COMPARATIVE STUDIES ON RESISTANCE TO ANTHRACYCLINE DERIVATIVES BETWEEN DOXORUBICIN-RESISTANT MOUSE LYMPHOBLASTOMA L5178Y CELLS AND MOUSE LEUKEMIA P388 CELLS

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When tumor cells are chronically treated with antitumor drugs such as vinca alkaloids or anthracyclines, they often acquire resistance not only to the same drugs but also to other antitumor drugs[1~3]. Although the mechanism of this pleiotropic drug resistance is not fully understood, decreased uptake and retention of doxorubicin (=adriamycin, ADM) and daunorubicin (=daunomycin, DNR) in drug resistant cell lines induced by ADM in mouse leukemia P388 cells and mouse lymphoblastoma L5178Y cells have been reported[4,5]. INABA *et al.*[5] observed the enhanced active transport of drugs outward plasma membrane in ADM-resistant P388 cells (P388/ADM). SUGIMOTO *et al.*[6] also proposed that the alteration in plasma membrane of ADM-resistant L5178Y cells (L5178Y/ADM) might be responsible for their lower sensitivity to antitumor drugs. Besides the membrane alteration, cytoplasmic functions were closely related to drug resistance. As compared to parental P388 cells (P388/S), P388/ADM showed a decreased content of cytochrome P-450 linked microsomal mixed-function oxidase components[7], with which the metabolism of antitumor drugs seems to be deeply associated.

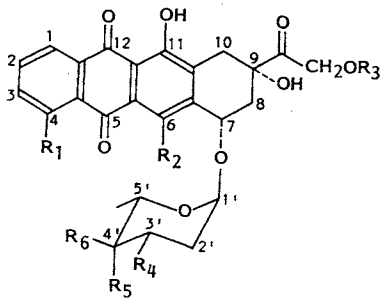
In a search for anthracyclines which are able to overcome drug resistance or highly toxic even to ADM-resistant cells, a total of 37 anthracyclines, including ADM (**1**) and DNR (**18**), have been evaluated for their growth inhibitory activity against parental and ADM-resistant L5178Y and P388 cells *in vitro*. The drug resistance mechanism in L5178Y/ADM is also discussed in comparison with that for P388/ADM.

Parental L5178Y cells (L5178Y/S) and L5178Y/ADM were supplied by Prof. N. TANAKA, Institute of Applied Microbiology, University of Tokyo, and cultured in Fischer's medium supplemented with 10% horse serum at 37°C in tightly capped test tubes. P388/S and P388/ADM were donated by Dr. M. INABA, Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, and maintained by weekly ip transplantation in DBA/2 mice. Cells were harvested from tumor-bearing mice and cultured in RPMI medium 1640 supplemented with 10% fetal calf serum, 100 μg/ml kanamycin and 5 μM hydroxyethyl disulfate at 37°C in an atmosphere of 5% CO_2 in air.

The cells were implanted in an appropriate growth medium at 3×10^4 cells/ml in the absence or the presence of each anthracycline. The number of cells was counted in a Coulter counter (Coulter Electronics Ltd., Beds, England) after 3 days' culture. The resistance factors for L5178Y/ADM and P388/ADM against the individual anthracyclines were defined as a ratio of ID_{50}s for the resistant cells to the parental cells. All anthracylines employed in this study are the gifts of Farmitalia Carlo Erba, Milan, Italy.

The results are summarized in Table 1. The resistance factors for L5178Y/ADM against ADM and DNR are 33 and 38, respectively, being fairly comparable to those previously reported by NISHIMURA *et al.*[4]. The resistance factors for L5178Y/ADM ranged from 0.5 against (7*R*,9*R*)-4-demethyl-11-deoxy-*N*-trifluoroacetyldaunomycin (**37**) to 23,000 against 4-*O*-demethyl-6-deoxyadriamycin (**12**). Anthracyclines, to which L5178Y/ADM shows much higher resistance than to ADM or DNR, are potent inhibitors of L5178Y/S but not of L5178Y/ADM with the exception of 3'-deamino-3'-(3-cyano-4-morpholinyl)adriamycin (MRA-CN, **6**). Though there exists significant difference in a range of distribution, the resistance factors for P388/ADM and L5178Y/ADM are apt to be proportionate to one another excluding the case of MRA-CN, as shown in Fig. 1. 3'-Deamino-3'-3'-(*epi*)-hydroxy-4'-deoxy-4'-aminodaunomycins (**24** and **25**) are poor inhibitors of both L5178Y/S and P388/S. The *N*-trifluoroacetyl derivatives of daunorubicin, *i.e.*, **23**, **29** and **32**, inhibited the growth of P388/S significantly, but these compounds are not necessarily potent inhibitors of L5178Y/S as was proven by the results for **29** and **32**. The ID_{50} of MRA-CN against L5178Y/ADM was as low as 7×10^{-6} μg/ml, in a marked contrast to those for the other anthracyclines (0.07 μg/ml or more). The resistance factor for L5178Y/ADM against MRA-CN was nevertheless 20-times higher than that

Table 1. Effects of anthracyclines on the growth of parental and ADM-resistant cell lines of mouse lymphoblastoma L5178Y and leukemia P388 cells *in vitro*.



