ANTITUMOR EFFECT OF A NEW DERIVATIVE OF BLEOMYCIN AGAINST A CELL LINE OF MULTIDRUG RESISTANT MURINE LYMPHOBLASTOMA

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It has been reported that antibiotic bleomycins and their derivatives exhibit antitumor effect against several types of tumors including Ehrlich carcinoma, squamous cell carcinoma *etc.*, but other types of tumors such as mouse ascites plasmacytoma, Friend ascites tumor, and rat ascites hepatoma are generally resistant to bleomycins. We present here the antitumor effect of aminopropylmethylpropyl-bleomycin (APMP-BLM), a new derivative of bleomycin, against a cell line of murine lymphoblastoma L5178Y resistant to aclarubicin (Y/ACR) which also expresses multidrug resistance[1], but not against the parent L5178Y cell (Y/S). As presented in Table 1, the maximum value of life span prolongation expressed by T/C (%) in a group of mice bearing tumors of Y/ACR cells treated with APMP-BLM at dose of 0.4 mg/kg was over 262, whereas that in a group of mice bearing tumors of Y/S cells treated with the agent was 127. The mean survival days in the host bearing tumors of Y/ACR cells was almost twice that of the host bearing parent tumor. We described previously that Y/ACR cells express a specific epitope on the cell surface which was demonstrated with a polyclonal antibody in rabbits (Y. HIRAKAWA; unpublished result) or a monoclonal antibody in a syngenic mouse DBA2 immunized with Y/ACR cells[2]. The results imply that the cell surface epitope on Y/ACR cells is recognized immunologically in mice bearing tumor of Y/ACR cells, and that the immune response targeting the surface antigen on the resistant cells mediates the resistance to transplantability[3]. In contrast to the results obtained *in vivo* which showed a selective inhibition of tumor growth in Y/ACR cell line by APMP-BLM, Y/ACR cell line cultured *in vitro* was more refractory to the agent than the parent Y/S cell line. The IC_{50} values of the agent against parent Y/S and Y/ACR cells were 0.2 and 0.7 μg/ml, respectively (data not shown). Again, the selective antitumor effect of the agent against Y/ACR cells was not due to selective cytotoxicity against the cell.

In an attempt to clarify the mechanism of selective antitumor effect of bleomycin against Y/ACR cells, we studied the effect of the agent

Table 1. Antitumor effect of APMP-BLM on the ascites tumor of murine lymphoblastoma L5178Y cells.

Cell	Drug (mg/kg/day)	MSD±SE (days)	T/C (%)
L5178Y/ACR	Control	39.7±1.6	100
	APMP-BLM 10	59.4±4.9[a]	149
	2	>90.8±20.2[b]	>228
	0.4	>103.9±10.1[a]	>262
	0.1	63.4±5.2[a]	159
L5178Y/S	Control	25.3±0.9	100
	APMP-BLM 10	31.1±3.0[a]	123
	2	32.1±2.1[a]	127
	0.4	32.0±2.6[a]	126
	0.1	31.9±4.4[a]	126

Antitumor activity toward ascitic tumors of Y/S and Y/ACR cells was evaluated by determination of survival time of mice. 2×10^6 cells cultured *in vitro* were ip implanted into 8-week old female DBA2 mice, in groups of 10, and APMP-BLM was administrated ip from day 1 to day 7 for 6 successive days once a day.

MSD: Mean survival days. Statistical analysis was performed by Student's t-test, and the marks [a] and [b] indicate $P<0.01$ and $P<0.05$, respectively.

Table 2. Effect of APMP-BLM on the immune response of tumor-bearing mouse of lymphoblastoma L5178Y cells resistant to aclarubicin.

The cytolysis of target cells by spleen cells.

E	T		E:T
	Y/ACR	Y/S	
1. Tumor-free	89	54	100:1
	42	41	50:1
2. Tumor-free	89	90	100:1
with APMP-BLM	71	62	50:1
3. Y/ACR-Bearing	34	0	100:1
	28	3	50:1
4. Y/ACR-Bearing	96	35	100:1
with APMP-BLM	64	14	50:1

Target cells of Y/ACR or parent Y/S labeled with [^{3}H]thymidine were exposed to spleen effector cells, incubated for 24 hours and [^{3}H]thymidine released from target cells was counted. The value was scored by the % lysis of [^{3}H]thymidine expressed by (experimental release − minimum release) dpm × 100/(maximum release − minimum release) dpm, where the maximum and minimum releases of [^{3}H]thymidine from the target cells were expressed by values in the presence or absence of 10% Triton X-100, respectively.

E: Effector cells of spleen from the tumor-free DBA/2 mice or the tumor-bearing DBA/2 mice at 2 weeks after implantation of Y/ACR cells. 1. Tumor-free; spleen cells from tumor-free DBA/2. 2. tumor-free with APMP-BLM; spleen cells from APMP-BLM treated DBA/2 mouse of tumor-free. 3. Y/ACR-bearing; spleen cells from Y/ACR tumor-bearing DBA/2 mouse. 4. Y/ACR-bearing with APMP-BLM; spleen cells from Y/ACR tumor-bearing DBA/2 mouse treated with APMP-BLM. APMP-BLM was administrated ip from day 1 to day 7 for 6 successive days once a day at dose of 0.2 mg/kg after implantation of 2×10^6 Y/ACR cells ip into DBA/2 mice. T: target cells labeled with [^{3}H]thymidine. Y/ACR: L5178Y cell resistant to aclarubicin. Y/S: parent L5178Y cell.

on immunological response in mice bearing Y/ACR tumors. The spleen cells from mice bearing Y/ACR tumor were found to have poor capability for targeting both Y/S and Y/ACR cells, while those of bleomycin-treated host were able to target and kill Y/ACR cells selectively to almost the same extent as normal host as shown in Table 2. The results indicate that immunosuppression induced in tumor bearing mice was restored to the level in normal mice by the administration of bleomycin, probably inducing killer T, NK cells or macrophages responsible for killing the target cell. We are speculating that the immunosupression in tumor bearing host was blocked by the administration of the agent followed by the reduction of suppressor T (Ts) cell population and the resistance of transplantability to Y/ACR cells.

Our results are certainly supported by the previous lines of evidence that immunosuppression is induced in tumor bearing host, that the cellular immunity is mostly involved in resistance of tumor development, and that the antibiotic enhances immune responses under certain conditions[4,5]. We have previously investigated the significant enhancement of delayed type hypersensitivity response of the tuberculin reaction in guinea pigs by bleomycin complex which mainly consists of bleomycin A_2 (unpublished result). It is likely that the selective antitumor effect of the agent against Y/ACR cell is mediated by immunopotentiation, especially of the cellular immune response. It is also possible that the selective antitumor effect of the agent against Y/ACR cells is exerted by synergistic action of both cytotoxicity and immunopotentiation.

We propose here the possibility that bleomycins and their derivatives are clinically useful to overcome problems of drug resistance in cancer chemotherapy treatment, especially to treat drug resistant tumors which exert multidrug resistance expressing the specific 170 k glycoproteins on the cell surface[6,7].

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