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Uptake of Free and DNA-bound Daunorubicin and Doxorubicin into Human Leukemic Cells

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Summary. *Leukemic cells from seven patients with acute nonlymphoblastic leukemia and granulocytes, and mononuclear cells from three healthy controls were isolated by centrifugation on metrozoate-dextran. The intracellular accumulation of both the free and DNA-bound forms of daunorubicin and doxorubicin was studied in vitro. The uptake of unbound daunorubicin was higher than that of doxorubicin. At drug concentrations of 1.75 μM and higher the uptake of the free drugs was greater than that of the bound forms, but at lower drug concentrations the uptake was about the same. This could at least partly be explained by a greater dissociation of the DNA-drug complexes at lower drug concentrations. The uptake into normal leukocytes was of the same order of magnitude as that into leukemic cells. There was a great interindividual variation in the accumulation of both free and DNA-bound drugs in the cells from leukemic patients. This variation might be of importance for the prediction of individual sensitivity to the different drugs.*

Introduction

Anthracycline antibiotics such as daunorubicin and doxorubicin probably exert their antitumoral effects by intercalation between the DNA base pairs in the nuclei, thereby preventing replication and transcription (Calendi et al., 1965; Pigram et al., 1972). Since the target is localized intracellularly, cellular uptake of the drugs is a decisive factor for their therapeutic effects.

Trouet in de Duve's group has introduced the concept of lysosomotropic cancer chemotherapy by linking daunorubicin and doxorubicin to DNA (Trouet et al., 1972). According to this idea, the DNA complexes enter the cell by endocytosis and reach the lysosomes. Since lysosomes contain DNA-digesting enzymes (de Duve et al., 1955), drug molecules can then be liberated and diffuse into other cellular compartments, e.g., the nucleus. Endocytosis is a very specific process (de Duve et al., 1974), which causes the possibility of a more selective action of the DNA complexes as compared with the free drugs. Evidence that the cardiotoxic effects of the drugs can be reduced by linking them to DNA (Ferrant et al., 1978) seems to support the above concept.

Daunorubicin and doxorubicin are effective in the treatment of acute leukemia but, due to their cardiotoxic effects, both the dose and the duration of treatment must be limited. There is also a great variation in the therapeutic response, some patients being resistant to treatment. Pretreatment investigations of the uptake of the free and bound drugs into leukemic cells might give information about the mechanism of resistance, and also about possible differences in effectiveness of bound and free drugs. Leukemic blood cells are particularly suitable for this type of cellular pharmacologic study, since they can be obtained in relatively pure suspensions (Böyum et al., 1968). In the present investigation, we have also studied the uptake into leukemic bone marrow cells.

Materials and Methods

Drugs. Daunorubicin hydrochloride (Cerubidin®) was obtained from AB Leo Rhodia (Helsingborg, Sweden) and doxorubicin hydrochloride (Adriamycin®) from Montedison läkemedel AB (Stockholm, Sweden). The DNA complexes were prepared from herring sperm DNA type VII (Sigma Chemical Co., St. Louis, USA). It was dissolved in 0.15 M NaCl and filtered through a Millipore filter (0.8 μ). Before use, the solution was autoclaved at 120° C for 15 min. After cooling, drug was added to give a final molar ratio (drug/mononucleotides) of 1 : 20. Concentrations of DNA-drug complexes given in the text refer to the drug.

Patients. Peripheral venous blood and bone marrow samples were obtained from seven patients with acute nonlymphoblastic leukemia with white blood cell counts between 8.6 × 10^9/l and 100 × 10^9/l

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(median 25 x 10^9/l) and blast cell counts between 3.3 x 10^9/l and 92 x 10^9/l (median 16 x 10^9/l). The bone marrow in all patients was dominated by the leukemic blast cells. Peripheral venous blood was also obtained from three normal healthy donors.

Isolation of Cells. White blood cells were isolated by centrifugation (400 *g* for 30 min) on metrozoate-dextran (Lymphoprep®, Nyegaard and Co., AS, Oslo, Norway) (Böyum, 1968). After isolation, the cells were suspended in RPMI 1640 to give a final cell concentration of 3 x 10^6–10^7 cells/ml.

Incubation Procedure. Incubation with the drugs was performed in open plastic tubes containing 2 ml of the cell suspension and an appropriate amount of the drug in a shaking water bath at 37° C. The incubation was terminated by the addition of 4 ml of ice-cold phosphate buffered saline (PBS, pH 7.4) followed by centrifugation (1000 *g* for 5 min at 4° C). The cells were then washed twice, resuspended in 2 ml of PBS, and stored at −20° C until analysis.