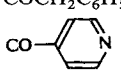
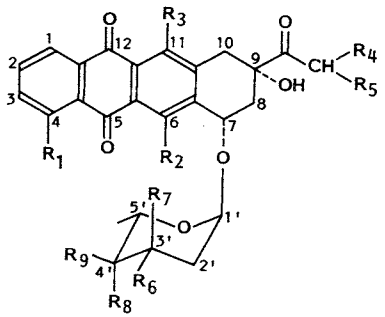
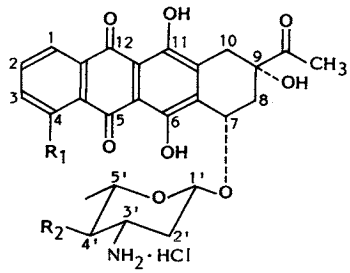
Compound	R_1	R_2	R_3	R_4	R_5	R_6	ID_{50} (μg/ml)		Resistance factor	ID_{50} (μg/ml)		Resistance factor
							L5178Y/S	L5178Y/ADM		P388/S	P388/ADM	
1 (ADM)	OCH_3	OH	H	$NH_2 \cdot HCl$	OH	H	0.063	2.1	33	8.6×10^{-5}	0.48	5.6×10^3
2	OCH_3	OH	H	$NH_2 \cdot HCl$	H	H	8.7×10^{-4}	0.88	1,000	4.8×10^{-9}	0.24	4.9×10^7
3	OCH_3	OH	H	$NH_2 \cdot HCl$	H	OH	6.2×10^{-3}	1.7	270	1.1×10^{-7}	2.1	1.9×10^7
4	OCH_3	OH	H	$NH_2 \cdot HCl$	I	H	3.4×10^{-3}	0.073	21	1.8×10^{-6}	0.0027	1.5×10^3
5	OCH_3	OH	H	OH	$NH_2 \cdot HCl$	H	4.0	16	4.0	0.36	8.7	25
6 (MRA-CN)	OCH_3	OH	H		OH	H	1.0×10^{-8}	7.2×10^{-6}	720	2.0×10^{-10}	3.7×10^{-10}	1.9
7	OCH_3	OH	COC_2H_5	$NH_2 \cdot HCl$	OH	H	0.016	2.3	140	2.2×10^{-4}	0.41	1.8×10^3
8	OCH_3	OH	$CO(CH_2)_6CH_3$	$NH_2 \cdot HCl$	OH	H	0.019	1.6	84	1.3×10^{-6}	0.37	2.8×10^5
9	OCH_3	OH	$COCH_2C_6H_5$	$NH_2 \cdot HCl$	OH	H	0.082	6.2	76	2.2×10^{-7}	0.32	1.4×10^6
10	OCH_3	OH		$NH_2 \cdot HCl$	OH	H	7.6×10^{-3}	6.0	790	0.0075	0.45	61
11	OCH_3	OH	COC_4H_9	$NHCOCF_3$	OH	H	0.31	7.1	23	0.017	0.63	36
12	OH	H	H	$NH_2 \cdot HCl$	OH	H	3.5×10^{-5}	0.818	23,000	2.0×10^{-5}	0.40	2.0×10^4
13	H	OH	H	$NH_2 \cdot HCl$	OH	H	6.9×10^{-4}	0.19	280	8.0×10^{-7}	0.16	2.0×10^5
14	H	OH	H	$NH_2 \cdot HCl$	H	H	0.016	0.088	5.5	2.8×10^{-7}	0.025	8.9×10^4
15	H	OH	H	$NH_2 \cdot HCl$	H	OH	2.4×10^{-4}	0.27	1,100	7.4×10^{-9}	0.27	3.7×10^7
16	H	OH	H	OH	OH	H	0.036	0.14	3.9	0.0016	0.045	29
17	H	NO_2	H	$NH_2 \cdot HCl$	OH	H	1.1	6.9	6.3	0.050	5.2	110

Table 1. (Continued)

