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Note

Identification of a chloroquine artifact by gas chromatography–mass spectrometry

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Chloroquine (CQ) is a derivative of the 4-aminoquinoline widely employed in the chemoprophylaxis and chemotherapy of malaria and chronic rheumatic diseases [1]. Several extraction and analytical methods have been reported for the analytical determination of CQ and its metabolites from biological fluids [2–8].

This paper describes the identification of an artifact detected in the urine of patients [9] and obtained by the treatment of CQ with different dilutions of acids during extraction of 100 and 10 μg/ml samples spiked with CQ base.

EXPERIMENTAL[a]

Apparatus

A Jeol mass spectrometer JMS-DX-300 with a JMA-3100 computer was used. An OV-17 bonded-phase capillary column (25 m × 0.21 mm I.D.) was used at 220–300°C with the temperature programmed to rise at 16°C/min. The splitless injection volume was 0.7 μl at 280°C injector temperature. The carrier gas (helium) flow-rate was 1.0 ml/min and the source ionization energy was 70 eV.

Chemicals and reagents

All chemicals were of analytical quality· HPLC-grade methanol, ethyl acetate,

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trifluoroacetic anhydride (TFAA) (Merck, Darmstadt, F.R.G.), double-distilled chloroform (Reactivul, Bucarest, Romania) and pharmacopoeial-grade chloroquine phosphate (National Industry of Pharmaceutical Products, Cuba).

Glassware and centrifuge tubes were precleaned with 0.1 *M* HCl in an ultrasonic bath and silanized with 2% dimethyldichlorosilane in 1,1,1-trichloroethane (BDH, Poole, U.K.).

The standard solutions of CQ base (100 and 10 μg/ml) were prepared in methanol. Sequential dilutions were prepared from the 1 mg/ml and 100 μg/ml solutions. The 0.1 and 1 *M* HCl and the 0.05 and 0.5 *M* H_2SO_4 dilutions were prepared from Fixanal ampoules (Riedel de Haen, F.R.G.).

Procedure

For the extractions, 2.0 ml of double-distilled water were combined with 2.0 ml of CQ base standard solution and 5.0 ml of the acid solution in a 20-ml screw-cap centrifuge tube. This solution was mixed for 5 min on a platform shaker, alkalinized with 0.5 ml of 60% (w/v) aqueous KOH, and 6.0 ml of chloroform were added. The aqueous solution was extracted by the organic phase on a platform shaker for 5 min. The tube was centrifuged for 10 min at 1500–2000 *g*, and the aqueous phase was discarded. The chloroform layer was evaporated to dryness at 45°C under nitrogen. The experimental control involved replacement of the acid solution with an equal volume of double-distilled water. Otherwise the tubes were treated identically. The residue was dissolved in 200 μl of methanol, transferred to a micro-tube and evaporated to dryness again. Then the residue was dissolved in 10 μl of methanol, and an aliquot was injected into the gas chromatographic–mass spectrometric (GC–MS) system. For comparison, a patient's urine sample was extracted and analysed as described previously [9].

RESULTS AND DISCUSSION

Fig. 1 shows the total-ion current chromatogram of a TFAA-derivatized urine sample from a *Plasmodium vivax*-infected patient 60 h after treatment with CQ

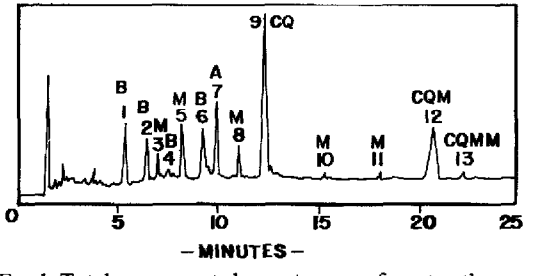

Fig. 1. Total-ion current chromatogram of a patient's urine sample Peaks: B = peak from blank urine, CQ = chloroquine, CQM = desethylchloroquine; CQMM = didesethylchloroquine, M = other probable chloroquine metabolites, A = artifact from exposure of chloroquine to an acid environment.

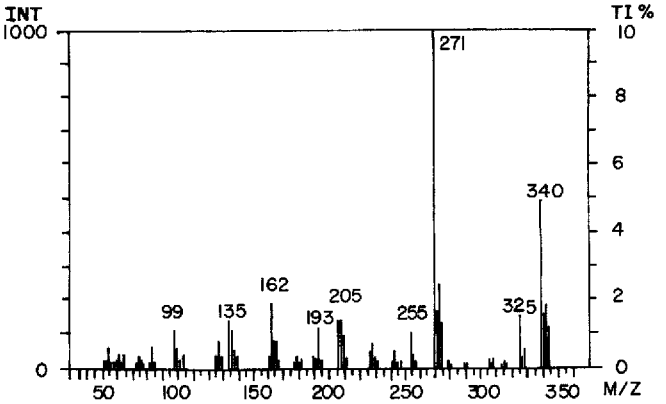

Fig 2 Mass spectrum of chromatographic peak 7 in Fig 1

phosphate was instituted. At least thirteen chromatographic peaks are observed, and CQ and its metabolites (M) are probably responsible for eight of them. Peak 9 was confirmed as chloroquine by comparison with the mass spectrum of the pure substance and by matching it with a reference spectrum [10]. Peak 12 was confirmed as the trifluoroacetate derivative of monodesethylchloroquine from its mass spectrum and by comparison with a previously published spectrum [3]. The identification of peak 13 as the difluoroacetyl derivative of didesethylchloroquine is inferred from its mass spectral fragmentation pattern and its similarities to spectra for chloroquine (peak 9) and its trifluoroacetylmonodesethyl metabolite (peak 12). Four peaks correspond to those in blank urine (B), and peak 7 is considered to be an artifact (A).

