ANTITUMOR ACTIVITY OF SPM VIII, A DERIVATIVE OF THE NUCLEOSIDE ANTIBIOTIC SPICAMYCIN, AGAINST HUMAN TUMOR XENOGRAFTS

MASARU KAMISHOHARA*,[a], HIROYUKI KAWAI[a], ATSUO ODAGAWA[a], TOSHIYUKI ISOE[a], JUN-ICHIRO MOCHIZUKI[a], TAKEŞHI UCHIDA[a], YOICHI HAYAKAWA[b], HARUO SETO[b], TAKASHI TSURUO[b] and NOBORU ŌTAKE[c]

[a]Pharmaceutical Research Laboratory, Kirin Brewery Co., Ltd., 3 Miyahara-cho, Takasaki-shi, Gunma 370-12, Japan
[b]Institute of Molecular and Cellular Biosciences, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113, Japan
[c]Department of Biosciences, Teikyo University, 1-1-1 Toyosatodai, Utsunomiya-shi, Tochigi 320, Japan

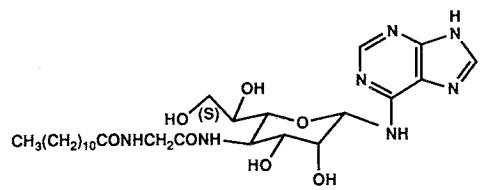
(Received for publication May 9, 1994)

The antitumor activity of spicamycin analogue SPM VIII against human stomach, breast, lung, colon and esophageal cancers was compared to that of mitomycin C (MMC) in the human tumor-nude mice xenograft model. Comparative studies of SPM VIII given iv at 6 mg/kg/day daily for 5 days and MMC given iv at 6.7 mg/kg on day 1 revealed that the antitumor spectrum of SPM VIII showed a different pattern from that of MMC and that SPM VIII caused tumor mass reductions in more tumors than did MMC in colon cancers (4/12 versus 1/11). In addition to this study, a comparative study of SPM VIII given iv at 12 mg/kg/day 8 times at 3- or 4-day intervals and 5'-deoxy-5-fluorouridine (5'-DFUR) given po at 185 mg/kg/day 5 days per week for 4 weeks showed that SPM VIII had the highest effect on SC-9 human stomach cancer and COL-1 human colon cancer among the 3 compounds, resulting in a significant reduction of tumor mass. Although other pharmacological studies are in progress, these results suggest that SPM VIII might be a novel antitumor compound effective for human cancers including cancer of the digestive organs.

Spicamycin is an antitumor antibiotic produced by *Streptomyces alanosinicus* 879-MT$_3$ and was obtained as a mixture with a variety of fatty acid moieties[1,2]. In the previous study, we reported that spicamycin showed antitumor effect on several human tumor xenografts[3]. The antitumor activity of semi-synthetic spicamycin analogues against human gastric cancer SC-9 was also examined and SPM VIII with a dodecanoic acid residue (Fig. 1) exhibited the highest antitumor activity among the tested spicamycin analogues. This activity was superior to that of mitomycin C (MMC) which is clinically used for many kinds of tumors[3]. We have thus pursued the antitumor activity of SPM VIII against other solid tumors with special emphasis on the spectrum of activity to determine its advantage over other drugs.

In this study we evaluated the antitumor activity of SPM VIII against nine stomach cancers, three breast cancers, three lung cancers, twelve colon cancers and one esophageal cancer by the human tumor-nude mice xenograft model which can predict the clinical efficiency of antitumor drugs[4~8] with MMC or 5'-deoxy-5-fluorouridine (5'-DFUR) as controls. The antitumor activities of these compounds were evaluated by their efficacy at reducing tumor volume in addition to activity on inhibition of tumor growth.

Fig. 1. Structure of SPM VIII.



Materials and Methods

Animals

Athymic female nude mice of 6- to 8-week-olds

(BALB/c nu/nu Slc) were obtained from Japan SLC, Inc., Shizuoka, Japan. Throughout the experiments, the mice were maintained in a laminar flow cabinet under specific-pathogen-free conditions. They were fed with irradiated basal CE-2 diet (CLEA Japan, Inc., Tokyo, Japan) and given water *ad libitum*.

