Determination of 8-Methoxypsoralen in Plasma by Scanning Fluorometry After Thin-Layer Chromatography

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A rapid and sensitive method is described for determining 8-methoxypsoralen in plasma. Plasma samples are acidified with 6 mol/liter and heated in a boiling water bath to release the plasma-bound drug nondestructively. It then is extracted into a solvent mixture consisting of benzene/ethyl acetate (9/1 by vol). The solvent phase is separated, evaporated, and an aliquot of the dissolved residue is thin-layer chromatographed, with benzene/ethyl acetate (9/1) as developing solvent. The plate is dried and the spots, made visible under ultraviolet light (320–400 nm), are scanned. The smallest amount detectable is 20 ng; the overall analytical recovery from plasma is 84%. We used the method to determine the drug in the plasma of rabbits after intravenous and oral administration of 10 mg, and in one patient after an oral dose of 30 mg.

Additional Keyphrases: methoxsalen • photochemotherapy • vitiligo • psoriasis • rabbits

Psoralen and 8-methoxypsoralen (8-MOP), plant furcoumarins, have been used in India and Egypt since antiquity for the treatment of vitiligo (1, 2). These naturally-occurring drugs, when activated by long-wave ultraviolet light (320–400 nm, with peak at 360–365 nm), are clinically effective in treating vitiligo, psoriasis, and mycosis fungoides (3–7). Patients receiving the drug orally are subsequently exposed to high-intensity ultraviolet light or sunlight, a type of treatment called photochemotherapy (5). Although 8-MOP can be spectrophotometrically determined in plasma,1 this method is too insensitive. A gas chromatographic method (8) and a high-pressure liquid-chromatographic method (9) have been reported for its determination in blood. Recently, Steiner et al. (10) reported a thin-layer chromatographic method for determination of 8-MOP in serum.

In the present method we used a combination of thin-layer chromatographic and scanning-fluorometric techniques.

Materials and Methods

Apparatus

The SD 3000 Chromatogram Analysis System (Schoeffel Instrument Corp., 24 Booker St., Westwood, N.J. 07665) was used to scan the thin-layer plates. The activation wavelength was 310 nm and the fluorescence, measured at 540 nm, was recorded by use of the reflectance mode. The range of sensitivity was set at 10, gain at 4, and strip-recorder speed at 10 cm/min. This system includes double-beam ratio recording, hence background subtraction is automatic.

Thin-layer Chromatography

Precoated glass thin-layer plates (no. 1011, 20 × 20 cm, with 250-μm silica gel G coat; Analtech, Newark, Del. 19711) were activated by heating at 100 °C for 20 min before use. A spotting guide (Fisher Scientific Co., Silver Spring, Md. 20910; cat. no. 4010) and disposable "Micropipettes" (Analtech, cat. no. MP-140), calibrated to contain 1 to 5 μl, were used for sample application on thin-layer plates. The plates were developed with benzene/ethyl acetate (9/1 by vol). A 4-W ultraviolet lamp (Ultraviolet Products, Inc., San Gabriel, Calif. 91778) was used to make the resulting spots visible.

All solvents and reagents used in this study were of Fisher certified grade or ACS grade (obtained from Fisher Scientific Co., Silver Spring, Md. 20910).

The human subject2 in this study was a patient undergoing photochemotherapy for vitiligo in our Department of Dermatology.

Procedure

To 5-ml samples of blood-bank plasma we added 8-MOP to give concentrations from 0 to 400 μg/liter, for use in measuring analytical recovery and as standards.

The drug was administered to rabbits either via the intravenous or oral route. Blood was sampled at the time of administration of 10 mg of 8-MOP and subsequently at 15 min and 30 min and 1, 1.5, 2, 4, 6, and 24 h from each of three rabbits. The blood was placed in heparinized test tubes and the plasma was separated.

Blood was drawn from the patient by venipuncture at the time of administration of 30 mg of 8-MOP (three 10-mg capsules; Paul B. Edler Co., Bryan, Ohio 43506) and subsequently at 30-min interval up to 4 h, and the plasma was separated by centrifugation.