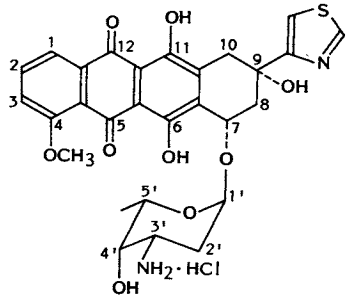


Compound	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	ID_{50} (μg/ml) L5178Y/S	ID_{50} (μg/ml) L5178Y/ADM	Resistance factor	ID_{50} (μg/ml) P388/S	ID_{50} (μg/ml) P388/ADM	Resistance factor
18 (DNR)	OCH_3	OH	OH	H	H	$NH_2 \cdot HCl$	H	OH	H	0.059	2.2	37	3.2×10^{-5}	0.31	9.4×10^3
19	OCH_3	OH	OH	H	H	$NH_2 \cdot HCl$	H	H	H	2.1×10^{-5}	0.17	8,100	1.9×10^{-7}	0.025	1.3×10^5
20	OCH_3	OH	OH	H	H	$NH_2 \cdot HCl$	H	H	OH	4.5×10^{-3}	1.6	360	5.7×10^{-6}	0.35	6.1×10^4
21	OCH_3	OH	OH	H	H	$NH_2 \cdot HCl$	H	I	H	0.033	0.073	2.2	6.2×10^{-9}	5.2×10^{-6}	8.3×10^2
22	OCH_3	OH	OH	H	H	$NHCOCH_3$	H	OH	H	1.6	25	16	0.44	3.1	7.0
23	OCH_3	OH	OH	H	H	$NHCOCF_3$	H	OH	H	6.0×10^{-3}	0.76	130	3.2×10^{-7}	0.036	1.1×10^5
24	OCH_3	OH	OH	H	H	OH	H	$NH_2 \cdot HCl$	H	1.8	6.6	3.7	0.25	2.1	8.7
25	OCH_3	OH	OH	H	H	H	OH	$NH_2 \cdot HCl$	H	1.4	6.7	4.8	0.060	3.0	51
26	OCH_3	OH	OH	Br	H	$NH_2 \cdot HCl$	H	H	OH	0.063	1.5	24	0.0025	1.2	470
27	OCH_3	OH	OH	OCH_3	OCH_3	$NH_2 \cdot HCl$	H	OH	H	1.7	33	19	0.19	4.5	24
28	OCH_3	H	OH	H	H	$NH_2 \cdot HCl$	H	OH	H	0.038	0.82	22	5.2×10^{-7}	0.21	4.1×10^5
29	OH	H	OH	H	H	$NHCOCF_3$	H	H	OH	1.2	4.4	3.7	1.5×10^{-5}	0.24	1.6×10^4
30	H	OH	OH	H	H	$NH_2 \cdot HCl$	H	OH	H	5.7×10^{-3}	0.18	32	3.8×10^{-7}	0.026	6.9×10^4
31	H	OH	OH	H	H	$NH_2 \cdot HCl$	H	H	OH	5.6×10^{-3}	0.21	38	2.1×10^{-6}	0.023	1.1×10^4
32	H	OH	OH	H	H	$NHCOCF_3$	H	OH	H	0.50	2.4	4.8	1.6×10^{-4}	0.18	1.1×10^3
33	H	OH	H	H	H	$NH_2 \cdot HCl$	H	H	OH	0.36	7.2	20	0.030	3.5	120

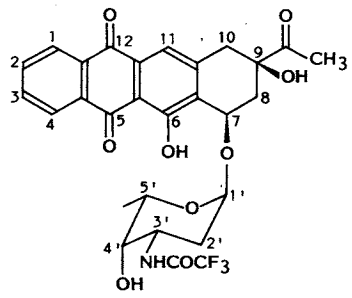
Table 1. (Continued)



Compound	R_1	R_2	ID_{50} (μg/ml)		Resistance factor	ID_{50} (μg/ml)		Resistance factor
			L5178Y/S	L5178Y/ADM		P388/S	P388/ADM	
34	OCH_3	OH	0.53	8.1	15	0.028	2.9	100
35	H	H	0.49	2.0	4.1	4.0×10^{-6}	0.040	99,000



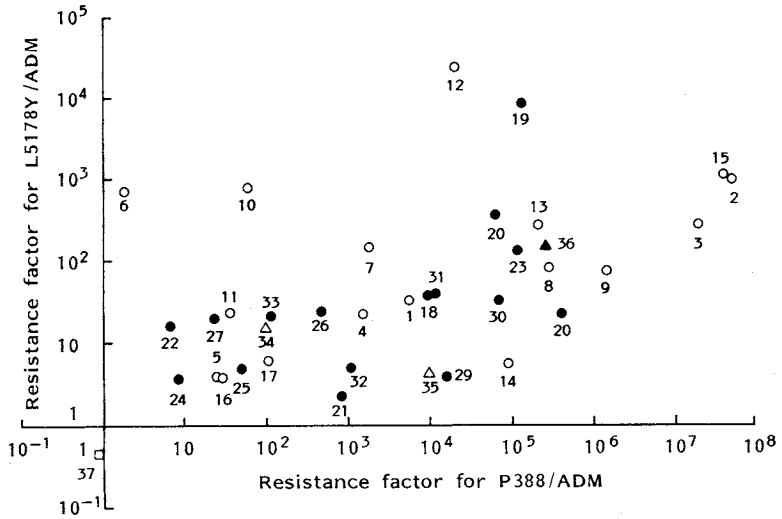
Compound	ID_{50} (μg/ml)		Resistance factor	ID_{50} (μg/ml)		Resistance factor
	L5178Y/S	L5178Y/ADM		P388/S	P388/ADM	
36	5.5×10^{-4}	0.086	160	1.2×10^{-6}	0.31	2.6×10^{5}