Drug Assay. The cell samples were sonicated (75 W, 20 KHz, 30 s) and the drugs were extracted with TCA (27%). This treatment releases the drugs from their association with cellular constituents (Noël et al., 1978). The drugs were assayed by fluorometry using a Shimadzu spectrofluorophotometer model RF-510 (excitation and emission wavelengths 485 and 560 nm, respectively). The drug concentration in each sample was calculated by comparison with identically treated standard solutions. Protein was determined according to the method of Lowry et al. (1951), using BSA as standard.

Results

The purity of the leukemic cells isolated from peripheral blood and bone marrow samples from patients with acute nonlymphoblastic leukemia was 80–90%.

There was a rapid intracellular accumulation of both free and DNA-bound daunorubicin and doxorubicin in leukemic cells from the peripheral blood (Fig. 1). At a concentration of 1.75 μM, the uptake of free drug always exceeded that of the bound form. The rate of accumulation of daunorubicin was more rapid than that of

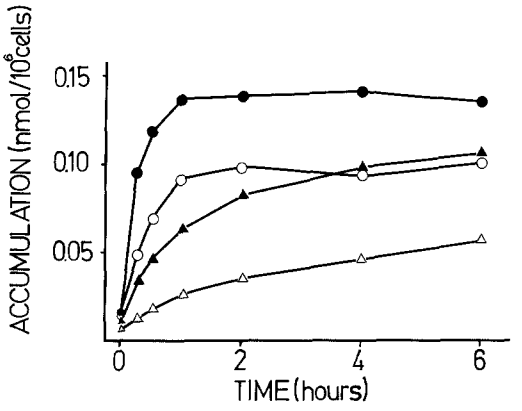

Fig. 1. Time course of the accumulation of free and DNA-bound daunorubicin and doxorubicin (1.75 μM) in leukemic cells from the peripheral blood of a patient with acute nonlymphoblastic leukemia. ● daunorubicin, ▲ doxorubicin, ○ daunorubicin-DNA, △ doxorubicin-DNA

doxorubicin and the steady-state level, reached within about 1 h, was higher for daunorubicin than for doxorubicin.

The relation between the cellular drug accumulation at steady-state and the drug concentration is shown in Fig. 2a and b. Above a concentration of 1 μM, there was a greater accumulation of the free drugs than of the bound forms. The difference in uptake increased with increasing drug concentration. On the other hand, at concentrations below 1 μM, the accumulation of the DNA-bound drugs equaled that of the free drugs. The low concentrations are comparable to those generally obtained in vivo during treatment with the drugs (Eksborg et al., 1978).

As demonstrated in Fig. 3a and b, there was a great interindividual variation in the drug accumulation of both the free drugs and the DNA-bound forms. In all

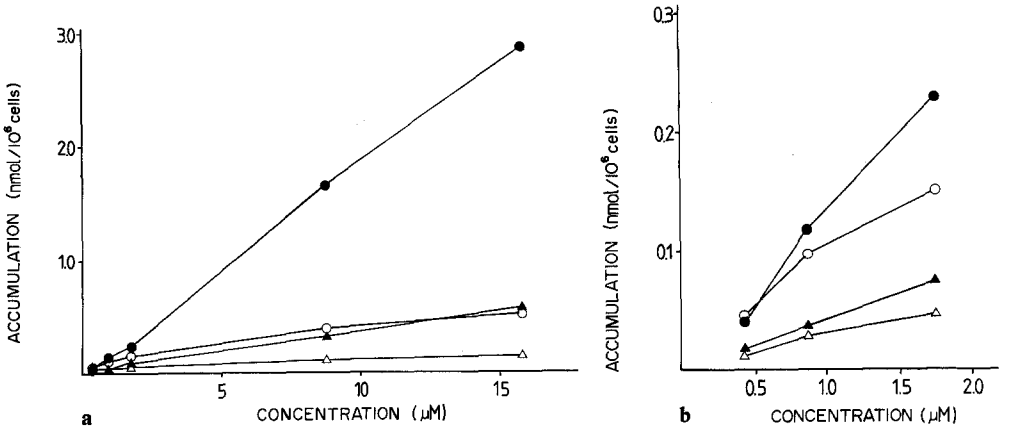

Fig. 2. a Drug accumulation at different concentrations of free and DNA-bound daunorubicin and doxorubicin in leukemic cells (2 h) from a patient with acute nonlymphoblastic leukemia. ● daunorubicin, ▲ doxorubicin, ○ daunorubicin-DNA, △ doxorubicin-DNA. **b** The same as **a** at concentrations below 1.75 μM

