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In Vitro Chemosensitivity Test of SK-302B on Human Colon Carcinoma Cell Lines

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SK-302B, an antibiotic purified from soil *Streptomyces sp.* 302, was structurally identified as echinomycin ($C_{50}H_{66}N_{11}S_2$). In the present experiment, the possibility of SK-302B as an anti-colon cancer agent was investigated by using chemosensitivity system (MTT assay, clonogenic assay). Treatment of SK-302B on various colon cancer cell lines resulted in a significant cytotoxicity and tumor colony formation inhibition. These studies showed that SK-302B had a potent inhibition on colon cancer cells.

Key words : SK-302B, Colon cancer cell line, Chemosensitivity

INTRODUCTION

Colon cancer is 4th to 5th cause of death due to cancers in Korean men and seems to be rapidly increasing (Ministry of Health and Social Affairs, 1989). Therefore, the development of effective chemotherapeutics as one of multimodal therapy against colon cancer is urgently in demand (Hutchison, 1989; Mitchell, 1989; Venditti, 1983). In clinical field, as a mean to overcome colon cancer, the best and widely accepted antitumuor agent is 5-fluorouracil (5-FU). 5-FU has been used to treat various stage of colon cancer patients in a sole or combined regimen. But, under recurrent or metastatic condition, 5-FU is far less effective than in a treatment of localized lesion (Zubrod, 1966). This made 5-FU limited in treating colon cancer with chemo-regimen. (Greco *et al.*, 1978; Lee *et al.*, 1990). In the course of screening an effective antitumor agent comparable to 5-FU or adriamycin, we purified SK-302B from soil *Streptomyces* sp. 302. Recently in our laboratory the structucre of SK-302B was determined as echinomycin ($C_{50}H_{66}N_{11}S_2$). In our previous report, we examined *in vitro* and *in vivo* antitumor activity using mouse cell lines with toxicity test. We also observed that SK-302B had excellent cytotoxicities with low toxicity. Interestingly, we found that SK-302B in screening test using human tumor panel, exerted strong inhibition on the growth of gastrointestinal tract cancer cells. Besides, cytotoxicity of SK-302B on colon cancer cells presumed to be notably higher than those of adriamycin. Thus, the present study was undertaken to evaluate if SK-302B inhibit growth and colony forming ability of colon cancer cell lines.

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MATERIALS AND METHODS

Drugs

SK-302B was purified from soil Streptomyces sp. 302 in our laboratory. Adriamycin was obtained from Sigma Co., Ltd(St. Louis, USA).

Cancer cell lines

The cancer cell lines for cytotoxicity test were as follows : HCT-15 (colon cancer, human), DLD-1 (colon cancer human), SW-480 (colon cancer, human). Colo 205 (colon cancer, human). Each cell line was maintained in RPMI 1640 medium supplemented with 10% fetal calf serum and incubated in a humidified 5% CO_2 chamber at 37°C.

Measurement of cytotoxicity

To evaluate cytotoxicity, modified MTT method was performed essentially as described previously (Carmichael *et al.*, 1987 ; Kim *et al.*, 1995). Briefly, monocellular susupension was seeded at 10^4 cells per well in 96 well plates with 100 μl of medium per well. To compare cytotoxicity between SK-302B and

adriamycin, they were added at varying concentrations and cultures were incubated for 72 hours in an incubator maintaining a highly humidified atmosphere, 5% CO_2 and 95% air. Fifty μl of the medium containing MTT (5 mg/ml) were added to each well. After 4 hours of exposure, the medium was removed and the wells were washed with PBS, and then 50 μl of DMSO were added to each well to solubilize the precipitates. The plates were transferred to an ELISA reader to measure absorbance at 570 nm. IC_{50} value, 50% inhibition of cell growth, was calculated by regression analysis (plotting the viablity versus the concentration of the test compound). All experiments were done at least 3 times, with 4 wells for each concentrations of test agents.

Measurement of tumor colony formation inhibition

Twenty-four well-clonogenic assay was done by modifying the 96 well based clonogenic assay (Salmon *et al.*, 1978 ; Shoemaker *et al.*, 1981). Layers of 0.5 ml, 0.5% noble agar in supplemented RPMI 1640 medium were prepared in a 24 well culture plate. Colon cancer cells to be tested were overlayered on basal agar in 0.5 ml of 0.3% agar containing 20% FCS, double strength-RPMI 1640 medium and various concentrations of the drugs. The final concentration of the cells in each culture was 5×10^3 per well. All experiments were done at least 3 times, with 4 wells for each concentrations of test agents. Colony formation inhibition was calculated as follows. C.F.E (colony forming efficiency) = [formed colony number/seeded colony number] × 100%

