POTENTIATION OF THE CYTOTOXICITY OF PEPLOMYCIN AGAINST EHRLICH ASCITES CARCINOMA BY BLEOMYCIN HYDROLASE INHIBITORS

Sir:

Bleomycin (BLM) displays remarkable therapeutic activity for squamous cell carcinoma and malignant lymphoma. Human and animal tissues contain BLM hydrolase, which hydrolyzes the carboxamide bond in the pyrimidoblamic acid moiety. The hydrolyzed antibiotic shows little antitumor activity. Therefore, inhibitors of BLM hydrolase are expected to increase the therapeutic activity of BLM. We have purified BLM hydrolase from rabbit liver using a monoclonal antibody and found that the enzyme activity is inhibited by thiol protease inhibitors, leupeptin[1] and E-64[2], suggesting that the enzyme is a thiol enzyme[3]. We have further examined the effect of BLM hydrolase inhibitors on the antitumor activity of peplomycin (PEP), an analogue of BLM, against Ehrlich ascites carcinoma. The results are presented in this communication.

Ehrlich ascites carcinoma cells (2×10^6 cells/mouse) were implanted into ICR female mice, weighing 20~25 g, on day 0 intraperitoneally. Drugs were given intraperitoneally 10 times every day, starting on day 1. As seen in Fig. 1, the combination therapy of PEP with leupeptin or E-64 exhibited significant increase of life span compared with PEP alone. PEP alone gave 150 T/C (%) and PEP plus leupeptin or E-64 gave 281 T/C (%) or 264 T/C (%), respectively. Leupeptin or E-64 alone had no antitumor activity (Table 1).

Aoyagi *et al.*[4] have previously reported that bestatin, an amino peptidase B inhibitor, increases the therapeutic activity of BLM against Ehrlich carcinoma inoculated to footpad. Sebti and Lazo[5] and we have found that the activity of BLM hydrolase is inhibited by leupeptin and E-64. We examined the potentiation of the cytotoxicity of PEP by leupeptin and E-64 *in vitro* and *in vivo*. Leupeptin and E-64 potentiated the cytotoxicity of PEP *in vivo* as described here, but they failed to show an apparent potentiation *in vitro* against Chinese hamster ovary cells and L5178Y murine lymphoma cells (data not shown). The reason why they do not potentiate the cytotoxicity of PEP to these cells *in vitro* is unclear. However, it might be due to existence of some constituents in the culture medium which block activity of

Fig. 1. Elongation of life span of Ehrlich ascites carcinoma-bearing mice treated with PEP plus leupeptin or E-64.

PEP alone at 1.25 mg/kg (●), leupeptin alone at 10 mg/kg (□), E-64 alone at 10 mg/kg (△), PEP at 1.25 mg/kg plus leupeptin at 10 mg/kg (■), PEP at 1.25 mg/kg plus E-64 at 10 mg/kg (▲) and control 0 mg/kg (○).

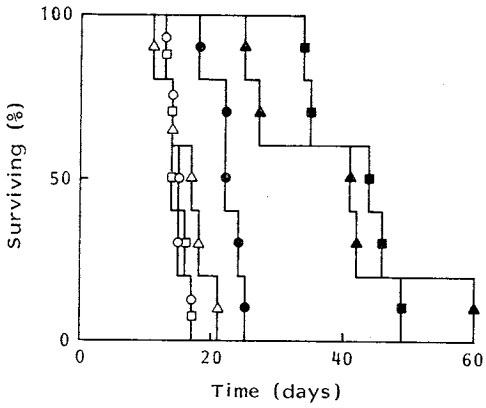

Table 1. Effect of BLM hydrolase inhibitors on the antitumor activity of PEP in Ehrlich ascites carcinoma-bearing mice.

Drug	Dosage (mg/kg/day)	Survival time[a] (days)	T/C (%)
PEP+leupeptin	1.25+10	41.6±6.7 ($P<0.01$)[b]	281
PEP+E-64	1.25+10	39.0±14.1 ($P<0.05$)[b]	264
PEP	1.25	22.2±2.7	150
Leupeptin	10	14.8±1.6	100
E-64	10	16.2±3.8	109
Control	0	14.8±1.5	100

[a] Mean±SD.

[b] Statistically significant by Student's t-test as compared with mice treated with PEP alone.

BLM hydrolase inhibitors.

The present results suggest that leupeptin and E-64 may be useful in combination therapy with PEP.

Acknowledgments

The authors express their deep thanks to the late Professor HAMAO UMEZAWA, Institute of Microbial Chemistry, Tokyo, for his generous advice and cooperation. They are also indebted to Dr. TOMOHISA TAKITA, Nippon Kayaku Co., Ltd., Tokyo, for kind supply of PEP. CHIAKI NISHIMURA acknowledges a fellowship from The Upjohn Pharmaceutical Ltd. The current work was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, Japan.

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(Received July 3, 1987)

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