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Note

Separation and identification of a plasma and urinary mono-acetylated conjugate of chloroquine in man by ion-pair high-performance liquid chromatography

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Acetylation in vivo in man of certain therapeutic drugs, especially those containing primary and secondary amino groups, is used as a biological marker for assessing the genetic polymorphism of an individual [1—3]. Human subjects are phenotyped as either rapid or slow acetylators. While it is not clear whether the recognizable phenotyped acetylator can be determined by the acetylation of chloroquine to its N-acetyl metabolite, results from this study showed that the process of acetylation does occur during the metabolism of chloroquine in man.

In this report, we describe an ion-pair high-performance liquid chromatographic (HPLC) method for detecting and quantifying an acetylated conjugate of chloroquine in plasma and urine specimens. Fig. 1 represents a metabolic scheme showing the formation of N-acetyl(mono)desethylchloroquine. A pretreated organic extract of plasma or urine is required prior to analysis. Amounts as low as 10 ng on-column are quantifiable, and the analysis requires 12 min per sample. The use of an isocratic system minimizes the analysis time between runs.

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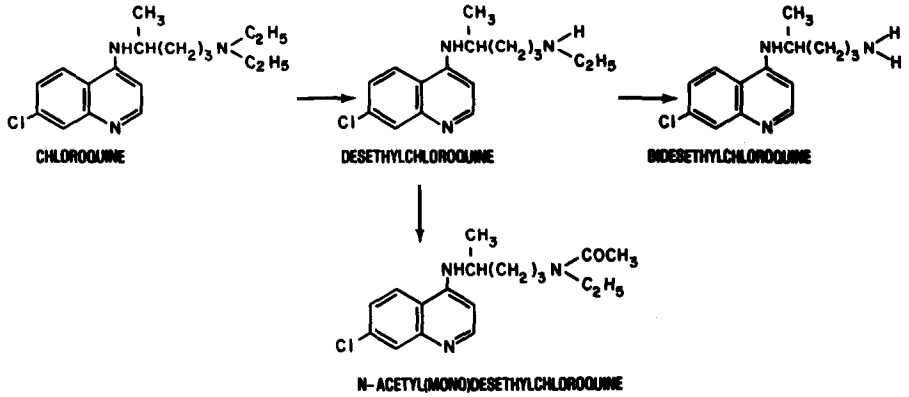

Fig. 1. Metabolic pathway for chloroquine in human subjects, showing the formation of N-acetyl(mono)desethylchloroquine.

We are currently employing this method in our laboratory for separating and identifying various 7-chloro-4-aminoquinoline metabolites associated with the metabolism of chloroquine in humans.

EXPERIMENTAL*

Apparatus

A Waters Assoc. (Milford, MA, U.S.A.) Model ALC/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 and a Model 730 data module was used to conduct this study.

Reagents

All solvents and chemicals used in this investigation were either of spectroquality or of analytical grade. Acetonitrile was obtained from Burdick and Jackson Labs. (Muskegon, MI, U.S.A.). PIC-B7 reagent (1-heptanesulfonic acid) was purchased from Waters Assoc. N-Acetyl(mono)desethylchloroquine was obtained as a gift from Dr. Frederick Churchill, III (CDC, Atlanta, GA, U.S.A.). Chloroquine disphosphate (Sigma, St. Louis, MO, U.S.A.), desethylchloroquine sesquioxalate, and bidesethylchloroquine hydrobromide (Inventory, Walter Reed Army Institute of Research, Washington, DC, U.S.A.) were used to prepare all working standards. Stock solutions containing 100 ng/ml of each compound were prepared in glass-distilled water. Working standards were prepared from the stock standards.

Procedure

A 300 mm × 3.9 mm I.D., 10-μm μBondapak C_{18} column (Waters Assoc.) was used to chromatograph chloroquine and its metabolites in standards and experimental samples. A description of the method has been published previously by our group [4].

*The manufacturers' names and products are given as scientific information only and do not constitute an endorsement by the United States Government.

Samples

Urine and plasma specimens were collected from two normal subjects. Each volunteer received a single 300-mg dose of chloroquine diphosphate. Biological specimens were taken before dosing (control) and 12 and 24 h after dosage. The extraction method used for our samples is described in an earlier report [4].

RESULTS AND DISCUSSION

The metabolism of chloroquine in humans has been widely discussed and debated by various research groups for many years, yet many questions on the pharmacokinetics of this drug remain unanswered. Recently, research by several groups [5, 6] has shown that HPLC can be utilized to separate and identify both major and minor metabolites present in physiological fluids. Work by our group has also shown that the mono- and di-ethylated metabolites of chloroquine were present in urine and plasma specimens of human subjects. At the same time, other 7-chloro-4-aminoquinoline analogues were suspected to be formed during this metabolic process. We now report the separation and identification of an N-acetyl conjugate of the primary metabolite of chloroquine in urine and plasma samples of man. N-Acetyl(mono)desethylchloroquine, the acetylated product of desethylchloroquine, was separated and quantified by HPLC and identified by chemical ionization mass spectrometry.

In utilizing this ion-pair reversed-phase HPLC method, various chloroquine analogues and experimental samples were chromatographed to determine the specificity and sensitivity of the procedure. Because of the similarities of chemical structures, separation of these 7-chloro-4-aminoquinolines is difficult. Results showed that the resolution for the series of compounds separated were excellent. At the same time, linearity was obtained for the various concentrations of each compound analyzed (5—200 ng).

