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Doxorubicin binds in a cooperative manner to myocardial cells

Two binding sites

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Summary. Experimental evidence indicates that the anthracycline antibiotic doxorubicin (adriamycin) localizes mainly in cell nuclei of cardiac cells and has a high affinity to several cellular constituents in addition to DNA. In the present study the cellular kinetics of doxorubicin in cultured rat myocardial cells were determined by measuring its uptake, its binding pattern over a concentration range of 0.1 m*M* to 80 μ*M*, and the cellular release by means of [14-^{14}C]doxorubicin. The binding kinetics of doxorubicin were compared with the doxorubicin-induced inhibition of [methyl-^{3}H]thymidine incorporation into DNA. It is demonstrated that at micromolar concentrations doxorubicin is readily taken up by myocardial cells and that myocardial cells have the ability to bind doxorubicin at two specific binding sites and that a noncooperative high-affinity/low-capacity type and a positive cooperative type of binding are involved, as indicated by the positive slope in the initial region of the binding isotherm (Scatchard plot). A dose-dependent inhibition of [methyl-^{3}H]thymidine incorporation into DNA is demonstrated. It is suggested that this is associated with the positive cooperative binding of doxorubicin. The cellular release of doxorubicin appeared to be biphasic, with estimated half-lives of about 5–6 h for the initial phase and 50–60 h for the terminal phase. The results of this study indicate that doxorubicin preferably binds to sites within myocardial cells and that the positive cooperative binding pattern is due to DNA as one of the binding sites. A relationship between the noncooperative high-affinity/low capacity binding and the pharmacological activity has yet to be determined.

Introduction

The anthracycline antibiotic doxorubicin (adriamycin) (DX) plays a prominent role in the treatment of a wide range of human malignant neoplasm [29]. However, the clinical administration of DX is limited by a dose-dependent development of irreversible congestive cardiomyopathy [1, 30].

The tissue distribution of DX has been studied in several animal species, showing a clear difference in drug concentration among tissues. Although it has been reported previously that DX is localized in the nuclei, DX is also able to bind to several tissue constituents in addition to DNA [12, 14, 18, 26]. The ability of cardiac cells to incorporate DX has led to studies of ultrastructural arrangements and biochemical interactions. It has been demonstrated that DX induces structural changes, inhibition of DNA synthesis, and biochemical alterations of contractile proteins in cardiac cells both in vivo and in vitro [2, 8, 12, 22, 27].

The availability of cultured cells from a wide range of normal and malignant tissues has made it possible to study the subcellular localization and effects of DX. Evidence exists concerning the interaction of DX with phospholipids of cardiac cell membranes and its affinity for actin, heavy meromyosin, mucopolysacchirdes, and nucleic acids [7, 9, 15, 25]. Although DX has been extensively investigated, the cellular kinetics of DX have not been elucidated in cardiac cells.

The present study is concerned with the cellular uptake, binding kinetics, and cellular release of DX in primary cultures of neonatal rat myocardial cells. In additions, the binding kinetics of DX are compared with the DX-induced inhibition of [^{3}H]thymidine incorporation into DNA as a specific target of action within the myocardial cells. It is revealed that myocardial cells have the ability to bind DX at two binding sites. An apparently high-affinity/low-capacity binding is followed by a positive cooperative binding process, which is presumably due to a cooperative binding of DX to DNA as a specific binding site within the cells.

Materials and methods

Materials

Neonatal Wistar rats (0–2 days old) were used. Hank's calcium- and magnesium-free balanced salt solution buffered with 10 m*M* Hepes (Gibco Bio-Cult) was used for washing during the cell separation procedures. Crude collagenase, 0.05% (Boehringer, Mannheim) was added during enzyme digestion.

The culture medium was Medium 199 (Gibco BioCult) with Hank's salts, 25 m*M* Hepes, and heat-inactivated newborn calf serum, 10%.

Hank's solution and Medium 199 were supplemented with penicillin (75 units/ml) and streptomycin (75 μg/ml); all solutions were sterile.

