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REVERSED-PHASE LIQUID CHROMATOGRAPHIC DETERMINATION OF IDARUBICIN AND ITS 13-HYDROXY METABOLITE IN HUMAN PLASMA

STAFFAN EKSBORG* and BIRGITTA NILSSON

Karolinska Pharmacy, P.O Box 60024, S-104 01 Stockholm (Sweden)

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SUMMARY

A method is given for the determination of idarubicin and its main metabolite, idarubicinol, in plasma from cancer patients. Idarubicin and idarubicinol are extracted from 2-ml samples of buffered plasma (pH 8.1) using chloroform–1-heptanol (9 1). After reextraction into phosphoric acid (0.1 *M*), separation is performed by reversed-phase liquid chromatography on a LiChrosorb RP-2 column (5 μm) with a mobile phase of acetonitrile–water, acidified with phosphoric acid. The absolute recovery in the range 5–100 ng/ml was greater than 83% with a precision better than 8% (relative standard deviation), using photometric detection at 484 nm. Proper handling of whole blood samples containing idarubidin is essential to avoid metabolic conversion into idarubicinol. Prolonged storage of the drug and its main metabolite under alkaline conditions should be avoided to prevent chemical degradation.

INTRODUCTION

Idarubicin (Imi 30; 4-demethoxydaunorubicin) is a synthetic anthracycline derivative (Fig. 1). Imi 30 is an active agent in acute leukaemia, malignant lymphoma and multiple myeloma [1–5]. In preclinical studies, Imi 30 has demonstrated several potential advantages over daunorubicin, including antitumour activity after oral administration and reduced cardiotoxicity [6]. Imi 30 is extensively metabolized to its 13-hydroxy derivatives (Imi 30-OH; 4-demethoxydaunorubicinol) (Fig. 1) [6]. Imi 30-OH appears to have a major role in the biological activity of Imi 30, since this metabolite has a high cytostatic activity in several in vitro test systems [7]. This observation is in accordance to what has previously been observed for several other anthracyclines in clinical use, e.g. adriamycin, daunorubicin and farmorubicin.

Several procedures for the determination of Imi 30 in plasma based on reversed-phase liquid chromatography (LC) in isocratic or gradient mode with

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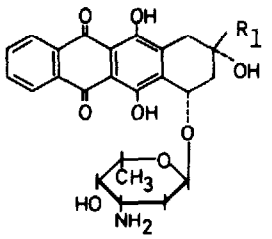

Fig. 1. Structural formulae of idarubicin ($R_1 = COCH_3$) and idarubicinol ($R_1 = CH(OH)CH_3$).

fluorometric detection have been described [3,8–10]. Conditions for the pre-chromatographic procedures, including the handling of patients' plasma samples, have, however, not been examined. The importance of the proper handling of plasma samples and optimization of the extraction procedures in the analysis of anthracyclines has previously been pointed out [11,12].

This paper gives a method for the determination of Imi 30 and Imi 30-OH in plasma from cancer patients, based on reversed-phase LC with photometric detection. The chemical stability of Imi 30 in buffers and the metabolic conversion of Imi 30 into Imi 30-OH in whole blood samples have been studied extensively, as have the extraction properties of Imi 30 in a two-phase system.

EXPERIMENTAL

Chemicals

Idarubicin and 13-hydroxy-idarubicin were kindly supplied by Farmitalia Carlo Erba (Täby, Sweden). Desipramine was kindly supplied by Hässle (Mölndal, Sweden). The organic solvents used were of Uvasol grade (Merck, Darmstadt, F.R.G.). The chromatographic support, 5-μm LiChrosorb RP-2 (Merck), was packed in 150 mm × 3.2 mm I.D. columns made of stainless steel.

Apparatus

The concentration of idarubicin in the partition experiments was measured by an Aminco-Bowman 4-8202 B spectrofluorometer, measuring at 436 nm/550 nm.

Absorption spectra were recorded on a Shimadzu UV-160 recording spectrophotometer. An Orion Research Model 701 digital pH meter equipped with an Ingold combined electrode (type 401) was used.

The chromatographic detectors used were the spectromonitor D from LDC/Milton Roy, operating at 484 nm and the Shimadzu RF-530 fluorescent HPLC monitor, operating at 254 nm/530 nm. A Rheodyne Model 7125 injection valve was used with a loop of 100 μl.

