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Short communication

Determination of idarubicin and idarubicinol in rat plasma using reversed-phase high-performance liquid chromatography and fluorescence detection

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Abstract

A reversed-phase high-performance liquid chromatographic method is described for the simultaneous determination of idarubicin and idarubicinol in rat plasma. Blood samples were analyzed from 16 rats which had received an intravascular dose of 2.25 mg kg^{-1} idarubicin. After deproteinization with acetonitrile, the separation was performed with a LiChrospher 100 RP-18 column (5 μm), using fluorescence detection (excitation: 485 nm/emission: 542 nm). The mean recovery was 95.6% for idarubicin and 90.7% for idarubicinol, respectively. The detection limit was 0.25 ng ml^{-1} using an injection volume of 50 μl. Daily relative standard deviation (RSD) was 3.2% (10 ng idarubicin/ml, $n=10$) and 4.4% (10 ng idarubicinol/ml, $n=10$).

Keywords: Idarubicin; Idarubicinol

1. Introduction

The anthracycline idarubicin (4-demethoxy-daunorubicin) is a derivative of daunorubicin. Anthracyclines are a group of antitumoral antibiotics which are widely used in clinical cancer therapy. The primary metabolite is idarubicinol (4-demethoxy-daunorubicinol), which shows similar activity to idarubicin in vitro. Besides a good review about idarubicin and its pharmacodynamic and pharmacokinetic properties published in 1991 by Hollingshead and Faulds [1], many HPLC methods for the determination of this substance were described [2–7]. In our study, we used a new reversed-phase HPLC method for the simultaneous determination of idarubicin and idarubicinol in rat blood samples. Our method is more sensitive (linear range started at 0.5 ng/ml) than previous methods (starting at 2 ng/ml [4], or 5 ng/ml [2,3,5,7]). The method is rapid (retention time for idarubicinol 4.5 min and for idarubicin 6.5 min) and shows very good separation (the methods [4] and [6] required about 10 min, method [2] about 24 min). Although higher concentrations of both substances were within the same sample, the peaks were still baseline separated. Moreover, the deproteinization with acetonitrile is

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much easier to handle than the extraction methods used in previous studies and the recoveries are higher [2–7].

The method described here is reliable, sensitive and easy to handle, and represents an improvement of our previous results [8,9].

2. Experimental

2.1. Chemicals

Acetonitrile, triethylamine (TEA), tetrahydrofuran (THF), H_3PO_4 and HCl were obtained from Merck, Darmstadt, Germany. Idarubicin (4-demethoxy-daunorubicin) and idarubicinol (4-demethoxy-daunorubicinol) were purchased from Pharmacia, Formitalia, Freiburg, Germany. The water used was distilled (NaNO pure, Wilhelm Werner, Bergisch Gladbach, Germany).

2.2. Instrumentation

The high-performance liquid chromatograph from Merck consisted of a pump (L-6200A), an auto-sampler (AS-2000A) and a fluorescence detector (RF-551) from Shimadzu, connected with a 50-μl external loop. Chromatographic separation was performed using a LiChrospher 100 RP-18 (5 μm; 250 mm×4 mm I.D., Merck). The guard column was a LiChrospher 100 RP-18 (5 μm; length: 4 mm, Merck). The excitation spectrum was scanned by an UV–Vis detector (Spectra Physics, UV3000-FOCUS-SM5000) and the emission spectrum by a fluorescence spectrophotometer F-3010 from Hitachi (Tokyo, Japan). A pH meter (WTW, pH537, Weilheim, Germany) was used for pH measurements.

2.3. Chromatographic technique

The mobile phase consisted of H_2O–acetonitrile–THF–H_3PO_4–TEA (312:165:20:1:2, v/v) and was adjusted to pH 2.2 with 5 *M* HCl. The filtered mobile phase was degassed for 5 min (Supersonic, Branson 2210, Danbury, CT, USA). At ambient temperature, a flow-rate of 1.0 ml min^{-1} was established. The excitation and emission wavelengths for fluorescence detection were set at 485 and 542 nm, respectively.