[14-^{14}C]Doxorubicin hydrochloride ([^{14}C]DX) with a specific radioactivity of 53.5 Ci/mol and doxorubicin hydrochloride (DX) were kindly supplied by Farmitalia Car-

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lo Erba, Milan, Italy. DX was dissolved in sterile water and further diluted in Medium 199 to the required concentrations.

[Methyl-^{3}H]Thymidine (^{3}H]TdR) with a specific radioactivity of 78.1 Ci/mmol (New England Nuclear) was diluted in Medium 199 to give a final activity of 0.250 μci/ml incubation medium.

Methods

Cardiac cell cultures. All dissections and preparation procedures were carried out in a laminar-flow hood at 37 °C, and primary cultures of cardiac cells were established.

The myocardial cells were isolated by step-wise enzyme digestion as previously described by Wassermann et al. [28], with minor modifications. In brief, heart ventricles were isolated from 0.2-day-old rats, and the tissue was cut into small pieces and washed twice. The tissue was placed in enzyme solution and gently agitated for five cycles of 15 min each. The harvest of the first cycle was discarded to avoid mesenchymal cells and debris.

The supernatant containing myocardial cells was centrifuged at 1000 rpm for 5 min in culture medium at 4 °C. After resuspension in Hank's solution the new suspension was centrifuged.

The cell pellets were resuspended in culture medium and transferred to a petri dish for cell cultures for 90 min. After gentle agitation the cells were transferred to a flask. The viable cells were counted in a hemacytometer after trypan blue staining.

The cells were plated in multidishes (Nunclon, Nunc, Denmark) coated with 0.01% gelatin, at 1.0 million cells/ml with 0.26 million cells/cm^2. The cultures were kept in a water-saturated (90%) incubator at 37 °C (Hotpack, model 351820, Philadelphia, Pa, USA) for 20 h and then washed twice with Hank's solution and incubated for 2 h in this solution. Hereafter Hank's solution was discarded and the cells were reincubated in Medium 199 for the assays required. The preincubation for 90 min in Medium 199 and the washing procedure with Hank's solution were performed to separate fibroblasts and endothelial cells from the myocardial cells [19].

To distinguish fibroblasts and endothelial cells from myocardial cells in the culture, PAS staining was performed according to Polinger [21]. The purity of the cultures with regard to muscle cells was 90%–95%.

Uptake experiments. After 48 h in culture, myocardial cells were incubated with [^{14}C]DX to determine the time to intracellular steady state. The uptake of DX was determined at two concentrations: 0.01 μ*M* (0.54 nCi/ml) and 0.1 μ*M* (5.40 nCi/ml). At the start of each experiment the appropriate amount of DX was added to duplicate cell chambers containing 0.5 million cells each, and incubated at 37 °C followed. The radioactive medium was aspirated and the cells rapidly washed three times with a 0.9% NaCl solution (4 °C). The cells were dissolved in 500 μl 0.5 *N* NaOH, and 2.5 ml scintilation liquid was added.

Binding of doxorubicin. After 48 h in culture, myocardial cells were incubated with [^{14}C]DX to determine the cellular binding pattern of the drug. The binding assay was determined over a concentration range of 0.1 n*M* to 80 μ*M* DX. At the start of each experiment the appropriate amount of DX was added to duplicate cell chambers containing 2.5 million cells if less than 0.1 μ*M* DX (with a pro rata reduction of radiolabeled [^{14}C]DX) or 0.5 million cells if more than 0.1 μ*M* DX (with 5.40 nCi/ml at all concentrations), and incubated at 37 °C. After incubation for 18 h the radioactive medium was aspirated and the cells rapidly washed three times with 0.9% NaCl solution (4 °C). The washed cells were dissolved in 1000 μl or 500 μl 0.5 *N* NaOH and 5.0 ml or 2.5 ml scintillation liquid, respectively, was added.

Binding of DX was also determined by incubation of myocardial cells with [^{14}C]DX for 18 h at 4 °C.

