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Note

Determination of mitomycin C in serum by high-performance liquid chromatography with dual-electrode coulometric detection

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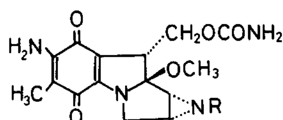
In recent years, mitomycin C (Fig. 1) has been widely used for cancer treatment, but its clinical applications are limited owing to inherent severe toxicity. The therapeutic efficacy of mitomycin C is closely related to the drug concentration in blood and tissues, depending on the dose, route and frequency of administration. Quantitation of mitomycin C in biological fluids was first attained by the bioassay technique [1]. Various other methods have been devised, such as enzyme immunoassay [2] and high-performance liquid chromatography (HPLC) with UV detection [3–5], differential pulse polarographic [6] and reduction-mode electrochemical detections [7]. However, development of a more feasible and sensitive assay method is needed for therapeutic



R = H : Mitomycin C (MMC)
R = CH_3 : Porfiromycin

Fig. 1. Structures of mitomycin C and porfiromycin.

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drug monitoring. This paper describes an HPLC method with a dual-electrode coulometric detection system for the determination of mitomycin C in serum.

EXPERIMENTAL

Materials

Mitomycin C was purchased from Nacalai Tesque (Kyoto, Japan). The natural standard porfiromycin (Fig. 1) was kindly donated by Kyowa Hakko Kogyo (Tokyo, Japan). A Molcut II® membrane filter was supplied by Millipore (Bedford, MA, U.S.A.). All other reagents and chemicals were obtained from Nacalai Tesque and purified by recrystallization or distillation prior to use.

Instruments

The apparatus used for HPLC was a Waters Model 510 chromatograph (Millipore Waters Chromatography Division, Milford, MA, U.S.A.) equipped with a Coulochem Model 5100A electrochemical detector (Environmental Science Assoc., Bedford, MA, U.S.A.). Detector potentials were set at -0.7 V (first electrode) and $+0.3$ V (second electrode) versus a palladium electrode. A test sample was injected using a Model 7125 injector (Rheodyne, Cotati, CA, U.S.A.) with an effective volume of 500 μl. HPLC was carried out using Develosil ODS-5 (5 μm particle size; 4 cm$\times$0.4 cm I.D.) as a precolumn and Develosil ODS-5 (5 μm particle size; 15 cm$\times$0.4 cm I.D.) as an analytical column (Nomura Chemical, Seto, Japan) at a flow-rate of 1.0 ml/min at ambient temperature. Acetonitrile–0.05 *M* sodium perchlorate (15:85, v/v) was used as the isocratic mobile phase.

Procedure for the determination of mitomycin C

Porfiromycin (50 ng) in methanol (20 μl) was added to serum samples (100–500 μl), and the mixture was filtered through a Molcut II membrane filter for deprotenization. The filtrate (100–500 μl) was then loaded onto the precolumn for elimination of interfering substances in serum. After thorough washing for 2 min, mitomycin C and porfiromycin were eluted from the precolumn and then led to the analytical column by a column-switching technique, using acetonitrile–0.05 *M* sodium perchlorate (15:85, v/v) as the mobile phase.

Recovery test for mitomycin C in serum

The spiked samples were prepared by addition of 20, 50, 70 and 100 ng each of mitomycin C to drug-free human serum specimens (100 μl). Pretreatment and subsequent HPLC with dual-electrode coulometric detection were carried out in the manner described above.

Drug administration and sampling

Mitomycin C was injected intraperitoneally to five guinea-pigs weighing ca. 800 g, at a dose of 4 mg/kg. Blood samples were drawn by heart puncture (1 ml) at 0.25, 0.5, 1, 2, 4, and 6 h after administration. Sera were separated by centrifugation at 1900 *g* for 15 min and stored at 0°C.

RESULTS AND DISCUSSION

Initially, the electrochemical properties of mitomycin C and porfiromycin were examined with a single working electrode. The hydrodynamic voltammograms of the two substances are illustrated in Fig. 2a. The half-wave poten-

