THE PERSISTENCE OF ADRIAMYCIN IN MAN AND RAT

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- 1 Adriamycin was administered as a single intravenous dose. The plasma concentration in man and rat decayed as a double exponential function of time.
- 2 A two compartment model was used to predict plasma and tissue concentrations in man and the rat. The validity of the tissue concentration predictions was confirmed experimentally in the rat.
- 3 Tissue components had a high capacity for the drug. There was evidence in the rat of preferential accumulation in the liver, spleen and bone marrow.
- 4 The large tissue capacity and the prolonged half time for elimination predispose to accumulation. This may be responsible for the greater toxicity of treatment schedules with short intervals between consecutive doses.

Introduction

Adriamycin is a glycoside antibiotic isolated from a mutant of *Strep. peucitus*. It is similar in structure to daunomycin differing only by the presence of an hydroxyl group on the acetyl radical of the aglycone moiety. On a molar basis it is less effective than daunomycin in inhibiting DNA synthesis (di Marco, Zunino, Silvestrini, Gambarucci & Gambetta, 1971). However, *in vivo* it has a higher therapeutic index (Sandberg, Howsden, di Marco & Goldin, 1970).

It was noted in early trials that frequent administration of daunomycin led to an increased incidence of toxic effects. Malpas & Scott (1969) observed a 50% incidence of fatal marrow aplasia when 2 mg/kg was given daily but this was reduced by increasing the interval between doses. Rusconi, di Fronzo & di Marco (1969) showed that rat tissues had a high capacity for daunomycin. This high capacity was predicted in man by di Fronzo & Bonadonna (1970) who suggested a dose interval of at least 2-3 days.

Bonadonna, Monfardini, de Lena & Fossati-Bellani (1969) noted with adriamycin that the incidence of toxic effects was increased by more frequent administration. di Fronzo, Gambetta & Bonadonna (1971) observed a slow decay of plasma concentration in man consistent with a high tissue capacity.

This paper describes the compartmental

analysis of adriamycin in man and compares the predicted distribution with that observed in the rat. It demonstrates that by studying data obtained from the plasma concentration/time curve it is possible to predict the pattern of tissue distribution and the likelihood of accumulation with successive doses.

Methods

Drugs

In the human study adriamycin was dispensed by the addition of water for injection to vials containing 10 mg of the hydrochloride freeze dried with lactose.

[³H]-adriamycin for the rat study was prepared by the Wilzbach technique at the Radiochemical Centre, Amersham, England. The crude product was purified as follows:

Cellulose powder (Whatman CF 11) was packed as a chloroform slurry in a 2 × 10 cm column. Aliquots of crude drug (10 mg) were dissolved in absolute methanol (2 ml), applied to the column and washed with chloroform (100 ml). The first 20 ml of the chloroform eluate was added to a second column to adsorb any drug carried through by the methanol present and rewashed with chloroform. Purified drug was then eluted from both columns with absolute methanol. The excess

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methanol was removed by distillation *in vacuo* at 4°C to a final volume of 5 ml.

Pure adriamycin was then precipitated by the addition of diethyl ether (100 ml). After centrifugation the supernatant was decanted, the precipitate washed twice with dry ether and the final product dried overnight in a desiccator.

The degree of purity was determined by ascending thin layer chromatography on 0.25 mm Silica gel plates (MN-Polygram Sil N-HR, Macherey-Nagel & Co.) using two solvent systems: (a) butanol : acetic acid : water (4 : 1 : 5, v : v : v); and (b) acetone : benzene : water (12 : 3 : 2, v : v : v). In each case the R_F value of the visible spot was identical to that of authentic standard; no other visible spot was identified. The total radioactivity associated with the visible spot was 84% and a further 9% lay within 5 mm of the edge of the spot. There was no evidence of a separate spot corresponding with labelled contaminants. The optical density of an aqueous solution of adriamycin was determined at 235 nm from which a specific activity of 78.14 $\mu\text{Ci}/\text{mg}$ was calculated. Pure tritiated adriamycin was then dissolved in 0.9% (w/v) NaCl to provide a final concentration of 2 mg/ml.

