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Note

Isotachophoretic determination of adriamycin and adriamycinol in human plasma

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Adriamycin (doxorubicin) is one of the potent anticancer drugs and used for the treatment of neoplasms such as malignant lymphoma, leukemia and lung cancer. However, since it shows severe side-effects when administered in excess, pharmacokinetic studies are necessary for therapeutic designs.

Several analytical methods have been reported for the determination of adriamycin in human plasma and urine. These methods are based on either fluorescence measurement [1, 2] or radioimmunoassay [3], but they have disadvantages due to the lack of specificity. The use of fluorescence scanning on thin-layer chromatograms [4] and of reversed-phase liquid column chromatography [5—7] made a considerable improvement in the specificity of assay.

In the present paper we report a method for the specific determination of adriamycin and its active metabolite (adriamycinol) in human plasma by means of isotachophoresis.

EXPERIMENTAL

Human plasma (1 ml) was diluted four times with water, and mixed vigorously with 4 ml of *n*-butanol for 5 min. The mixture was then centrifuged at 2000 *g* for 10 min and the resultant butanol layer was concentrated to dryness in a flask under vacuum at 40°C. The wall of the flask was washed twice with 0.5 ml of methanol and the solvent was transferred to a pyrex tube and evaporated under nitrogen gas. The dried residue was dissolved in 50—100 μl of 90% methanol and was subjected to isotachophoresis. The recovery of adriamycin extraction from plasma was 89.1 ± 0.94% (mean ± S.E.M.).

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Isotachophoretic separations were performed in a 23 cm × 0.5 mm I.D. capillary using the LKB 2127 Tachophor. One or 10 μl of the sample were injected. The separations were done at a constant current of 100 μA. The light transmission at 254 nm was recorded at a chart speed of 6 cm/min. The leading electrolyte was 10 m*M* sodium acetate—acetic acid buffer in 60% methanol (pH 6.0) and the terminator was 10 m*M* β-alanine in 60% methanol.

Adriamycin was obtained from Kyowa Hakko Co., Tokyo, Japan. Adriamycinol (13-hydroxydoxorubicin) and adriamycin aglycones were purified by thin-layer chromatography from the incubation mixture of adriamycin with the supernatant fraction (105,000 *g*, 60 min) of mouse kidney homogenate by the method of Bachur et al. [8]. Ampholine (pH 3.5—10) was purchased from LKB, Bromma, Sweden.

RESULTS AND DISCUSSION

Fig. 1 shows the isotachophoretic pattern of adriamycin. A typical isotachophoretic run of the butanol extract of plasma containing no adriamycin

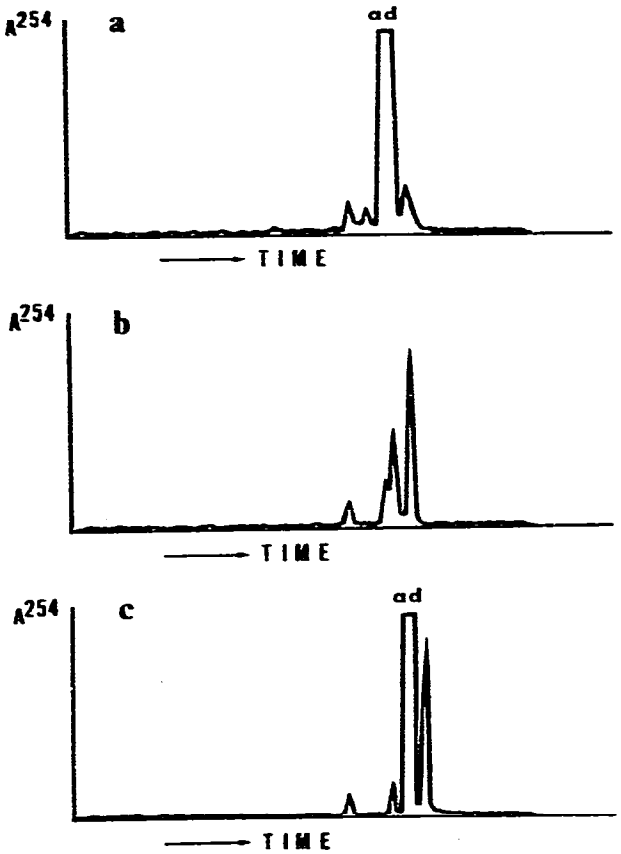

Fig. 1. Isotachopherograms of adriamycin. Ten microliters of the sample were injected. (a) Adriamycin (200 μg/ml of saline); (b) *n*-butanol extract of plasma; (c) *n*-butanol extract of plasma containing 200 μg/ml adriamycin. ad = adriamycin.

