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Determination of Chloroquine and Monodesethylchloroquine in Hair

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ABSTRACT; Using thin-layer and gas chromatography and mass spectrometry, chloroquine and its major metabolite (monodesethylchloroquine) were identified in hair samples of numerous patients who received this antimalarial drug for several months. In two patients the amounts of chloroquine were, respectively, 310 and 145 mg/kg hair and those of the monodesethylchloroquine 23 and 11 mg/kg. The respective proportions (93 and 7%) are the same in the two subjects. The chloroquine percentage was near those in the spleen or stomach wall after poisoning. Other metabolites in hair are being identified. Hair analysis may provide a good toxicologic and forensic science complement to the blood, urine, and tissues. It may be useful for the control of chloroquine therapy.

KEYWORDS: toxicology, chloroquine, therapeutic control, hair, monodesethylchloroquine

Distribution of chloroquine in human tissues was studied, especially in cases of fatal poisonings. Generally the chloroquine levels decrease in the following order: liver and spleen, stomach wall, kidney, lung, heart, muscle, pancreas, and brain [*1*]. Numerous studies were made on animals. In 1975, Fletcher et al [*2*], after intramuscular administration of 10 mg/kg per body weight to rhesus monkeys, found chloroquine and its monodesethylated metabolite in urine, liver, and kidney; one carboxylic acid metabolite was also present in the urine, but only in trace amounts. Previously, McChesney et al [*3*], 96 h following intramuscular injection to the rhesus monkey of a single dose of ^{14}C-chloroquine (4 mg/kg per body weight), determined radioactivity in various tissues; in "skin-hair," not separately considered (estimated as 12% of body weight), they found a mean of 1.3 μg/g (expressed as the chloroquine equivalent), but distribution between skin and hair was not indicated. According to our knowledge, only these authors pointed out the presence of chloroquine in animal "skin-hair." No investigation of this kind was made in humans.

The presence of various substances in hair, even their accumulation, was particularly noted for use in the diagnosis of possible exposures or poisonings. Baumgartner et al found morphine

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in hair of mice treated with this alkaloid and in hair of heroin users [*4*], and phencyclidine in hair of users of this drug [*5*]. Klug [*6*] confirmed the existence of morphine in hair of chronic opiate users. Barbiturates were found in skin and hair of guinea pigs one month after the administration of these compounds [*7*] and in hair of a patient on long-term phenobarbital therapy [*8*]. Harrison et al [*9*] showed the presence of radioactivity in the hair of guinea pigs following administration of ^{14}C-labeled amphetamine and dopamine. Witherspoon and Trapani [*10*] and Maugh [*11*] also remind of possible accumulation of trace elements in hair at concentrations that are often ten times higher than the blood or urine content; hair analysis may provide a continuous record of exposure to "heavy metals." The same authors otherwise emphasize the possible relation between the hair concentrations of metals and some diseases.

Our own investigations were carried out on numerous human hair samples from patients on chloroquine treatment for several months. We report here the first results obtained with various chromatographic techniques and mass spectrometry (MS).

Experimental Procedure

Reagents and Standards

All reagents were analytical grade. The detergent RBS 25 was from F.L.I. (Fourniture pour Laboratoires et Industries, Vitrolles, France). Diethylether and 1,2-dichloroethane were purified by Solvants, Documentation, Synthèses (Peypin, France).

Other chemicals and solvents were of the highest purity available commercially: potassium hydroxide, ammonia 20%, methanol, isoamyl alcohol, ethylacetate, and isopropyl alcohol.

To make the iodoplatinate spray-reagent, 97 mL of distilled water and 100 mL of potassium iodide 6% solution are added to 3 mL of platinum chloride 10% solution; before use the reagent is diluted with two volumes of 1*N* hydrochloric acid.

Standard solutions of chloroquine sulfate (100 and 10 μg/mL of chloroquine base) and of papaverine (internal standard) (200 and 20 μg/mL of papaverine base) were prepared in methanol. They remain stable for at least 15 days when stored in a refrigerator at +4°C.

Instruments

Thin-Layer Chromatography—Glass plates (20 by 20 cm) with silica-gel G (thickness 0.25 mm) were used. Developed in the mixture were:

ethyl acetate, 79 mL,
isopropyl alcohol, 15 mL, and
20% ammonia, 6 mL.

Gas Chromatography—The techniques used were as previously described [*12*]. The method employs a nitrogen-selective detector and papaverine as internal standard. Under the analytical conditions, chloroquine and papaverine have retention times of 3 min 50 s and 13 min 10 s, respectively.