Incubation of whole blood samples

To 4-ml whole blood samples (heparinized) were added 100 μl of 10^{-2} *M* phosphoric acid containing idarubicin (50 μg/ml). After the addition of 100 μl of 10^{-2} *M* sodium hydroxide the samples were incubated at 0, 20 and 37°C for 5 min to 6 h. At the end of the incubation period the samples were centrifuged at 4000 *g*

for 10 min. The plasma fractions were carefully aspirated and stored at −70 °C until analysis for Imi 30 and Imi 30-OH.

Partition experiments

Imi 30 was initially dissolved in aqueous (citrate) buffers to an initial concentration of 10^{-7} *M*. The partition experiments were performed in centrifuge tubes using equal phase volumes (10 ml) and mechanical shaking for 30 min in a thermostatted bath (25.0 ± 0.2 °C). After centrifugation, the phases were separated by a capillary siphon. The concentration of Imi 30 was measured fluorometrically in both phases; in the aqueous (buffer) phase directly and in the organic phase (chloroform–1-pentanol, 9:1) after reextraction into 0.1 *M* phosphoric acid. The spectrofluorometer was standardized against a solution of Imi 30 prior to each measurement (cf. ref. 13). The symbols used in the evaluation of constants are given in ref. 13.

Stability of idarubicin in buffer solutions

The stability of Imi 30 (1 μg/ml) was studied in citrate (pH 4.01, 5.40), phosphate (pH 2.31, 7.01–8.89, 9.28) and carbonate (pH 9.04) buffers with an ionic strength (μ) of 0.1 The experimental temperature was 25.0 ± 0.2 °C. All solutions of Imi 30 were protected from daylight during the experiments.

Drug administration

The drug was dissolved in distilled water and administered as a 3.0-min intravenous infusion, or orally in capsules at doses of 10 mg/m^2 and 35 mg/m^2, respectively.

Plasma samples from patients

Blood samples (5–7 ml) from patients treated with Imi 30 were collected in glass test-tubes (Vacutainer®) containing 250 I.U. of heparin (freeze-dried) immediately prior to and at appropriate times after start of drug administration. The samples were immediately cooled in an ice-bath and centrifuged at 4000 *g* for 10 min. The plasma fraction was carefully aspirated and stored at −70 °C until analysis.

Analytical procedure

Extraction procedure. A 2.00-ml sample of plasma was mixed with 0.2 ml of phosphate buffer (pH 8.1, $\mu = 1.0$), and extracted with 7.00 ml of cloroform–1-heptanol (9:1) for 10 min. After centrifugation, 5.00 ml of the organic phase was reextracted with 300 μl of 0.1 *M* phosphoric acid containing 5 μg/ml of desipramine. The aqueous (upper) phase from the extraction procedure was transferred to a centrifuge tube with a tapered base (0.2 ml) containing 2 ml of hexane. (This step is included to facilitate the transfer of the aqueous phase into the chromatographic column without contamination with organic phase.)

Liquid chromatographic isolation and quantification. Part of the aqueous (lower) phase (100 μl) was injected into the chromatographic column (5-μm LiChrosorb RP-2; mobile phase, acetonitrile–water–0.1 *M* phosphoric acid (32:58:10); mo-

bile phase flow-rate, 0.6–0.8 ml/min). The eluate was monitored photometrically at 484 nm, with quantification based on peak-area measurements and external standardization.

RESULTS AND DISCUSSION

Chemical stability

The chemical stability of Imi 30 in aqueous buffers is strongly dependent on pH (Fig. 2). The half-life of Imi 30 at pH 2.3 is ca. 45 days and at pH 7.7 ca. 8.3 h. The degradation of Imi 30 under alkaline conditions is somewhat faster than the degradation of adriamycin [12]. Under the conditions used in the extraction procedure (pH 8.1), 80% of Imi 30 remains intact after storage for 1 h.

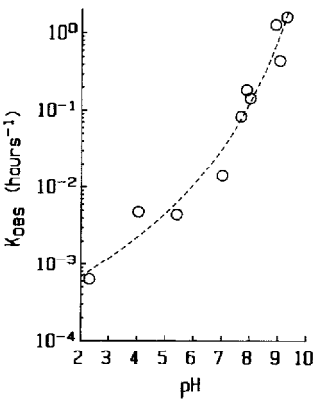

Fig. 2. Dependence of the stability of idarubicin on pH: temperature, 25.0 ± 0.2°C. (K_{OBS} is the observed pseudo first order degradation rate constant.)

