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Note

Determination of aclacinomycin A by reversed-phase high-performance liquid chromatography

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Aclacinomycin A (ACM) is an anthracycline antibiotic isolated from *Streptomyces galilaeus* by Oki et al. [1]. Its chemical structure is shown in Fig. 1. Antitumor activity has been shown in several experimental tumor models as well as in phase I and phase II clinical trials in man [2, 3]. It was the aim of the following study to develop a sensitive detection method for ACM in biological material.

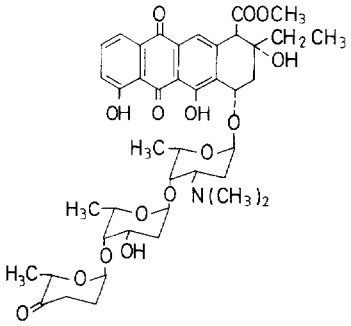

Fig. 1. Chemical structure of aclacinomycin A.

METHOD

Extraction

ACM (Behring, Marburg, F.R.G.) was added to pooled human plasma at concentrations of 10 μg/ml (12.3 μM) and 500 ng/ml (0.62 μM) and allowed to equilibrate for 1 h at room temperature. Plasma was deproteinized by adding an

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equal volume of 300 m*M* trichloroacetic acid (Merck, Darmstadt, F.R.G.). After centrifugation at 9980 *g* for 2 min the serum was applied to a C_{18} Sep-Pak® cartridge (Waters, Königstein, F.R.G.) which was then flushed with 5 ml of water and 5 ml of methanol–water (1:4, v/v). ACM was extracted with acetonitrile–methanol (1:1, v/v) (Merck) in a volume of 0.7 ml; 200-μl aliquots of the eluate were injected onto the column.

Patient samples (after bolus injection of 25 mg of ACM per m^2 body surface) were prepared by the same procedure, 10 ml of serum being applied to the C_{18} cartridge.

Liquid chromatography

The chromatographic system consisted of a μBondapak C_{18} column (300 × 4 mm, particle size 10 μm, Waters) as the support and acetonitrile–methanol–water (40:90:70, v/v) acidified with orthophosphoric acid (85%, 1.5 ml per 100 ml) as the mobile phase. The flow-rate was 2 ml/min, the temperature was ambient (about 25°C). Detection was performed at 254 nm. Quantitation is based on peak area as well as on peak height measurement.

Associated equipment comprised an M-45 solvent delivery system, an absorbance detector M 440 (Waters), and a BBC Metrawatt 10 mV writer (BBC, Nürnberg, F.R.G.).

RESULTS AND DISCUSSION

Under the conditions described ACM had a retention time of 5.9 min. As the ACM in 10 ml of serum is concentrated on the C_{18} cartridge the detection limit of ACM has been found to be 10.8 ng = 13.3 pmol per ml plasma.

A linear correlation between peak area as well as peak height and ACM injected from 25 ng to 5 μg (30.6 pmol, 6.2 nmol) has been demonstrated.

As the chromatographic system used is a modification of another one which was developed for the identification of other anthracyclines such as doxorubicin and daunorubicin [4], a good separation between these drugs and ACM could be established. This allowed the use of daunorubicin as internal standard (Fig. 2a and b).

The extraction method described had a recovery of 85 ± 7% in a series of six experiments in which ACM was added to pooled human plasma (concentration 10 μg/ml = 12.3 μM). The recovery for 500 ng/ml (= 0.62 μM) was 81 ± 9%. The inter-assay coefficient of variation for the ACM determination in general was 12.7% for 500 ng/ml (0.62 μM) samples (n = 14) and 6.9% for 10 μg/ml (12.3 μM) samples (n = 9). The intra-assay values were 8.5% (n = 6) and 4.7% (n = 6), respectively.

The advantage of using the preformed C_{18} cartridges is the rapid sample preparation. Moreover, concentration of ACM from serum samples containing low amounts of the drug is possible. This results in an improved sensitivity for pharmacokinetic studies.

In conclusion, the HPLC detection method as well as the extraction procedure described in this paper allow the collection of pharmacokinetic data in patients undergoing chemotherapy with ACM (Fig. 2b).

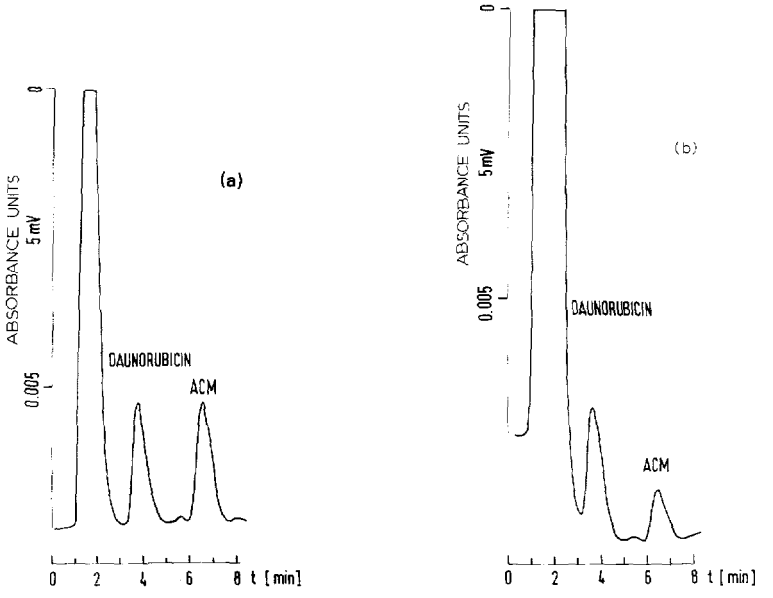

Fig. 2. (a) 1 ml of pooled human plasma was spiked with 750 ng (1.3 nmol) of daunorubicin and 400 ng (0.5 nmol) of ACM. After deproteinization and preparation on the C_{18} cartridge as described in Methods, a 200-µl aliquot was injected. (b) 10 ml of a patient serum, drawn 24 h after bolus injection of 25 mg per m^2 body surface, was spiked with 750 ng (1.3 nmol) of daunorubicin. The total 10-ml sample was deproteinized and prepared on the C_{18} cartridge; 200 µl of the eluate were injected. The ACM peak represents a plasma concentration of 17.2 ng/ml (21 pmol/ml).

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REFERENCES

1 T. Oki, Y. Matsuzawa, A. Yoshimoto, K. Numata, J. Kitamura, S. Hori, A. Takamatsu, H. Umezawa, M. Ishizuka, H. Naganawa, H. Suda, M. Hamada and T. Takeuchi, J. Antibiot., 28 (1975) 830.
2 T. Sakano, N. Okazaki, T. Ise, K. Kitaoka and K. Kimura, Jap. J. Clin. Oncol., 8 (1978) 49.
3 G. Mathe, M. Bayssas, J. Gouveia, D. Dantchev, P. Ribeaud, D. Machover, J.L. Misset, L. Schwarzenberg, C. Jasmin and M. Hayat, Cancer Chemother. Pharmacol., 1 (1978) 259.
4 R. Erttmann, C. Drax and S. Myhr, Naunyn-Schmiedeberg's Arch. Pharmacol. Suppl., 321 (1982) R59.