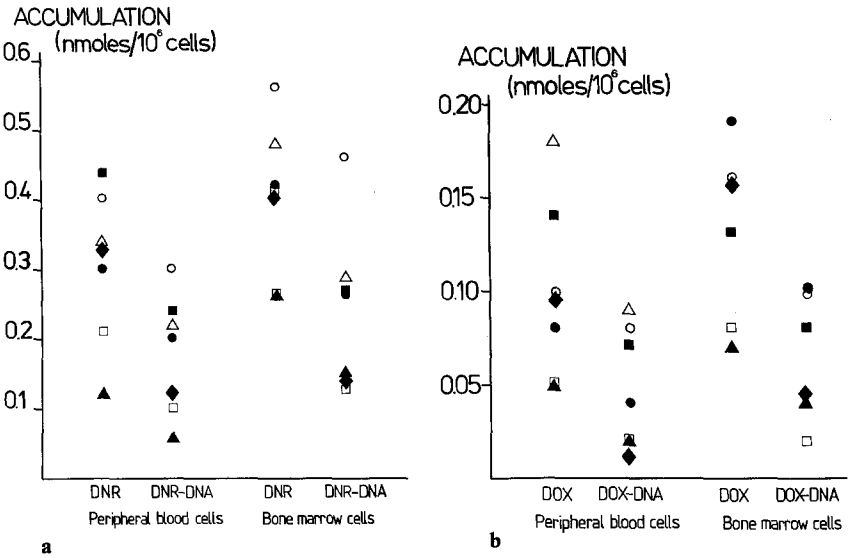

Fig. 3. **a** Accumulation of free and DNA-bound daunorubicin in leukemic cells from the peripheral blood and the bone marrow cells from seven patients with acute nonlymphoblastic leukemia (1.75 μM, 2 h). Each symbol represents one patient. **b** The same experiments with the same patients as in **a** with free and DNA-bound doxorubicin

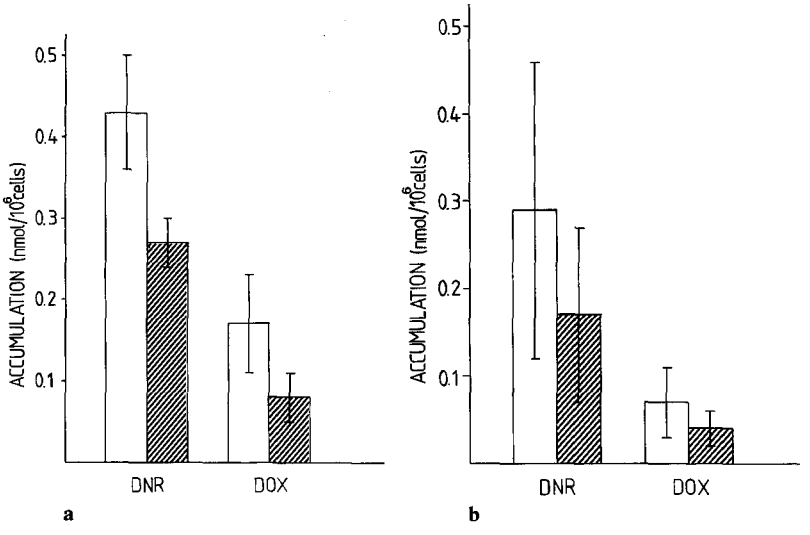

Fig. 4. Accumulation of free (*open bars*) and DNA-bound (*hatched bars*) daunorubicin and doxorubicin (1.75 μM, 2 h) in normal mononuclear cells (**a**) and granulocytes (**b**). Means ± SD of three experiments

patients the uptake of free drug exceeded that of the DNA-bound drug, but in some patients the difference was small. There was a tendency for a higher accumulation in bone marrow cells than in peripheral blood cells. However, this was not statistically significant.

The uptake of free and DNA-bound drugs in normal mononuclear cells and granulocytes is demonstrated in Fig. 4a and b. At a concentration of 1.75 μM at steady-state the uptake of free daunorubicin exceeded that of the DNA-bound form and the uptake of free or bound daunorubicin exceeded that of free or bound doxorubicin, respectively. These differences applied both to granulocytes and to mononuclear cells. However, there was a tendency for the drug accumulation in mononuclear cells to be higher than that in granulocytes. The uptake into normal leukocytes was of the same order of magnitude as the uptake into leukemic cells.

Discussion

In the present study, isolated leukemic cells from patients with acute nonlymphoblastic leukemia have been used to study the accumulation of free and DNA-bound daunorubicin and doxorubicin. These drugs are not metabolized to a significant extent during in vitro incubation (Baurain et al., 1978). The accumulation of daunorubicin markedly exceeded that of doxorubicin. This observation is consistent with the results of other studies in various in vitro systems (Chervinsky and Wang, 1976; Bachur, 1976; Skovsgaard, 1977; Noël et al., 1978).

