Journal of Chromatography, 345 (1985) 209—214
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2784

Note

Chloroquine metabolism in man: urinary excretion of 7-chloro-4-hydroxyquinoline and 7-chloro-4-aminoquinoline metabolites

NESBITT D. BROWN*

Division of Biochemistry, Walter Reed Army Institute of Research, Washington, DC 20307-5100 (U.S.A.)

BING T. POON

Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC 20307-5100 (U.S.A.)

and

JEFFREY D. CHULAY

U.S. Army Medical Research Unit-Kenya, Box 401, Army Post Office, New York, NY 09675 (U.S.A.)

(First received March 26th, 1985; revised manuscript received July 9th, 1985)

Data published during the past 37 years [1–4] show that while chloroquine and desethylchloroquine are the major excretory by-products of urine and feces in man, many minor but still important metabolites are also present in these physiologic specimens. In all but a few cases, the identities of these 7-chloro-4-aminoquinoline compounds remain unknown. Brown et al. [5] have recently identified a mono-acetylated conjugate of chloroquine in human plasma and urine.

In this report, we describe a reversed-phase ion-pair high-performance liquid chromatographic (HPLC) method for separating and detecting 7-chloro-4-hydroxyquinoline, 7-chloro-4-aminoquinoline and 4-(4′-hydroxy-1′-methylbutylamino)-7-chloroquinoline in urine specimens. Organic extracts of the samples are prepared, prior to analysis, with separation performed on a semi-preparative C_{18} column. Amounts as low as 2 ng on-column are detectable. Correlation coefficients for the three compounds assayed ranged between 0.929 and 0.941. Utilization of an isocratic mobile phase minimizes equilibration time between runs.

0378-4347/85/$03.30

With the adoption of this method, the separation, identification and quantification of the various 7-chloro-4-aminoquinoline metabolites have produced a clearer picture for gaining greater insight into this metabolic process.

EXPERIMENTAL*

All separations were made using a Waters Model APC/GPC-204 liquid chromatograph, equipped with two Model 6000A high-pressure pumps, a 660 solvent programmer, a U6K loop injector, a Model 440 absorbance detector, set at 340 nm, a Houston Instrument Omni-Scribe A5000 dual-pen recorder and a Columbia Industries Supergrator-3 integrator.

Spectroquality acetonitrile (Burdick & Jackson Labs., Muskegon, MI, U.S.A.) mixed with PIC B-7 reagent (1-heptanesulfonic acid, Waters Assoc., Milford, MA, U.S.A.) was used as the mobile phase. 7-Chloro-4-hydroxyquinoline, 7-chloro-4-aminoquinoline hydrate and 4-(4′-hydroxy-1′-methylbutylamino)-7-chloroquinoline (Walter Reed Army Institute of Research's Inventory, Washington, DC, U.S.A.) were used to prepare all stock standards. Stock standard solutions, containing 100 ng/μl of each compound were used to prepare working standards.

Procedure

A 300 mm × 7.8 mm I.D. 10-μm μBondapak C_{18} column (Waters Assoc.) was employed to chromatograph all compounds used in this study. The mobile phase consisted of a 0.02 *M* solution of PIC B-7 reagent in water, mixed with acetonitrile. The 0.02 *M* PIC B-7 solution was prepared by dissolving 40 ml of the pre-package reagent into 460 ml of glass-distilled water. The pH of the solution was 3.4. Acetonitrile—0.02 *M* PIC (35:65) was used in an isocratic mode. The flow-rate was 2.0 ml/min. Column pressures ranged between 76 and 84 bar. All separations were performed at ambient temperature. Sample volumes (30 μl) were introduced into the column through a continuous flow loop injector. Detection limit of the method was 2 ng on-column. Peak areas were measured by an on-line computing integrator.

Samples

Urine specimens were collected from two normal subjects (treated), who received a 300-mg dose of chloroquine diphosphate every 48 h during a two-week study period. Urine specimens were taken before dosing and at various time intervals after dosing during the two-week collection period (short-term study). Urine samples were also collected from ten additional volunteers within the indigenous population (long-term study). These subjects were on a one to three year regimen of chloroquine.