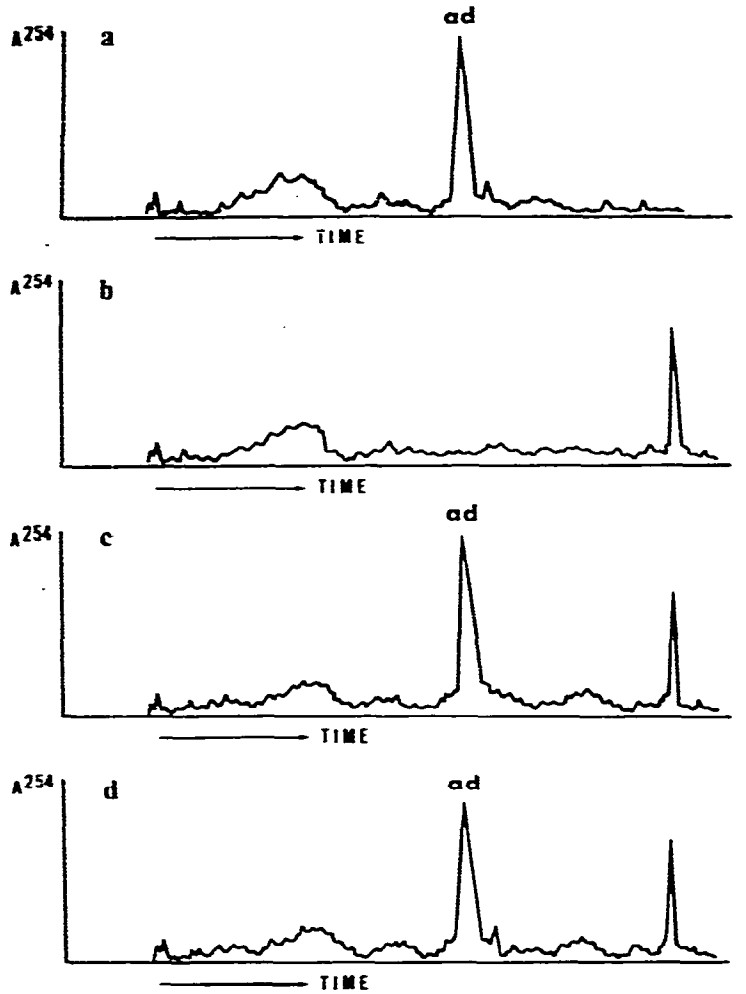

Fig. 2. Isotachopherogram of adriamycin using Ampholine as a spacer. Ten microliters of sample were mixed with 1 μl of 1% Ampholine (pH 3.5—10) and injected. (a) Adriamycin (10 μg/ml of saline); (b) *n*-butanol extract of plasma; (c) *n*-butanol extract of plasma to which adriamycin (10 μg/ml) was added before injection; (d) *n*-butanol extract of plasma containing 10 μg/ml adriamycin. ad = adriamycin.

(Fig. 1b) shows two UV-negative zones and three UV-positive spikes. When adriamycin was added to the extract, the UV-positive zone of adriamycin was positioned just behind the second UV-positive spike (Fig. 1c). Since the UV-positive spikes disturbed the detection of small amounts of adriamycin, Ampholine (pH 3.5—10, final concentration 0.1%) was added to the extract and electrophoresed. As shown in Fig. 2, the peak corresponding to adriamycin was well separated from the distinct UV-positive spike in blank plasma. Adriamycinol, a major metabolite with antineoplastic activity [9], co-migrated with adriamycin (data not shown). A linear relationship [y (mm^2) = 11.2 x (ng) — 0.3; r = 0.999] was obtained between the integrated peak area (y) and the injected amount of adriamycin (x) within the range 2.5—100 ng. Since adriamycinol has a molar extinction coefficient at 254 nm essentially

identical to that of adriamycin [8], this concentration range almost covers combined levels of the drug and its active metabolite in plasma in ordinary therapeutic designs [10].

Eksborg et al. [7] and Pierce and Jatlow [6] have studied the use of reversed-phase liquid chromatography for the determination of adriamycin and adriamycinol. The detection sensitivity of our method was not better than but as good as that of their procedures. The recovery of the drug and its hydroxyl metabolite was higher than that reported by Pierce and Jatlow and was almost the same as that found by Eksborg et al.

Aglycones of adriamycin, which are pharmacologically inactive metabolites [9], did not move into the capillary and stayed at the origin. The present method, therefore, gives the levels of the active compounds in plasma, avoiding the contamination by the inactive aglycones which in the usual fluorescence method interferes with the determination of the active compounds due to their similar fluorescence spectra.

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