[^{3}H]Thymidine incorporation. After 48 in culture, myocardial cells were incubated in duplicate with DXover a concentration range of 0.1 n*M* to 80 μ*M* for 18 h at 37 °C. After the DX treatment, the cells were reincubated with 0.250 μCi ^{3}H-TdR/ml for 4 h at 37 °C. At the end of the 4-h incubation period the radioactive medium was aspirated and the cells rapidly washed three times with a 0.9% NaCl solution (4 °C). The acid-soluble material was extracted twice into 0.5 ml cold 4% trichloracetic acid for 10–15 min [1]. The extracted cells were washed twice with ethanol and dissolved in 500 μl 0.5 *N* NaOH. The radioactivity of [^{3}H]TdR in this solute originated in DNA-incorporated [^{3}H]TdR [1] was measured after addition of 2.5 ml scintillation liquid.

Efflux experiments with doxorubicin. After 48 h in culture, myocardial cells were preloaded with DX at an incubation concentration of 0.1 μ*M* for 18 h at 37 °C. Radiolabeled [^{14}C]DX (5.40 nCi/ml) was used as a tracer to measure the amount of intracellularly bound DX. After incubation the cells were washed three times with 0.5 ml Medium 199 at 4 °C to remove extracellular drug and then reincubated in duplicate in DX-free medium at 37 °C. After varying incubation periods the medium was aspirated and the cells were rapidly washed three times with a 0.9% NaCl solution (4 °C). The washed cells were dissolved in 500 μl 0.5 *N* NaOH and 2.5 ml scintillation liquid was added for radioactive measurements.

Efflux experiments with [^{3}H]thymidine. After 48 h in culture medium the cells were incubated with DX at a concentration of 0.1 μ*M* for 18 h at 37 °C. At the end of the DX treatment the cells were reincubated in duplicate with 0.250 μCi [^{3}H]TdR/ml for 8 h at 37 °C. After the 8-h incubation period the cells were washed three times with 0.5 ml Medium 199 at 4 °C to remove extracellular [^{3}H]TdR and then reincubated in radioactive-free medium at 37 °C. The medium was aspirated and the cells rapidly washed three times with 0.9% NaCl solution (4 °C). Hereafter the washed cells were extracted and dissolved as described in *"[^{3}H]Thymidine incorporation"*.

Determination of [^{14}C]DX, [^{3}H]TdR, and cellular protein. In experiments with [^{14}C]DX or [^{3}H]TdR the radioactivity was determined in an LKB 1216 Rackbeta liquid scintillation counter using Opfi-Fluor (Packard) as scintillation liquid.

Cellular protein was chosen as a reproducible parameter to which [^{3}H]TdR incorporation into DNA and cellularly bound [^{14}C]DX were standardized. Cellular protein was determined in cells dissolved in 0.5 *N* NaOH using the method of Lowry et al. [13]. Serum albumin was used as a standard.

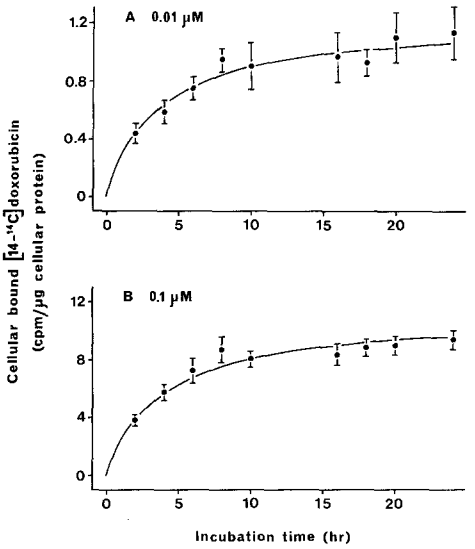

Fig. 1 A, B. Time course of uptake of doxorubicin by cultured myocardial cells incubated at an extracellular drug concentration of 0.01 μ*M* **(A)** and 0.1 μ*M* **(B)**. Experimental conditions as described in *Materials and methods.* Each *point* represents the mean value each bar, the SEM for cellularly bound [14-^{14}C]doxorubicin (specific activity 53.5 Ci/mol) as counts/minute per microgram of cellular protein from five individual experiments. The *curves* have been fitted by means of the Lineweaver-Burk equation.