RESULTS AND DISCUSSION

To develop the antitumor agent from the broth of soil *Streptomyces* sp. 302, the anti-tumor activity was examined under *in vitro* and *in vivo* system (Geran *et al.*, 1972; Venditti, 1983). Prior to *in vitro* chemosensitivity assay, we determined optimal cell concentration and incubation time. The optimal cell number and culture duration for *in vitro* tumor cell cytotoxicity (TCC) and colony formation inhibition assay is 5×10^3~1×10^4 cells/well (TCC) and 2.5×10^3~5×10^3 cells/well (CFIA), also 3 days (TCC) and 9~13 days (CFIA), respectively, for all colon cancer cell lines (Table 2, a part of the data not shown). SK-302B showed dose-dependent cytotoxicity against all tested colon cancer cell lines. In this paper we presented the data exemplified by SK-302B on DLD-1 celll line (Fig 1). The cytotoxic activities of SK-302B against colon cancer cell lines were evaluated by MTT method (Carmichael *et al*, 1986) and the results were shown as IC_{50} value in Table I. Interestingly, SK-302B showed higher cytotoxicity against all tested colon cancer cell line than adriamycin. Among the tested cell lines, DLD-1 and SW-480 colon cancer cell lines were the most sensitive to SK-302B (Table I). In colony formation inhibition assay, colony forming abilities after treatment of SK-302B on colon cancer cells were lower than those of adriamycin (Table III). Some restrospective study indicated that the clonogenic assay could predict in vivo chemosensitivity with approximately 50% to 70% accuracy and in vivo resistance with greater than 85% accuracy (Von Hoff *et al.*, 1981). Therefore, effective inhibition in colony formation by SK-302B may be interpreted to have the possibility to be active *in vivo*. Actually, we proved *in vivo* efficacy by SK-302B, adopting *in vivo* mouse syngeneic tumor (P388, L1210, 3LL and B16) model (Kim and Koh, 1993; Koh *et al.*, 1994) In view of the

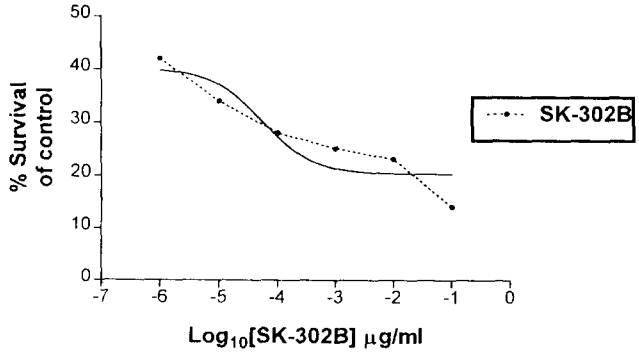

Fig. 1. Survival curve of DLD-1 cell line treated with SK-302B

Table I. 50% Inhibitory concentrations of SK-302B[a] on colon cancer cell lines measured by MTT assay

IC_{50} (μg/ml)[b]	Cell line	
	SK-302B	ADM
HCT-15	0.003	0.2
COLO-205	0.0002	0.1
DLD-1	0.00001 >	0.2
SW-480	0.00001 >	0.15

[a]Measured by MTT assay
[b]IC_{50} value which is defined as the concentration that caused 50% inhibition of cell growth

Table II. Clonogenicity of colon cancer cell lines by 24 well-assay

Cell line	% C.F.E[a]			
	1250 cells	2500 cells	5000 cells	10000 cells
HCT-15	30	67	156	356
COLO-205	47	81	142	269
DLD-1	N.D	N.D	131	325
SW-480	N.D	N.D	156	256

[a]Percent of colony forming efficiency
N.D : Not done

Table III. Percent colony formation of colon cancer cell lines after treatment with SK-302B

Cell line	% C.F.E[a]										
	SK-302B (μg/ml)					ADM (μg/ml)					Control
	1	0.1	0.01	0.001	0.0001	10	1	0.1	0.01	0.001	
HCT-15	0	0	0	12	70	0	2	67	-	-	100
COLO-205	0	7.2	34	78	-	0	5	34	67	-	100
DLD-1	0	10.8	49.4	80.1	-	1.4	18.5	60	78.5	-	100
SW-480	0	0	0	0	0.7	0	0	0	11.4	49.1	100

[a]Percent of colony forming efficiency
- : Same or over grown number as colonies of control plate

results so far obtained, SK-302B could be regarded to have the significant growth inhibition against colon cancer cells. Recently, echinomycin, chemically identical to SK-302B was reported to be comparably as effective as 5-FU on recurrent and metastatic colorectal cancer patients (Wadler *et al.*,1994). It could be analyzed that this report closely related to the data obtained from *in vitro* chemosensitivity tests. Now, we are conducting studies on *in vivo* efficacy in nude mice, structural modification and biochemical mechanism. Consequently, the advance to preclinical test by SK-302B would be necessary to establish clinical application.

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