Compound	ID_{50} (μg/ml)		Resistance factor	ID_{50} (μg/ml)		Resistance factor
	L5178Y/S	L5178Y/ADM		P388/S	P388/ADM	
37	4.3	2.1	0.49	3.7	3.2	0.87

Fig. 1. Correlation between the resistance factors for the ADM-resistant L5178Y cells and the ADM-resistant P388 cells.

○ Compounds **1** to **17**, ● **18** to **33**, △ **34** and **35**, ▲ **36**, □ **37**.



against ADM. The lack of resistance to **37** shown by L5178Y/ADM and P388/ADM as well as a poor potential of **37** as a growth inhibitor implies that (7*S*,9*S*)-configuration, which is prevailing among naturally occurring anthracyclines, is recognized by all four cell lines.

MOSHER *et al.*[8] synthesized 3′-deamino-3′-(4-morpholinyl)adriamycin (MRA) by the reductive alkylation of ADM with diglycol aldehyde and sodium cyanoborohydride, in which MRA-CN was recovered as a byproduct from a nonbasic fraction[9]. According to ACTON *et al.*[9], MRA-CN was one of the most potent antitumor agents being one hundred to one thousand times more potent than ADM against tumor cells *in vitro* and *in vivo*. The drug resistance of P388/ADM might depend on the function of membrane transport systems. The lipophilic compounds such as MRA-CN and MRA could be taken up by cells at the increased rates, resulting in rapid intracellular accumulation of the drugs and overcoming the resistance of P388/ADM *in vitro*[10]. In fact, the ID_{50}s of MRA-CN for P388/S and P388/ADM were not distinguishable from each other; 2.0×10^{-10} and 3.7×10^{-10} μg/ml, respectively (resistance factor = 1.9). These results are in good agreement with the previous findings by the other group[10,11].

It was recently reported that MRA-CN produces DNA interstrand crosslinks, whereas equitoxic concentrations of ADM and MRA produced no significant levels of DNA-DNA crosslinks[12~14]. This suggests that the cyano-morpholinyl group may act as an alkylating moiety like the α-cyanoamine groups carried by the antibiotics with alkylating potential such as saframycin A[15~17], cyanocycline A[18], naphthocyanidine[19] and cyanonaphthyridomycin[20,21]. A putative mechanism for covalent alkylation of DNA by MRA-CN may account for pronounced antitumor activity of this compound as compared with that of MRA.

Cross resistance of P388/ADM was related to the specific classes based on mechanism of action rather than to chemical nature. The best example of this is the complete resistance of P388/ADM to emetine as compared to the sensitivity of P388/ADM to emetine mustard[22]. The substitution of the tetrahydroisoquinoline nitrogen of emetine with the alkylating moiety renders the derivatives inactive as an inhibitor of protein synthesis and results in an alkylating agent. A subline of human ovarian carcinoma ES-2 was developed with a 10-fold resistance to MRA-CN[23]. This subline ES-2/MR was highly cross resistant to the alkylating agents melphalan and carmustine but relatively sensitive to ADM and vinblastine. It is possible that alkylating agents do not share the efflux transmembrane mechanism which functions for DNA intercalators, vinca alkaloids and protein synthesis inhibitors. Alternatively, the rapid reaction of alkylating agents with DNA does not require the extended period of intracellular drug content. Due to either one of these two mechanism or both, MRA-CN can overcome

the drug resistance of P388/ADM which is probably as sensitive to alkylating agents as P388/S.

The extremely low ID_{50}s of MRA-CN for both L5178Y/S and L5178Y/ADM could also be accounted for by its increased lipophilicity and alkylating ability. On going from ADM to MRA-CN, however, the resistance factor for L5178Y/ADM increased from 31 to 730. Although the poor drug uptake for certain antitumor agents by L5178Y/ADM (owing to decreased influx), which has been reported to be the basis of its resistance to anthracyclines[4,6], could be nullified by the lipophilicity of MRA-CN as in the case of P388/ADM, the persistent resistance to MRA-CN might result from its potential resistance to alkylating agents. This proposed additional mechanism for the drug resistance of L5178Y/ADM is now under investigation in our laboratory.

Acknowledgments

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