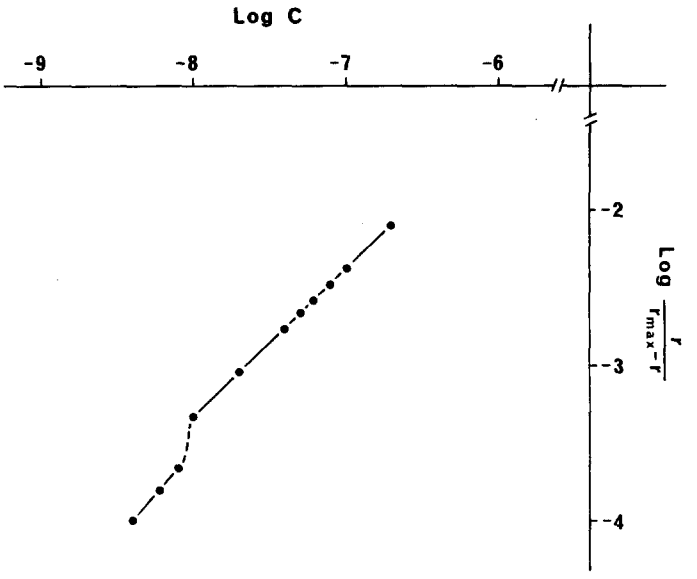

Fig. 3. Hill plot of the binding of doxorubicin to cultured myocardial cells. The binding pattern is presented over an incubation concentration range of 4 n*M* to 0.2 μ*M* doxorubicin. Each *point* represents the mean value from six individual experiments. Experimental conditions as described in Fig. 2

Results

The time-course of the uptake of DX by myocardial cells exposed to two different drug concentrations is shown in Fig. 1. At both concentrations the uptake pattern was characterized by an initial linear phase, after which the rate of uptake gradually decreased. The initial rate of drug uptake was calculated as 0.084 ± 0.01 cpm/h and 0.81 ± 0.1 cpm/h after incubation with 0.01 μ*M* and 0.1 μ*M* DX, respectively. The time to reach 95% of cellular steady state level at both concentrations was calculated as approximately 70 h after continuous DX exposure; after 18 h incubation the cellular level had reached approximately 85% of the steady state level at both concentrations.

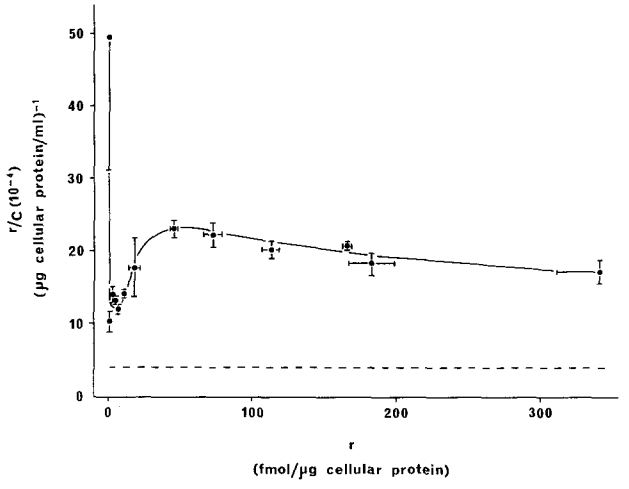

Fig. 2. Scatchard plot of the binding of doxorubicin to cultured myocardial cells. The binding isotherm was obtained by incubation of myocardial cell cultures over a concentration range of 0.1 n*M* to 80 μ*M* doxorubicin for 18 h. [14-^{14}C]Doxorubicin (specific activity 53.5 Ci/mol) was used as a tracer, as described in *Materials and methods.* The binding pattern is presented as the sum curve of specifically and nonspecifically bound doxorubicin over a concentration range of 0.1 n*M* to 0.2 μ*M* doxorubicin. The nonspecifically bound doxorubicin is shown by the *dashed line.* Each *point* represents the mean value each *bar*, the SEM from six individual experiments. *r*, femtomoles per microgram of cellular protein; *C*, molar concentration of free doxorubicin in the incubation medium after 18 h incubation

