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Plasma Levels of Doxorubicin After IV Bolus Injection and Infusion of the Doxorubicin-DNA Complex in Rabbits and Man

Comparison with Free Doxorubicin

R. Baurain, D. Deprez-De Campeneere, A. Zenebergh, and A. Trouet

Université Catholique de Louvain and International Institute of Cellular and Molecular Pathology, 75, Avenue Hippocrate, B-1200 Brussels, Belgium

Summary. *When injected rapidly IV into rabbits, the plasma levels of free DOX decreased biphasically and the drug was distributed in a volume greater than the body volume. When given as a DNA complex, the area under the concentration versus time curve was increased 10-fold and the distribution volume reduced more than 100-fold. The DOX-DNA complex infused both in rabbits and in human patients reached steady state-concentrations 10 and 20 times higher, respectively, than free DOX infusion, and the distribution volumes were reduced accordingly. These results confirm that the observed lower cardiotoxicity of the DOX-DNA complex arises despite higher plasma concentrations of the drug.*

Introduction

When doxorubicin (DOX) is bound to high-molecular-weight DNA, the drug becomes more active against experimental murine L1210 leukemia and less toxic [6, 15]. The lower uptake by heart tissue of the DOX-DNA complex which has been recorded previously in experimental models [4, 16] is most probably related to its lower cardiotoxicity. Clinical results obtained from more than 560 patients treated with DOX-DNA, 94 patients having received more than 500 mg DOX/m^2, tend to confirm the lower cardiotoxicity of the complex without concomitant decrease in therapeutic activity [14].

In the present study we therefore compared the pharmacokinetic properties of DOX and DOX-DNA both in an experimental model using rabbits and in man.

Materials and Methods

Doxorubicin (DOX) was provided by Farmitalia Benelux, Brussels. DNA was extracted from herring sperm (type VII, Sigma Chemicals, St. Louis, USA). The anthracycline-DNA complex was prepared at a nucleotide/drug molar ratio of 20 [15].

Plasma levels of DOX and high-molecular-weight DNA were followed in New Zealand white rabbits (NZW; Creal, S.A., Loupoigne, Belgium) after an IV bolus injection or infusion of the free or DNA-bound drug at 3.5 mg/kg. After various times 2–3 ml blood was collected from the opposite ear into EDTA as anticoagulant and centrifuged at 4° C for 10 min at 2,500 rpm (model PR 6000, rotor 259, Damon/IEC, International Centrifuge, Needham Heights, Mass., USA).

Reprint requests should be addressed to R. Baurain

The drugs were detected in the plasma by high-pressure liquid chromatography (HPLC) and fluorometry as described previously [2, 3]. For the complexes, DNA was ^{125}I-labelled according to the method of Commerford [5] as modified by Orosz and Wetmur [9]. The amount of TCA-soluble ^{125}I-DNA was determined in the plasma samples after addition of ice-cold TCA at 40% and of bovine serum albumin (Poviet Producten, N.V., Amsterdam, Holland) at 10% as co-precipitant. After 1 h the tubes were centrifuged in a Damon/IEC International Centrifuge at 2,400 rpm for 45 min at 4° C. The radioactivity of the supernatant was subtracted from the total radioactivity to obtain the amount of TCA-insoluble ^{125}I-DNA. The labelled DNA used contained less than 1.4% of TCA-soluble fragments.

Data Analysis. Plasma concentrations versus time data, obtained for each separate individual either after bolus injection or after the end of infusion, were fitted to an exponential or a bi-exponential equation. From the parameters of these equations, the pharmacokinetic parameters were calculated as follows:

From the exponential equation: $\ln C = \ln (A \cdot e^{-\alpha t})$

V_d = volume of distribution = $\dfrac{\text{administered dose}}{A}$,

k_{el} = elimination constant = α,

$t_{½_{el}}$ = half-life of elimination = $\dfrac{\ln 2}{\alpha}$,

$\int_0^\infty C \cdot dt$ = area under the C × t curve = $\dfrac{\text{dose}}{\alpha \cdot V_d} = \dfrac{A}{\alpha}$;

From the bi-exponential equation: $\ln C = \ln (A \cdot e^{-\alpha t} + B \cdot e^{-\beta t})$ the pharmacokinetic parameters being calculated using the HP67 program described by Niazi [8].