The extraction method used for all samples analyzed in this investigation is described in our earlier report [6]. An alternative procedure described by Kuroda [7] was also used to extract phenolic and amphoteric metabolites from

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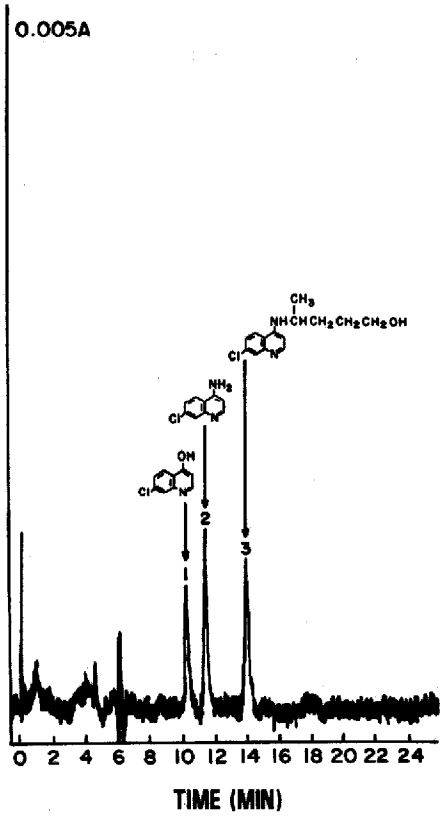
the specimens. Organic extracts from the two procedures were combined, evaporated and reconstituted in a mixture of methanol—0.1 *M* phosphoric acid (1:1).

RESULTS AND DISCUSSION

Early investigators, studying the metabolic fate of chloroquine in man, have agreed that while chloroquine and its desethyl congeners are the prime constituents of excretion in humans, the formation and elimination of more subtle 7-chloroquinoline compounds are also important aspects of this metabolic process. Both Kuroda [7] and McChesney et al. [8] focused much of their research efforts on this area of study. Earlier methods used by these investigators for detecting and measuring many of these metabolites proved successful. At the same time, other analogues of chloroquine, suspected to have been present in urine and fecal samples, could not be detected.

With the development of more specific methods, along with more sensitive



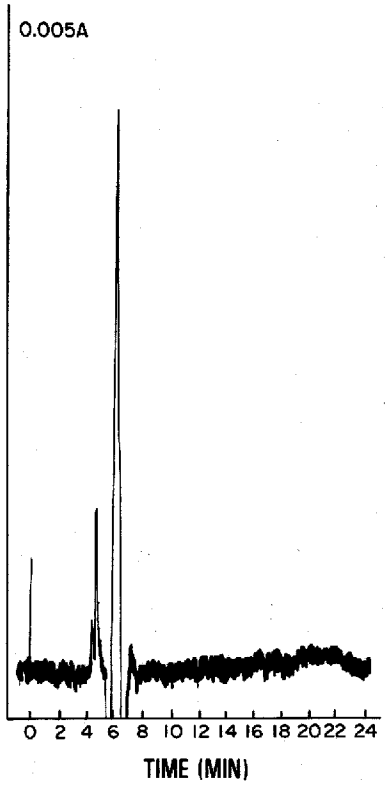


Fig. 1. Separation of a standard solution containing (1) 50 ng of 7-chloro-4-hydroxyquinoline; (2) 25 ng of 7-chloro-4-aminoquinoline; and (3) 25 ng of 4-(4′-hydroxy-1′-methylbutylamino)-7-chloroquinoline. Column: 30 cm × 7.8 mm I.D. μBondapak C_{18}. Mobile phase: 0.02 *M* PIC B-7—acetonitrile (65:35). Flow-rate: 2 ml/min. Column temperature: ambient.

Fig. 2. Chromatogram of a normal subject's urine, prior to chloroquine adminstration (0 h). Peak, unknown. Sample volume: 30 μl.

instrumentation, the task of identifying and confirming some of the more elusive metabolites has been made much easier.

In this paper, we describe a method for separating and assaying metabolites of chloroquine, which are present in trace amounts, or levels requiring ultrasensitive detection systems. A semi-preparative μBondapak C_{18} column, having a large loading capacity and high theoretical plate values, was used to enhance the separations.

The resolution and selectivity in such a system produced peaks, whereby the smaller peaks of the minor constituents, as well as the larger peaks of chloroquine and de-ethylated chloroquine, are separated.

