Cancer Chemother Pharmacol (1981) 5: 139–144

Cancer Chemotherapy and Pharmacology



*Original Articles*

# Fluorescence Assays and Pharmacokinetic Studies of 4′-Deoxydoxorubicin and Doxorubicin in Organs of Mice Bearing Solid Tumors

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***Summary.*** *The pharmacokinetic of 4′-deoxydoxorubicin, a new analog of doxorubicin, was compared with that of its parent compound in mice treated with equal and equiactive doses. The levels of total fluorescence due to the initial drugs and to metabolites were determined in tissue extracts by fluorometry. 4′-Deoxydoxorubicin reached the same tissue levels as doxorubicin in all the organs tested except in spleen and lung, where a higher peak was found in the animals treated with the new analog. The rate of elimination of 4′-deoxydoxorubicin from the organs tested was higher than that of doxorubicin.*

## Introduction

4′-Deoxydoxorubicin (4′-deoxyDX) is a new analog of doxorubicin (DX) and is characterized by the lack of the hydroxylic group in position 4′ of the aminosugar. It has been reported that this compound is as active as DX, and more potent than DX in some experimental tumor systems [1]. Cardiotoxicity studies on rabbits and mice [5] have shown that 4′-deoxyDX is less cardiotoxic than DX. The higher potency together with the lower cardiotoxicity of 4′-deoxyDX compared with DX make this new analog very interesting and prompted us to investigate its pharmacokinetic behavior in mice and compare it with that of DX.

## Materials and Methods

*Drugs*

DX and 4′-deoxyDX were supplied as hydrochlorides by Ricerca Chimica, Farmitalia Carlo Erba Research Laboratories, Milano, Italy. Aqueous drug solutions were freshly prepared immediately before use and injected IV in a volume of 10 ml/kg body weight. 4′-DeoxyDX was given at an equal dose (10 mg/kg) and at an equiactive dose (5 mg/kg), since 4′-deoxyDX is twice as potent as DX [5].

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*Animals and Tumors*

Adult male BALB/c mice were supplied by Charles River Breeding Laboratories, Calco, Italy. The animals were 2–3 months old, weighed 20–30 g, and were maintained under standard laboratory conditions. The animals received IM injections of $10^4$ MS-2 sarcoma cells/mouse [8] into the right hind leg. The animals were used for tissue distribution studies when the tumors were palpable.

Four animals were used for each experimental point. Animals were treated by IV injection and killed with ether. Tissues were removed, rinsed in saline, and stored at −70° C until drug extractions. The gallbladder was always removed from the liver, and the small intestine was always emptied before being rinsed in saline. Blood was collected via the eye plexus into cold heparin-coated glass tubes. After the blood had been centrifuged, the plasma was removed and stored at −70° C until analysis.

*Biochemical Assay of the Drugs*

The levels of DX and 4′-deoxyDX in plasma and organs were estimated by fluorometry. This method does not differentiate between parent drugs and fluorescent metabolites, and therefore drug concentrations are expressed as drug equivalents (μg/g wet tissue and μg/ml plasma). The extraction of the drugs from tissue homogenates and plasma was performed with *n*-butyl alcohol after the addition of $AgNO_3$ to release drugs bound to DNA and RNA and to precipitate proteins, as described by Schwartz [10]. Reference standard curves, set up for plasma and each organ with both drugs, were linear in the range investigated. The sensitivity of the assay was 0.5 μg/g for tissues and 0.05 μg/ml for plasma. The fluorescent intensities of the extracted drugs were read on a Perkin-Elmer MPF 44A spectrofluorometer at the optimal excitation wavelength of 470 nm and at the maximum emission wavelength of 592 nm for both compounds.

*Pharmacokinetic Analysis*

The experimental results, represented by the averages of the concentrations for four animals per time, were analyzed

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according to a single-compartment or a two-compartment model by means of programs implemented on a UNIVAC 1106 computer, which calculates the pharmacokinetic parameters by means of an iterative calculation process where the convergence is obtained by a 'steepest descent' method [9].

The comparisons of overall elimination parameters of the different treatment groups were carried out by means of an appropriate '*t*' statistical test [4].

For data concerning the tumor, the areas under the concentration-versus-time curves (AUC) of levels from 0 to 72 h were calculated for each treatment group. For the comparison of areas the variance of each area was calculated and the differences were tested by Sheffé's test, as previously described [7].