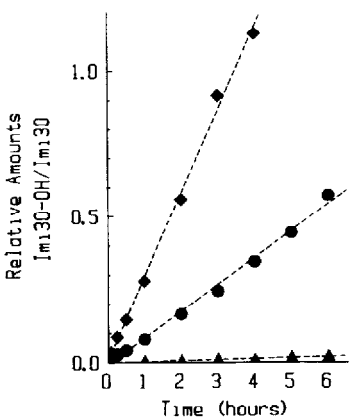

Fig. 3. Time course for metabolic transformation of Imi 30 into Imi 30-OH. ▲ = 0°C; ● = 20°C; ◆ = 37°C. The initial concentration of Imi 30 was 1 μg/ml.

Metabolic degradation of Imi 30 in whole blood samples

The results from incubation studies in whole blood samples at 0, 20 and 37°C are presented in Fig. 3. Imi 30, like adriamycin and daunorubicin, is enzymically reduced in whole blood samples [11]. The rate for the metabolic conversion of Imi 30 into Imi 30-OH is of the same order of magnitude as previously been observed for the conversion of daunorubicin into daunorubicinol [11]. The transformation of Imi 30 in blood samples from patients can be arrested by immediate cooling of the samples in an ice-bath, followed by isolation of the plasma fraction by centrifugation.

Liquid–liquid partition of Imi 30 in a two-phase system

The liquid–liquid distribution of the anthracyclines in a two-phase system is strongly dependent on the pH in the aqueous phase and also on the concentration of the compounds in the aqueous phase, as the result of dimerization and tetramerization processes [13]. Under the conditions used for the extraction experiments in the present study, however, Imi 30 can be assumed to be present as monomer only. A plot of $1/D$ versus ^{a}h (Fig. 4) showed a linear relationship. From the slope was calculated $\log k_d k'_2 = -4.55$, i.e. equal amounts of Imi 30 are present in the organic and aqueous phases at pH 4.55 in partition experiments using equal phase volumes. A comparison with extraction data in ref. 13 indicated that Imi 30 is more lipophilic than daunorubicin.

To avoid an interfering peak in the chromatogram caused by 1-pentanol, chloroform–1-heptanol was used for the extraction of Imi 30 and Imi 30-OH. This modification probably does not affect the magnitude of the distribution constants [14].

Calculations based on the determined distribution ratio showed that more than 99% of Imi 30 is transferred to the organic phase in the initial extraction step, and ca. 95% is reextracted into the acidic aqueous phase. However, to avoid adsorption losses in the reextraction step addition of desipramine was essential [14].

The amount of Imi 30-OH available was insufficient for extraction experiments. Calculations based on determined distribution ratio for Imi 30 and data

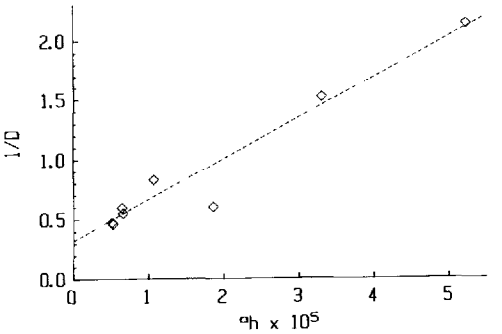

Fig. 4. Evaluation of the distribution constant for idarubicin. Organic phase, chloroform–pentanol (9:1); aqueous phase, buffers (μ=0.1); D=distribution ratio (C_{org}/C_{aq}).

in ref. 13 suggest that Imi 30-OH is quantitatively extracted under the analytical conditions used.

Liquid chromatography

Anthracyclines with only minor differences in chemical structure can easily be separated by reversed-phase LC in isocratic mode [15]. For the separation of intact drugs and their corresponding 13-hydroxy metabolites, LiChrosorb RP-2 with a mobile phase of acetonitrile–diluted phosphoric acid was more suitable. The retention of Imi 30 and Imi 30-OH, expressed as the capacity factor, was strongly dependent on the concentration of acetonitrile in the mobile phase (Fig. 5). Baseline separation of the drug and the metabolite was possible within 8 min, using a mobile phase containing 32% of acetonitrile.

Imi 30 and Imi 30-OH have identical absorption spectra in the range 300–550 nm. The high molar absorptivity ($\epsilon = 1.0 \cdot 10^4$ l mol^{-1} cm^{-1}) at the absorbance maximum at 484 nm gives a high detection sensitivity and selectivity. No interfering chromatographic peaks were observed in blank plasma samples from cancer patients immediately prior to treatment with Imi 30.