Patients and dosage

Six patients with malignant disease who were about to receive adriamycin in a therapeutic trial were selected for study. Each had advanced disease and conventional treatment had become unsuccessful.

Adriamycin in a single dose of 60 mg/m² was administered by injection into the tubing of a fast running saline drip; the duration of the injection did not exceed 3 minutes. Serial blood samples (10 μl) were collected in tubes containing sodium edetate (20 mg). The plasma was separated and stored at -20°C. Urine samples collected from 0-12, 12-24 and 24-48 h were stored in a like manner.

Drug concentrations were determined by the daunomycin method of Bachur, Moore, Bernstein & Liu (1970) as modified by Alberts, Bachur & Holtzman (1971). The activation spectrum of adriamycin was determined using a Farrand spectrophotofluorimeter. The optimum activating wavelength was 470 nm and the optimum emission wavelength 595 nm. These values were used for all estimations. The activation and emission spectra of the drug in fresh urine were identical with those in plasma.

Standard solutions of adriamycin in plasma were used to construct a calibration curve; to plasma (1 ml) was added absolute methanol (1 ml) and concentrated hydrochloric acid (0.05 ml).

Samples were centrifuged for 10 min and the fluorescence of the supernatant determined. All estimations were performed in triplicate.

The relationship between the concentration and the current output (mA) of the fluorimeter was linear. A fresh calibration curve was performed for each set of estimations. Calibration curves obtained when the drug was added to plasma (0.05-5 $\mu\text{g}/\text{ml}$) were identical with curves obtained by adding adriamycin directly to plasma supernatant obtained after protein precipitation. Thus, even at the lowest plasma concentrations encountered in this study, all protein bound drug was freed and estimated by the method. The concentrations in urine were measured similarly.

Animal study

[³H]-adriamycin (2 mg/kg) was administered via the caudal vein to male Wistar rats weighing 200-260 g. At selected time intervals venous blood samples were obtained from the inferior vena cava at open thoracotomy of the anaesthetized animal. Tissues and organs were dissected, rinsed in 0.9% (w/v) NaCl and stored at -20°C. Urine and faeces collected over 0-12, 0-24 and 0-48 h were obtained from animals housed separately in metabolic cages.

Total radioactivity in the plasma and tissues was determined by the method of Mahin & Lofberg (1966). Triplicate samples were counted in a Nuclear Chicago liquid scintillation spectrometer. Radioactive quenching was determined by external standardization. Total faecal activity was determined by homogenizing stool samples in distilled water (50 ml) and assaying samples of 0.2 ml in triplicate. Urine was diluted (1 : 1, v : v) with acidified methanol, added to 10 ml of scintillant (PPO 10 g; POPOP 0.1 g; naphthalene 160 g; ethyl alcohol 682 ml; dioxane 772 ml; xylene 772 ml) and counted.

The proportion of adriamycin present in muscle, bone and fat was obtained from the product of the individual mean tissue concentrations and their appropriate proportion of the total body weight (Neville & de Lucca, 1966).

Autoradiographs of selected tissues were obtained by dipping standard histological sections in Ilford L4 autoradiographic emulsion. After exposure for ten days the slides were stained with Giemsa stain and examined for the presence of grains.

Compartmental analysis

The individual human plasma concentration/time curves were fitted by the equation for a double exponential function (Riggs, 1963). The intercepts and dispositional rate constants were obtained by

least squares regression analysis of each log concentration/time curve. The above values were used to calculate the dimensions of two compartment open models using the equations of Riegelman, Loo & Rowland (1968). The corresponding calculations for the rat data were based on the group mean plasma concentration/time curve.

The means of the estimates of the distribution volumes and clearances were used in a digital computer model to reproduce the experimental mean concentration/time curves and to predict the distribution of the drug between a central compartment and a peripheral compartment.

Results

The plasma concentration/time curve for man is shown in Fig. 1a, and for the rat in Figure 1b. The characteristics of the two exponential functions of each curve are compared in Table 1. The half time of the fast phase in man was 7 min and of the slow phase 31 h; the corresponding values in the rat were 20 min and 61 hours.