In Fig. 2 the binding isotherm obtained for the interaction of DX with myocardial cells over a concentration range of 0.1 n*M* to 0.2 μ*M* is demonstrated in a Scatchard plot with *r/C* versus r [23]; where *r* represents moles of bound drug per microgram of cellular protein and *C* is the molar concentration of free DX in the incubation medium after 18 h incubation. An apparently linear relationship was demonstrated between *r* and *r/C* for low *r* values ($r < 5$ fmol/μg cellular protein) foolowed by a positive cooperative binding or allosteric effect, as illustrated by the positive slope in the lower *r* region of the binding isotherm ($r > 5$ fmol/μg cellular protein). The curve reached a maximum at a value of $r \approx 45$ fmol/μg cellular protein, corresponding to an incubation concentration of 0.02 μ*M* DX. Beyond this point a declining slope was observed at higher *r* values.

This cooperative binding is also visualized in a Hill plot [5], which displays nonlinearity at the same concentration range as that of the Scatchard plot.

In order to correlate the apparently specific binding sites of DX, as illustrated by the binding isotherm of DX (Fig. 2), with a specific target of action within the myocardial cells, [^{3}H]TdR incorporation into DNA was determined in myocardial cells pretreated with DX over a concentration range of 0.1 n*M* to 80 μ*M*.

An increased incorporation of approximately 10%–20% of [^{3}H]TdR into DNA in cells pretreated with DX below 0.01 μ*M* was observed (Fig. 4). The incorporation then decreased and a linear correlation between inhi-

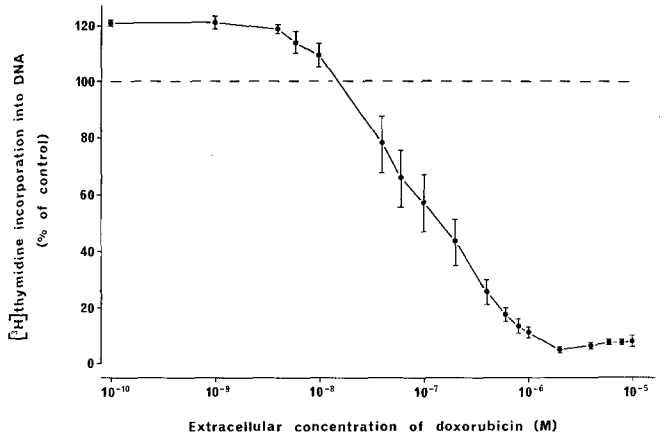

Fig. 4. Incorporation of [^{3}H]thymidine (specific activitiy 78.1 ci/mol) into DNA in doxorubicin-treated myocardial cell cultures. Cultures were incubated with doxorubicin over a concentration range of 0.1 n*M* to 80 μ*M* for 18 h before [^{3}H]thymidine incubation. Experimental conditions as described in *Materials and methods*. Each *point* represents the mean value and each *bar*, the SEM [^{3}H]thymidine incorporation into DNA as a percentage of control from five individual experiments. The log dose-response curve is presented over a concentration range of 0.1 n*M* to 10 μ*M* doxorubicin