Samples from two human subjects were chromatographed. Chromatograms shown in Fig. 2A and B depict two time frames for subject A. N-Acetyl(mono)-desethylchloroquine was observed in both the 12- and 24-h urine specimen. In calculating the total urinary output of chloroquine and its metabolites for subject A over the 24-h period, only 17% of the administered dose was recovered. Of this, 1.4% was N-acetyl(mono)desethylchloroquine. Bidesethylchloroquine (4.66%), desethylchloroquine (22.83%) and chloroquine (69.03%) constituted the remaining 7-chloro-4-aminoquinoline compounds present in the 24-h urine collection. Fig. 3 represents the 12-h plasma sample of subject A. N-Acetyl(mono)desethylchloroquine was also observed in this specimen. Chromatographic data of the 24-h plasma sample of subject A failed to show the presence of the N-acetylated metabolite.

The chromatograms shown in Fig. 2C and D show the separation of the urine specimen of subject B. Even though the excreted urinary volume for subject B was 50% greater than that of subject A, quantifiable amounts of N-acetyl(mono)desethylchloroquine were measurable in the 12-h urine sample. It represented 1.1% of the administered dose of chloroquine diphosphate. The 24-h urine specimen did not show a corresponding peak for the N-ace-

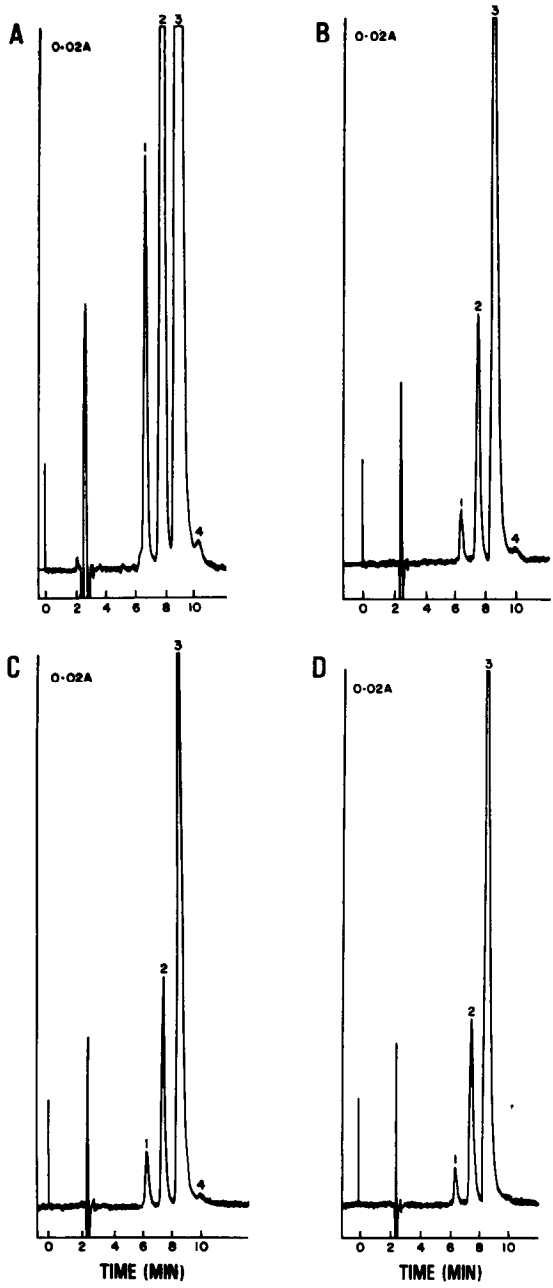

Fig. 2. Chromatograms of (A) the 12-h urine sample from human subject A; (B) the 24-h urine sample from human subject A; (C) the 12-h urine specimen from human subject B; (D) the 24-h urine specimen from subject B. Conditions: column: 300 mm × 3.9 mm I.D., μBondapak C_{18}; mobile phase: 0.02 *M* PIC-B7 reagent—acetonitrile (66:34); flow-rate: 1.0 ml/min; column temperature: ambient; sample volume: 10 μl; detection wavelength: 340 nm. Peaks: 1 = bidesethylchloroquine; 2 = desethylchloroquine; 3 = chloroquine, and 4 = N-acetyl(mono)desethylchloroquine.

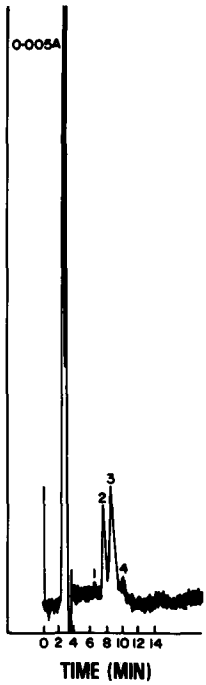

Fig. 3. Chromatogram of a 12-h plasma extract from subject A. Sample volume: 10 μl. Peaks: 1 = bidesethylchloroquine; 2 = desethylchloroquine; 3 = chloroquine; and 4 = N-acetyl(mono)desethylchloroquine.

tylated metabolite. However, the recoverable amounts of chloroquine and its two de-ethylated metabolites were measured and their amounts were similar to the total 24-h urinary values of subject A. Recovered percentages for these compounds were as follows: 5.8% for bidesethylchloroquine, 22.48% for desethylchloroquine, and 68.88% for chloroquine. The 12- and 24-h plasma sample of subject B did not contain any detectable amounts of N-acetyl(mono)desethylchloroquine.

While the experiment we describe in this study should have obvious importance in probing the metabolic fate of chloroquine in humans, many of the aspects involving the metabolism of this drug are far from being completely understood.

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