Fig. 1 depicts the separation of a standard solution, containing 7-chloro-4-hydroxyquinoline, 7-chloro-4-aminoquinoline and 4-(4′-hydroxy-1′methylbutylamino)-7-chloroquinoline, detected at 0.005 a.u.f.s. All concentrations used in this study (2—50 ng) produced linearity for each compound assayed.

In order to produce peaks from the experimental samples collected in this study, 400 μl of urine containing the metabolites were extracted, concentrated to 30 μl, and injected onto the column. A chloroquine-free urine sample (0 h)

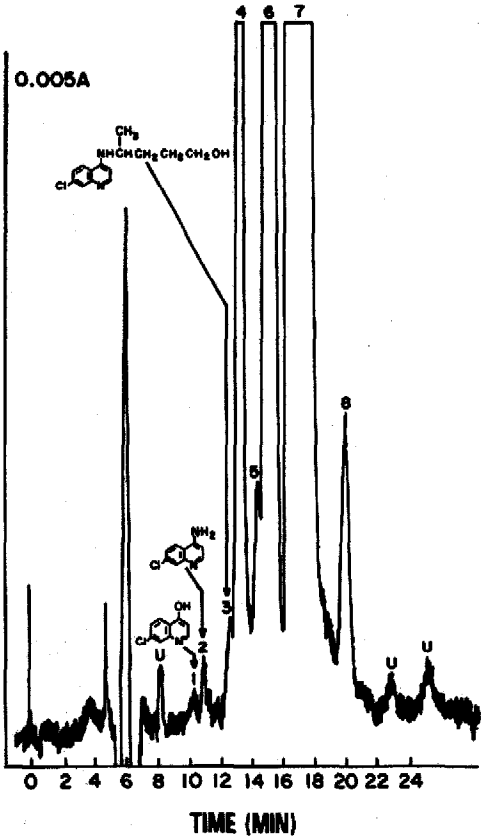


Fig. 3. Chromatogram of a 15-h treated subject urine sample. Peaks: 1 = 7-chloro-4-hydroxyquinoline; 2 = 7-chloro-4-aminoquinoline; 3 = 4-(4′-hydroxy-1′-methylbutylamino)-7-chloroquinoline; 4 = bidesethylchloroquine; 5 = hydroxychloroquine; 6 = desethylchloroquine; 7 = chloroquine; 8 = N-acetyl-(mono)desethylchloroquine; U = unknown metabolites. Sample volume: 30 μl. Detection wavelength: 340 nm.

TABLE I

7-CHLORO-4-HYDROXYQUINOLINE AND 7-CHLORO-4-AMINOQUINOLINE METABOLITES IN CHLOROQUINE-TREATED SUBJECTS

+ = Present; – = absent.

Subject*	7-Chloro-4-hydroxyquinoline	7-Chloro-4-aminoquinoline	4-(4'-Hydroxy-1'-methylbutylamino)-7-chloroquinoline
ST-1	+	+	+
ST-2	+	+	+
LT-1	–	+	+
LT-2	–	–	+
LT-3	–	–	–
LT-4	–	+	–
LT-5	+	+	+
LT-6	–	+	+
LT-7	–	+	+
LT-8	–	–	+
LT-9	–	–	–
LT-10	–	+	+

*ST = Short-term; LT = long-term.

was chromatographed to determine the possibility of interference from endogenous material (Fig. 2). No peaks were found which had retention times similar to those of the treated subjects. Fig. 3 represents the separation of a 15-h urine sample, collected from a chloroquine-treated subject. Ten additional urine samples were similarly analyzed in this study.

For all of the specimens analyzed, only three samples contained all three metabolites of interest. Two were observed in our treated subjects, while only one long-term subject had all three. A combination of the other two metabolites was displayed in the chromatographic profiles of the other subjects. Table I shows the distribution scheme of the 7-chloroquinoline metabolites for all twelve subjects.

Where measurable amounts of the metabolites were found, no more than 0.20—0.25% of the administered dose of chloroquine was recovered in the 24-h urine specimens.

From these studies, we were able to separate and measure small amounts of 7-chloroquinoline metabolites in urine. At the same time, three unknown metabolites were also separated. In the near future, efforts will be made to identify these unknown compounds.

ACKNOWLEDGEMENT

We express our sincere appreciation to Miss Laura Watson for her excellent secretarial assistance in the preparation of this manuscript.

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