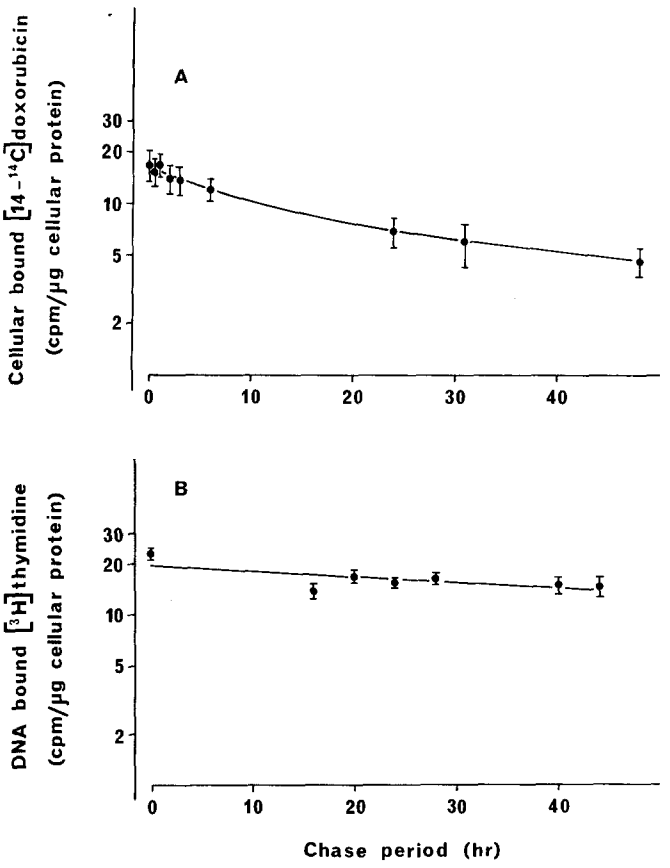

Fig. 5A, B. Efflux of doxorubicin from myocardial cell cultures **(A)** and changes in [^{3}H]thymidine incorporated into DNA in doxorubicin-treated myocardial cell cultures **(B)** during chase incubation. Cell cultures for efflux experiments with doxorubicin were preloaded with doxorubicin at an incubation concentration of 0.1 μ*M* for 18 h and [14-^{14}C]doxorubicin (specific activity 53.5 Ci/mmol) was used as a tracer. Experimental conditions as described in *Materials and methods*. Each *point* represents the mean value and each *bar*, the SEM for cellularly bound [14-^{14}C]doxorubicin and DNA-bound [^{3}H]thymidine as counts/minute per microgram of cellular protein from four individual experiments

bition of [^{3}H]TdR incorporation and DX concentration was demonstrated; the incorporation gradually decreased to 5% in cells pretreated with 2 μ*M* DX for 18 h, indicating simple saturation kinetics. The K_d value (ED_{50}) of DX with respect to the inhibitory effect on [^{3}H]TdR incorporation into DNA was calculated to be 1.37×10^{-7} *M*.

Efflux experiments were carried out as pulse-chase experiments in which myocardial cells were preloaded with either [^{14}C]DX at incubation for 18 h or with [^{3}H]TdR in DX-treated cells (0.1 μ*M* for 18 h) and then chased in a radioactive-free medium.

The unidirectional efflux of DX was biphasic (Fig. 5a), with an initially more rapid exit of drug and an estimated half-life of 5.3 h, after which the rate of release became very slow, with an estimated half-life of about 50–60 h. In comparison, the release of [^{3}H]TdR from myocardial cells in the pulse-chase experiments with DX-pretreated cells was linear and the estimated half-life was about 80–90 h (Fig. 5b).

Discussion

It is believed that DX enters cells by passive diffusion, with the rate of entry directly proportional to the external concentration of the drug [4, 10, 20]. The process appears to be unidirectional, and the drug appears to be trapped in the cell, probably by binding to nucleic acids and proteins, where it accumulates against a concentration gradient [14, 18]. Furthermore, in most studies, cellular accumulation is a linear function of time [10, 24]. However, intracellular DX does not distribute uniformly throughout the cells but accumulates predominantly in the nuclei and in the lysosomes, as observed in cultured fibroblasts and cardiac myocytes [12, 18]. A plausible assumption is that the subcellular localization of DX is due to specific binding sites within the cells.

In the present study, we examined primary cultured myocardial cells to determine the cellular kinetics and the binding isotherm of DX. Our findings support previous studies demonstrating the uptake pattern as a linear function of time and the rate of entry as directly proportional to the concentration of drug in the medium. However, previous studies conducted in embryonic chick cardiac myocytes demonstrated a much faster incorporation of [^{14}C]DX, followed by more rapid achievement of steady state [12].

Numerous studies concerning the binding properties and subcellular distribution of DX have demonstrated exclusive nuclear localization and a good correlation between the tissue binding of DX and the tissue DNA concentration [26]. Since DX has a high-affinity mode of binding to nucleic acids, probably involving an intercalation process, the DNA concentration-dependent localization in the nuclei seems evident. The binding isotherm obtained for the interaction of DX with cultured myocardial cells after long-term incubation in medium containing [^{14}C]DX revealed that the cells had the ability to bind DX at two binding sites. For low *r* values in the Scatchard plot apparently noncooperative high-affinity/low-capacity binding

was observed, followed by positive cooperative binding characterized by a higher affinity and a high binding capacity of DX.