Tumors

A total of 28 cancers including nine human stomach cancers, three human breast cancers, three human lung cancers, twelve human colon cancers and one human esophageal cancer were used as shown in Table 1. SC-2, SC-6, SC-7, SC-9, St-4, St-15, St-40, 4-1ST, NS-8, MX-1, MC-2, MC-8, LX-1, Lu-24, Lu-99, COL-1, COL-5, COL-8 and Co-3 were supplied by the Center Institute for Experimental Animals, Kanagawa, Japan. CH-5 and EH-10 were supplied by the Research Institute for Nuclear Medicine and Biology, Hiroshima University, Hiroshima, Japan. HT-29 and WiDr were supplied by the Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Tokyo, Japan. COLO205, LS-180, LS-174T, LoVo and DLD-1 were purchased from Dainippon Seiyaku Co., Ltd., Osaka, Japan. HT-29, WiDr, COLO205, LS-180, LS-174T, LoVo and DLD-1 were maintained *in vitro* in RPMI 1640 Medium (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) supplemented with 10% heat-inactivated fetal calf serum (Gibco Laboratories, Grand Island, NY, U.S.A.), 100 units/ml of penicillin and 100 units/ml of streptomycin (both from Meiji Seika Kaisha, Ltd., Tokyo, Japan). Other tumors were maintained by serial sc transplantations of about $2 \times 2 \times 2$ mm cubic fragments in the right subaxillary region of the nude mice.

Antitumor Agents

MMC was purchased from Kyowa Hakko Kogyo Co., Tokyo, Japan. 5'-DFUR was purchased from Nippon Roche Co., Ltd., Tokyo, Japan. SPM VIII semi-synthesized in our laboratory[3] was dissolved in DMSO and an equal volume of Cremophor EL (Sigma Chemical Co., St. Louis, Mo., U.S.A.) was added. A 0.85% NaCl solution was added to make the final concentrations of DMSO and Cremophor EL 1% each.

Chemotherapy

Cells of COLO205, LS-180, LS-174T, LoVo, HT-29, WiDr, or DLD-1 at 1×10^6, or about $2 \times 2 \times 2$ mm cubic fragments of SC-2, SC-6, SC-7, SC-9, St-4, St-15, St-40, 4-1ST, NS-8, MX-1, MC-2, MC-8, LX-1, Lu-24, Lu-99, COL-1, COL-5, COL-8, Co-3, CH-5 or EH-10 were inoculated sc in the right subaxillary region of the nude mice[9,10]. When the tumor volume reached $100 \sim 300\,mm^3$, the tumor bearing mice were randomly allocated to several experimental groups each consisting of five animals and chemotherapy was started.

SPM VIII was injected iv daily for five days at the dosage level of 6 mg/kg/day, and MMC was injected iv only on day 1 at 6.7 mg/kg in the comparative experiment of SPM VIII and MMC[9]. In the comparative experiment of SPM VIII and 5'-DFUR, SPM VIII was injected iv at 12 mg/kg/day 8 times at 3- or 4-day intervals and 5'-DFUR was given at 185 mg/kg/day by the po route daily for 5 days a week for 4 weeks[11].

Measurement of Tumor Size

The tumor size was measured and calculated as described in a previous report[3]. From the start of the injections, the tumor volume (V) was calculated once or twice a week for 3 weeks in the comparative experiment with SPM VIII and MMC, or for 6 weeks in the comparative experiment with SPM VIII and 5'-DFUR. The tumor growth inhibition rate (TGIR) was determined as $TGIR = (1 - T/C) \times 100$, where T is the mean of relative tumor volume in the treated mice and C is the mean of relative tumor volume in the control mice.

Evaluation

The antitumor activity was evaluated from two perspectives. An "Effective" rating in the TGIR was based on the maximum TGIR (%) of 50% or more showing statistical significance as determined by the Mann-Whitney U-test ($p < 0.05$, one sided) without any mouse death. An "Effective" rating in the ability to reduce the tumor mass (expressed in Tables 2 and 3 as "Remission") was based on the minimal relative tumor volume of less than 1 without any mouse death.