## Results

Drug equivalent concentrations found in several organs at different times after IV administration of 5 mg 4′-deoxyDX/kg and of equal (5 mg/kg) and equiactive (10 mg/kg) doses of DX and the relative curves fitted by means of the calculated parameters are shown in Fig. 1. The fit of the experimental data was good, since the standard errors of the estimates gave a percentage error ranging between 1% and 20%, mostly below 10%.

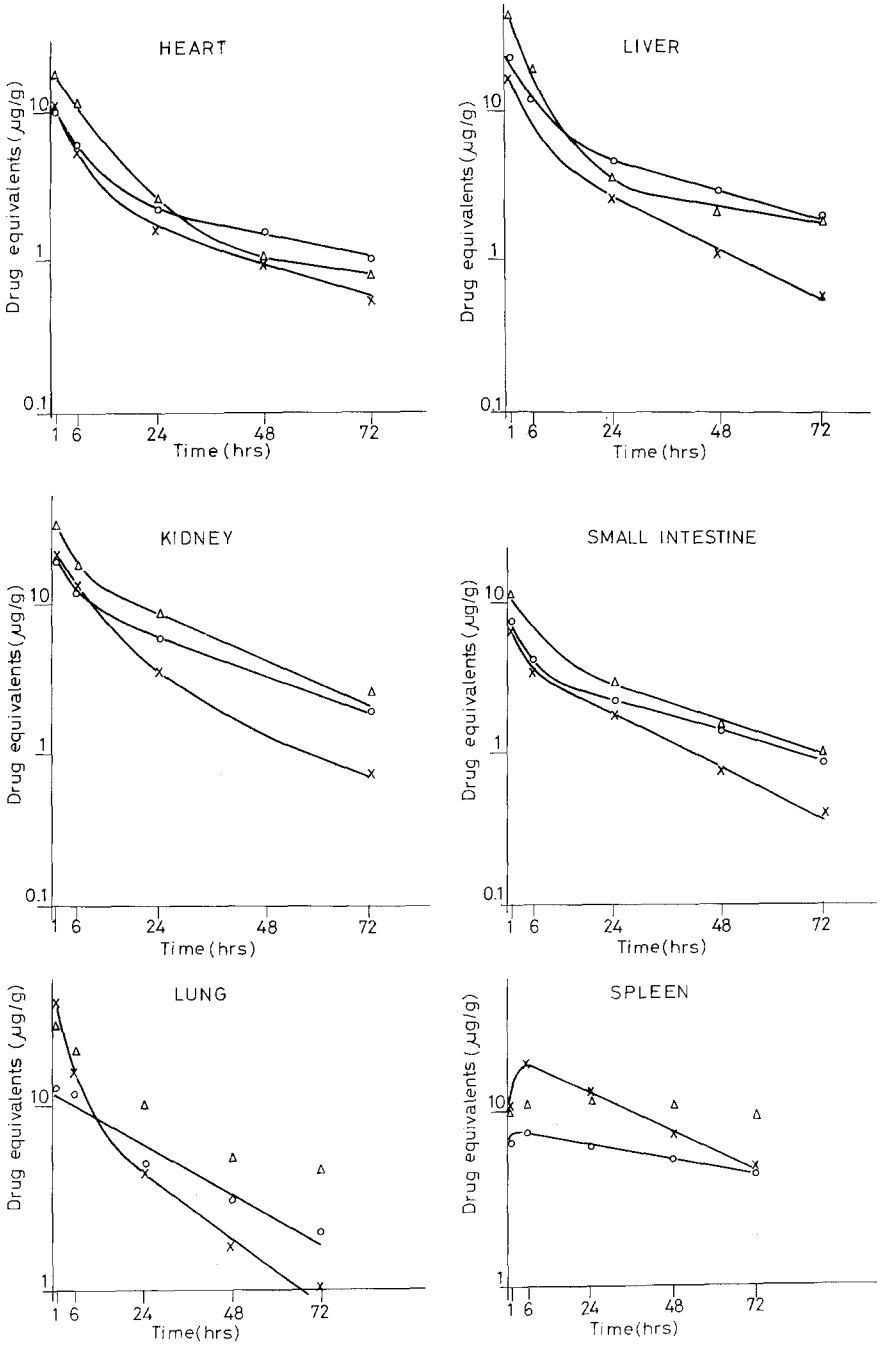


**Fig. 1.** Drug equivalent levels and relative fitted curves found in organs of BALB/c mice bearing MS-2 sarcoma treated with 5 mg 4′-deoxyDX/kg (×), 5 mg DX/kg (○), and 10 mg DX/kg (△). Drug equivalent levels found after 10 mg DX/kg (△) were not fitted by curves in lung and in spleen