Gas Ghromatography/Mass Spectrometry—A Hewlett-Packard 5985 mass spectrometer was used. Parameters were: electron energy, 145 eV; emission, 300 μA; source temperature, 150°C; pressure, 133 Pa (1 torr); and reactant gas, methane.

The gas chromatography (GC) parameters were: capillary column methyl-silicone (12 m by 0.2 mm inner diameter); carrier gas: helium, column pressure 50 kPa (0.5 bar); programmation temperature 190 to 220°C (15°/min); and solid injector, temperature 250°C.

Identification Procedure

A hair sample was washed with RBS that had been diluted tenfold, rinsed with distilled water, and dried on air flow at room temperature. Fifty to one hundred milligrams were placed

into a centrifuge tube with 2 mL of 60% potassium hydroxide solution. The tube was maintained for 5 to 10 min in boiling water until dissolution of the sample. After cooling, 3 mL of distilled water was added. Two 5-mL extractions were made with diethylether. The ether phases were combined and evaporated under nitrogen and vacuum. The residue was dissolved into 100 μL of methanol. After shaking, the methanolic solution (100 μL) was applied on a thin-layer plate, beside a standard of chloroquine. After elution with the above-mentioned solvent, the plate was examined on ultraviolet light at 350 nm. The fluorescent spots were located and the corresponding silica zones were removed. The silica samples were placed into tubes and shaken twice with 3 mL of methanol. After centrifuging, the supernatent solutions were removed and concentrated to a small volume and assayed by gas chromatography/mass spectrometry (GC/MS).

A second hair sample (50 to 100 mg) was treated the same way. The final extract was assayed by thin-layer chromatography (TLC) beside a standard of chloroquine. After visualization by an ultraviolet light, zones were developed with iodoplatinate reagent.

Determination Procedure

A third hair sample (50 to 100 mg), washed with diluted RBS, rinsed and dried, was assayed by the GC technique previously described [*12*]. The internal standard (100 μL of the 200 μg/mL papaverine solution) was added after potassium hydroxide hydrolysis, cooling, and dilution with 3 mL of distilled water.

We took into consideration the possible interference of one of the chloroquine metabolites and the possible low levels encountered in the samples. For these reasons, instead of lowering the column temperature, which would not be sufficient for better resolution of two peaks, we developed the following procedure. The total (chloroquine + monodesethyl metabolite) was first determined by GC [*12*] using 2 to 3 μL of the final extract obtained into 100 μL of the 1,2-dichloroethane-isoamyl alcohol (9:1) mixture, then the residual solution was submitted to TLC for separating chloroquine from its metabolites. The spots were located by ultraviolet light and the silica zones corresponding to chloroquine and to its major metabolite were scraped and separately eluted with methanol. Papaverine (internal standard) has the same R_f as chloroquine and it was present in the same eluate. After centrifuging, the methanolic extracts were evaporated under nitrogen and vacuum. The residue containing chloroquine, and also papaverine, was dissolved into 100 μL of methanol, and assayed by GC to determining the chloroquine ratio in the total "chloroquine + metabolite." The monodesethyl metabolite ratio was obtained by measuring the difference and then confirmed by GC following another addition of internal standard (100 μL of the 20 μg/mL papaverine solution) to the corresponding extract, evaporating and dissolving the residue into 100 μL of methanol.

Results

Hair samples from 20 patients on long-term chloroquine therapy were analyzed. They all contained chloroquine and its metabolites. Equivalent hair samples from a drug free individual were treated as above and observed as negative controls.

The TLC of the hair extracts showed by ultraviolet light seven spots whose R_f values were: 0.09, 0.16, 0.27, 0.34, 0.42, 0.59, and 0.86. Three of these spots had a yellow fluorescence (R_f 0.09, 0.27, and 0.42). The four others had a bluish fluorescence. Two were quantitatively the most important: R_f 0.59 and 0.86. The later had the same R_f (0.86) as the spot of chloroquine standard. Figure 1 was obtained after use of the iodoplatinate reagent. Similarities existed between the hair and urinary extracts from the same patient.[3] Five spots (R_f 0.09, 0.16, 0.34,

[3]The urinary extract was obtained by the same way as the hair extract but without hydrolysis.

