Journal of Chromatography, 274 (1983) 434—440
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 1613

Note

Determination of razoxane by high-performance liquid chromatography

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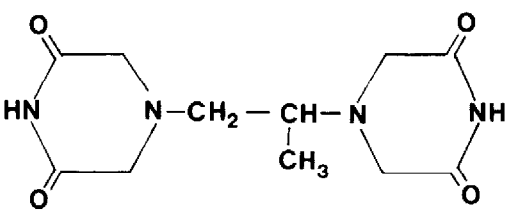
(First received October 1st, 1982; revised manuscript received December 13th, 1982)

Pharmacokinetic studies of antineoplastic drugs have contributed to an understanding of the relationship between drug concentration, intensity of pharmacological effects and appearance of adverse effects.

The anticancer agent razoxane (ICRF 159; NSC 129943; (±)-1,2-bis(3,5-dioxopiperazin-1-yl)propane; Fig. 1) has been in clinical use for over a decade but to date no simple and accurate assay has been available to permit measurements of important pharmacokinetic parameters.



Razoxane

Fig. 1. Structure of razoxane.

Apart from assays using the radiolabelled drug [1, 2] the only sensitive technique reported for the measurement of razoxane has been the gas—liquid chromatography—mass fragmentography (GLC—MF) method of Sadeé et al. [3]. This assay however, involves a long and potentially explosive methylation step with diazomethane and is therefore unsuitable for extensive in-

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0378-4347/83/$03.00

vestigations. These difficulties led us to examine the suitability of high-performance liquid chromatography (HPLC) to detect razoxane in plasma and serum samples from animals and patients.

An HPLC assay method has recently been developed for the *d*-enantiomer of razoxane, ICRF 187, by Earhart and Tutsch [4] and the method described below for razoxane was adapted from that for ICRF 187. It has been found to be simple, sensitive to nanogram levels of razoxane as well as accurate and reproducible. However it differs from the method of Earhart and Tutsch in the use of an ultrafiltration step for plasma and serum samples prior to HPLC analysis.

EXPERIMENTAL

HPLC separation was carried out on a Waters Assoc. (Milford, MA, U.S.A.) liquid chromatograph consisting of two Model 6000A solvent pumps, a 660 programmer, a U6K injector and an LKB Bromma 2138 UV detector. Signals from the detector were recorded by an LKB Bromma 2210 two-channel recorder. All separations were carried out on a Varian MCH-10 analytical column (C_{18} reversed-phase bonded on 10-μm silica gel; 30 cm × 4 mm) preceded by a guard column filled with Whatman ODS reversed-phase pellicular packing.

Materials

Millipore-filtered, degassed distilled water was used in all experiments. Potassium phosphate, sodium hydroxide (BDH, Poole, Great Britain) were all of analytical grade. Methanol (Rathburn Chemicals, Edinburgh, Great Britain) was HPLC grade. Razoxane was a gift from Imperial Chemical Industries (Macclesfield, Great Britain).

Animal studies

Rat plasma. Female Sprague—Dawley rats weighing 200 g were starved overnight and dosed with a single dose of 21.75 mg/kg razoxane in neutralized aqueous solution either orally or intravenously.

Blood was obtained by cardiac puncture at various times after razoxane administration using three rats per time point. The blood was centrifuged in heparin at 7000 *g* for 5 min at 4°C to obtain plasma which was assayed for razoxane.

Rat serum. Female Sprague—Dawley rats weighing 200 g were starved overnight and dosed with a single dose of 21.75 mg/kg razoxane orally or 10.9 mg/kg razoxane intravenously in a neutralised aqueous solution. Blood was taken by cardiac puncture at various times after razoxane administration and allowed to clot at 4°C. The pooled serum obtained at each time point was assayed for razoxane.

Rat cerebrospinal fluid. Razoxane (100 mg/kg) was administered intraperitoneally to rats of approx. 200 g weight. At various times after they were anaesthetised with sodium pentobarbital (40 mg/kg), blood and cerebrospinal fluid (CSF) were taken and the animals killed. Blood was removed and plasma prepared as described; CSF was obtained by percutaneous sampling from the cisterna magna [5] and placed in solid carbon dioxide prior to analysis.

Human studies

Patients with colorectal carcinoma received one tablet containing 125 mg razoxane and blood was taken via an indwelling intravenous cannula at 0, 1, 2, 4 and 24 h following razoxane administration. The blood was allowed to clot at 4°C to obtain serum, which was then assayed for razoxane.

