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Note

Determination of the biological response modifier MVE-2 (AD-022) in biological fluids by high-performance liquid chromatography

MICHAEL G. ROSENBLUM*, EVAN M. HERSH and TI LI LOO

Department of Clinical Immunology and Biological Therapy and The Department of Developmental Therapeutics, The University of Texas, M.D. Anderson Hospital and Tumor Institute at Houston, Houston, TX 77030 (U.S.A.)

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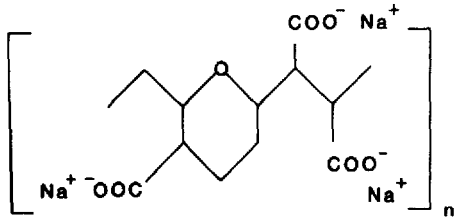
Maleic vinyl ether (MVE) copolymers are polyanionic macromolecules with broad molecular weights ranging from 1000 to 1,000,000 daltons. They have been shown to possess antitumor activity against several murine tumors [1—6] as well as antiviral activity against vaccina virus, herpes simplex virus, and influenza type A virus [7]. The putative mechanism of antitumor action of these agents is through the stimulation of the reticuloendothelial system and enhancement of endogenous interferon production [8—12].

Five MVE fractions having a discrete molecular weight range (from 12,500 to 52,600 daltons) were synthesized in 1974 and designated MVE-1 through MVE-5 [13]. Among these, MVE-2 (AD-022, Fig. 1), with an average molecular



MALEIC ANHYDRIDE - DIVINYL ETHER COPOLYMER
(AD-022, MVE-2)

Fig. 1. Structure of MVE-2.

weight of 15,500 daltons, was found to have antitumor and antiviral activity in murine model systems [14, 15] and is currently undergoing phase I

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clinical trials. In concert with these trials, we have developed a high-performance liquid chromatographic (HPLC) method for the analysis of MVE-2, as well as all of the other MVE polymers, in biological fluids in preparation for pharmacokinetic and metabolism studies of this agent in man.

MATERIALS AND METHODS

All materials purchased from regular commercial suppliers were of reagent grade or higher. High-purity water and distilled-in-glass methanol were purchased from Burdick and Jackson Labs. (Muskegon, MI, U.S.A.). All solvents were filtered and vacuum-degassed immediately before use. ^{14}C-Labeled MVE-2 (0.47 μCi/mg) was a generous gift from Adria Laboratories (Columbus, OH, U.S.A.).

Chromatography

All analyses were performed with a Waters Assoc. (Milford, MA, U.S.A.) liquid chromatograph consisting of a Model 710B sample processor, two Model M6000A pumps, a Model 720 system controller, a data module, and a Model 450 variable-wavelength UV detector. An analytical reversed-phase (5 μm particle size, silica matrix) short alkyl chain (C2,3) column (200 × 4.6 mm I.D.) from Custom L.C. (Houston, TX, U.S.A.) was used for all analyses. Solvent A consisted of 0.02 *M* Na_2HPO_4 adjusted to pH 4.0 with H_3PO_4. Solvent B consisted of 0.02 *M* Na_2HPO_4 (pH 4.0) in 80% methanol. The flow-rate was 2 ml/min, and a nonlinear gradient (curve 7) was generated from 0% B to 100% B over 25 min. The column eluate was monitored for UV absorbance at 220 nm.

Preparation of biological fluids

Plasma. Specimens (4 ml) containing MVE-2 were placed in 10-ml plastic centrifuge tubes and admixed rapidly with a 400-μl aliquot of 0.2 *N* sodium hydroxide and 4 ml of hot water (ca. 85°C). The samples were allowed to stand for 5 min and applied to mini-columns constructed from 1-ml blue plastic pipette tips (Eppendorf). Washed macroporous anion-exchange resin (AGMP-1, 100—200 mesh, from Bio-Rad Labs., Richmond, CA, U.S.A.) was added to a bed size of 2.0 × 0.5 cm. A small plug of glass wool prevented loss of the resin. After the samples were loaded, the columns were washed with 3 ml of water previously adjusted to pH 11 with sodium hydroxide. The columns were washed with 4 ml of distilled water, 2 ml of 1 *M* formic acid, and 3 ml of 8.5 *M* formic acid. MVE-2 was eluted from the column with 2.5 ml of 14 *M* formic acid. The 14 *M* acid wash was collected in plastic test tubes and reapplied to mini-columns containing washed Bio-Rex 70 cation-exchange resin (100—200 mesh, bed size 2.0 × 0.5 cm). The samples were washed through with 0.5 ml of distilled water. The column eluates were collected in plastic centrifuge tubes, a 50-μl aliquot of 0.1 *N* sodium hydroxide was added and the samples were lyophilized to dryness. The samples were reconstituted with 250 μl of buffer A and chromatographed as described above.

Urine. Urine specimens (4 ml) were similarly prepared without the addition of hot water. Samples were lyophilized, reconstituted and chromatographed as above.

Radiochemical techniques

The HPLC column eluates were collected at 30-sec intervals directly in glass scintillation vials after injection of radiolabeled MVE-2. Aquosol scintillant (12 ml, New England Nuclear, Boston, MA, U.S.A.) was added to each vial. The vials were counted in a Packard Tri-Carb liquid scintillation spectrometer Model 2650 to determine ^{14}C activity.