V_1 = volume of central compartment = $\dfrac{\text{dose}}{A + B}$,

k_{21} = transfer constant from peripheral compartment to central compartment = $\dfrac{A \cdot \beta + B \cdot \alpha}{A + B}$,

k_{el} = elimination constant = $\dfrac{\alpha \cdot \beta}{k_{21}}$,

k_{12} = transfer constant from central to peripheral compartment = $\alpha + \beta - k_{21} - k_{el}$,

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$$V_2 = \text{volume of peripheral compartment} = V_1 \cdot \frac{k_{12}}{k_{21}},$$

$$\int_0^\infty C \cdot dt = \text{area under the C} \times \text{t curve} = \frac{A}{\alpha} + \frac{B}{\beta},$$

$$V_{d_{\text{area}}} = \text{volume of distribution} = \frac{\text{dose}}{\beta \cdot \int C \cdot dt}.$$

During the infusion, the area under the curve is calculated by numerical integration and the value added to the area calculated post-infusion by the previous equations. For the infusion data, the $V_{d_{\text{area}}}$ is calculated according to:

$$V_{d_{\text{area}}} = \frac{\text{infusion rate}}{k_{el} \times C_p^{ss}}.$$

Results

After IV bolus administration at 3.5 mg/kg to NZW rabbits, the plasma levels of DOX decreased biphasically. As shown in Fig. 1a, DOX remained the major fluorescent compound but two metabolites were also present in the plasma in lower amounts: doxorubicinone (DOX-ONE) and doxorubicinol (DOX-OL). Table 1 shows the pharmacokinetic parameters calculated assuming an open two-compartment model. When administered as free drug, DOX was distributed in a volume of 336 ml and was eliminated from the rabbit with a half-life of 1.3 min.

When given at the same dose but as a DNA complex, DOX dissociated partly from the high-molecular-weight DNA (Fig. 1b) and was eliminated with a half-life of 15.4 min. Both DOX and DNA were distributed in a volume of about 120 ml (Table 1).

After infusion of free DOX at 3.5 mg/kg to NZW rabbits over 69 min, the steady-state concentration reached a value of 1.8 μg/ml (Fig. 2a). In the case of DOX-DNA infusion both the steady-state concentration and the area under the concentration versus time (c × t) curve were 12 times higher (Fig. 2b and Table 2) than after free DOX infusion. DOX and DNA distributed in about 95 ml, a volume 10 times smaller than that after free DOX infusion.

The plasma levels of doxorubicin after infusion of the free drug at a dose of 75 mg/m² have been followed in three patients

Table 1. Pharmacokinetic parameters obtained following IV bolus administration to NZW rabbits of 3.5 mg free DOX or DOX-DNA/kg

	Free DOX ($n = 3$)[a]	DOX-DNA ($n = 4$)	
		DOX	DNA
V_1 (ml)	336 ± 134[b]	105 ± 8	122 ± 2
V_2 (ml)	4,260 ± 1,860	–	–
$V_{d\,\text{area}}$ (ml)	12,180 ± 5,360	105 ± 8	122 ± 2
k_{12} (min^{-1})	0.277 ± 0.007	–	–
k_{21} (min^{-1})	0.023 ± 0.007	–	–
k_{el} (min^{-1})	0.517 ± 0.102	0.045 ± 0.010	0.018 ± 0.001
$\int_0^\infty C \cdot dt$ (μg · min · ml^{-1})	104 ± 3	1,610 ± 380	39,200 ± 5,400
$V_{d\,\text{area}}$ (% of weight)	238 ± 69	4,9 ± 0.4	5.6 ± 0.3
$\int_0^\infty C \cdot dt$ (for 1 kg)	22 ± 6	760 ± 190	17,900 ± 1,300

[a] n, number of rabbits
[b] Mean ± SD

Table 2. Pharmacokinetic parameters obtained following infusion to NZW rabbits of 3.5 mg free or DNA-bound DOX/kg