The response rate in the evaluation by TGIR was calculated as the number of tumors evaluated as

effective according to the criteria for TGIR/number of evaluated tumors. In the evaluation of tumor remission, the response rate was calculated as the number of tumors showing tumor mass remission/number of tumors evaluated. Tumor lines which showed TGIR of 50% or more with dead mice, or a reduction in tumor mass with dead mice, were omitted from the calculation of response rate.

Statistics

Statistical comparisons treated with and without antitumor agents were performed by the Mann-Whitney U-test. Others were performed by the Student t test.

Results

Human tumor lines and doubling time of the tumor mass in nude mice used in this study are listed in Table 1. The nine stomach cancers, three breast cancers, three lung cancers, twelve colon cancers and one esophageal cancer required 3.5 to 11.2 days, 4.2 to 10.0 days, 6.2 to 8.1 days, 4.2 to 11.5 days, and 5.8 days, respectively, to double their tumor mass.

Prior to the comparison study with MMC, we examined the optimal schedule for chemotherapy with SPM VIII. The result indicated that daily injection for 5 days was one of the most effective schedules for SPM VIII (data not shown), and the maximum tolerated dose (MTD) on this schedule was 6 mg/kg/day. On the other hand, another optimal schedule was examined for the comparison study with 5'-DFUR to adapt the span of chemotherapy to that of 5'-DFUR. As a result SPM VIII was injected twice a week for 4 weeks at an MTD of 12 mg/kg/day. MMC was injected by the optimal schedule described in the reference[9] and 5'-DFUR was injected according to the schedule reported by FUJITA *et al.*[11]. In both schedules, vehicle of SPM VIII did not show any effect on the tumor growth or any toxicity.

The effects of SPM VIII and MMC on human tumor xenografts are listed in Table 2. The antitumor activity of SPM VIII against SC-9, COL-1, COL-5, COL-8, LS-174T and EH-10 was superior to that of MMC. On the other hand, MMC had higher antitumor activity against SC-6, St-40, MX-1, LX-1, Lu-99, Co-3, LS-180 and WiDr than that of SPM VIII. Other than these tumors, SC-2, St-15, NS-8, MC-2, COLO205 were evaluated as sensitive to SPM VIII by TGIR and/or remission. SC-7, St-4, 4-1ST, MC-8, Lu-24, CH-5, LoVo, HT-29 and DLD-1 did not respond to SPM VIII and/or MMC. The response rates

Table 1. Profiles of human cancers used in this study.

Tumor lines	Origin	Tumor mass doubling time (day)	Reference	Tumor lines	Origin	Tumor mass doubling time (day)	Reference
SC-2	Stomach	3.5	18)	Lu-99	Lung	8.1	21)
SC-6	Stomach	7.3	18)	COL-1	Colon	7.7	
SC-7	Stomach	11.2	18)	COL-5	Colon	6.9	
SC-9	Stomach	4.9	18)	COL-8	Colon	8.0	
St-4	Stomach	6.2	18, 19)	Co-3	Colon	4.2	19)
St-15	Stomach	6.9	18)	CH-5	Colon	6.5	
St-40	Stomach	6.4	18, 19)	COLO205	Colon	6.5	22)
4-1ST	Stomach	10.2	18)	LS-180	Colon	5.4	23)
NS-8	Stomach	8.5	18)	LS-174T	Colon	5.0	23)
MX-1	Breast	5.4	20)	LoVo	Colon	5.8	24)
MC-2	Breast	4.2	20)	HT-29	Colon	7.3	25)
MC-8	Breast	10.0	20)	WiDr	Colon	8.5	26)
LX-1	Lung	6.2	21)	DLD-1	Colon	11.5	27)
Lu-24	Lung	8.1	21)	EH-10	Esophagus	5.8	

Table 2. Comparative study of the effects of SPM VIII and MMC on human tumor xenografts.