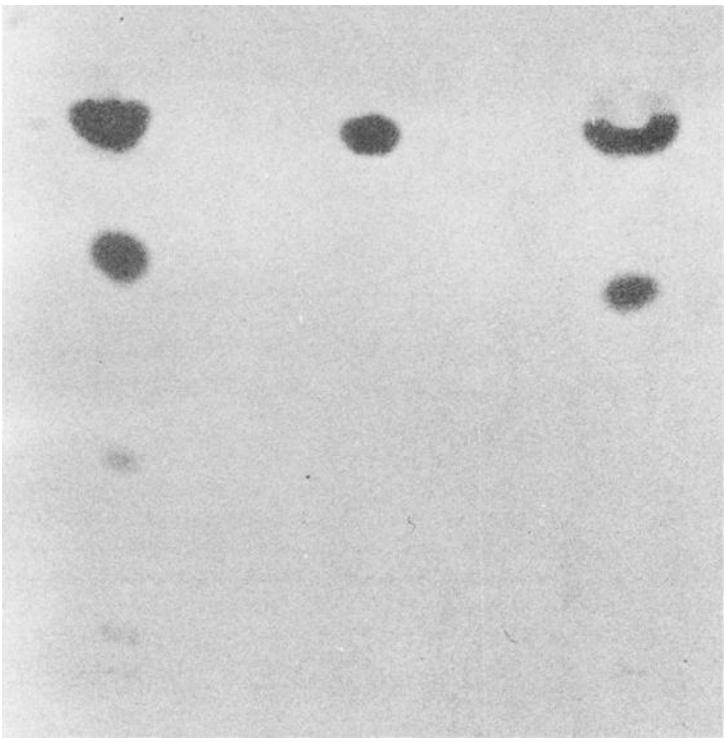

FIG. 1—*Thin-layer separation of biological extracts obtained from a patient on chloroquine therapy (iodoplatinate spraying reagent). Left to right: urine. chloroquine standard, and hair.*

0.59, and 0.86) were developed with iodoplatinate by the urinary extract, only three (R_f 0.09, 0.59, and 0.86) by the hair extract.

MS studies were carried out on the two most important compounds. In the chemical ionization mode (with methane reagent gas, whose ionization becomes H^+, $C_2H_5^+$, and $C_3H_5^+$), the mass spectrum of the first substance (R_f 0.86) was that of chloroquine itself (molecular weight 319); it showed four distinct ions (Fig. 2): $(M + H)^+ = 320$ (relative intensity 100%), $(M + 29)^+ = 348$, $(M + 41)^+ = 360$, and $(MH + 2)^+ = 322$ (relative intensity 31%). The mass spectrum of the second compound (R_f 0.59) (Fig. 2) agreed with the monodesethyl metabolite of chloroquine (molecular weight 291); its four distinct ions were: $(M + H)^+ = 292$ (relative intensity 100%), $(M + 29)^+ = 320$, $(M + 41)^+ = 332$, and $(MH + 2)^+ = 294$ (relative intensity 31%). Other metabolites in hair are in the process of being identified.

Hair locks of two patients were sampled as entire lengths from several areas of the head and assayed according to the described procedure. The quantitative data are summarized in Table 1. The total amount (chloroquine + monodesethyl metabolite) was different for each patient, but the respective proportions of chloroquine and its metabolite (93 and 7%) were the same for each subject.

Discussion

The first results in this study show the quantitative importance of chloroquine and its metabolites in hair of patients who chronically received the antimalarial drug. Chloroquine and its major metabolite (monodesethylchloroquine) were identified and we are continuing the characterizations of the other metabolites.