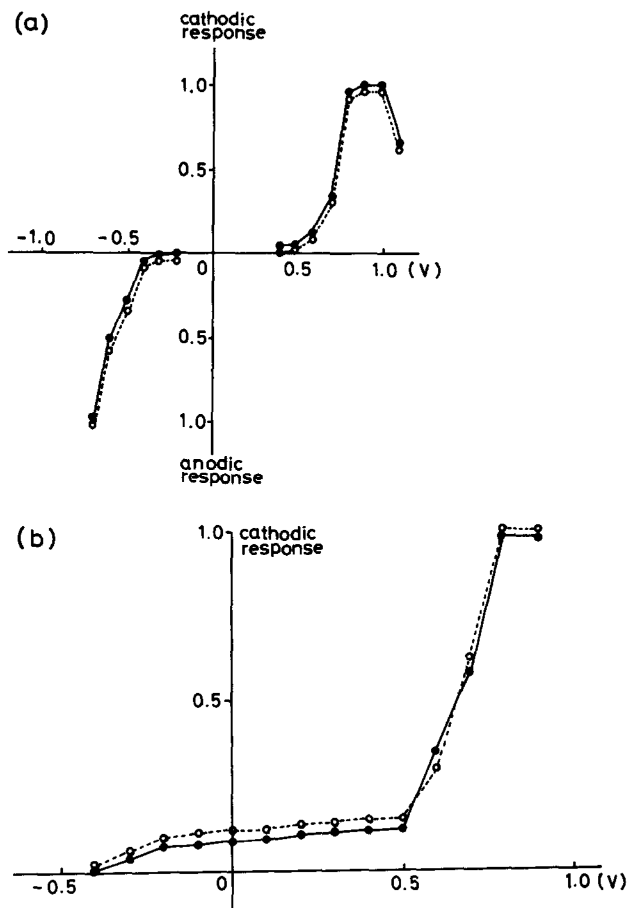

Fig. 2. Hydrodynamic voltammograms of mitomycin C and porfiromycin. (a) Cathodic and anodic responses obtained with a single electrode; (b) anodic response obtained by the serial use of the first electrode at −0.7 V.

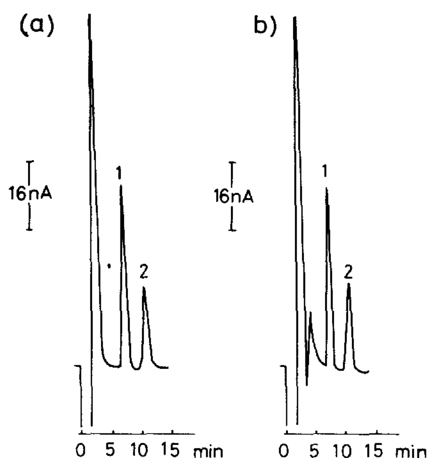

Fig. 3. Chromatograms of mitomycin C and profiromycin. (a) Authentic standards; (b) human serum. Peaks: 1 = mitomycin C (1000 ng/ml); 2 = porfiromycin (500 ng/ml).

tials ($E_{1/2}$) of both mitomycin C and porfiromycin were observed at +0.7 and −0.6 V in the cathodic and anodic responses, respectively. The hydrodynamic voltammograms with the second electrode combined with the serial use of the first electrode at −0.7 V are shown in Fig. 2b. The *p*-quinone structure in mitomycin C was quantitatively reduced with ease on the first electrode at −0.7 V to the *p*-hydroquinone, which in turn was oxidized to the *p*-quinone on the second electrode between −0.3 and +0.5 V. Mitomycin C can thus be detected with high selectivity even in the presence of electroactive compounds. The detection limit of mitomycin C was 1 ng (signal-to-noise ratio = 5 at 16 nA full-scale) at the applied potential of +0.3 V versus a reference electrode.

On the basis of these data, we attempted to determine mitomycin C in biological fluids by the direct injection method employing a column-switching technique. Typical chromatograms of mitomycin C and porfiromycin spiked into a buffer solution and drug-free human serum are illustrated in Fig. 3. No significant interfering peaks derived from biological substances were observed in the chromatogram. When the amount of mitomycin C was plotted against the peak-height ratio relative to porfiromycin, a linear relationship was observed in the range 10–100 ng of mitomycin C, the regression equation being $y = 0.0245x + 0.053$ ($n = 5$). The recovery rates of mitomycin C added to the control serum at four levels (200, 500, 700 and 1000 ng/ml) were examined according to the standard procedure. It is evident from the data in Table I that the proposed procedure is satisfactory with respect to accuracy and precision.

The present method was then applied to the determination of mitomycin C in serum from guinea-pigs given an intraperitoneal injection of mitomycin C. The changes in the serum level of mitomycin C during 6 h after administration are shown in Fig. 4: it reached a maximum of 670 ± 91 ng/ml at 15–30 min. The

TABLE I

RECOVERY OF MITOMYCIN C ADDED TO HUMAN SERUM

Concentration (ng/ml)		Recovery (mean ± S.D., $n=5$) (%)
Added	Found	
200	224 ± 14	110.0 ± 7.2
500	534 ± 61	106.8 ± 12.2
700	704 ± 36	100.5 ± 5.1
1000	1016 ± 84	101.6 ± 8.4

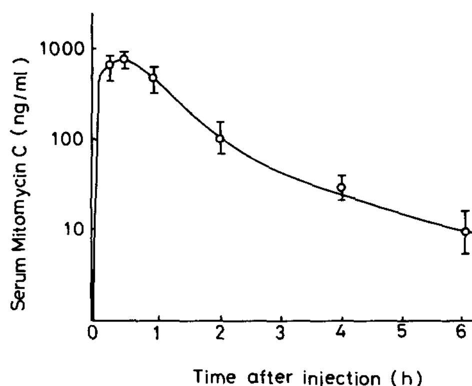

Fig. 4. Changes in the serum level of mitomycin C in guinea-pigs following intraperitoneal injection.

half-lives for drug elimination in α- and β-phases were calculated to be 18 and 105 min, respectively.

The proposed method for the determination of mitomycin C in serum proved to be satisfactory with respect to feasibility, sensitivity and reproducibility. Note that the use of a dual-electrode coulometric system involving an initial reduction followed by oxidation at +0.3 V would favour selective detection, eliminating interfering peaks, and hence the direct injection technique. Also, the sensitivity obtainable by this procedure is ten times better than that by UV detection.

Further applications to therapeutic drug monitoring for patients are being investigated in these laboratories, and the details will be reported elsewhere.

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