Ultrafiltration procedure

Plasma and serum samples were filtered prior to HPLC analysis in Chemlab Ultrafiltration cells (Model C10) (Chemlab, Cambridge, Great Britain) through UM05 Diaflow membranes (Amicon, MA, U.S.A.) at 2 bar nitrogen pressure.

Rat CSF which contains minimal amounts of protein required no ultrafiltration prior to assay.

HPLC analysis

A 20-μl aliquot of the ultrafiltrate was injected onto the column. Samples were eluted in 20% methanol in 0.01 *M* phosphate buffer, pH 7.1 (helium degassed) at 98 bar pressure and a flow-rate of 1 ml/min. The absorbance of the eluate at 206 nm was recorded, razoxane peak heights were measured and values were read off standard curves constructed for plasma, serum and CSF samples.

RESULTS AND DISCUSSION

Quantitative aspects of the method

A typical separation of razoxane is illustrated in Fig. 2. Razoxane had a retention time of 5.4 min under these conditions of assay and the detection limit was 0.1 μg/ml. The Model 2138 UV detector response was linear over the range of razoxane concentrations studied in these experiments (0—250 μg/ml).

Recovery of razoxane was investigated by the addition of known concentrations of razoxane to 1-ml aliquots of rat serum or plasma samples immediately prior to ultrafiltration and prior to direct sample injection onto the HPLC column. This showed the razoxane loss during the ultrafiltration step to 38.6%. This poor recovery may be attributed to non-specific binding of the drug to the material of the membrane. Samples were assayed in quadruplicate and could be assayed with a reproducibility of ± 3.3%.

No loss of razoxane was seen following storage of the samples for up to 12 weeks at —70°C prior to analysis.

Animal studies

No razoxane was detectable in plasma, serum or CSF at time zero. Fig. 3 shows the plasma levels of razoxane following administration of 21.75 mg/kg razoxane by the oral and intravenous routes against time.

Following intravenous administration of the drug, plasma levels of razoxane appeared to follow a biphasic pattern of decay (Fig. 3). The initial and terminal phase half-lives were calculated to be 11.7 ± 2.4 min and 40.3 ± 3.8 min, respectively. These values correlated well with work of Sadeé et al. [3] who demonstrated a plasma elimination half-life of 40—45 min for razoxane, and