To 5 ml of plasma in 50-ml glass-stoppered tubes, 5 ml of distilled water and 5 ml of 6 mol/liter HCl were added and the contents were mixed. The tubes were placed in a boiling water bath for 30 min. After cooling, 10 ml of a mixture of benzene/ethyl acetate (9/1 by vol) was added. The tubes were then stopped and shaken on a 115-V mechanical shaker for 20 min at 160 strokes/min. After centrifugation (1465 × g for 20 min) the supernatant solvent phase was transferred into appropriately marked test tubes, 10 ml of fresh solvent mixture was added to each extraction tube, and the extraction was completed.

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2 Human subject in this study was a patient undergoing photochemotherapy for vitiligo at the Howard University Hospital. Institutional permission for use of human subjects for this study was obtained from the Human Research Review Committee of Howard University.
repeated. The combined supernatant fractions were evaporated in a gentle stream of air in a hood. The residue in the test tubes was reconstituted in 20 μl of methylene chloride and 10 μl was spotted on thin-layer plates, dried with a warm-air blower, and developed with benzene/ethyl acetate (9/1 by vol). When the solvent front had moved to about 1 cm from the top, the plates were removed from the developing tank and allowed to dry in a hood. On each thin-layer plate one standard 8-MOP spot (1 μl, 1 μg), a spot of an impure preparation (E17975, Paul B. Elder Co.; 1 μl or 2 μg), and a spot of methylene chloride only were applied for checking the Rf of 8-MOP and the quality of separations of components on the thin-layer plates.

Preparation of Standard Curves and Results

Onto thin-layer plates we applied 100, 200, 500, 1000, and 2000 ng of 8-MOP (in 10 μl) and developed the plates. The 8-MOP spots were made visible under long-wavelength ultraviolet light, scanned, and recorded. The peak heights, in millimeters, were plotted vs. the amount of 8-MOP, in nanograms. Each point was determined from an average of six such determinations for each amount of 8-MOP. A straight-line relationship was obtained (Figure 1, solid line). The broken line in Figure 1 shows the standard curve for the drug added to and recovered from plasma.

Figure 2 shows 8-MOP concentrations in the plasma of rabbits weighing 5 kg after intravenous and oral doses of 10 mg. The concentration is greatest in 15 min in the case of intravenous injection and in 60 min in the case of oral administration of the drug. The drug evidently is completely metabolized in 24 h in rabbits; 8-MOP could not then be detected in the plasma. The maximum concentration peak after an intravenous dose is threefold that for the same dose orally.

The 8-MOP concentration in the plasma of the human subject after a 30-mg oral dose of the drug reached a maximum in 2 h and had declined sharply by 3 h. The peak plasma concentration (3 mg/liter) was at 2 h. Table 1 shows that the analytical recovery of the drug from plasma is much lower if the heat and acid treatments are omitted.

The identity of the measured compound from rabbit plasma was demonstrated by scraping the appropriate spot from the thin-layer plates, extracting the scrapings with methylene chloride, chromatographing the extract in two dimensions in the systems benzene/ethyl acetate (9/1 by vol) and CHCl3/CH3OH (50/1 by vol), and comparing the results with those for authentic 8-MOP. In addition, the authentic 8-MOP and the compound isolated from rabbit plasma were subjected to gas-chromatographic and mass-spectrometric study and both had similar elution and fragmentation characteristics (Figure 3). Figure 3c shows a single peak of the product from rabbit plasma eluted from gas-chromatographic column at 220 °C; Figure 3d shows the mass-spectrometric fragmentation pattern of the same. The gas-chromatographic and mass-spectrometric elution pattern for authentic 8-MOP are shown in Figure 3a and b. The latter is similar to the published mass-spectral fragmentation pattern of the compound (11).
Discussion

Development of a regular fluorometric method for 8-MOP is not feasible because of solvent quenching. We found that 40 to 60% of 8-MOP is tightly bound to plasma proteins and cannot be extracted into a solvent or dialyzed out completely except after the acid and heat treatments described. Fortunately, furocoumarins are stable under these conditions (11). The method is reproducible and applicable to determination of 8-MOP in plasma. Its highest concentration in plasma was at 2 h after the oral dose to the human subject, a finding consistent with the usual photochemical response in man. We did not attempt to identify metabolites of 8-MOP. Analysis for 8-MOP in plasma will permit establishment of correlation between therapeutic dosage, plasma 8-MOP concentration, and clinical response in phototherapies.

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References