Graves and Krugh [9] have demonstrated that DX exhibits cooperative binding to both native DNA and synthetic copolymer systems at ionic concentrations comparable to physiological levels. Previous studies have demonstrated a DX-induced inhibition of [^{3}H]TdR incorporation into DNA [8, 22, 28]. However, an increased incorporation of [^{3}H]TdR into DNA was observed in myocardial cells pretreated with DX at concentrations corresponding to *r* values below the maximum of the cooperative binding curve. This phenomenon is a paradoxical effect of DX, which may be due to partial agonism with an apparently intrinsic activity at very low concentrations. A comparable observation has been made with low concentrations of DX [1–10 ng), which enhanced the DNA synthesis of growing mouse embryonic fibroblasts [6]. After pretreatment with increasing concentrations of DX corresponding to the *r* values beyond the maximum of the cooperative curve, a decreased and dose-dependent incorporation of [^{3}H]TdR into DNA was observed. In the light of this coincidence between the cooperative binding of DX and the dose-dependent inhibition of [^{3}H]TdR incorporation into DNA, together with the findings of Graves and Krugh [9] and the reported exclusive localization of DX in the nuclei, it seems likely that the observed cooperative binding pattern could be due to DNA as one of the binding sites of DX within the myocardial cells.

DX has a high-affinity mode of binding to membrane-bound cardiolipin [7], which is exclusively localized in the inner mitochondrial membrane [3]. It has been suggested that cardiolipin is a prerequisite as a receptor for the rebinding of mitochondrial creatine phosphokinase to the inner mitochondrial membranes [16], and that DX selectively abolishes this interaction, presumably by interfering at the receptor site by binding to cardiolipin [16, 17]. Thus, the noncooperative high-affinity/low-capacity binding could reflect this binding of DX to cardiolipin. Further studies, however, are in progress to elucidate this problem.

The rapid phase of the efflux of DX represents the exit of reversibly bound drug, whereas the very slow phase may represent the release of more tightly bound drug. This may be localized to DNA in the nucleus, and the slow efflux phase may represent the decay or turnover of the DNA within the nucleus compatible with the estimated long half-life of [^{3}H]TdR in the pulse-chase experiments in DX-treated cells. The binding of DX at this site is regarded to be almost irreversible.

With an incubation time of 18 h, the present study was presumably performed at an equilibrium state for the binding site with the rapid release of drug, and with only a small amount of drug bound to the other binding site with a much longer half-life for the efflux.

