Journal of Chromatography, 164(1979) 404—406
Biomedical Applications

CHROMBIO. 402

Note

Determination of mitomycin C in biomedical specimens by high-performance liquid chromatography

AKIRA KONO*, YASUHIRO HARA, SANETOMI EGUCHI and MUTSUKO TANAKA

Kyushu Cancer Center, Notame, Minamiku, Fukuoka 815 (Japan)

and

YOSHIKAZU MATSUSHIMA

Faculty of Pharmaceutical Sciences, Kyushu University 62, Higashiku, Fukuoka 812 (Japan)

(Received May 15th, 1979)

Mitomycin C (MMC), an antitumor antibiotic, is currently used in the treatment of various tumors [1]. The therapeutic effectiveness of MMC should be related to its concentration in blood, body fluids and tissues, which in turn depends on the dose, method, and schedule of administration. Although a number of clinical investigations have proved its effectiveness against cancer in the intestinal region, monitoring its concentration in the time course of the drug therapy has hardly been explored. A rapid and sensitive method of its assay is required for such monitoring.

High-performance liquid chromatography (HPLC) is widely used in the separation, identification and determination of a large number of compounds. Recently HPLC was used to separate MMC from other mitomycins and its polar conversion products [2]. We have been working on the analysis of anticancer agents in biomedical specimen by means of HPLC [3] and have found this technique can be applied with much success in the monitoring of MMC.

The present paper describes the HPLC analysis of MMC in biomedical specimens, which would give the necessary information for the optimal administration schedule.

MATERIALS AND METHODS

Mitomycin C used in this investigation was kindly supplied by Kyowahakkokogyo (Tokyo, Japan). All solvents for HPLC and chemicals were certified grade and products of Wako (Osaka, Japan).

*To whom correspondence should be addressed.

A Waters Assoc. high-pressure liquid chromatograph Model 204 was used. The chromatograph was equipped with a Model 6000 solvent delivery system, a Model U6K universal injector, and a Model 440 UV detector. A Waters Assoc. μBondapak C_{18}/Porasil (8—10 μm particle size, 300 × 3.8 mm I.D.) reversed-phase column was used. The mobile phase was methanol—water (35:65). The pump was generally run at a flow-rate of 1.0 ml/min at a pressure of 1800 p.s.i.

Standard curves obtained by plotting the peak heights against the amounts of tain 1.0—25.0 ng MMC per μl. Samples were injected into the chromatograph in aliquots of exactly 1.0 μl with a 10-μl Hamilton syringe.

Human serum, ascites and urine for analysis were collected from patients administered MMC. MMC-free samples were from a healthy adult man. To 1.0 ml of material, 0.1 ml of 0.5 *M* NaH_2PO_4 and 8 ml of ethyl acetate were added and the sample was extracted with vigorous shaking. The organic layer was separated by centrifugation and evaporated to dryness using a water-bath at 30° and a water-pump vacuum. The residue was dissolved in 40—100 μl of methanol for analysis by HPLC.

RESULTS AND DISCUSSION

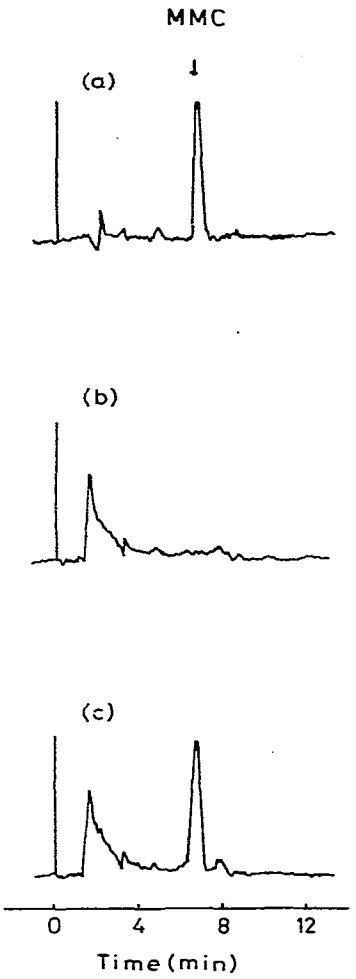
Fig. 1 shows chromatograms of (a) a standard MMC solution (10 ng/μl), (b) an extract of MMC-free serum, and (c) an extract of serum from a patient administered 10 mg of MMC. A sharp peak with a retention time of 6.6 min is readily identified as that of MMC. Column effluents were monitored at 365 nm. Comparable elution profiles were obtained by monitoring at 254 nm, though there was a slight interfering peak arising from endogenous serum components. Since detection at 365 nm gave higher absorbance and better resolution, the UV detector was operated at this wavelength for the determination of MMC.

