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Interaction of epirubicin with other cytotoxics and anti-emetic drugs

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Epirubicin is usually administered in combination with other cytotoxics. Few pharmacological studies address whether relevant clinical interactions occur *in vitro* between these drugs. This study investigated whether epirubicin interacted with other cytotoxics or anti-emetics. The following drugs were prepared at pharmacological concentrations, etoposide (200 μg/ml), 5-fluorouracil (120 μg/ml), cisplatin (100 μg/ml), vincristine (100 μg/ml) and cyclophosphamide (1 μg/ml) respectively were admixed with epirubicin (1 μg/ml). Epirubicin was analysed by high performance liquid chromatography using in-line UV and fluorescence detectors. Experiments were performed in quadruplicate. No significant interactions were noted. The experiments were repeated for stemetil and maxolon. Maxolon did not interact with epirubicin but stemetil produced an interfering peak in the assay. We conclude that interaction studies are an important step in the workup of chemotherapy regimens.

Key words: Anti-emetics, cytotoxics, epirubicin, pharmacokinetics.

Introduction

Adriamycin, an anthracycline cytotoxic, is an important drug in the treatment of a number of tumors.[1] Recently, epirubicin, an epimer of doxorubicin having a substitute hydroxyl in the C-4′ position of the sugar daunosamine, has become increasingly used clinically because of the lower incidence of toxicity noted in animal and clinical trials.[2,3] Despite the reduced toxicity, prospectively randomized trials comparing epirubicin with adriamycin in breast cancer have demonstrated similar response rates.[4]

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Epirubicin is commonly prescribed in combination with other cytotoxics. Despite the number of different regimens to which epirubicin has been added (in place of doxorubicin), few pharmacological studies[5,6] have addressed the question of whether clinically important interactions between epirubicin and epirubicin with other cytotoxics or anti-emetics occur *in vitro*. We have previously demonstrated that cisplatin reacted with indomethacin in *in vitro* studies.

This paper describes our modified method for analyzing epirubicin and its metabolites: 13-dihydro-4-epirubicin, 4-*O*-β-glucuronyl-4-epirubicin and 4-*O*-D-glucuronyl-13-dihydro-4-epirubicin in plasma. The possible *in vitro* interactions of epirubicin with other cytotoxics were investigated.[6]

Materials and methods

Chemicals

Epirubicin, 13-dihydro-4-epirubicin (13-OH), 4-*O*-β-glucuronyl-4-epirubicin (GLUC-2) and 4-*O*-D-glucuronyl-13-dihydro-4-epirubicin (GLUC-1) were kindly provided by Farmitalia Carlo Erba (Melbourne). Methotrexate (MTX) was obtained from Lederle Laboratories (Nottinghill, Victoria).

High performance liquid chromatography (HPLC) was performed on a BAS PM-60 pump (Hart Analytical, Collingwood, Victoria) a FS-970 LC Fluorometer (Schoeffel Instruments, USA) in sequence with a LC-6 UV/vis Absorbence detector (Hart Analytical, Collingwood, Victoria). A 3 μm ODS 100 mm × 3.2 mm column (Hart Analytical, Collingwood, Victoria) was used. Sep-Pak C_{18} columns (Hart Analytical Pty Ltd, Collingwood, Victoria) were used for sample preparation and a vacuum system was used for sample extraction.

Extraction procedure

Initially, 35% perchloric acid was used to precipitate the plasma proteins. This produced a low recovery rate of 12.5% for epirubicin. Perchloric acid also produced a late peak on the chromatogram.

The extraction technique was changed to solid phase extraction using a Sep-Pak C_{18} pre-column following a previously described method.[8]

One ml of phosphate buffer (0.05 M pH 8.85, 5 mM EDTA) and 100 μl of internal standard (MTX 2.5 mg/ml), were added to 1.0 ml plasma and vortexed for 2 min. Sep-Pak C_{18} pre-columns were prepared for the application of the above mixture by the following sequence of washes: 10 ml of methanol followed by 10 ml 0.05 M phosphate buffer, pH 8.85 (5 mM EDTA). The plasma samples were applied to the pre-column. The flow rate through the pre-columns was controlled using a constant pressure vacuum system. The columns were washed with another 1.0 ml of phosphate buffer, and epirubicin and its metabolites were eluted using 1 ml acidified methanol (pH 3). Initially, methanol was used to elute the compounds but acidified methanol (pH 3) increased the recovery to 71.3%. The eluent was dried under nitrogen gas at 40°C. The residue was reconstituted in 200 μl of 0.02 M HCl and 20 μl was injected into the HPLC system.