2.4. Animal experiments

In brief, male Wistar rats, weighing 200–250 mg, were used in all experiments. They were intraperitoneally anaesthetised with ketaminhydrochloride (50 mg kg^{-1}). A catheter was either placed into the right atrium (via V. iugularis interna) or into the aorta (via A. carotis communis), depending on the site of application (pre- or post-pulmonal). Animals of both groups received a dose of 2.25 mg kg^{-1} idarubicin by constant infusion over 5 min. Blood samples were collected from the left V. femoralis 6, 15, 30, 90, 180, 270 min and 24 h after the start of the idarubicin administration.

2.5. Sample preparation

The samples were stored until analysis at −20°C. For HPLC determination, 100 μl of plasma were deproteinized with 100 μl acetonitrile. After centrifugation (16000 *g*/5 min; centrifuge 5416, Eppendorf, Engelsdorf, Germany) the supernatant was injected onto the column.

2.6. Calibration curve

An external calibration curve with nine points was established (1.0, 2.5, 5.0, 10.0, 25.0, 50.0, 100.0, 250.0, 500.0 ng ml^{-1}). The standard solutions were prepared daily by adding idarubicin and idarubicinol to human plasma and were deproteinized as described above.

3. Results and discussion

The excitation and emission spectra of idarubicin and idarubicinol were scanned with a fluorescence spectrophotometer from Hitachi and an UV–Vis detector from Spectra Physics (see Section 2.2). The observed excitation (250 nm, 285 nm, 417 nm, and 485 nm) and emission (542 nm) maxima were similar for both substances. The scans were performed on mobile phase solutions spiked with idarubicin and idarubicinol, respectively. The best

Table 1
Calibration curve data for idarubicin and idarubicinol in human plasma

Idarubicin [ng/ml]	Peak-area found [mV]	Peak-height found [mV]	Idarubicinol [ng/ml]	Peak-area found [mV]	Peak-height found [mV]
1.0	9960	446	1.0	11487	547
2.5	28234	1443	2.5	27361	1432
5	60156	3136	5	49061	2290
10	74923	3524	10	104729	4478
25	188752	8627	25	252087	10794
50	394754	18277	50	482032	21397
100	850301	38410	100	896727	40720
250	2239331	102912	250	2271865	104569
500	4280693	197818	500	4491137	207073
Linear regression coefficient [r^2]	0.99970	0.99972	Linear regression coefficient [r^2]	0.99995	0.99998

signal–noise ratio was achieved at an excitation wavelength of 485 nm and an emission wavelength of 542 nm. Using these wavelengths, the method had a limit of detection for both substances of 0.25 ng ml^{-1} (at a signal–noise ratio of 3) and was linear between 0.5 and 500 ng ml^{-1}. The coefficient of determination for the calibration curve (peak-area) was 0.9997 for idarubicin and 0.99995 for idarubicinol, respectively (Table 1). The rat plasma samples were deproteinized with acetonitrile and, after centrifugation, the supernatant was injected onto the column. The calculated recovery was about 95.63% (idarubicin) and 90.70% (idarubicinol) (Table 2). The within-run relative standard deviations (RSD) were always less than 7.68% (mean: 4.21%) (Table 2), and between days less than 6.96% (mean: 5.41%), respectively. Using a flow-rate of 1.0 ml min^{-1}, the retention time for idarubicinol was about 4.5 min and for idarubicin about 6.5 min (at a column pressure of about 125 bar). Fig. 1 shows a chromatogram of human plasma, spiked with 25 ng ml^{-1} of idarubicin and idarubicinol. Fig. 2 depicts a

Table 2
Analytical recovery of idarubicin and idarubicinol in human plasma

Amount added [ng/ml]	Found after deproteinization (mean±SD) [n=10]	Recovery [%]	RSD [%] within-run [n=10]	RSD [%] between-run [n=4]
Idarubicin				
2.5	2.260±0.173	90.40	7.683	6.853
10	9.041±0.288	90.41	3.183	n.d.
50	47.681±0.950	95.36	1.993	4.646
100	106.364±5.587	106.36	5.253	n.d.
Mean:	–	95.63	4.528	5.749
Idarubicinol				
2.5	2.139±0.113	85.57	5.285	6.963
10	9.317±0.410	93.17	4.398	n.d.
50	43.987±1.129	87.97	2.566	3.195
100	96.082±3.209	96.08	3.340	n.d.
Mean:	–	90.70	3.897	5.079

RSD=Relative standard deviation.
n.d.=not determined.