Standard curves obtained by plotting the peak heights against the amounts of MMC injected were linear in the range 1.0—25.0 ng. It is estimated that 1.0 ng of MMC can easily be detected using the available detector. In the determination of MMC in solutions containing 10 ng, the standard deviation was 0.41 ng (n=7).

Appropriate amounts of MMC were added to MMC-free serum and these spiked standards were carried through the procedure. Plots of the peak heights against the amounts of spiked MMC gave straight lines, and recoveries of 93.8 ± 2.3% were obtained from comparison of the slopes with that obtained with the standard methanol solutions.

The present method permits the accurate determination of MMC in biological fluids at concentrations as low as 40 ng/ml and is suited for monitoring the drug in the therapeutic dose range (2—10 mg). The simple procedures allow a large number of analytical samples to be handled.

Fig. 2 shows examples of the time—concentration curves in the sera of a patient administered 10 mg MMC. These pharmacokinetic measurements of MMC in tumor-bearing patients are continuing. Correlation of the therapeutic effects to its concentrations in body fluids and tissues is under investigation. The details will be reported elsewhere.



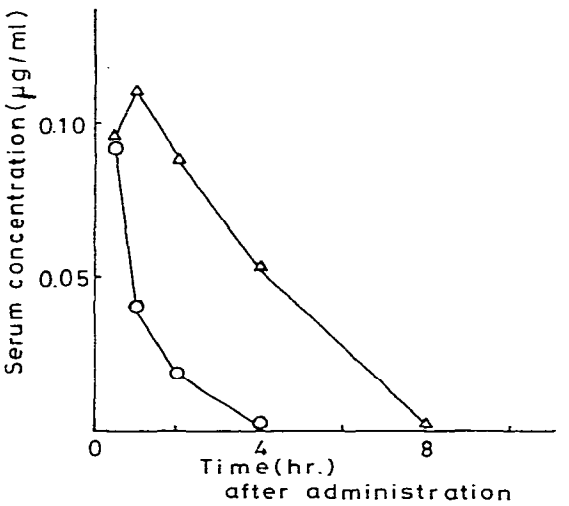


Fig. 1. Chromatograms of (a) methanolic solution of MMC (10 ng/μl), (b) an extract of MMC-free serum, and (c) an extract of serum collected 3 h after the intraperitoneal infusion of 10 mg of MMC to an adult man with intestinal cancer.

Fig. 2. Serum concentration of mitomycin C at various times after intravenous (○) or intraperitoneal (△) administration (10 mg) to a patient.

ACKNOWLEDGEMENT

This work was supported by a Grant-in-Aid for Cancer Research from the Ministry of Health and Welfare, Japan, for which we are grateful.

REFERENCES

1 S.T. Crooke and W.T. Bradner, Cancer Treat. Rev., 3 (1976) 121.
2 S.C. Srivastava and U. Hornemann, J. Chromatogr., 161 (1978) 393.
3 A. Kono, M. Tanaka, S. Eguchi, Y. Hara and Y. Matsushima, J. Chromatogr., 163 (1979) 109.