	Free DOX ($n = 1$)[a]	DOX-DNA ($n = 4$)	
		DOX	DNA
C_p^{ss} (μg/ml)	1.75	20.6 ± 3.6[b]	296 ± 16
k_{el} (min^{-1})	0.110	0.058 ± 0.003	0.043 ± 0.002
$V_{d\,\text{area}}$ (ml)	966	91 ± 16	95 ± 5
$\int_0^\infty C \cdot dt$ (μg · min · ml^{-1})	81.5	1,190 ± 230	21,740 ± 1,460
Infusion time (min)	69	68.5 ± 9.6	
$V_{d\,\text{area}}$ (% of weight)	28.9	4.7 ± 0.9	4.2 ± 0.1
$\int_0^\infty C \cdot dt$ (for 1 kg)	24.4	600.4	9,710 ± 900

[a] n, number of rabbits
[b] Mean ± SD

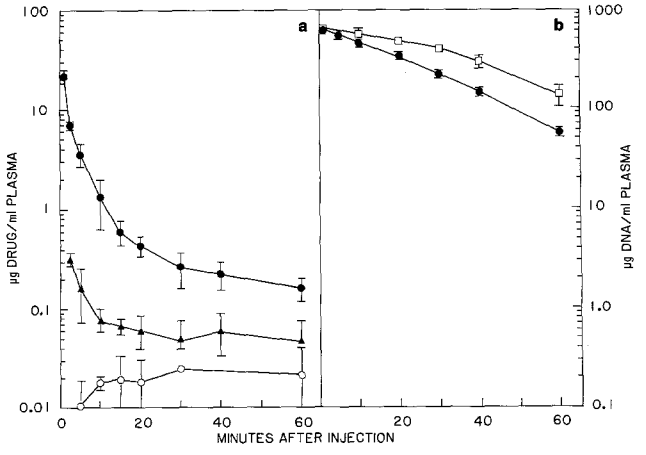

Fig. 1a and b. Plasma levels after IV bolus administration of free (**a**) and DNA-bound (**b**) DOX at 3.5 mg/kg to NZW rabbits. Mean ± SD of at least three experiments in each case. **a** DOX (●), DOX-OL (○), and DOX-ONE (▲) after DOX administration; **b** DOX (●) and TCA-precipitable DNA (□) after DOX-DNA administration

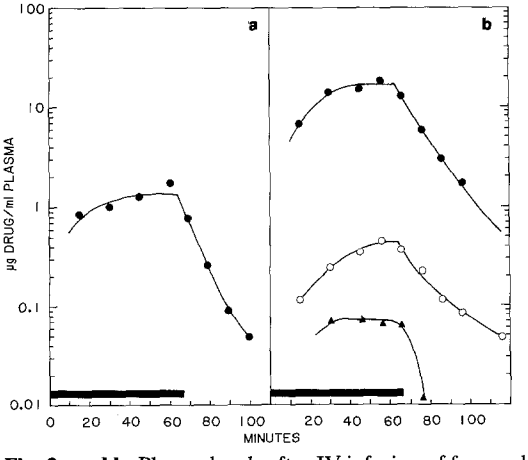

Fig. 2a and b. Plasma levels after IV infusion of free and DNA-bound DOX at 3.5 mg/kg to NZW rabbits over 69 min. **a** DOX (●) after free DOX infusion; **b** DOX (●), DOX-OL (○), and DOX-ONE (▲) after DOX-DNA infusion

Table 3. Pharmacokinetic parameters obtained following infusion of free DOX and DOX-DNA complex at 75 mg/m^2 to patients

	DOX			DOX-DNA
	PG	RM	CD	CD
C_p^{ss} (μg/ml)	0.048	0.068	0.075	1.79
k_{el} (min^{-1})	0.069	0.016	0.029	0.031
$V_{d\,area}$ (ml)	122,000	391,000	198,000	9,230
$\int_0^\infty C \cdot dt$ (μg · min · ml^{-1})	9.6	16.5	14.3	327
Infusion time (min)	225	210	205	205

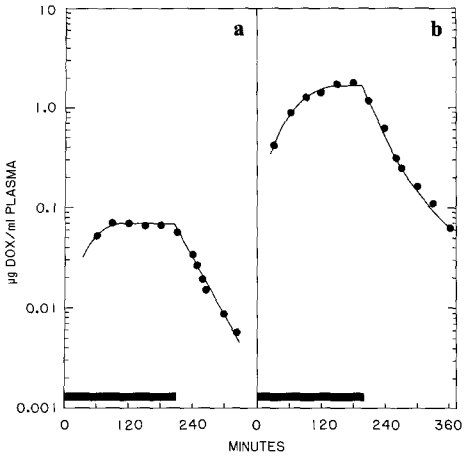

Fig. 3a and b. Plasma DOX levels in patient CD after infusion of either free (**a**) or DNA-bound (**b**) DOX at 75 mg/m^2 over 205 min

