 2, 1992.)

Acta Derm Venereol (Stockh) 1992; 72: 352-357.

U. Runne, Zentrum Dermatologie u. Venerologie, Klinikum d. J. W. Goethe-Universitaet, Theodor-Stern-Kai 7, D - 6000 Frankfurt/M 70, Germany.

Human hair is a unique specimen for the detection of metals, addictive narcotics and drugs. Since incorporated substances can be detected retrospectively, even after months or years, hair is a valuable tool for forensic medicine. This holds true for metals such as arsenic, thallium and lead, which can already be detected in the picogram range, and, in recent years, also for organic substances such as therapeutic and narcotic drugs (1).

Chloroquine can be detected in hair, not only in therapeutic doses, but also after overdosage in white hair (2, 3). Previous studies have examined total hair in full length. The objective of this study was to determine whether the sequential analysis of hair allows conclusions as to the doses taken and the time of treatment. For this purpose we examined 5 patients with different models of chloroquine-intake.

MATERIAL AND METHODS

Patients and dosage

Five patients with five different models of chloroquine intake were studied (2 males, 3 females; single chloroquine dose between 70 mg and 10 g). None of them had evidence of pathological hair loss.

Patient 1: Continuous dosage; 23 years, female, chronic discoid lupus erythematosus (CDLE), chloroquine therapy for 90 days, daily dosage 250 mg/day.

Patient 2: Intermittent weekly low dose for malaria prophylaxis; 29 years, male, healthy, malaria prophylaxis, chloroquine therapy for 60 days, 500 mg once weekly corresponding to 70 mg/day.

Patient 3: Long term therapy with varying dosage; 69 years, female, CDLE, chloroquine for 270 days; the first 120 days 125 mg/day were prescribed, the next 150 days 250 mg/day. After confronting the patient with the results of the chloroquine concentration in her hair, the patient admitted to having actually taken only 60 mg/day for 120 days, 100 mg/day for the next 30 days and 125 mg/day for the last 120 days.

Patient 4: Intermittent therapy; 23 years, male, CDLE, chloroquine for 244 days; initially 500 mg/day for 51 days, no therapy for 176 days, 17 days 500 mg/day.

Patient 5: Suicidal attempt; 24 years, female, healthy, single dose of 10 g chloroquine 1 year before taking the hair.

Method

The examinations were made on patients 1-4 still under therapy, in patient 5, 1 year after a single overdose. About 50 occipital hairs were epilated (patients 2-5); on patient 1 they were cut just over the scalp's skin. After partitioning them into segments of 1 cm length, 45 single samples were analyzed.

The specimens were weighed, then dissolved with 1.0 ml of 15% potassium hydroxide in a waterbath for a maximum of 2 min. The samples were immediately cooled, diluted with 4 ml aqua dest., and extracted, twice each, with diethylether and subsequently a mixture of dichloromethane and ether (70:30;v/v). The extracts were analyzed by gaschromatography with a nitrogen specific detector after separation on a packed glass column, filled with 5% SE 30 on chromosorb W-AW DMCS, 80-100 mesh (gaschromatograph: Hewlett-Packard, type 5880; gas: helium 20 ml/min; temperature-programme: 180°C 1 min isotherm, increase-rate 10°C/min, end-temperature 280°C). The quantitative analyses were repeated at least twice. The lower detection limit was 1-2 ng absolutely. As we examined specimens from clinical patients, the results could not be corrected in respect to yield.

To confirm the results of gaschromatography, single extracts were analyzed by gaschromatography/mass spectrometry with a GC-MS

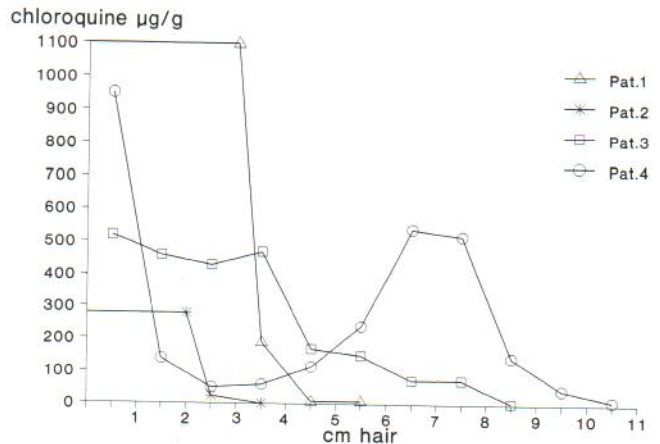


Fig. 1. Results of hair analyses in patients 1-4, for dosages see "Material and Methods".

* Abstract: Arch Dermatol Res 281 (1989) 121.

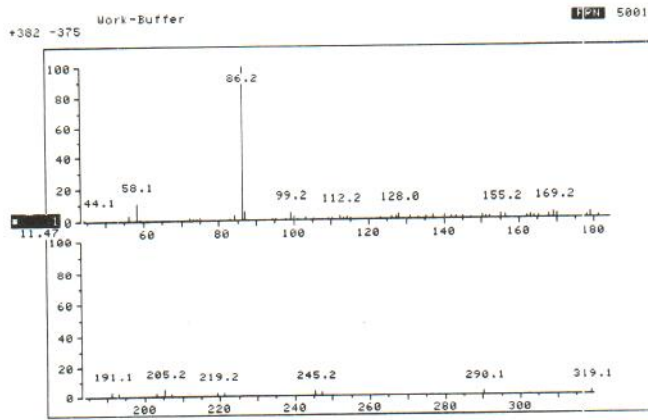


Fig. 2. Mass spectrogram of the hair sample of patient 2 (500 mg chloroquine/week).

device Hewlett-Packard type 5985 B. The separation column was a WCOT fused silica column (0.53 mm), type CP-Sil-5 CB. The specimen was delivered without split, and the separation took place with a temperature-programme (140/12/270°C) and helium gas (10 ml/min).