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References

1. Adams RLP (1980) Use of radioactive isotopes in cell culture. Cell culture for biochemists. In: Laboratory techniques in biochemistry and molecular biology, vol 8. Elsevier/North-Holland Biomedical, Amsterdam, p 181
2. Bertazzoli C, Bellini O, Magrini U, Tosana MG (1979) Quantitative experimental evaluation of adriamycin cardiotoxicity in the mouse. Cancer Treat Rep 63: 1877
3. Comte J, Maisterrena B, Gautheron DC (1976) Lipid composition and protein profiles of outer and inner membranes from pig heart mitochondria. Comparison with microsomes. Biochim Biophys Acta 419: 271
4. Dalmark M, Storm HH (1981) A fickian diffusion transport process with features of transport catalysis. Doxorubicin transport in human red blood cells. J Gen Physiol 78: 349
5. De Lean A, Rodbard D (1979) Kinetics of cooperative binding: In: O'Brien RD, (ed) The receptors: a comprehensive treatise. Plenum, New York, p 143
6. Di Marco A (1983) Anthracyclines in cancer chemotherapy. Drugs Exp Clin Res 9: 751
7. Duarte-Karim M, Ruysschaert JM, Hildebrand J (1976) Affinity of adriamycin to phospholipids – a possible explanation for cardiac mitochondrial lesions. Biochem Biophys Res Commun 71: 658
8. Fialkoff H, Goodman MF, Seraydarian MW (1979) Differential effect of adriamycin on DNA replicative and repair synthesis in cultured neonatal rat cardiac cells. Cancer Res 39: 1321
9. Graves DE, Krugh TR (1983) Adriamycin and daunorubicin bind in a cooperative manner to deoxyribonucleic acid. Biochem J 22: 3941
10. Inaba M, Johnson R (1978) Uptake and retention of adriamycin and daunorubicin by sensitive and anthracycline-resistant sublines of P388 leukemia. Biochem Pharmacol 27: 2123
11. Kobrinsky NL, Ramsay NKC, Krivit W (1982) Anthracycline cardiomyopathy. Ped Cardiol 3: 265
12. Lewis W, Galizi M, Puszkin S (1983) Compartmentalization of adriamycin and daunomycin in cultured chick cardiac myocytes. Effects on synthesis of contractile and cytoplasmic proteins. Circ Res 53: 352
13. Lowry OH, Rosebrough NJ, Raff AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193: 265
14. Marafino BJ, Giri SN, Siegel DM (1981) Pharmacokinetics, covalent binding and subcellular distribution of ^{3}H-doxorubicin after intravenous administration in the mouse. J Pharmacol Exp Ther 216: 55
15. Menozzi M, Arcamone F (1978) Binding of adriamycin to sulphated mucopolysaccharides. Biochem Biophys Res Commun 80: 313
16. Müller M, Moser R, Cheneval D, Carafoli E (1985) Cardiolipin is the membrane receptor for mitochondrial creatine phosphokinase. J Biol Chem 260: 3839
17. Newman RA, Hacker MP, Fagan MA (1982) Adriamycin-mediated inhibition of creatine phosphokinase binding to heart mitochondrial membrane. Biochem Pharmacol 31: 109
18. Noël G, Peterson C, Trouet A, Tulkens P (1978) Uptake and subcellular localization of daunorubicin and adriamycin in cultured fibroblasts. Eur J Cancer 14: 363
19. Pal K, Grover PL (1983) A simple method for the removal of contaminating fibroblasts from cultures of rat mammary epithelial cells. Cell Biol Int Rep 7: 779
20. Peterson C, Trouet A (1978) Transport and storage of daunorubicin and doxorubicin in cultured fibroblasts. Cancer Res 38: 4645
21. Polinger IS (1973) Identification of cardiac myocytes in vivo and in vitro by the presence of glycogen and myofibrils. Exp Cell Res 76: 243
22. Rosenoff SH, Brooks E, Bostick F, Young RC (1975) Alterations in DNA synthesis in cardiac tissue induced by adriamycin in vivo-relationship to fatal toxicity. Biochem Pharmacol 24: 1898
23. Scatchard G (1949) The attractions of proteins for small molecules and ions. Ann NY Acad Sci 51: 660

24. Skovsgaard T (1977) Transport and binding of daunorubicin, adriamycin and rubidazone in Ehrlich ascites tumour cells. Biochem Pharmacol 26: 215
25. Someya A, Akiyama T, Misumi M, Tanaka N (1978) Interaction of anthracycline antibiotics with actin and heavy meromycin. Biochem Biophys Res Commun 85: 1542
26. Terasaki T, Iga T, Sugiyama Y, Sawada Y, Hanano M (1984) Nuclear binding as a determinant of tissue distribution of adriamycin, daunomycin, adriamycinol, daunorubicinol and actinomycin D. J Pharm Dyn 7: 269
27. Tobin TP, Abbott BC (1980) A stereological analysis of the effect of adriamycin on the ultrastructure of rat myocardial cells in culture. J Mol Cell Cardiol 12: 1207
28. Wassermann K, Møllgaard K, Steiness E (1985) Similar changes in cardiac morphology and DNA synthesis induced by doxorubicin and 4'-epi-doxorubicin. Cancer Chemother Pharmacol 15: 244
29. Young RC, Ozols RF, Myers CE (1981) The anthracycline antineoplastic drugs. N Engl J Med 305: 139
30. Zähringer J (1982) Clinical features of the adriamycin cardiomyopathy. Heart Bull 13: 87

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