Journal of Clinical Laboratory Analysis 11:232–234 (1997)

Unusual Interference From Primary Collection Tube in a High-Performance Liquid Chromatography Assay of Amiodarone

Vadiraja V. Murthy

Department of Pathology, Albert Einstein College of Medicine, The Bronx, New York

We describe an unusual interference in our routine high-performance liquid chromatography (HPLC) assay of amiodarone and its active metabolite, desethylamiodarone, used to quantify the parent drug and the active metabolite in serum from the primary sample collection tube. The interfering peak had a retention time very similar to that of the authentic desethylamiodarone. Substitution of Corvac tubes with Vacutainer tubes for the collection and transportation of serum samples eliminated the source of interference. We routinely suggest the use of Vacutainer collection tubes for obtaining blood samples of cardiac patients undergoing amiodarone therapy. J. Clin. Lab. Anal. 11:232–234, 1997. © 1997 Wiley-Liss, Inc.

Key words: assay interference from primary collection tube; HPLC; amiodarone assay; desethylamiodarone, active metabolite

INTRODUCTION

Amiodarone (2-butyl-3-[3,5-diiodo-4-β-diethylamino-ethoxy benzoyl] benzofuran; AMIO), an antiarrhythmic drug, exhibits a long half-life in humans. It is metabolized in vivo to a therapeutically active metabolite, desethylamiodarone (DESAMIO). Optimal therapeutic efficacy is achieved slowly within a few days to weeks after an oral dose of 200–600 mg/day and continuous antiarrhythmic control can persist for days and even weeks after discontinuance of treatment. AMIO with its cumulative therapeutic effect was thought to be an ideal drug for the treatment and prevention of a wide variety of atrial and ventricular arrhythmias (1). However, AMIO, which contains 37% iodine by weight, can present a considerable thyroid load since as much as 18 mg/day of iodine may be released from AMIO. Although generally well tolerated, the main side effects of the drug include thyrotoxicosis, hypothyroidism, peripheral neuropathy, changes in hepatic enzymes, photosensitive skin rash, and in extremely rare instances, pulmonary fibrosis (2). Owing to its extremely slow elimination from the body, continuous monitoring of serum AMIO concentrations is very important. A good therapeutic monitoring method for measuring AMIO levels in serum must also be able to distinguish AMIO from its active metabolite, DESAMIO, and to quantify their respective concentrations in the sample. We routinely measure AMIO and DESAMIO concentrations in serum after removing the protein in serum by addition of saturated $ZnCl_2$ and CH_3CN. The deproteinized sample is assayed by the HPLC method with UV detection at 254 nm as described earlier (3).

HPLC assay is performed with a 15 × 0.42 cm (i.d.) Nucleosil-5 C18 column and a mobile phase, containing acetonitrile (600 mL), 0.1 Mole/L phosphoric acid (1 mL), and diethylamine (0.5 mL) in 1 L of distilled water. It is delivered at a flow rate of 1 mL/min with a ConstaMetric pump (LDC, Riviera Beach, FL) after sample injection with a Perkin-Elmer ISS-1000 auto-injector (Norwalk, CT). The column effluent is monitored by absorbance at 254 nm with a Milton Roy CM-4000 programmable UV detector (Rochester, NY). The detector signals are processed through a PE Nelson data station (Cupertino, CA) equipped with the Turbochrom program. The linear range of our method is between 0 and 5 mg/L for both AMIO and DESAMIO, with a minimum detection limit of 0.1 mg/L (3).

Of the 66 samples taken from 20 patients and analyzed for AMIO by HPLC in our laboratory during June 1994, assays of 22 samples from 8 patients received in the last 2 weeks exhibited an extraneous peak with a retention time of 6.2 minutes in the vicinity of the DESAMIO peak, in addition to the expected peaks representing AMIO and DESAMIO with characteristic retention times of 8.6 and 5.9 minutes. The interfering peak made it difficult to quantify the DESAMIO concentration in these samples, thus necessitating a careful examination of the cause of interference in our sample assay procedure.

Representative chromatograms of an aqueous solution of AMIO and DESAMIO standards (trace A), a patient sample obtained earlier during the month, not showing the interference

*Correspondence to: Dr. V. V. Murthy, Special Chemistry Laboratories, Room 2S-11, Nurses Residence Building, Jacobi Medical Center, The Bronx, NY 10461.

Received 18 November 1996; Accepted 22 November 1996

peak (trace B), and a later sample from the same patient exhibiting the interfering peak (trace C) are shown in Figure 1.

A plausible cause for the interference peak was sought by repeating the assays of suspect serum samples by a) employing a freshly prepared mobile phase, b) replacing the C18 column in use with a freshly packed column, or c) using both a fresh mobile phase and column. In all 22 samples taken from the same 8 patients, the interference persisted irrespective of any substitutions in the HPLC assay protocol, confirming no methodological problems. We also repeated the analyses of representative samples received a month earlier from the same patient population along with the suspect samples received later. The assay results indicated that the interfering peak was present only in the chromatograms obtained after HPLC assays of the batch of samples received in Corvac Monoject® tubes during the later part of the month while confirming the absence of such an interfering peak in the chromatograms of samples received in Vacutainer tubes (Beckton-Dickinson), during the first 2 weeks of the month.