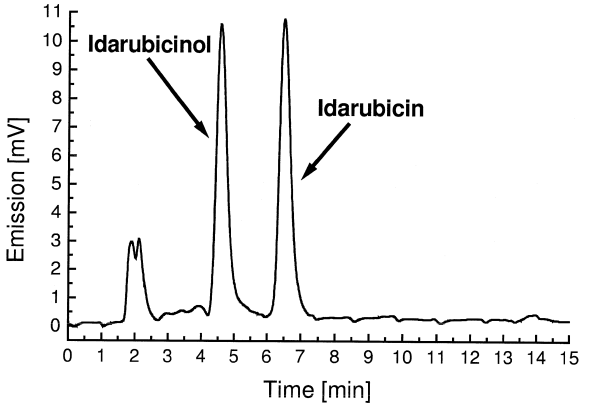

Fig. 1. HPLC chromatogram, using fluorescence detection (485/542 nm) of human plasma, spiked with 25 ng ml^{-1} of idarubicin and idarubicinol.

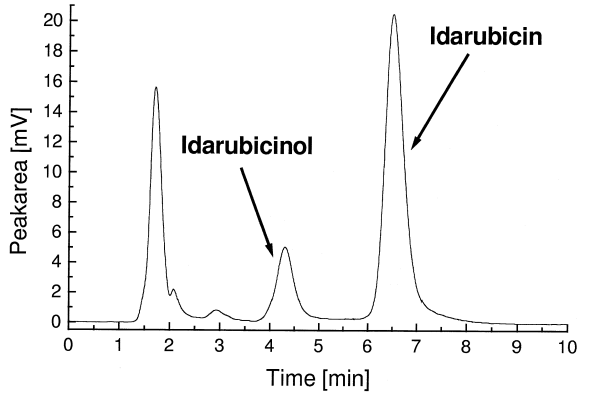

Fig. 2. HPLC chromatogram, using fluorescence detection (485/542 nm), of rat plasma after intravenous injection of 2.25 mg kg^{-1} idarubicin (V. iugularis interna). Sampling time was 90 min after injection. The calculated concentrations were 14.6 ng ml^{-1} idarubicinol and 57.6 ng ml^{-1} idarubicin, respectively.

HPLC chromatogram of rat plasma after injection of 2.25 mg kg^{-1} idarubicin intravenously (V. iugularis interna). Sampling time was 90 min after injection. The calculated concentrations were 14.6 ng ml^{-1} idarubicinol and 57.6 ng ml^{-1} idarubicin, respectively. The concentration vs. time profile of this rat is presented in Fig. 3. The pharmacokinetic aspects of our study will be discussed elsewhere [10].

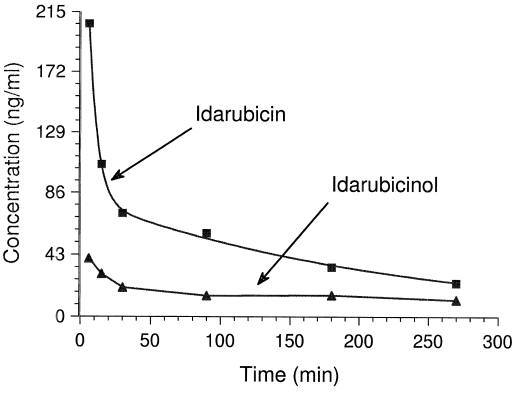

Fig. 3. Concentration–time profile of idarubicin and idarubicinol in rat plasma after intravenous injection of 2.25 mg kg^{-1} idarubicin (V. iugularis interna).

In conclusion, the described reversed-phase HPLC method has shown its practical applicability and is suitable for pharmacokinetic studies. The method is rapid, adequately sensitive, selective and is easy to handle.

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