Chromatography conditions

The mobile phase consisted of acetonitrile, 0.05 M potassium dihydrogen phosphate (22:78) and 5 mM EDTA at pH 3.5. The pump flow rate was 0.8 ml/min. Epirubicin, its metabolites and MTX (internal standard) were detected by in-line fluorescence (260 nm excitation/550 nm emission) and visible (372 nm) spectrum detectors, respectively.

Reproducibility

The reproducibility of the assay was determined by calculating the coefficient of variation for repeated injections of the same sample ($n = 10$). Also, samples ($n = 10$) spiked with epirubicin to produce a concentration of 50 μg/ml were extracted and injected into the HPLC system. The coefficient was calculated using a standard formula.

Recovery

The recovery of the extraction technique was calculated by the following formula:

$$\text{Recovery} = A/B \times K \times 100 \times C/D$$

where A is the peak height of the extracted compound at concentration X, B is the peak height of the unextracted compound at concentration X, C is the concentration of the extracted compound (X), D is the concentration of the unextracted compound (Y) and K is the concentration factor.

In vitro interference of drugs with epirubicin

Etoposide, 5-fluorouracil (5-FU), cisplatin, vincristine, cyclophosphamide and stemetil were added to saline (pH 7.4) to produce concentrations of 200, 120, 10, 100, 100 and 1 μg/ml, respectively. These concentrations were chosen as these represent the peak concentrations measured in patients. Samples were initially injected onto the HPLC system to ensure that they did not produce interference peaks.

The drugs listed above were mixed with epirubicin (1 μg/ml), vortexed for 1 min and incubated at 37°C in a water bath protected from light for 3 h. Experiments were performed in quadruplicate. On completion of the incubation, the concentration of epirubicin was determined by HPLC. The results were analyzed by analysis of variance and $p < 0.05$ was accepted as statistically significant.

Results

HPLC assay

The chromatogram of blank plasma is demonstrated in Figure 1. There are no interfering peaks within the period of interest with the detector set at the lowest setting. The chromatograms for plasma spiked with epirubicin and its metabolites are demonstrated in Figure 2. The retention times for GLUC-1, 13-OH, GLUC-2 and epirubicin were 5.0, 6.4, 9.4 and 13.0 min, respectively.

Daunorubicin was initially used as the internal standard. However, this substantially increased the run time as daunorubicin's retention time was found to be 20 min. A spectrophotometric scan of MTX revealed that it had an absorption band at 372 nm and under the above chromatographic

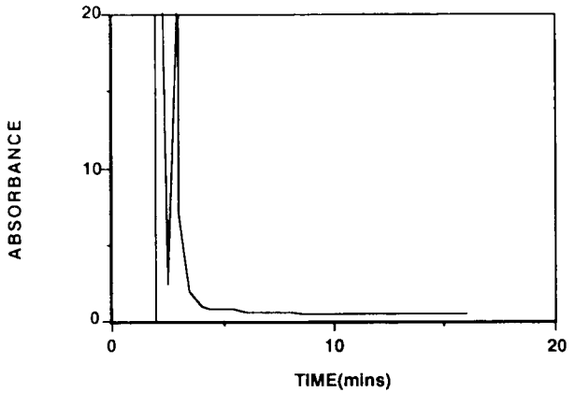

Figure 1. Chromatogram of blank plasma.

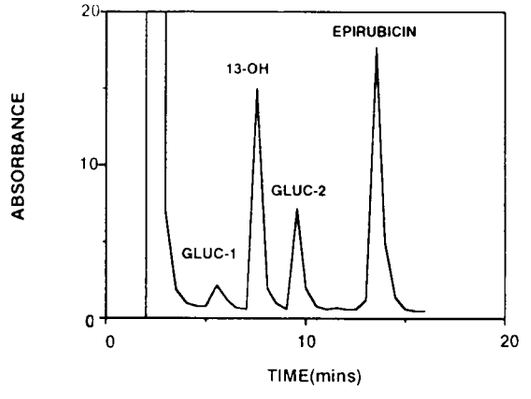

Figure 2. Chromatograph of spiked plasma with epirubicin and its metabolites.

conditions an elution time of 1–2 min. Epirubicin and its metabolites did not absorb significantly in this region. Epirubicin, its metabolites and MTX were analyzed by in-line fluorescence and UV detectors. The recovery of MTX using the above extraction procedure was 50% and the coefficient of variation was 1.8%. Figures 1 and 2 demonstrate combined chromatograms for epirubicin, its metabolites and MTX.

The recovery, limits of detection and coefficient of variation for the assay are demonstrated in Table 1.

In vitro interference of drugs with epirubicin

MTX, cyclophosphamide, etoposide, vincristine, cisplatin and 5-FU did not produce an interfering peak with epirubicin using this HPLC assay. Maxolon did not produce an interfering peak, but quinidine, quinine and Stemetil produced interfering peaks at pharmacological concentrations.