Substitution of the intercepts and rate constants A and B and α and β into the equations of Riegelman *et al.* (1968) gave the dimensions of the two compartment models which are shown in Table 2. The ratio of the mean central : peripheral compartment volume in man was 1 : 18 and in the rat 1 : 9.

The predicted concentrations in the two compartments for man are shown in Fig. 1a and for the rat in Figure 1b. In man the concentration in the peripheral compartment equalled that in the central compartment 80 min after infusion. Thereafter, the concentrations in the two compartments decayed exponentially the greater concentration being in the tissues. In the rat equilibration was slower; the concentrations in the two compartments did not coincide until 2.5 h after injection.

The patients excreted in the urine $12.6 \pm 1.3\%$ of the injected dose during the first 48 h (predicted total elimination 73%). Extraction and

Fig. 1 Decay of plasma adriamycin concentration ($\mu\text{g/ml}$) (mean $\pm$ s.e. mean) during the first 48 h after the intravenous administration to (a) man (6 patients; 60 mg/m^2) and (b) rat (groups of 4: 2 mg/kg). The continuous line represents the central compartment concentration and the interrupted line the peripheral compartment concentration predicted by the model.

thin layer chromatography confirmed that the major proportion of this was unchanged drug. The rats excreted $46 \pm 3\%$ during the first 48 h (predicted total elimination 42%). Approximately equal amounts were excreted in the urine and faeces.

Table 1 Characteristics of the two exponential components of the plasma adriamycin concentration/time curve in man and rat

		Man	Rat
		(mean $\pm$ s.e. mean, n = 6)	(groups of 4)
First	A ($\mu\text{g/ml}$)	4.99 ± 0.46	10.83
Exponential	$\alpha (\text{h}^{-1})$	5.88 ± 0.42	2.20
Component	$T_{1/2} (\text{h})$	0.12 ± 0.009	0.32
Second	B ($\mu\text{g/ml}$)	0.24 ± 0.03	1.14
Exponential	$\beta (\text{h}^{-1})$	0.024 ± 0.003	0.011
Component	$T_{1/2} (\text{h})$	30.8 ± 3.9	61.6

Fig. 2 Tissue concentration ($\mu\text{g/g}$) [^3H]-adriamycin in the rat (groups of 4) between 3 and 48 h after the intravenous administration of 2 mg/kg. Vertical bars represent s.e. mean. Key: (1) lung; (2) liver; (3) spleen; (4) kidney; (5) small intestine; (6) bone marrow; (7) plasma; (8) heart; (9) fat; (10) large intestine; (11) muscle; (12) bone; (13) brain.

The concentration of [^3H]-adriamycin in individual rat tissues is shown in Figure 2. The organs with the highest concentration were liver, lungs and spleen; very low concentrations were found in bone and brain.

The proportion of [^3H]-adriamycin present in the rat tissues at 3 and 48 h is shown in Figure 3.

Labelled adriamycin was located by autoradiography in the Kpffer cells of the liver (Fig. 4) and the histiocytes of the bone marrow, lung and spleen.

Discussion

The plasma concentration of adriamycin in man decayed as a double exponential function of time. This was explained by postulating that the drug was distributed within a two compartment system. The plasma concentration/time curve predicted by this hypothesis lay within one standard error of the mean experimental concentrations except at the 1 h point (Figure 1a). The concentration at zero time corresponded with rapid distribution in

Table 2 Dimensions of hypothetical two compartment models which fit the plasma adriamycin concentration/time curves in man and rat

	Man (mean $\pm$ s.e. mean, $n = 6$)	Rat (groups of 4)
Central compartment volume (ml)	19,300 $\pm$ 1,600	40
Peripheral compartment volume (ml)	350,000 $\pm$ 43,000	353
Transfer clearance (ml/min)	1,600 $\pm$ 63	1.25
Elimination clearance (ml/min)	156 $\pm$ 21	0.066

Fig. 3 The distribution of [^3H]-adriamycin in the rat (groups of 4) 3 and 48 h after the intravenous administration of 2 mg/kg.