These findings indicated that either the collection or the sample processing was the possible source of interference! Upon further inquiry from the customer, it was found that SST® tubes (no. 6510 Beckton Dickinson, Rutherford, NJ), which were used routinely to collect and transport samples, had been replaced with Corvac Monoject® tubes (Sherwood Medical, St. Louis, MO). The interfering peak was first encountered during the latter part of June 1994, which was about the time that the Corvac Monoject tubes were being used. The samples sent for analyses were usually transported in primary collection tubes with gel separators. Prolonged contact with the gel separator was probably the cause of interference; serum samples, separated and poured off from Corvac Monoject tubes into clean glass tubes immediately after processing, exhibited the interference peak (see chromatogram C in Fig. 1). This confirmed that the interfering material was present even in freshly processed serum, only when the blood was collected in Corvac Monoject tubes. We therefore concluded that the source of interference was the Corvac Monoject tubes used for blood collection. It was not possible to ascertain whether the gray stopper used to seal the tubes also contributed to the observed interference.

Blood samples collected from the same 8 patients prior to June 1994 did not exhibit the interference peak in our HPLC assay. However, subsequent samples collected from the same group of patients during and after the third week of June 1994 exhibited the interference. This observation confirmed our suspicion that Corvac Monoject tubes were indeed the source of the interference. Therefore, the HPLC assay results of samples exhibiting the interference peak were reported as being incomplete without the result for the metabolite concentration. They were highlighted with a comment, "interfering substance present," with a request for a fresh sample collected in a Vacutainer tube.

Blood transported in Corvac tubes somehow become contaminated and hence are unsuitable for HPLC assays. We would like to caution the readers about this potential interfer-

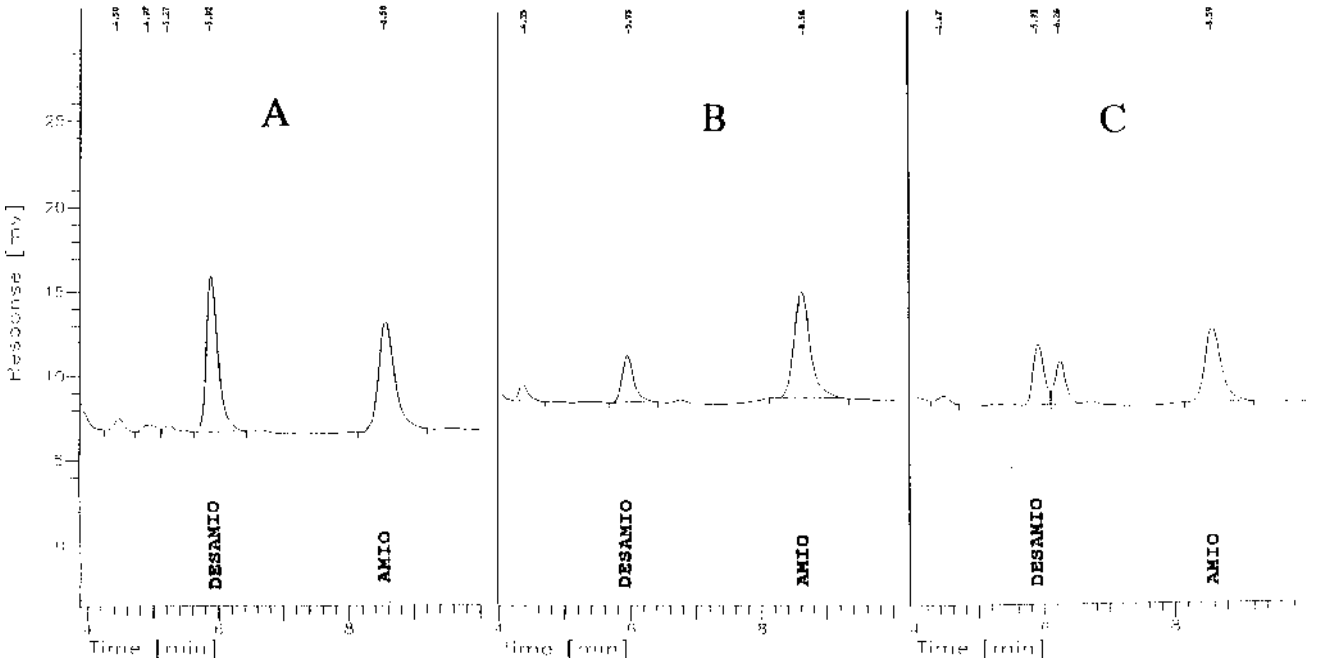

Fig. 1. Chromatograms of **(A)** an aqueous standard solution with a concentration of 5 mg/L each of amiodarone (AMIO) and desethylamiodarone (DESAMIO); **B:** an earlier serum sample; and **C:** a later sample from the same patient.

ence in HPLC assays. As we were able to correct the problem of interference, we did not pursue the project any further to determine whether or not the interference could be attributed to any particular lot of Corvac tubes.

ACKNOWLEDGMENTS

I gratefully acknowledge the technical assistance of Marcel Zoontjens and Ana Ignat.

REFERENCES

1. Rosenbaum MB, Chiale PA, Halpern MS, Nau GJ, Przybylski J, Levi RJ, Lazzari JO, Elizari MW: Clinical efficacy of amiodarone as an antiarrhythmic agent. *Am J Cardiol* 38:934–944, 1976.
2. Kennedy RL, Griffiths H, Gray TA: Amiodarone and the thyroid. *Clin Chem* 35:1882–1887, 1989.
3. Lam S: High performance liquid chromatography of amiodarone and desethylamiodarone in serum after microscale protein precipitation. *J Chromatogr* 381:175–178, 1986.