(Table 3). One of the patients received an infusion of DOX-DNA containing the same dose of DOX 3 weeks later (Fig. 3). During free DOX infusion a steady-state plasma level of 0.065 μg/ml was reached after 90 min, and at the end of the infusion the drug plasma level decreased with a half-life of 23.9 min. When the DOX-DNA complex was administered under the same conditions, the steady-state concentrations of DOX reached a level 26 times greater and the apparent volume of drug distribution was 26 times smaller than after infusion of free DOX. The total plasma drug exposure, measured by the area under the concentration versus time curve, was increased 23 times when the drug was given as a DNA complex.

Discussion

After administration of free or DNA-bound DOX to NZW rabbits, the parent drug remained the main fluorescent compound in the plasma, only small amounts of doxorubicinol and doxorubicinone being formed. The low amounts of DOX-OL and DOX-ONE found in the plasma confirm that DOX is poorly metabolized in vivo [1] and that our HPLC method avoids any artefactual transformation or metabolism of the drug during the extraction procedure. Usually less than 30 min elapses between blood collection and the end of the plasma analysis.

When DOX was given as an IV bolus injection into NZW rabbits, the drug apparently distributed in a volume greater than the total body volume. Although the distribution volume has no physiological significance, that it is greater than the total body volume suggests that the drug is segregated and concentrated in concealed compartments. It has been shown previously that DOX is taken up by various cell lines and concentrated intracellularly more than 100 times [18]. After infusion of free DOX to rabbits, no DOX-OL could be detected and the plasma exposure (area under the concentration versus time curve) was the same as after IV bolus administration.

The dissociation of various anthracycline-DNA complexes in the plasma has been shown to depend on the concentration and on the affinity constant of the drug for DNA [11]. When DOX-DNA was given as an IV bolus injection to rabbits, the drug partly dissociated from the high-molecular-weight DNA. However, by comparison with free DOX administration higher plasma concentrations were maintained for longer times. Zenebergh et al. [18] have shown that DOX-DNA is less thoroughly taken up than DOX by various cell lines, the binding to DNA preventing the drug from entering by permeation through the cell membrane unless DOX dissociates from the DNA. The drug escaping from the high-molecular-weight DNA does not seem to be taken up mainly by the extravascular compartments, since the volume of distribution of both DOX and DNA was not significantly different from the plasma volume, results which suggest that the drug leaving the DNA complex is eliminated from the body.

The decreased exposure of various organs of mice and rats, including the heart, despite higher plasma concentration of DOX when associated to DNA has been used to explain the lower general toxicity and cardiotoxicity of the DOX-DNA complex [4, 6, 13, 16].

Administration of DOX-DNA by either IV bolus injection or infusion afforded values of distribution volume, plasma exposure, and elimination constants for the drug which were not significantly different. Therefore from a pharmacokinetic point of view there is no difference in the disposition of DOX-DNA according as whether it is injected rapidly IV or infused.

When DOX was infused at a dose of 75 mg/m^2 to three patients over about 210 min the steady-state plasma concentration was not greater than 0.08 μg/ml and the apparent volume of distribution was greater than the total body volume. After administration of the DOX-DNA complex to the same patient (CD) under the same conditions and with the same drug dose 3 weeks after infusion of free DOX, both the steady-state plasma concentration and the plasma exposure were increased 25-fold, while the distribution volume was reduced 20-fold.

These results confirm that the decreased cardiotoxicity observed in more than 560 patients treated with the DOX-DNA complex in Belgium [14] and in fewer cases in Norway [7] and Sweden [10], arise despite higher plasma coincentrations of the drug, as shown also by Lie et al. [7].

In conclusion, the use of DOX-DNA complex results in higher plasma exposure and probably prevents the drug from entering the cells by permeation unless it dissociates from the DNA. The possibility that part of the drug escapes from the complex and reaches the cardiac muscle cells as free drug could not, however, be excluded.