Our provisional interpretation of the mass spectrum of peak 7 (Fig. 2) includes a probable molecular ion at *m/e* 340, suggesting that the molecule probably has only two nitrogen atoms. We suggest that the ionic fragment *m/e* 325 results from the loss of a methyl group from the molecular ion. The fragment *m/e* 271 is suggested to arise from the loss of CF_3 from the molecular ion. The *m/e* 162 fragment corresponds to $(C_9H_5NCl)^+$, and *m/e* 135 corresponds to the loss of HCN from *m/e* 162. Retention of Cl in all of these fragments is suggested from

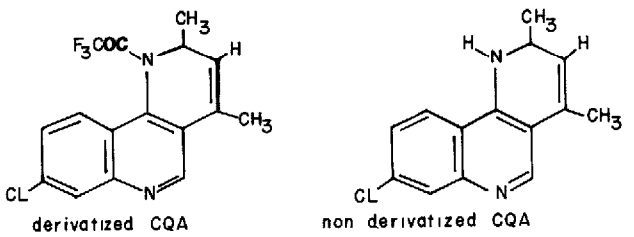

Fig. 3. Structures proposed for the chloroquine artifact (CQA) in free and trifluoroacetylated forms

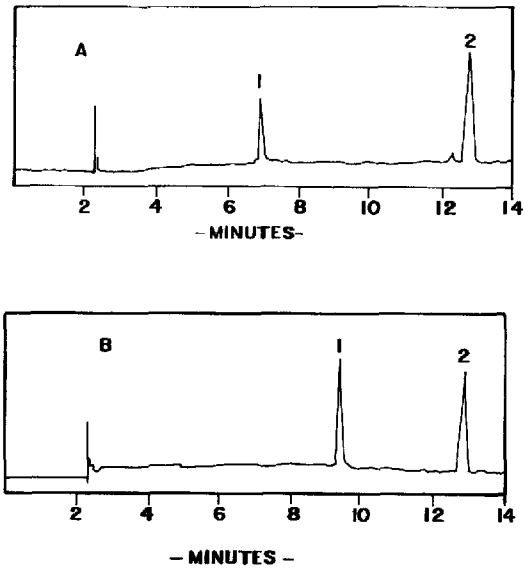

Fig. 4. Total-ion current chromatograms from samples spiked with CQ base and exposed to 0 05 M H_2SO_4 prior to solvent extraction. (A) Non-derivatized sample; (B) sample derivatized with TFAA. Peaks: 1 = CQ artifact, 2 = CQ

the natural isotope abundance ratio for this element. Fig. 3 illustrates the molecular structure we propose for this artifact, which is produced when CQ is exposed to an acid environment. However, the final structure for the CQ artifact remains to be confirmed by other methods.

To confirm that peak 7 in Fig. 1 is an artifact produced during the extraction procedure, duplicate extractions of CQ (100 and 10 μg/ml) in double-distilled

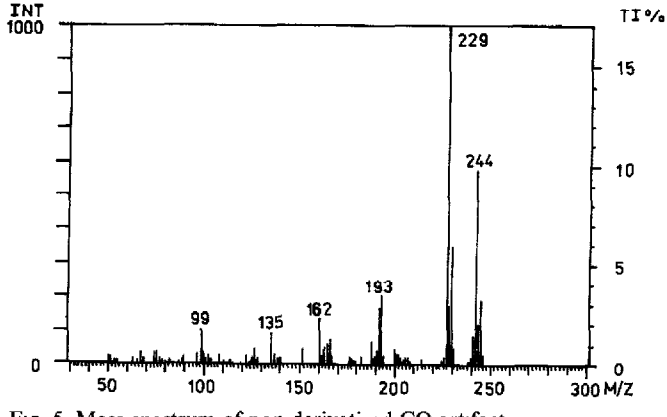

Fig. 5. Mass spectrum of non-derivatized CQ artifact.

water were performed using 0.1 and 1 *M* HCl and 0.05 and 0.5 *M* H_2SO_4 solutions. The appropriate control samples were extracted at the same time. One of each duplicate was derivatized with TFAA to determine the potential influence of this step on our observations. The analytical studies performed demonstrated that both the derivatized and non-derivatized samples treated with acid showed CQ and an additional chromatographic peak (Fig. 4). This second peak in the derivatized samples has a mass spectrum identical with the one obtained for peak 7 in the patient's urine sample (Fig 2).The peak in the total-ion current chromatogram from the non-derivatized samples has a mass spectrum with a probable molecular ion *m/e* 244 (M^+) and a base peak *m/e* 229 ($[M-15]^+$) (Fig. 5).

These experiments corroborated our supposition that peak 7 (Fig. 1) identified in the urine samples is an artifact that appears when CQ is treated with acids during the extraction process, and is not caused by the derivatization with TFAA.

CONCLUSION

The formation of an artifact during the treatment of CQ with acids is demonstrated, and a tentative structure for it is proposed. This reaction may be important in the accurate quantitation of CQ levels in biological fluids.

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