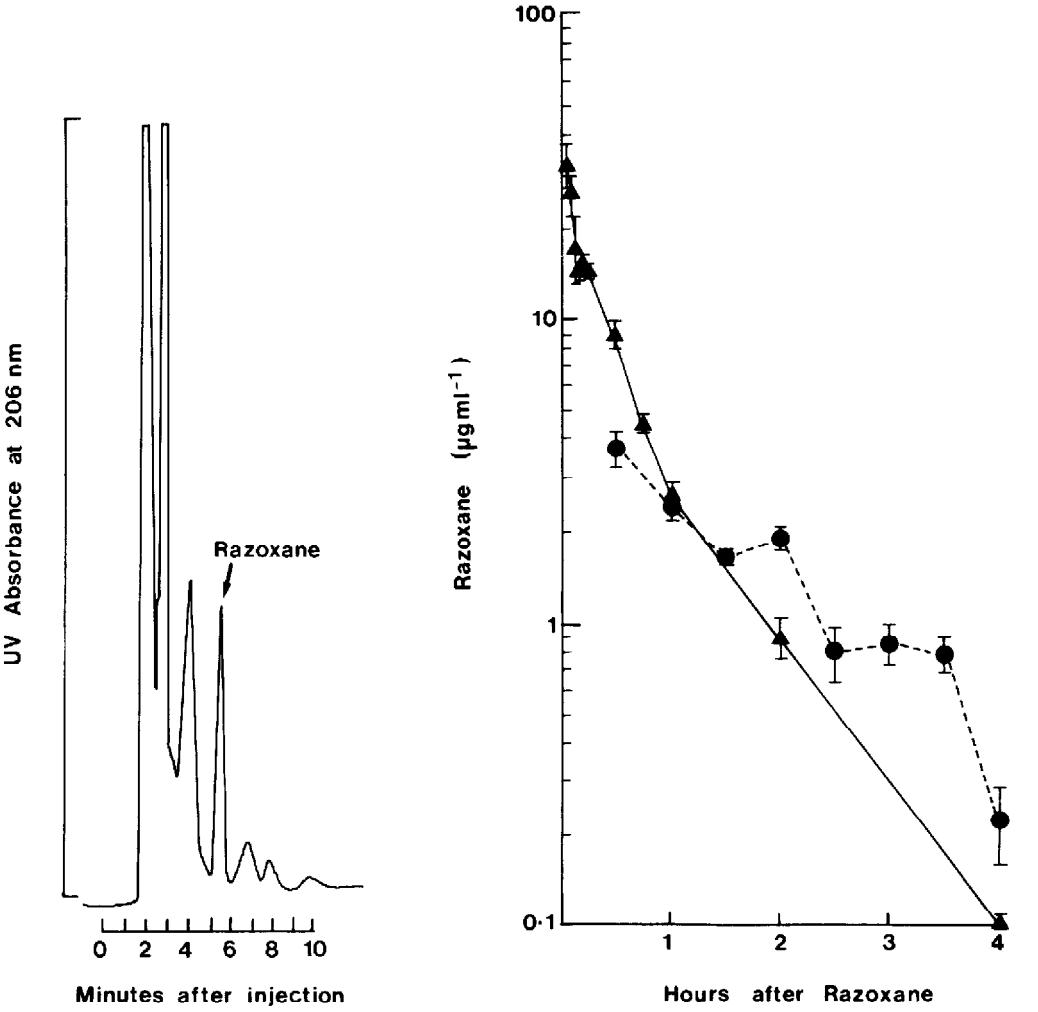

Fig. 2. Typical chromatogram of an ultrafiltrate of plasma/serum following razoxane administration.

Fig. 3. Plasma concentration of razoxane at various times after intravenous (▲) or oral (●) administration of 21.75 mg/kg razoxane in neutralised aqueous solution. The data presented are mean values ± S.E.M. for three rats per time point.

similar studies by Field et al. [2] in which a plasma half-life of about 30 min was found. Complete elimination of razoxane occurred by 8 h.

Following oral administration of an equal quantity of razoxane, a peak plasma level of 3.7 μg/ml was seen within 30 min of drug treatment. The plasma levels then declined monoexponentially with a half-life of 78.4 ± 6.8 min; complete elimination occurred by 8 h.

The ratio of the areas under the plasma concentration time curves for oral versus intravenous administration of razoxane is 0.53 suggesting that the bioavailability of oral razoxane is limited.

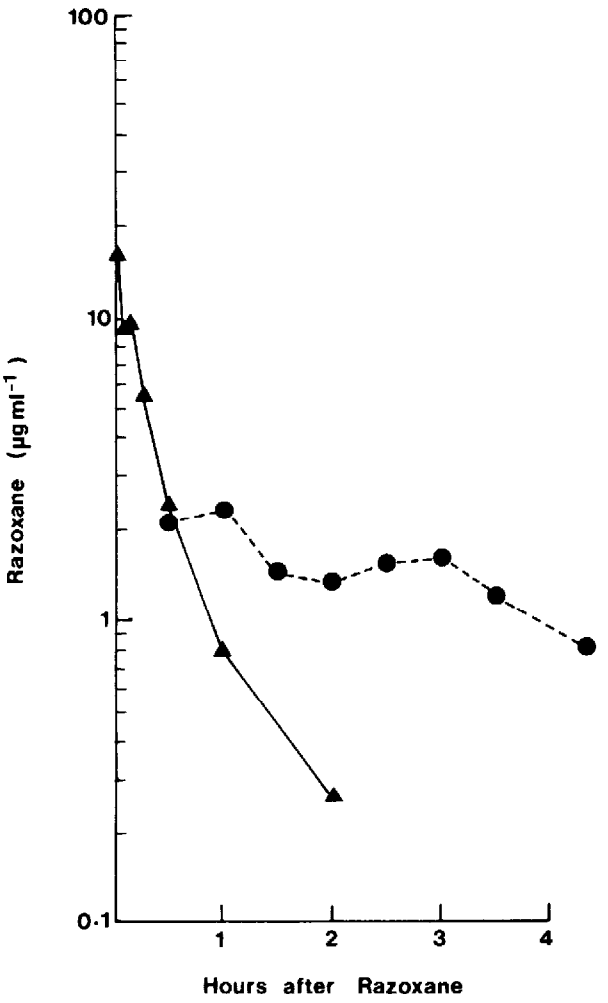

Fig. 4. Serum concentration of razoxane at various times after intravenous administration of 10.9 mg/kg razoxane (▲) or oral administration of 21.75 mg/kg razoxane (●) in neutralised aqueous solution. The data presented are values from pooled samples of four rats per time point.