Fig. 4 Autoradiograph of rat liver 24 h after the intravenous administration of [^3H]-adriamycin (20 mg/kg). The encircled areas represent cells with high grain counts.

a central compartment with a volume of the same order as that of extracellular fluid. The initial rapid decay was explained by redistribution into a less accessible peripheral compartment. The long plasma half time was attributed to the large capacity of this compartment. The model predicted that 85% of the dose was in the peripheral compartment at 1 h, 50% at 24 h and 25% at 48 hours.

The large tissue capacity and the prolonged half time for elimination predispose to accumulation. When a hypothetical 30 mg dose was administered to the model at intervals of 1 day there was progressive accumulation. When the fourth dose

was due 27 mg was already present, mainly in the peripheral compartment. Accumulation of this order may explain the high incidence of toxicity observed by Mathé, Amiel, Hayat, De vassel, Schwarzenberg, Schneider, Jasmin & Rosenfeld (1972).

The predicted mass eliminated by the model exceeded that of the observed urinary excretion. This can be explained partly by faecal excretion which may account for 35% of the total dose in 48 h (di Fronzo *et al.*, 1971). Metabolism may account for the remainder.

There is evidence that daunomycin is metabolized in man and other species (Alberts *et al.*,

1971; Yesair, Schwartzbach, Shuck, Denine & Asbell, 1972) but the extent of adriamycin metabolism is a matter for conjecture. There is evidence of reductive cleavage of adriamycin *in vitro* (Asbell, Schwartzbach, Bullock & Yesair, 1972) but this has not been confirmed *in vivo*. Yesair *et al.* (1972) found no evidence of adriamycin metabolism in the rat. di Fronzo, Gambetta & Lenaz (1971) found no metabolites of adriamycin in mouse tissue extracts, whereas daunomycin metabolites accounted for 45% of the administered dose. In man there are contradictory reports of adriamycin metabolism. Rosso, Ravazzani, Esposito, Sala & Santi (1972) found no evidence of drug metabolism; Benjamin, Riggs & Bachur (1973) presented chromatographic evidence for the existence of unidentified metabolites in plasma.

The validity of the above predictions could not be confirmed experimentally in man and therefore confirmation was sought from animal studies. The plasma concentration decay curve of adriamycin in the rat was similar to that in man and to that obtained in the rat by other workers (Arena, d'Alessandro, Dusonchet, Gebbia, Gerbasi, Palazzoadriano, Raineri, Ransa & Tubaro, 1971; Yesair *et al.*, 1972). Thus, a two compartment system was again postulated and the predicted plasma concentrations lay within one standard error of the observed values.

The measured amounts of adriamycin present in the rat tissues were compared with those predicted by the model and are shown in Figure 5. One hour after injection 82% of the dose was present in the tissues (predicted value 76%). Thus, the initial rapid decay of plasma concentrations was in fact associated with redistribution of the drug between the plasma and the tissues. At 24 h the tissues contained 63% and at 48 h 55% of the injected dose. Despite the very low mass of drug in plasma a considerable proportion of the injected dose persisted in the tissues. The predicted mass of drug present in the peripheral compartment lay

Fig. 5 Tissue content of adriamycin (% of dose) between 1 and 48 h after intravenous administration to the rat (2 mg/kg). The continuous line represents the drug mass present in the peripheral compartment predicted by the two compartment computer model and the points the experimental values ($\pm$ s.e. mean, $n = 4$).

within one standard error of the observed values. Thus, the validity of the model was confirmed.

The predicted high capacity of the peripheral compartment in man and the observed concentration of adriamycin in the tissues of the rat suggest binding of the drug to tissue components. The organs showing the highest concentration of adriamycin were liver, lung and spleen which together contained 52% of the dose at 3 h and 41% at 48 h (Figure 2).

The persistence of the drug in these tissues has been noted by other workers (Arena *et al.*, 1971; di Fronzo, Gambetta & Lenaz, 1971) and may be due to preferential uptake of adriamycin by the reticuloendothelial system. This may be of therapeutic importance if the phenomenon also occurs in the neoplastic reticular cell.

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