In heart, liver, kidney, and small intestine, the drug equivalent levels observed after administration of 5 mg 4′-deoxyDX/kg and 5 and 10 mg DX/kg were best described by a two-compartment model. Similar findings were observed in the lung of animals treated with 4′-deoxyDX. The lung levels observed after administration of 5 mg DX/kg were best described by a single-compartment distribution, while levels found after 10 mg DX/kg did not fit either the two- or the one-compartment model.

The drug equivalent levels found in the spleen showed a slow absorption phase followed by an

**Table 1.** Pharmacokinetic parameters of 4′-deoxydoxorubicin and doxorubicin in organs of BALB/c mice bearing MS-2 sarcoma

| | Dose (mg/kg) | $\alpha$[a] | $\beta$[b] | Co[c] | $t_{50\%}$ | |
|---|---|---|---|---|---|---|
| | | | | | $\alpha$ phase | $\beta$ phase |
| Two-compartment model | | | | | | |
| Heart | | | | | | |
| 4′-DeoxyDX | 5 | 0.20874 | 0.02326 | 12.9 | 3.31 | 29.79 |
| DX | 5 | 0.15203 | 0.01391 | 11.4 | 4.56 | 49.82 |
| DX | 10 | 0.10871 | 0.01087 | 20.4 | 6.37 | 63.75 |
| Liver | | | | | | |
| 4′-DeoxyDX | 5 | 0.21580 | 0.03309 | 19.2 | 3.21 | 20.94 |
| DX | 5 | 0.16270 | 0.01709 | 22.9 | 4.25 | 40.55 |
| DX | 10 | 0.18180 | 0.01032 | 49.4 | 3.81 | 67.15 |
| Small intestine | | | | | | |
| 4′-DeoxyDX | 5 | 0.48970 | 0.03381 | 8.7 | 1.41 | 20.49 |
| DX | 5 | 0.28660 | 0.01980 | 8.9 | 2.41 | 35.00 |
| DX | 10 | 0.17212 | 0.02251 | 12.2 | 4.02 | 30.78 |
| Kidney | | | | | | |
| 4′-DeoxyDX | 5 | 0.11660 | 0.02686 | 23.7 | 5.94 | 25.80 |
| DX | 5 | 0.22039 | 0.02399 | 22.3 | 3.14 | 28.88 |
| DX | 10 | 0.30833 | 0.02896 | 39.3 | 2.24 | 23.93 |
| Lung | | | | | | |
| 4′-DeoxyDX | 5 | 0.22760 | 0.02758 | 38.1 | 3.04 | 25.13 |
| One-compartment model | | | $k_{el}$[e] | | | $t_{50\%}$ |
| Spleen | | | | | | |
| 4′-DeoxyDX | 5 | | 0.02187 | 21.3 | | 31.68 |
| DX | 5 | | 0.00787 | 7.6 | | 88.05 |
| Lung | | | | | | |
| DX | 5 | | 0.02640 | 11.6 | | 26.25 |

[a] $\alpha$, distribution slope ($h^{-1}$)
[b] $\beta$, overall elimination slope ($h^{-1}$)
[c] Co, calculated concentrations at time 0 (μg/g)
[d] $t_{50\%}$, half life of distribution ($\alpha$) and elimination ($\beta$) phases (h)
[e] $k_{el}$, overall elimination slope ($h^{-1}$)

**Table 2.** Comparison of elimination constants

| Treatment (mg/kg) | Heart | Liver | Small intestine | Kidney | Spleen |
|---|---|---|---|---|---|
| 4′-DeoxyDX (5) vs DX (5) | 4′-DeoxyDX** > DX | 4′-DeoxyDX > DX | 4′-DeoxyDX > DX | not different | 4′-DeoxyDX > DX |
| 4′-DeoxyDX (5) vs DX (10) | 4′-DeoxyDX > DX | 4′-DeoxyDX > DX | 4′-DeoxyDX* ⩾ DX | not different | – |
| DX (5) vs DX (10) | not different | DX (5) > DX (10) | not different | DX (10) > DX (5) | – |