A similar pattern was seen for razoxane levels in rat serum (Fig. 4). Following administration of 10.9 mg/kg razoxane intravenously values of $t_{½}$ α of 8.1 min and $t_{½}$ β of 42.2 min correlated well with the values of these parameters in plasma. Complete elimination occurred by 4 h.

Following oral administration of 21.75 mg/kg razoxane, a peak serum level of 2.3 μg/ml was achieved within 1 h. Serum levels then declined monoexponentially with a half-life of 157 ± 27 min.

Fig. 5 shows razoxane plasma and CSF concentrations following intraperitoneal administration of the drug. Razoxane was absorbed rapidly from the peritoneal cavity reaching a peak plasma concentration of 12 μg/ml within 15 min. Razoxane levels then declined monoexponentially with a half-life of 96 min; complete elimination occurred by 8 h.

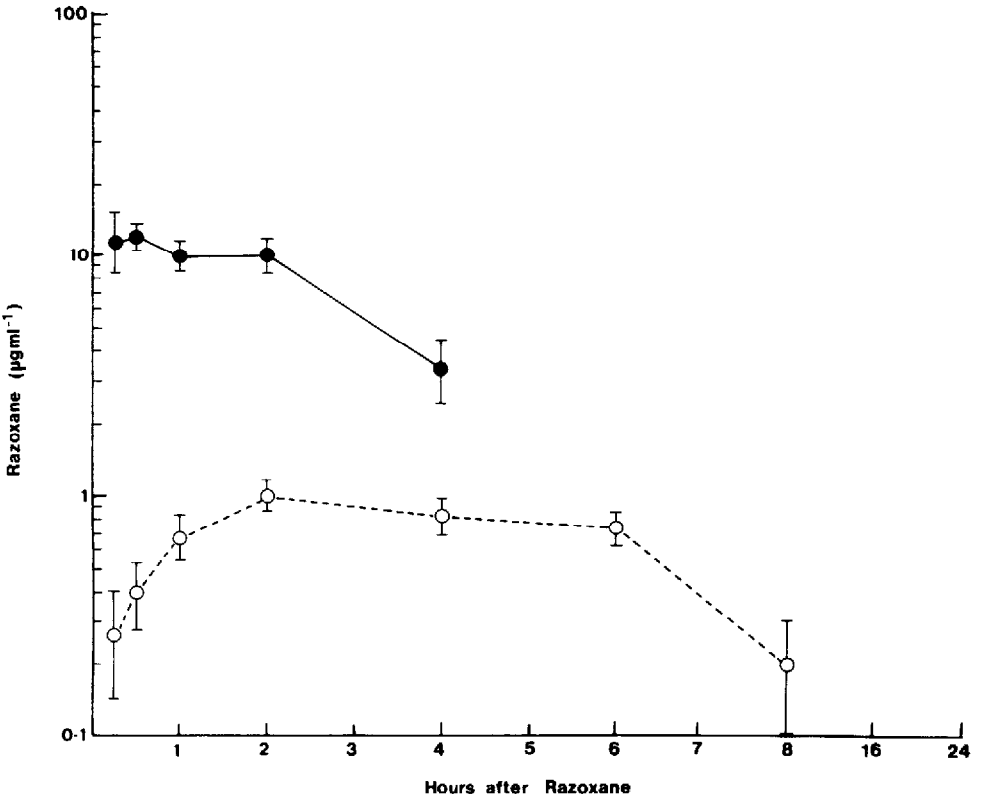

Fig. 5. Plasma (•) and cerebrospinal fluid (○) concentrations of razoxane after intraperitoneal administration of 100 mg/kg razoxane suspended in 0.5% carboxymethylcellulose in 0.9% sodium chloride. The data presented are mean values ± S.E.M. for 2—4 rats per time point.

Human studies

Preliminary experiments to measure razoxane levels in the serum of cancer patients treated with razoxane have so far been unsuccessful because the serum levels of razoxane seen were below the detection limit of the assay. Further attempts are being made to measure razoxane levels following administration of higher doses of razoxane under more controlled conditions, such as starving the patients and the use of patients with no prior drug therapy.

Thus, the use of a method involving HPLC and ultrafiltration has enabled the measurement of razoxane levels in the plasma, serum and CSF of rats. The method is easy to use, gives reproducible results and appears suitable for use in establishing pharmacokinetic data for razoxane. It also has potential for use in routine clinical investigations.

ACKNOWLEDGEMENT

The authors wish to thank Mr. D. Newell for his assistance in analysis of the pharmacokinetic data for razoxane.

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