NOTES

DRUG SENSITIVITY AND SOME CHARACTERISTICS OF A BLEOMYCIN-RESISTANT SUBLINE OF MOUSE LYMPHOBLASTOMA L5178Y CELLS

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Cellular resistance to bleomycin is important in the treatment of neoplasms. However, the mechanism of bleomycin resistance has been not well investigated. The resistance may be attributed to alterations of membrane transport, binding to DNA or other cell components, an inactivating enzyme, and/or a DNA repair system. MIYAKI *et al.* have examined the binding of [^{14}C]bleomycin to DNA in bleomycin-sensitive and -resistant rat ascites hepatoma cells, and found that the resistance is due to increased activity of a bleomycin-inactivating enzyme but not to the transport barrier. BRABBS and WARR[2] have reported a bleomycin-resistant clone of Chinese hamster ovary cells, in which a membrane change has been suggested.

We have recently isolated a bleomycin-resistant subline of mouse lymphoblastoma L5178Y cells, and found that the resistance cannot be attributed to alteration of bleomycin-inactivating activity. The drug sensitivity and some characteristics of the resistant cell subline is presented in this publication.

Bleomycin A_2 (copper-free), peplomycin (pepleomycin), macromomycin and aclacinomycin A were generously given by Dr. HAMAO UMEZAWA, Institute of Microbial Chemistry. Mitomycin C and adriamycin were kindly supplied by Kyowa Hakko Kogyo Co., Ltd. Neocarzinostatin was a product of Kayaku Antibiotics Research Co., Ltd., blasticidin S of Kaken Chemical Co., Ltd., and fusidic acid of Leo Pharmaceutical Products, Ballerup, Denmark. The cells were grown in FISCHER's medium supplemented with 10% horse serum.

A bleomycin-resistant subline of L5178Y cells was isolated by repeated injection of 10 mg/kg of bleomycin A_2 to CDF_1 (BALB/cAnNCrj × DBA/2NCrj) female mice, 7~8 weeks of age, bearing L5178Y tumors. After 9 successive transplants, colonies of resistant cell sublines were selected in FISCHER's medium supplemented with 15% horse serum, 0.15% agar, and 10 μg/ml bleomycin A_2.

As illustrated in Fig. 1, the resistant cells grew a little more slowly than the parental cells. The

Fig. 1. Effects of bleomycin A_2 on growth of parental and bleomycin-resistant L5178Y cells.

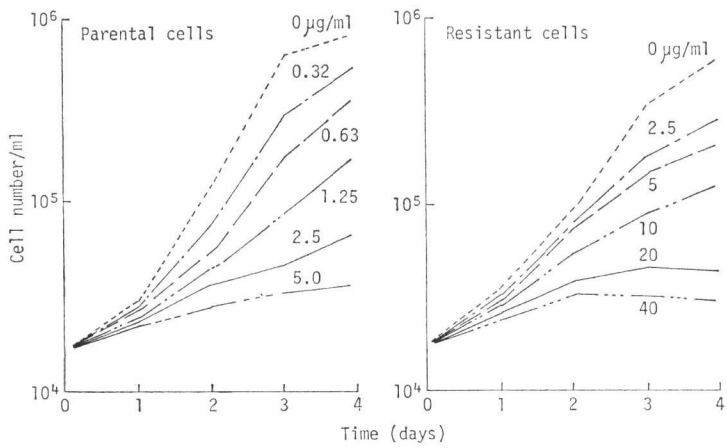

Table 1. Drug sensitivity of a bleomycin-resistant subline of L5178Y cells in comparison with that of the parental cells.

	Antibiotic	IC_{50}(μg/ml) for		Degree of resistance*
		Parental	Resistant	
Experiment 1	Bleomycin A_2	0.48	8.4	17.5
	Neocarzinostatin	0.01	0.02	2.0
	Macromomycin	0.015	0.028	1.8
	Mitomycin C	0.064	0.094	1.5
	Adriamycin	0.07	0.08	1.1
	Aclacinomycin A	0.047	0.058	1.2
	Blasticidin S	3.4	0.94	0.28
	Fusidic acid	35	52	1.5
Experiment 2	Bleomycin A_2	0.82	8.5	10.4
	Peplomycin	0.45	3.4	7.7

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

doubling time of the former was *ca.* 14.7 hours, and that of the latter *ca.* 11.3 hours. In this experiment, 50% inhibitory concentration (IC_{50}) of bleomycin A_2 on day 3 was *ca.* 3.2 μg/ml for the resistant cells, and 0.28 μg/ml for the parental cells: *i.e.* the degree of resistance was *ca.* 11.4 fold. The degree of resistance varied from one experiment to another in a range of 10~20 fold.

The inhibitory effects of various antibiotics on growth of the bleomycin-resistant subline and parental cell line were determined on day 3. The results are presented in Table 1. IC_{50} for the bleomycin-resistant cells of DNA-binding antibiotics (neocarzinostatin, macromomycin, mitomycin C, adriamycin, and aclacinomycin A) were similar to those of the parental cells, indicating that these drugs show no significant cross resistance with bleomycin in this type of resistant cells. No significant cross resistance was observed with fusidic acid, an inhibitor of protein synthesis or elongation factor 2.

On the contrary, for peplomycin, a novel derivative of bleomycin[3], the subline cells exhibited cross resistance with bleomycin A_2. But, the degree of resistance for peplomycin was slightly less than that for bleomycin A_2.

The bleomycin-resistant cells were more sensitive to blasticidin S than the parental cells. However, the mechanism of the collateral sensitivity remains to be determined.

For the purpose of studying bleomycin inactivation, the parental or bleomycin-resistant cells (1.3×10^8 cells) were homogenized in 4.6 ml of 70 mM phosphate buffer, pH 7.0, with a Teflon homogenizer, and then briefly sonicated. The cell lysate was centrifuged at 105,000 *g* for 60 minutes, and the supernatant was assayed for the inactivating enzyme by the method of UMEZAWA *et al.*[4] The residual bleomycin was determined by a paper disc method, using *Bacillus subtilis* ATCC 6633 as test organism.

Table 2. Bleomycin-inactivating activity in the parental and bleomycin-resistant cell lines.

Cell line	Bleomycin A_2 inactivated ($\mu g/10^7$ cells/min)
Parental	1.03
Resistant	0.91

The level of enzymic activity seemed to be less than that of rat hepatoma AH66 reported by MIYAKI *et al*[1]. No significant difference in bleomycin-inactivating activity was found between the parental and resistant cells (Table 2). The result indicated that the resistance may not be due to the increase of bleomycin-inactivating enzyme.

An alternative assumption, concerning the mechanism of resistance, is decreased uptake or retention of bleomycin in the resistant cells by alteration of plasma membrane. We have observed changes of membrane-associated enzyme activities in the resistant cells[5]. The finding supports the hypothesis that the resistance is attributable to alteration of the plasma membrane.

The bleomycin-resistant Chinese hamster ovary cells, isolated by BRABB and WARR[2], were re-

ported to show around twice as resistant as the wild type cells and cross resistance with vinblastine, puromycin and some other agents. The bleomycin-resistant L5178Y cell subline, obtained in the current experiment, is 10~20 fold resistant to bleomycin and exhibits no significant cross resistance with unrelated antitumor antibiotics. This subline may be useful for studying the transport system of bleomycin group antibiotics. Since bleomycin causes single strand scission of DNA[6], studies on repair of single strand breaks in the sensitive and resistant cells would be of interest.

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