For LC determination of Imi 30 and Imi 30-OH, fluorimetric detection has exclusively been used with the aim of optimizing the detection sensitivity and selectivity [3,8–10]. However, comparison of the signal-to-noise ratio of the photometric detector with fluorometric detection at 254 nm/530 nm showed that the relative signal-to-noise ratio was 1.02 ± 0.07 was (mean $\pm$ S.D.). Thus, photometric detection gives almost identical sensitivity.

Recovery and precision

Plasma concentrations of Imi 30 and Imi 30-OH were evaluated from standard curves (peak area versus plasma concentration) obtained by analysis of spiked blank plasma samples. The absolute recovery of Imi 30 from spiked plasma samples (5–100 ng/ml) was in the range 83.1–96.2%, with a precision better than 8.9% (coefficient of variation, Table I). A plasma sample containing 1 ng/ml of

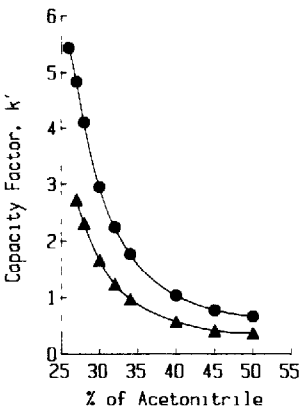

Fig. 5. Chromatographic retention and mobile phase composition. Support, LiChrosorb RP-2 (5 μm); mobile phase, acetonitrile–10^{-2} M phosphoric acid; samples: ● = Imi 30; ▲ = Imi 30-OH.

TABLE I

PRECISION AND ABSOLUTE RECOVERY

Plasma concentration (ng/ml)	Absolute recovery (%)	Relative standard deviation (%)	Number of samples
100	83.1	5.4	20
50	83.6	5.3	17
10	87.2	8.9	15
5	96.2	5.5	19

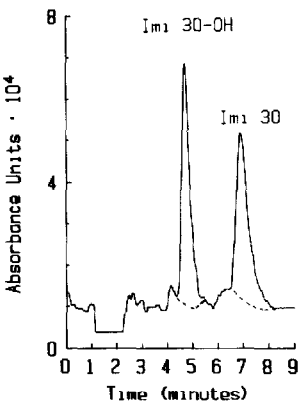

Fig. 6. Chromatogram of a patient's plasma sample. Support, LiChrosorb RP-2 (5 μm); mobile phase, acetonitrile–10^{-2} *M* phosphoric acid (32·68). The plasma sample contained ca. 15 ng each of Imi 30 and Imi 30-OH. The blank chromatogram is given by the dashed line.

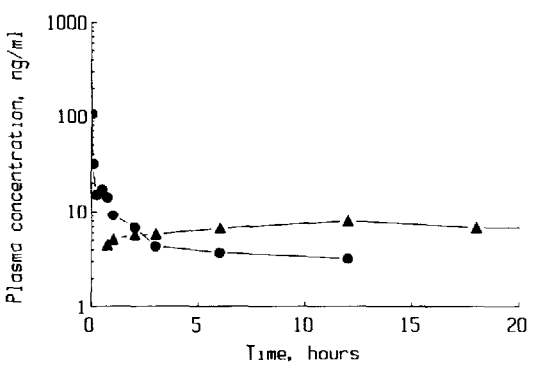

Fig. 7. Plasma concentration–time curves after intravenous administration of 17 mg of Imi 30 as a 5-min infusion. ● = Imi 30; ▲ = Imi 30-OH.

Imi 30 gave a signal-to-noise ratio of ca. 1.5. The analytical procedure given here thus offers a detection sensitivity of the same order of magnitude as those previously reported [3,8–10].

No data on the recovery and precision of the analysis of Imi 30-OH could be

obtained for reasons stated above. Previous experience of the analysis of anthracyclines using liquid–liquid extraction as the work-up procedure have demonstrated that the recovery and precision of the 13-hydroxy metabolites are very similar to the results obtained with the parent drugs [8,14,16].

Clinical application

A chromatogram of a plasma sample from a patient treated with Imi 30 is shown in Fig. 6. Plasma concentration–time curves for Imi 30 and Imi 30-OH after intravenous administration of idarubicin (Fig. 7) demonstrate the applicability of the proposed analytical method.

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