RESULTS AND DISCUSSION

The MVE-2 polymer is composed of macromolecules with a variety of molecular weights ranging from a few thousand to several hundred thousand daltons. However, the majority of molecular weights center at approximately 15,500 daltons. Nevertheless, as shown in Fig. 2 (top), this polymeric mixture can be chromatographed and eluted as a discrete peak (retention time 14.5 min) using a short alkyl chain reversed-phase column and the elution buffers and gradients described. The curved line in Fig. 2 shows the gradient profile. Injection of an equal amount of MVE-4 (M.W. 34,000 daltons) in this

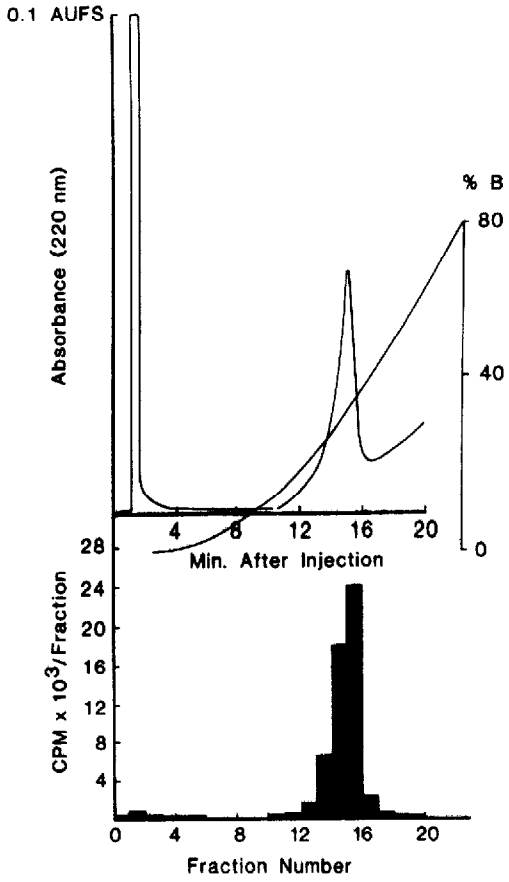

Fig. 2. HPLC analysis of MVE-2. Top panel shows the UV profile (220 nm) of the column eluate after an injection of unlabeled MVE-2 (100 μg total injected). Bottom panel shows the radioactive profile of the column eluate after injection of ^{14}C-labeled MVE-2. Recovery of injected radioactivity was 98 ± 4%.

chromatographic system resulted in an identical peak at 14.5 min, suggesting that this system may be useful for analysis of the entire MVE polymeric series. The chromatographic separation used in this analysis may consist of size exclusion, ion-exchange, normal-phase and reversed-phase partition modes. The relative contribution of each mode is unknown.

Co-injection with ^{14}C-labeled MVE-2, fractionation, and scintillation counting of the column eluate showed that most (96%) of the injected counts were in the MVE-2 peak (Fig. 2, bottom panel). The radioactivity in fractions 2—4 may represent unreacted ^{14}C-labeled maleic acid. Total recovery of injected radioactivity was 98 ± 4%.

A method for the extraction of MVE-2 from plasma and urine was developed using ion-exchange chromatography (see Materials and methods). This method was utilized in conjunction with the HPLC analysis of MVE-2. Standard curves for MVE-2 were constructed by the addition of various amounts of MVE-2 standard to plasma and urine aliquots. The lowest limits of detection for MVE-2 by this method were approximately 10 μg and 20 μg per ml plasma and urine, respectively. The recovery rate of MVE-2 from plasma was 73—85%, while the recovery rate from urine was 40—65%. For plasma, standard curves were linear ($Y = 0.35X + 3.07$, $r^2 = 0.99$) over the concentration range tested. Standard curves for MVE-2 in urine were also linear ($Y = 0.16X + 0.91$, $r^2 = 1$) over the concentration range tested. Intra-assay variability of triplicate samples averaged ±8% while inter-assay variability was ±10%. Incubation of MVE-2 with plasma and urine at 37°C for 24 h produced no evidence of drug degradation.

Analysis of both a plasma blank and a patient plasma sample drawn 5 min after MVE-2 administration (Fig. 3, 650 mg/m^2 dose) showed that the method described for MVE-2 analysis is sensitive enough for monitoring patient samples after drug administration. Studies are ongoing to determine the plasma pharmacokinetics and urinary excretion of MVE-2 in man. This method may

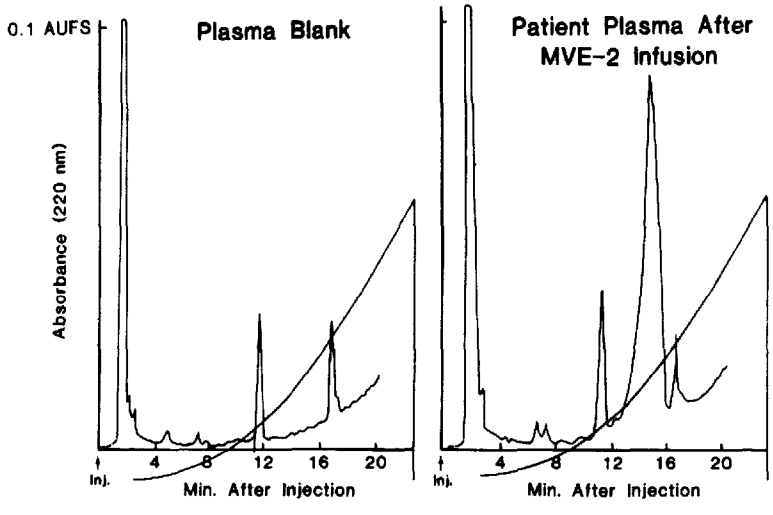

Fig. 3. Chromatographic profile of patient plasma prior to and after infusion of MVE-2. The concentration of MVE-2 in this patient sample withdrawn 5 min after the end of MVE-2 infusion was 100 μg/ml.

also be applicable to clinical pharmacology studies of other MVE polymers.

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