Biochemical Pharmacology, Vol. 37, No. 9, pp. 1863–1864, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00
Pergamon Press plc

INTRACELLULAR DRUG CONCENTRATION AND DNA DAMAGE IN HUMAN CHILDHOOD LEUKEMIC CELLS EXPOSED TO DOXORUBICIN

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INTRODUCTION

The mode of action of anthracyclines appears related to inhibition of DNA-Topoisomerase II. Like other DNA-intercalating antitumor agents they stabilize DNA-topoisomerase II complexes which, on treatment with denaturants, are converted to DNA-single strand breaks (DNA-SSB) and DNA-double strand breaks (DNA-DSB) (1,2). Doxorubicin (DX) induced fewer DNA breaks in some cell lines selected for resistance to anthracyclines or other topoisomerase II inhibitors than in the parental cell lines. Qualitative or quantitative changes in DNA topoisomerase II may be involved in the anthracycline resistance (3,4), though in some murine and human cancer cell lines selected for resistance to anthracyclines and to vinca alkaloids, epipodophyllotoxins and Act-D (multi-drug resistance), intracellular drug retention was decreased (5).

Data on the mechanisms of resistance to antineoplastic agents in freshly isolated cancer cells is scant; we therefore measured intracellular DX concentrations and DX-induced DNA breaks in human childhood leukemic cells from 14 children with acute lymphoblastic (11 cases) and non-lymphoblastic (3 cases) leukemia, at the onset of the disease with no previous treatment ("potentially responsive", 10 cases) or after at least two relapses ("clinically resistant", 4 cases).

MATERIALS AND METHODS

Leukemic cells from peripheral blood or bone marrow were sedimented on a Ficoll-Hypaque gradient, resuspended in RPMI 1640 (0.5-2 x 10^6 cells/ml) then exposed for 4 h to DX at concentrations from 1.25 to 40 ug/ml. Drug retention was evaluated by flow cytometry using a Becton Dickinson FACS IV. Intracellular drug fluorescence was analyzed with excitation at 488 nm and emission above 530 nm (6). Dead cells were excluded on the basis of their scatter signal. The mean frequency distribution was normalized taking the value for fixed lymphocytes treated with DX (10 ug/ml) as 100 fluorescence units (F.U.). The results were corrected for the mean autofluorescence of untreated cells. In a few cases more than one peak was resolved and the mean F.U. value was scored for each.

A standard procedure was used for DNA index and cell cycle analysis. Cells were stained for 1 h with 2 ml of a solution containing 50 ug/ml propidium iodide in 0.1% sodium citrate, 6 ug/ml RNase, 0.015% nonidet P 40.

DX-induced DNA-SSB were determined by the alkaline elution method (7), with fluorimetric detection of eluted DNA. 3-4 x 10^6 cells were layered on polycarbonate filters, 2 um pore size and 47 mm diameter (Nucleopore Corp., Pleasanton, USA). Cells were lysed with 10 ml of a solution of 2 M NaCl/ 0.04 M Na_2 EDTA/0.2% Sarkosyl, pH 10.0. The funnel outlets were plugged and the filters were incubated with 5 ml proteinase K (0.4 mg/ml) in the dark at room temperature for 1 h. Then the filters were washed with 10 ml of 0.02 M Na_2 EDTA, pH 10.0. DNA was eluted from the filters with 0.02 M Na_2EDTA, pH 12.4. Elution rate was 2 ml/h for 15 h, and 3-h fractions were collected. The DNA in each fraction and that remaining on the filter was assayed by fluorimetric detection using Hoechst 33258, as described by Cesarone et al. (7).

RESULTS AND DISCUSSION

Intracellular DX concentrations were similar in cells from "potentially responsive" and "clinically resistant" patients exposed to drug concentrations of 1.25,5 and 40 ug/ml for 4 h (Table 1). In 4 out of 13 cases two cell populations were resolved, indicating heterogeneous drug retention in cells from the same patient.

TABLE 1. Intracellular DX concentration (expressed in F.U.[a]) in human leukemic cells after 4h drug exposure.

	1.25 ug/ml		5 ug/ml		40 ug/ml	
	S[b]	R	S	R	S	R
No. of patients	9	4	5	1	9	4
No. of cell populations[c]	12	4	6	1	12	5
Median	2.2	3.2	9.7	6.2	74	69
Range	0.6-5.0	1.5-4.9	4.3-26	-	20-152	24-124

[a]By determining ^{14}C-DX uptake and DX fluorescence by flow cytometry it was estimated that 1 F.U. corresponds to approximately 10 fg DX per cell. [b]S: cells from potentially responsive patients; R: cells from clinically resistant patients. [c]Since some cases presented two leukemic cell populations the medians and ranges were calculated, taking all cell populations into account.

Although the number of cases investigated is too small to permit any firm conclusion, it would appear that the resistance of leukemic cells to DX is not necessarily due to lower intracellular drug accumulation. These findings contrast with previous data for several tumor cell lines (5). Caution is therefore needed when using cell lines to investigate drug resistance of human tumors and more studies on tumoral cells freshly obtained from patients are essential.

DX-induced DNA-SSB were almost undetectable in cells from "potentially responsive" and from "clinically resistant" patients (Table 2). However, in these cases the fraction of S phase cells was 6 ± 1% (range 1-17%). The small fraction of cycling cells probably explains the few DNA breaks, as drug-induced DNA-topoisomerase II cleavable complexes are related to cell proliferation (9). DNA breaks may have been present only in the small proportion of proliferating cells and were therefore too "diluted" to be detectable. DX probably induces more DNA damage in sensitive than in resistant proliferating leukemic cells. To verify this, we have planned studies in which leukemic cells from "potentially responsive" and "clinically resistant" patients are stimulated with appropriate mytogens before exposure to topoisomerase II inhibitors.

TABLE 2. DX-induced DNA-SSB in human leukemic cells after 4 h drug exposure

			1.25 ug/ml		40 ug/ml	
			S	R	S	R
No. of cases	-	-	10	4	10	3
Mean values*	0.028	0.214	0.045	0.016	0.078	0.012
SE	0.004	0.040	0.009	0.007	0.017	0.004
Range	0.003-0.051	0.090-0.4	0.012-0.110	0.002-0.032	0.012-0.171	0.006-0.02

*DNA damage was assessed by the alkaline elution method; values are -log (retention) of DNA on filter after 12 h elution (4 fractions) taking retention after collection of the first fraction as 1.0.

ACKNOWLEDGEMENTS

The generous contribution of Ms. G. Necchi Campiglio is gratefully acknowledged.

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