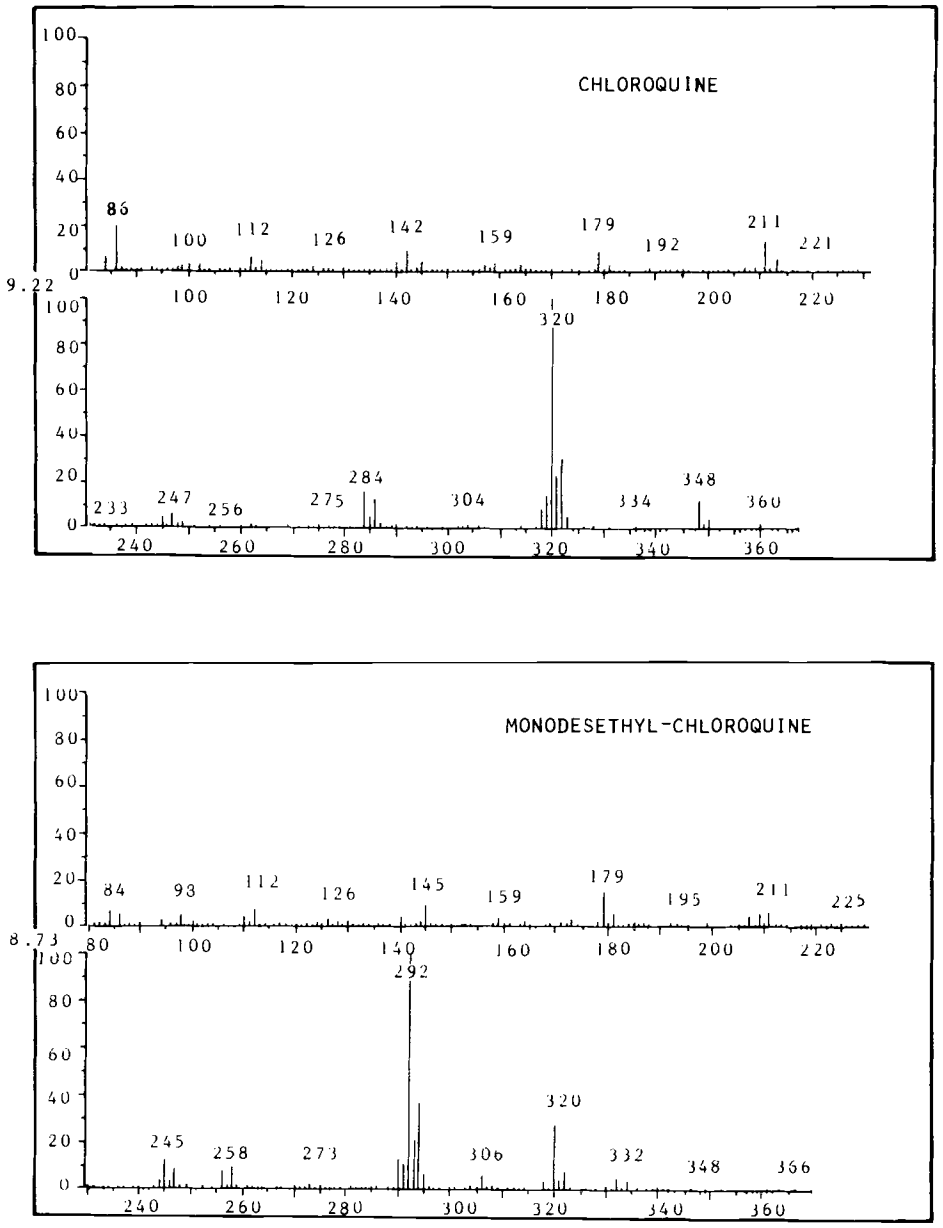

FIG. 2—*Mass spectra of chloroquine and monodesethyl-chloroquine obtained from hair sample of a patient on chloroquine treatment.*

The respective percentages of chloroquine and monodesethylchloroquine found in man by other authors in urine and blood were: 95 to 97,6 and 2.4 to 5 (urine) [*2,14*] and 90 and 10 (blood) [*15*] for a single dose and 61 to 75 and 25 to 39 (urine) [*3,13*] and 50 and 50 (blood)[4] for repeated doses.

[4]F. C. Churchill, personal communication, Department of Health and Human Services, Centers for Disease Control, Atlanta, GA 30333.

TABLE 1—*Determination of chloroquine and monodesethylchloroquine in human hair.*

Patient	Chloroquine Dosage	Total Chloroquine + Monodesethylchloroquine (Expressed as Chloroquine Equivalent mg/kg Hair)	Chloroquine, mg/kg Hair	Monodesethylchloroquine (Expressed as Chloroquine Equivalent mg/kg Hair
M. adult man	100 mg (one tablet) daily six days in one week (for three years)	333	310 (93%)	23 (7%)
J. (girl, 14 years)	100 mg (one tablet) daily six days in one week (for several months)	156	145 (93%)	11 (7%)

We found 83 and 17%, respectively, in blood samples of a patient on long-term chloroquine therapy. In urine as well as in blood, the percentage of unchanged chloroquine seem to be lower in cases of chronic treatment than after administration of single doses. These observations are to be compared with those made by some of us [*1, 16*] on the distribution of chloroquine and its metabolites in twelve cases of poisoning: into the total amount "chloroquine + its metabolites" the relative proportions of chloroquine itself were found between 60 and 91% in biological fluids (urine and blood) and tissues (brain, heart, stomach wall, liver, lung, spleen, and kidney).

The total hair chloroquine content as determined on the two patients was appreciably different. Its percentage seems to come near to the proportions found in some tissues like spleen (83 to 91%) or stomach wall (88%) after poisoning or in blood and urine after administration of single doses. Additional hair samples must be analyzed to obtain more precision.

Nevertheless hair samples offer several advantages. They are easily collected and can be stored without damage. They will be useful for the control of chloroquine treatments. If the time between the first dose and the occurrence of the drug and its metabolites in hair is well known, by analyzing hair portions cut from the origin toward the tip, it may be possible to determine the time from and during which chloroquine was administered, and this procedure can indicate the exposure to certain metallic or metalloidic pollutants. Also, hair analysis may provide a good toxicologic and forensic science complement to the diagnostic information obtained from blood and urine, even from tissues.

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