SELECTIVE CYTOTOXIC ACTIVITY OF BREFELDIN A AGAINST HUMAN TUMOR CELL LINES

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One of the difficult problems in the screening of antitumor agents is the difference of sensitivity between murine experimental tumors and human tumors towards antitumor agents. Therefore, a drug which has strong antitumor activity towards murine experimental tumors is not always active against human tumors in the clinic. In fact, almost all the antitumor drugs exhibit stronger cytotoxic activity against murine tumor cells than human tumor cells *in vitro*. We therefore started to search for drugs from the fermentation broth of microorganisms which show stronger cytotoxic activity towards human cell lines than murine cell lines.

Such activity was found in the culture filtrate of *Eupenicillium* sp. As a result of purification and structure elucidation, the active substance was revealed to be brefeldin A[1].

The antitumor activity of brefeldin A was already reported[1], but its selective cytotoxic activity towards human tumor cells was not. Therefore, we studied the cytotoxic spectrum of brefeldin A compared with doxorubicin as a reference antitumor drug.

In this study, five murine cell lines and nine human tumor cell lines were used. All cells were cultivated with RPMI-1640 medium supplemented with 10% fetal calf serum and 10 μM of 2-hydroxyethyldisulfide (growth medium) at 37°C under 5% CO_2 in air. Cytotoxic activity was determined as follows; cells were seeded to 96-well flat-bottomed microtiter plate (Falcon, No. 3002) 3,000 cells/well in 140 μl of growth medium. After 24 hours incubation, 20 μl of sample solution was added and then the mixture was further incubated for 72 hours. Viable cell fraction was measured by a modified MTT assay[2,3] and 50% inhibitory concentration (IC_{50}) value was calculated by probit method. All experiments were carried out for three times and the mean values were shown.

Table 1 shows the results of cytotoxic activity of brefeldin A and doxorubicin. The range of full IC_{50} values of brefeldin A for murine and human cell lines was 1.9~7.1 μM and 0.08~1.2 μM, respectively and that of doxorubicin for murine and human cell lines was 0.022~0.65 μM and 0.038~0.66 μM, respectively. Brefeldin A showed stronger cytotoxic activities towards human cell lines than murine cell lines, although doxorubicin showed almost equal cytotoxic activity towards human and murine cell lines. Table 1 also shows the IC_{50} ratio of brefeldin A to doxorubicin. The range of brefeldin A/doxorubicin ratio was 0.27~4.2 in human cell lines and 5.5~86 in murine cell lines showing that cytotoxic activity of brefeldin A was almost equal to that of doxorubicin against human cell lines but weaker than doxorubicin against murine cell lines.

Brefeldin A has various biological activities such as antitumor[1], antifungal[1] and antiviral activity[4] as well as inhibition of intracellular transport of several proteins[5,6]. It was suggested that antiviral activities are partialy based on its inhibiting activity of intracellular transport of viral proteins[7]. However the mechanism of antitumor activity of brefeldin A is unknown at present. To study antitumor mechanisms of brefeldin A might be helpful to clarify the mechanisms of difference of sensitivity between murine and human tumor cells to antitumor drugs. Brefeldin A seems to be a good

Table 1. Cytotoxic activity of brefeldin A and doxorubicin.

Cell line	IC_{50} value (μM)		IC_{50} ratio
	BFDA	DXR	BFDA/DXR
Murine cell line			
P388 leukemia	1.9	0.022	86
L1210 leukemia	3.6	0.65	5.5
B16 melanoma	3.6	0.65	5.5
Meth A fibrosarcoma	7.1	0.17	42
NIH3T3 fibroblast	3.0	0.082	37
Human cell line			
K562 leukemia	0.11	0.064	1.7
CCRF-CEM leukemia	0.14	0.038	3.7
HL60 leukemia	0.16	0.038	4.2
PC14 lung carcinoma	1.2	0.66	1.8
MKN-1 gastric carcinoma	0.40	0.30	1.3
KATOIII gastric carcinoma	0.080	0.30	0.27
OST osteosarcoma	0.11	0.60	0.18
T24 bladder carcinoma	0.16	0.19	0.84
HeLa S_3 cervix carcinoma	0.55	0.42	1.3

BFDA: Brefeldin A, DXR: doxorubicin.

tool to study the difference of chemotherapeutic sensitivity between human and murine tumors.

The results of our study suggest that brefeldin A might possibly exhibit stronger antitumor activities against human tumors implanted in nude mice than murine experimental tumors. This hypothesis could not be tested because of its poor solubility in water. Therefore, we will continue to search for water soluble compounds showing selective cytotoxic activity towards human cells. The results will be reported elsewhere.

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