MTX, cyclophosphamide, etoposide, vincristine, cisplatin and 5-FU did not produce any change in the epirubicin peaks when incubated with epirubicin at 37°C for 2 h (Table 2). The anti-emetic Maxolon did not produce any interaction; however, because stemetil produced an interfering peak, no attempt to assess an interaction was made on this system. Statistical analysis of the results demon-

Table 1. Comparison of the coefficient of variation, recovery, injection volume and limit of detection for epirubicin and its metabolites in our HPLC system and that of Tjuljandin *et al.*[3]

	HPLC	Tjuljandin *et al.*[3]
Column	3 μm ODS(100 mm × 3.2 mm)	Bondapak phenyl
Mobile phase	acetonitrile/phosphate buffer, pH 5.8	acetonitrile/phosphate buffer, pH 2.6
Detector	vis + fluorescence (260/550 nm)	fluorescence (480/550 nm)
Limit of detection	1 ng/ml	0.6 ng/ml
Recovery	71.3 ± 9.8%	—
Injection volume	20 μl	—
Coefficient of variation		
(1) for the HPLC system ($n = 10$)	GLUC-1 = 5.0% 13-OH = 2.4% GLUC-2 = 4.2% epirubicin = 3.4%	—
(2) of the extraction technique ($n = 10$)	GLUC-1 = 9.3% 13-OH = 1.5% GLUC-2 = 8.1% epirubicin = 4.8%	—
Internal standard	MTX	daunorubicin

Table 2. Demonstration that there was no significant interaction between epirubicin and the other drugs investigated

	Peak height (mean ± SEM)	Number	Probability *p* value
Epirubicin alone	23.0 ± 0.6	4	—
Epirubicin + MTX	22.8 ± 1.5	4	≥0.05
Epirubicin + etoposide	23.0 ± 1.0	4	≥0.05
Epirubicin + 5-FU	24.0 ± 0.0	4	≥0.05
Epirubicin + cisplatin	22.7 ± 0.7	4	≥0.05
Epirubicin + vincristine	23.4 ± 3.2	4	≥0.05
Epirubicin + cyclophosphamide	22.7 ± 0.7	4	≥0.05

The absolute peak height did not alter when epirubicin was mixed with the other drugs investigated. The *p* value was calculated by analysis of variance.

strated no difference between the different drugs with a p value of 0.05.

Discussion

This study describes a modified HPLC assay for epirubicin and its metabolites.[3] The assay was modified by initially changing the internal standard to MTX and thereby reducing the run time to under 15 min per sample. The combination of UV and fluorescence detection was a useful modality that resulted in a reduction in the retention time of the assay. Furthermore, by having an early rather than late retention time for the internal standard it reduced the coefficient of variation and thereby improved the reproducibility of the assay. The limits of detection of our assay were satisfactory for clinical use as compared with reported studies.[3]

This study investigated the possible interaction of epirubicin, other cytotoxics and anti-emetics. The following cytotoxics did not produce an interfering peak in the HPLC assay: MTX, 5-FU, cisplatin, vincristine and cyclophosphamide. Metoclopramide, a commonly used anti-emetic in cancer treatment, did not produce any interfering peaks. However, stemetil produced an interfering peak at pharmacological doses which prevented analysis of epirubicin or metabolite peaks. It was noted that quinidine and quinine also produce interfering peaks at pharmacological concentrations which interfered with the analysis of the metabolites of epirubicin. These results indicate that in this assay stemetil needs to be avoided while performing pharmacokinetic studies.

The wide variation in plasma concentration noted in patients receiving cytotoxics has been attributed to a number of causes. However, *in vitro* interactions have not been studied in great detail.[5] We have previously demonstrated a small interaction between indomethacin and cisplatin.[7] Furthermore, it has been demonstrated that cyclosporin affects anthracycline and etoposide pharmacokinetics.[10] The co-administration of an anthracycline or etoposide with cyclosporin results in changes in the pharmacokinetics of the cytotoxic drugs. These studies suggest that pharmacokinetic interactions should be excluded before differences in pharmacodynamic effects are attributed to molecular events. For the case of cyclosporin and adriamycin the different pharmacodynamic profile noted *in vitro* may be partly explained by changes in the plasma concentration of adriamycin.

This study investigated any possible *in vitro* interaction between epirubicin, other cytotoxics and anti-emetics. The *in vitro* interaction studies performed did not demonstrate any interaction between epirubicin and the other cytotoxics studied. *In vitro* testing of possible interaction is an important step as it may predict differences in protein binding and subsequent pharmacodynamic effects. The exclusion of *in vitro* interactions between drugs is a necessary step in the evaluation of pharmacokinetic data.

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