DRUG SENSITIVITY OF AN ADRIAMYCIN-RESISTANT MUTANT SUBLINE OF MOUSE LYMPHOBLASTOMA L5178Y CELLS

Sir:

Anthracycline-resistant sublines have been isolated in a number of cell lines and cross-resistance with actinomycin, vinca alkaloids and other inhibitors have been demonstrated. Drug resistance has been suggested to be due to alteration of the cell membrane, resulting in permeability barriers to drugs, or to impaired retention of antibiotics.[1~6]

We have isolated adriamycin-resistant mutant sublines of mouse lymphoblastoma L5178Y cells by treating the culture with N-methyl-N'-nitro-N-nitrosoguanidine. However, even though they showed a tendency to revert to sensitivity, some maintained resistance after serial *in vitro* transfer. A resistant subline, thus obtained, exhibited properties different from those previously reported[1~6]. It was observed that the adriamycin-resistant mutant subline exerted an unique cross-resistance pattern, and the presence of amphotericin B did not induce drug sensitivity in the resistant cells. The results are presented in this communication.

Mouse lymphoblastoma L5178Y cells were grown in FISCHER's medium, supplemented with 10% horse serum. Growth was observed after incubation at 37°C for 70 hours by counting the cell number in a Coulter counter; and the concentration of drugs, exhibiting 50% growth inhibition, (IC_{50}) was determined.

Adriamycin-resistant mutant cell sublines were obtained by incubating cells ($3\sim4\times10^4$/ml) at 37°C for 6 hours with N-methyl-N'-nitro-N-nitrosoguanidine 0.1 μg/ml; they were washed and collected by centrifugation. After incubation in FISCHER's medium at 37°C for 48 hours, cells were transferred to FISCHER's medium containing 0.15% agar, 15% horse serum and adriamycin 0.05 μg/ml. The colonies were further selected in soft agar medium containing 0.5 μg/ml adriamycin; and a mutant cell subline maintained in the drug-free medium, was used in this experiment. The doubling time of the adriamycin-resistant subline was observed to be approximately 12 hours. It was slightly longer than that of the parental cell line with an 11-hour doubling period.

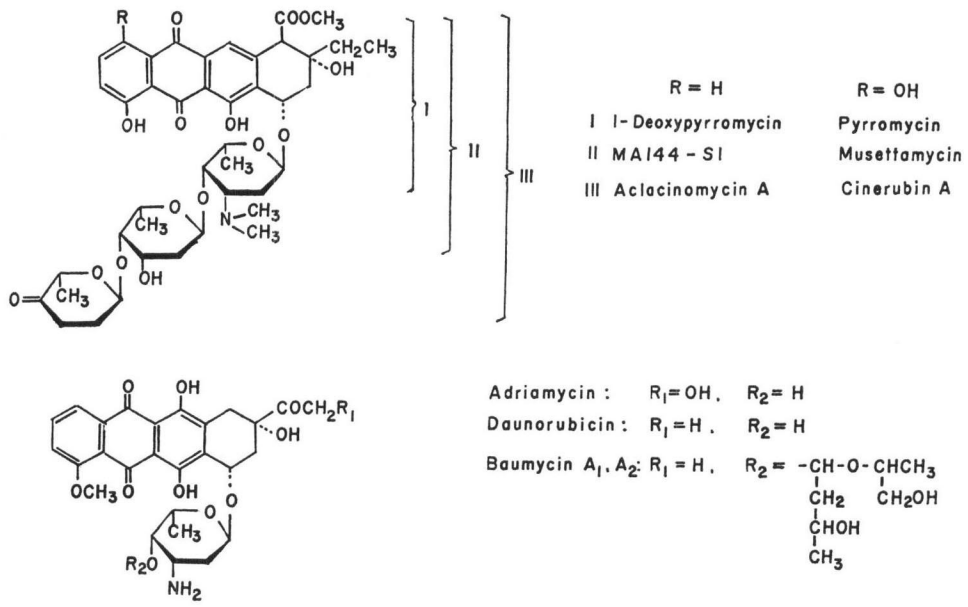
The inhibitory effects of anthracyclines and other antibiotics on growth of the adriamycin-resistant subline and parental cell line are summarized in Table 1. The average concentrations exhibiting 50% inhibition of growth (IC_{50}), were determined by the observations in seven repeated experiments. The resistant mutant cell subline was found to exhibit no cross-resistance with chemically different antitumor antibiotics: bleomycin A_2, mitomycin C, actinomycin D, neothramycin, and blasticidin S. Since a number of new anthracycline antibiotics have been described recently[7], we compared the sensitivity of the adriamycin-resistant subline to these and other anthracyclines (Fig. 1). The resistant mutant subline showed complete cross-resistance to daunorubicin, and baumycins A_1 and A_2 (daunorubicin derivatives) and partial cross-resistance with other anthracyclines. The 1-deoxy group of antibiotics (1-deoxypyrromycin, MA144-S1, aclacinomycins A, B and Y) exhibited a higher degree of partial cross-resistance than the cor-