** > Significant difference ($P < 0.05$); * ⩾ Significant difference ($0.05 < P < 0.10$)

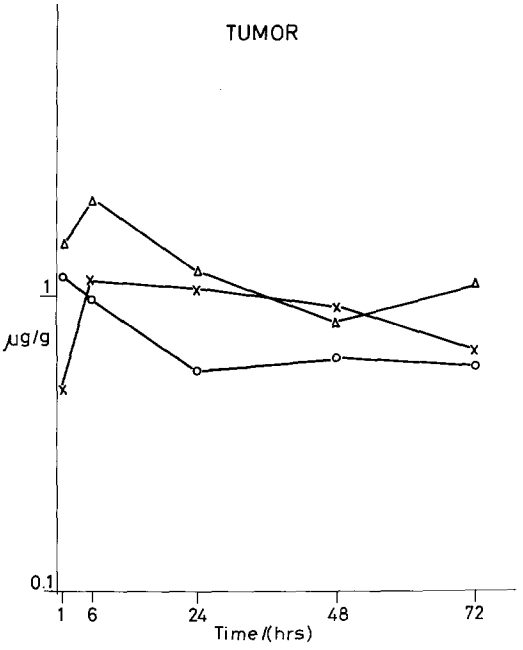


**Fig. 2.** Plasma levels of 4′-deoxyDX given in dose of 5 mg/kg (×), and DX given in doses of 5 mg/kg (○) and 10 mg/kg (△), to BALB/c mice bearing MS-2 sarcoma

**Table 3.** Areas under the concentration versus times curves (0–72 h) found in tumors

| Treatment | mg/kg | AUC ± SD (µ/g × h) |
|---|---|---|
| 4′-DeoxyDX | 5 | 67.26 ± 3.90[a] |
| DX | 5 | 48.85 ± 4.07 |
| DX | 10 | 87.27 ± 5.11[b] |

[a] Statistically higher than AUC after DX 5 mg/kg ($P < 0.01$)
[b] Statistically higher than AUC after DX 5 mg/kg and 4′-deoxyDX 5 mg/kg ($P < 0.01$)

elimination phase, which fitted the single-compartment model well, in animals treated with 4′-deoxyDX and 5 mg DX/kg. No statistical regression of drug levels in relation to time was found in animals treated with 10 mg DX/kg.

The distribution and overall elimination slopes ($\alpha$ and $\beta$), the drug concentrations at time 0 ($C_0$), and the half-life ($t_{50\%}$) of distribution and elimination phases ($\alpha$ and $\beta$ phases) of the investigated organs are reported in Table 1. 4′-DeoxyDX and DX given at equal doses (5 mg/kg) had similar initial levels ($C_0$) in heart, liver, small intestine, and kidney. In the same organs, higher initial drug levels were obtained after administration of DX at a dose of 10 mg/kg. In lung, 4′-deoxyDX reached higher initial levels than DX administered at the same dose, and the patterns of distribution were different, since the former followed a two-compartment model and the latter a one-compartment model, as previously described. The peak reached in spleen after administration of 4′-deoxyDX was much higher than that reached by DX given at equal and equiactive doses.

The results of the statistical comparison of the elimination constants in the differently treated groups ($\beta$ for the two-compartment model and $k_{el}$ for the one-compartment model) are reported in Table 2. The rate of elimination of 4′-deoxyDX from heart, liver, and small intestine was statistically higher than that of DX given at either dose. In spleen, the rate of elimination of 4′-deoxyDX was higher than that of DX given at an equal dose, while a comparison with DX given at 10 mg/kg was not possible owing to lack of regression of drug concentrations during the period of the study. The elimination constant of DX given at 5 mg/kg was significantly higher in liver and lower in kidney than that of DX at 10 mg/kg.