Nevertheless, only a covalent linkage of DOX to a high-molecular-weight carrier could prevent the drug from being completely released in the bloodstream. The stability of the drug-carrier conjugate in the bloodstream is one of the criteria which must be fulfilled to achieve cancer-targeted chemotherapy, the other criteria being [12]: the recognition

and endocytosis of the carrier by the target cells and the reversibility of the link between the drug and its carrier inside the lysosomes after endocytosis of the conjugate. Such a linkage between DNR and proteins has recently been described [17].

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References

1. Bachur NR, Hildebrand RC, Jaenke RS (1974) Adriamycin and daunorubicin disposition in the rabbit. J Pharmacol Exp Ther 191: 331–350
2. Baurain R, Deprez-De Campeneere D, Trouet A (1979a) Determination of daunorubicin, doxorubicin and their fluorescent metabolites by high-pressure liquid chromatography: plasma levels in DBA_2 mice. Cancer Chemother Pharmacol 2: 11–14
3. Baurain R, Deprez-De Campeneere D, Trouet A (1979b) Rapid determination of doxorubicin and its fluorescent metabolites by high-pressure liquid chromatography. Anal Biochem 94: 112–116
4. Blanchard JC, Schneider Y-J, Baurain R, Trouet A (1981) Accumulation, metabolism and subcellular localization of daunorubicin, doxorubicin and their DNA-complexes in rat heart ventricles. Eur J Cancer 17: 297–305
5. Commerford SL (1971) Iodination of nucleic acids in vitro. Biochemistry 10: 1993–1999
6. Deprez-De Campeneere D, Baurain R, Huybrechts M, Trouet A (1979) Comparative study in mice of the toxicity, pharmacology and therapeutic activity of daunorubicin-DNA and doxorubicin-DNA complexes. Cancer Chemother Pharmacol 2: 25–30
7. Lie SO, Lie KK, Glomstein A (1979) Clinical and pharmacological studies with adriamycin-DNA complex in children with malignant disease. Cancer Chemother Pharmacol 2: 61–66
8. Niazi S (1979) Multicompartment pharmacokinetic analysis and simulations using a programmable calculator. Int J Biomed Comput 10: 245–255
9. Orosz JM, Wetmur JG (1974) In vitro iodination of DNA. Maximizing iodination while minimizing degradation. Use of buoyant density shift for DNA-DNA hybrid isolation. Biochemistry 13: 5467–5473
10. Paul C, Lönnqvist B, Gahrton G, Peterson C (1981) Reducing the cardiotoxicity of anthracyclines by complex-binding to DNA: report of three cases. Cancer 48: 1531–1534
11. Schneider Y-J, Baurain R, Zenebergh A, Trouet A (1979) DNA binding parameters of daunorubicin and doxorubicin in the conditions used for studying the interactions of anthracyclines-DNA complexes with cells in vitro. Cancer Chemother Pharmacol 2: 7–10
12. Trouet A (1978) Increased selectivity of drugs by linking to carriers. Eur J Cancer 14: 105–111
13. Trouet A, Deprez-De Campeneere D (1979) Daunorubicin-DNA and doxorubicin-DNA: a review of experimental and clinical data. Cancer Chemother Pharmacol 2: 77–79
14. Trouet A, Sokal G (1979) Clinical trials with daunorubicin-DNA and adriamycin-DNA complexes: a review. Cancer Treat Rep 63: 895–898
15. Trouet A, Deprez-De Campeneere D, de Smedt-Malengreaux M, Atassi G (1974) Experimental leukemia chemotherapy with a "lysosomotropic" adriamycin-DNA complex. Eur J Cancer 10: 405–411
16. Trouet A, Deprez-De Campeneere D, Baurain R, Huybrechts M, Zenebergh A (1979) Desoxyribonucleic acid as carrier for anti-tumoral drugs. In: Gregoriadis G (ed) Carriers in biology and medicine. Academic Press, London, pp 87–105
17. Trouet A, Masquelier M, Baurain R, Deprez-De Campeneere D (1982) A covalent linkage between daunorubicin and proteins that is both stable in serum and reversible by lysosomal hydrolases, as required for a lysosomotropic drug-carrier conjugate: in vitro and in vivo study. Proc Natl Acad Sci USA 79: 626–630
18. Zenebergh A, Schneider Y-J, Trouet A (1980) Comparative studies of the accumulation of doxorubicin and doxorubicin-DNA in various cell lines. Biochem Pharmacol 29: 1035–1040

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