Table 1. Drug sensitivity of an adriamycin-resistant subline of mouse lymphoblastoma L5178Y cells in comparison with that of the parental cell line.

Antibiotics	IC_{50} (μg/ml) for		Degree of* resistance
	Parental	Resistant	
Adriamycin	0.01	0.2	20
Daunorubicin	0.01	0.2	20
Baumycin A_1	0.004	0.08	20
Baumycin A_2	0.004	0.08	20
1-Deoxypyrromycin	0.03	0.12	4
MA144-S1	0.01	0.12	12
Aclacinomycin A	0.01	0.02	2
Aclacinomycin B	0.01	0.02	2
Aclacinomycin Y	0.01	0.02	2
Pyrromycin	0.04	0.04	1
Musettamycin	0.004	0.008	2
Cinerubin A	0.006	0.006	1
Bleomycin A_2	0.7	0.8	1
Mitomycin C	0.06	0.08	1
Actinomycin D	0.0005	0.001	2
Neothramycin	0.15	0.15	1
Blasticidin S	1.0	1.2	1

* The resistance is expressed as ratio of IC_{50} values for resistant to parental cell line.

Fig. 1. The structure of the anthracycline antibiotics.



responding 1-hydroxyl analogs (pyrromycin, musettamycin, and cinerubin A). The results suggested that the 1-hydroxyl group as well as the sugar moiety may be related to this type of partial cross-resistance. This is in accord with the report by JOHNSON *et al.*[4] of an adriamycin-resistant subline of P388 leukemia lacking cross-resistance to cinerubin A.

Amphotericin B, which causes damage to the cytoplasmic membrane of mammalian cells, has been reported to induce sensitivity to actinomycin and other antitumor agents in drug-resistant cells by impairing the permeability barrier[3]. The effects of adriamycin in combination with amphotericin B (Fungizone, Squibb) on growth of the parental and adriamycin-resistant cell lines of L5178Y were investigated. Cell growth was partially blocked by amphotericin B, 5~10% inhibition at a concentration of 2.5 μg/ml and 15~30% at 5 μg/ml. The adriamycin-resistant mutant was observed to be slightly more sensitive to amphotericin B than the parental cell line. Amphotericin B, at a concentration of 2.5 and 5 μg/ml, was found to induce no significant sensitivity to adriamycin in both parental and resistant cell lines (Table 2). The results suggest that adriamycin resistance may not be attributed to a mutational alteration of the permeability barrier of the cell membrane. This assumption seemed to be supported by the lack of cross-resistance of the resistant mutant cells to chemically different antibiotics (Table 1).

Table 2. The effects of adriamycin on growth of the resistant and parental cell lines in the presence of amphotericin B.

Cell lines	Amphotericin B (μg/ml)		
	0	2.5	5
Parental (sensitive)	0.01*	0.01	0.01
Adriamycin-resistant	0.21	0.21	0.20

* The number represents the concentration of adriamycin, showing 50% inhibition of cell growth (IC_{50}).

The mechanism of drug resistance of the mutant subline remains to be determined. One possibility is that the resistance may be due to metabolic alteration, with respect to inactivation or activation of the antibiotic. Alternatively, a change(s) in chromatin or a nuclear protein which interferes with the binding of adriamycin to DNA, may be responsible for the resistance to adriamycin. Finally, there remains the possibility that resistance may be due to alteration of the cell membrane. The enhanced sensitivity to amphotericin B, although the degree was low, may be indicative of change(s) in the cytoplasmic membrane of the resistant mutant cells.

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