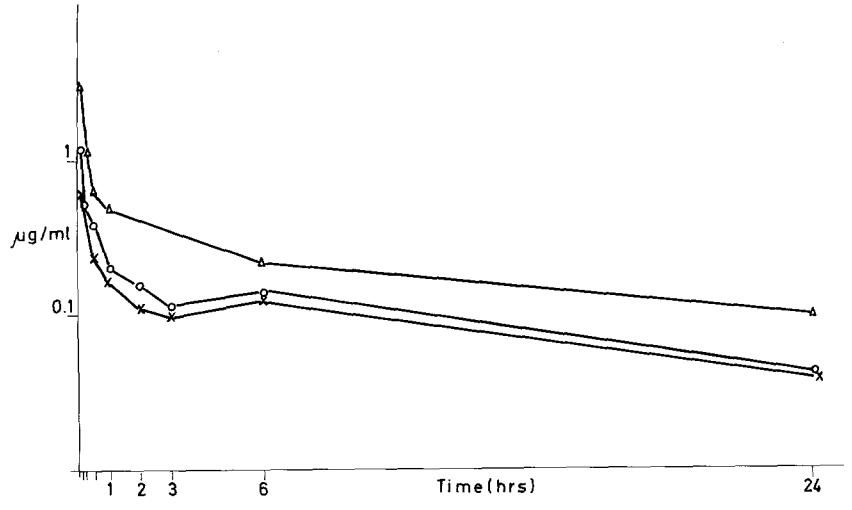


**Fig. 3.** Drug equivalent levels found in MS-2 sarcomas transplanted in BALB/c mice treated with 5 mg 4′-deoxyDX/kg (×), 5 mg DX/kg (○), 10 mg DX/kg (△)

Drug concentrations in the tumor did not fit either the two- or the one-compartment model. The equivalent drug concentrations found in tumors at different times after drug administration and the calculated areas under the curves (AUC) are given in Fig. 2 and in Table 3. The AUC obtained after the administration of 10 mg DX/kg is statistically larger than that following 5 mg DX/kg or 5 mg 4′-deoxy-DX/kg. The AUC of 4′-deoxyDX concentrations is statistically larger than that obtained after administration of an equal dose of DX.

Plasma drug levels, reported in Fig. 3, were investigated only up to 24 h, since the sensitivity of the fluorescence assay does not make it possible to demonstrate the drug after long periods (48–72 h), and it was therefore not possible to evaluate the elimination phases of the two drugs. From the results obtained it seems that 4′-deoxyDX reaches the same drug equivalent levels as DX given at an equal dose (5 mg/kg), and higher drug levels were found after administration of 10 mg DX/kg.

## Discussion

The effects that the lack of the hydroxylic group in position 4′ of the aminosugar of DX brings about on the biochemical activity of this new compound (4′-deoxyDX) have been previously investigated. In cell-free systems no substantial differences were found between DX and 4′-deoxyDX: 4′-deoxyDX showed the same apparent binding constant (Kapp) to DNA as DX and the same effect on RNA polymerase, and it was only slightly more active than DX on DNA polymerase [6]. In cell cultures, 4′-deoxyDX was more active than DX against HeLa cells treated for 8 h, and its uptake in L1210 cells was much higher than that of DX, in parallel with the higher partition coefficient [6].

From the results reported here, it can be seen that in mice 4′-deoxyDX, given at the same dose as DX, reached initial drug levels equal to those of DX in all the organs tested except in lung and spleen, where higher concentrations were found in the animals treated with the new analog. In general, 4′-deoxyDX was eliminated more rapidly than DX from the tissues.

In the spleen, unlike other organs, an absorption phase was found with both drugs, indicating a slower uptake for this organ than for other organs, as already also seen for daunorubicin [2, 11]. In this organ 4′-deoxyDX reached much higher drug levels than DX; this was followed by a rapid elimination, so that 72 h after treatment its concentration was similar to that of DX. This pharmacokinetic behavior, which brings about higher levels in the spleen, appears to correlate with the higher acute toxicity of 4′-deoxy-DX than of DX [5]. In fact, acute toxicity of this class of compounds is mainly due to an inhibiting effect on cellular reproduction, which is particularly evident in proliferative organs like spleen [3].

In the tumor, higher concentrations of 4′-deoxy-DX than DX were found after administration of an equal dose, as shown by a comparison of the areas under the curves up to 72 h after treatment. This could be correlated with the higher potency of 4′-deoxyDX than of DX: in solid sarcoma 180, the same antitumor activity seen with DX can be achieved with 4′-deoxyDX given at half doses [1].

It has been reported that 4′-deoxyDX is less cardiotoxic than DX both in rabbits and in mice [5]. Since the higher rate of elimination of 4′-deoxyDX than of DX has also been found in the heart, this might be one of the reasons for the lower cardiotoxicity of this new analog compared with its parent compound. However, since our data refer to total fluorescence due to both initial drugs and metabolites, different rates of metabolism and different intracellular drug distributions could also account for the biological differences observed in vivo between DX and this new derivative.

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Received May 16/Accepted November 3, 1980