In each instance we compared the spectrum found with the spectrum of pure chloroquine in the reference spectrum library.

RESULTS

The results of the quantitative chloroquine determinations are presented in Figs. 1–3.

Patient 1 (Fig. 1): In the first 3 cm the chloroquine concentration was 1100 µg/g hair, in the 4th cm 190 µg/g hair. By mass spectrometry, the substance detected by gaschromatography could definitely be assigned to chloroquine (Fig. 2).

Patient 2 (Fig. 1): The chloroquine concentration was about 280 µg/g hair, i.e. lower by a factor of 3.6 according to the lower dose of intake.

Patient 3 (Fig. 1): The concentrations found initially did not correspond to the prescribed dosages. Especially the enormously delayed increase was inexplicable. Intensive questioning revealed that the patient had taken half the recommended dosage on her own account (see patient and dosage). The chloroquine concentrations in the hair correlated with the actual doses taken.

Patient 4 (Fig. 1): The curve shows a prompt rise at the beginning of therapy and a slow decline after the therapy ended, lasting for over 6 months. After reinstatement of chloroquine an accelerated increase of the chloroquine concentration in hair was observed.

Patient 5 (Fig. 3): The hair analysis showed a chloroquine maximum between 12–13 cm, and a slow decline in the following 4 cm.

DISCUSSION

Scalp hair is a unique specimen for investigation. Due to the short generation time of the matrix cells of only 23 h (4), the

high growing fraction and the continuous hair-production, circulating substances are incorporated into the hair-shaft. These remain in the shaft – in contrast to blood and urine – over months and years. In this respect, hair is not only in its structure a kind of tachogram (5), more importantly it is a *pharmacological and toxicological tachogram*.

Since samples of hair can be taken at any time, stored without problems and examined at any time, they are of experimental, toxicological and forensic importance. Especially by sequential examination of single hair sections, it is possible to assess the intake of the substance retrospectively.

Chloroquine is incorporated into and preserved in hair. The aim of our study was not to compare absolute chloroquine concentrations in serum and hair but to investigate the relations of different concentrations of chloroquine in hair to different models of intake. The following characteristics were found:

1. Even low chloroquine dosages, e.g. typical malaria-prophylactic doses (500 mg once a week), can be detected in hair (Fig. 1).
2. Higher chloroquine dosages lead to higher concentrations in hair (Fig. 1).
3. A characteristic time course was found: at the beginning of therapy there was a continuous rise; after the end of therapy, however, a protracted decline occurred. This characteristic of the chloroquine concentration in hair correlates with the pharmacokinetics of the substance and its tendency to be stored in deep compartments (Fig. 3).
4. The chloroquine concentration incorporated in hair correlates with the temporal course of the chloroquine dosage ingested (Fig. 1).
5. Even after 1 year a single chloroquine dosage can be detected and placed in time (Fig. 3).

The chloroquine concentration in hair apparently depends on the serum concentration over the time. The concentrations found correspond to the pharmacokinetics of chloroquine. Initially, deep compartments are slowly filled. Due to a long half-life of 6–33 days, a steady state is not reached before 3

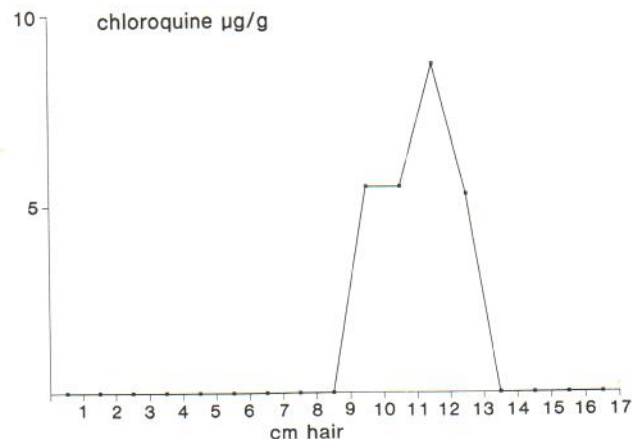


Fig. 3. Results of hair analysis in patient 5 (10 g chloroquine in an attempted suicide 1 year ago; the hair was analyzed in segments of 1 cm).

weeks, i.e. not before 3 to 4 half-lives. After withdrawal of the drug, the serum concentration declines very slowly, i.e. over months (Fig. 1), due to the accumulation in deep compartments (distribution volume 116–880 l/kg bodyweight) (6–10). After reinstatement of the drug the concentration in serum and hair rises much faster, as the deep compartments are still filled to a large extent (Fig. 1).

After a single application of a toxic dose chloroquine is incorporated into hair and can reliably be detected after 1 year (Fig. 3). The concentration in hair, however, is rather low. This is the result of a fast and successful therapeutic intervention, leading to extremely low serum concentrations. The position of the chloroquine maximum, found 1 year after intake of the drug, corresponds to the growth rate of human hair of about 0.36 mm/d. The incorporation phase, which lasts 4 months, shows that chloroquine is accumulated in deep compartments even after a single intake, from where it is released over several months.

The sequential examination of scalp hair allows retrospective conclusions concerning the initiation, duration and dose of chloroquine intake. As the chloroquine concentration measured over time depends on the pharmacokinetic profile of the substance, hair can serve as a semi-quantitative pharmacotoxicological indicator for chloroquine. Chloroquine serum concentrations can be monitored retrospectively in hair by highly specific and sensitive analytical methods. Thus hair analysis gives retrospective evidence for unreliable intake, overdose in attempted suicide or poisoning.

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