In cultured fibroblasts, daunorubicin and doxorubicin are exclusively localized to nuclei and lysosomes, and the higher uptake of daunorubicin is due to a greater accumulation of this drug in the lysosomes, while daunorubicin and doxorubicin accumulate to the same

extent in the nuclei (Noël et al., 1978). So far, we have no information about the intracellular localization of the drugs in human leukemic cells. However, since our results indicate the same differences between daunorubicin and doxorubicin accumulation as found in fibroblasts, it is possible that the same difference in lysosomal capacity to store the two drugs explains the differences in uptake into leukemic cells.

At higher extracellular drug concentrations, the uptake of the free drugs was much higher than that of the DNA-bound drugs. At low drug concentration, corresponding more closely to the in vivo situation (Eksborg et al., 1978), the uptake of the DNA-bound drugs was found to be similar to that of the free drugs. This is in accordance with the hypothesis that the DNA complexes are taken up by endocytosis, which is a saturable process. However, another explanation must be considered. Since the binding of daunorubicin to DNA is reversible, there is a certain fraction of free daunorubicin present. According to the equilibrium reaction (daunorubicin + DNA $\rightleftarrows$ daunorubicin-DNA), that fraction increases with decreasing concentrations of daunorubicin and DNA (at a constant molar ratio). Preliminary experiments on the uptake and digestion of ^{125}I-labeled DNA in human leukemic cells support this explanation.

There was a great interindividual variation in the uptake of both free and DNA-bound drug in leukemic cells. This variation may be of great clinical importance, since it might be correlated to differences in drug sensitivity and development of drug resistance. Work is in progress to find out whether the in vitro uptake is correlated to the clinical effect and thereby can be used to predict the therapeutic response.

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References

Bachur, N. R., Steele, M., Meriwether, W. D., Hildebrand, R. C.: Cellular pharmacodynamics of several anthracycline antibiotics. J. Med. Chem. **19,** 651 (1976)

Baurain, R., Zenebergh, A., Trouet, A.: Cellular uptake and metabolism of daunorubicin as determined by high pressure liquid chromatography: Application to L 1210 cells. J. Chromatogr. (In press, 1978)

Böyum, A: Isolation of leucocytes from human blood. Further observation. Scand. J. Clin. Lab. Invest. [Suppl.] **97,** 1 (1968)

Calendi, E., Marco, A., Reggiani, M. di, Scarpinati, B., Valenti, L.: On physico-chemical interaction between daunomycin and nucleic acids. Biochim. Biophys. Acta **103,** 25 (1965)

Chervinsky, D. S., Wang, J. J.: Uptake of adriamycin and daunorubicin in L 1210 and human leukemia cells: A comparative study. J. Med. **7,** 63 (1976)

Duve, C. de, Pressman, B. C., Gianetto, R., Wattiaux, R., Appelmans, F.: Intracellular distribution patterns of enzymes in rat-liver tissue. Biochem. J. **60,** 604 (1955)

Duve, C. de, Barsy, T. de, Poole, B., Trouet, A., Tulkens, P., Hoof, F. van: Lysosomotropic agents. Biochem. Pharmacol. **23,** 2495 (1974)

Eksborg, S., Ehrsson, H., Andersson, B., Beran, M.: Liquid chromatographic determination of daunorubicin and daunorubicinol in plasma from leukemic patients. J. Chromatogr. **153,** 211 (1978)

Ferrant, A., Hulhoven, R., Bosly, A., Cornu, G., Michaux, J. L., Sokal, G.: Clinical trials with daunorubicin-DNA and adriamycin-DNA in acute lymphoblastic leukemia and bronchogenic carcinoma. Cancer Chemother. Pharmacol. **2,** 67–71 (1979)

Lowry, O. M., Rosebrough, N. J., Farr, A. L., Randall, R. J.: Protein measurement with the folin phenol reagent. J. Biol. Chem. **193,** 265 (1951)

Noël, G., Peterson, C., Trouet, A., Tulkens, P.: Uptake and subcellular localization of daunorubicin and adriamycin in cultured fibroblasts. Eur. J. Cancer **14,** 363 (1978)

Pigram, W. J., Fuller, W., Hamilton, L. D.: Stereochemistry of intercalation interaction of daunomycin with DNA. Nature [New Biol.] **235,** 17 (1972)

Skovsgaard, T.: Transport and binding of daunorubicin, adriamycin and rubidazone in Ehrlich ascites tumor cells. Biochem. Pharmacol. **26,** 215 (1977)

Trouet, A., Deprez-de Campeneere, D., Duve, C. de: Chemotherapy through lysosomes with a DNA-daunorubicin complex. Nature [New Biol.] **239,** 110 (1972)