Tumor lines	SPM VIII, 6 mg/kg/day × 5			MMC, 6.7 mg/kg/day × 1		
	TGIR[a]	Remission[b]	No. of dead mice[c]	TGIR	Remission	No. of dead mice
SC-2	69*,[d]	−	−	N.D.[f]	N.D.	N.D.
SC-6	69**	+	−	93**	+	−
SC-7	23	−	−	(55**)	(+)	1/5
SC-9	91**	+	−	84**	+	−
St-4	27	−	2/5	12	−	−
St-15	62	+	−	N.D.	N.D.	N.D.
St-40	66**	+	−	96**	+	−
4-1ST	32	−	−	46**	−	−
NS-8	49**	+	−	68*	−	−
MX-1	63**	−	−	100**	+	−
MC-2	71**	+	−	(96**)	(+)	2/5
MC-8	32	−	1/5	N.D.	N.D.	N.D.
LX-1	38	−	−	91**	+	−
Lu-24	42**	−	−	N.D.	N.D.	N.D.
Lu-99	60*	+	−	84**	+	−
COL-1	99**	+	−	43	−	−
COL-5	66**	+	−	42**	−	−
COL-8	77**	+	−	53**	−	−
Co-3	26	−	1/5	67**	−	−
CH-5	15	−	3/5	35	−	−
COLO205	46	+	−	70	(+)	1/5
LS-180	55*	−	−	80**	−	−
LS-174T	74**	−	−	76	−	1/5
LoVo	22	−	−	32	−	−
HT-29	(50**)[e]	−	1/5	46	−	−
WiDr	55**	−	−	85**	+	−
DLD-1	19	−	−	45	−	−
EH-10	78**	−	−	1	−	−

Tumor cells or fragments were implanted sc into nude mice. When the tumor volume reached 100~300 mm^3, 6 mg/kg/day of SPM VIII was given iv daily for 5 days or 6.7 mg/kg of MMC was given iv on day 1. From the start of drug injection, the tumor volume was measured once or twice a week for several weeks.

[a] Maximum TGIR through the experimental period; * $p<0.05$ compare to control, ** $p<0.01$.

[b] +, Minimum mean relative tumor volume in the experimental period was less than 1; −, 1 or more.

[c] −, All mice survived through the experimental period; Fraction, Number of dead mice/number of treated mice.

[d] Underlined values were evaluated as "Effective" according to the criteria described in "Materials and Methods".

[e] Parenthesized data was negated because of toxicity.

[f] Not determined.

of all human tumor xenografts to SPM VIII were approximately equal to those of MMC (52% versus 50% by TGIR and 39% versus 33% by remission, Table 3). The response rates of colon cancer to SPM VIII, however, were significantly higher than those of MMC (55% versus 33% by TGIR, 33% versus 9% by remission, Table 3). On the other hand, SPM VIII did not show advantage over MMC in breast, lung and esophageal cancers in this study. The results of the comparative study with SPM VIII and 5'-DFUR are listed in Table 4. SPM VIII showed a strong effect on SC-9 and COL-1 resulting in a significant reduction of tumor masses. 5'-DFUR had no effect on these two tumors.

In the two comparative studies, SPM VIII showed the highest TGIR against SC-9 and COL-1 among the 3 drugs (Tables 2, 4). The growth curves of COL-1 treated with SPM VIII or MMC are shown in Fig. 2. SPM VIII significantly reduced the tumor volume from the beginning of chemotherapy, and the

Table 3. Response rates of human tumors to SPM VIII and MMC.

Tumor origin	Response rates			
	SPM VIII		MMC	
	TGIR[a]	Remission[b]	TGIR	Remission
Stomach	4/ 9 (44%)	5/ 9 (56%)	4/ 6 (67%)	3/ 6 (50%)
Breast	2/ 3 (67%)	1/ 3 (33%)	1/ 1 (100%)	1/ 1 (100%)
Lung	1/ 3 (33%)	1/ 3 (33%)	2/ 2 (100%)	2/ 2 (100%)
Colon	6/11 (55%)	4/12 (33%)	4/12 (33%)	1/11 (9%)
Esophagus	1/ 1 (100%)	0/ 1 (0%)	0/ 1 (0%)	0/ 1 (0%)
Total	14/27 (52%)	11/28 (39%)	11/22 (50%)	7/21 (33%)

The response rates were calculated as [a] the number of tumors evaluated as effective according to the criteria for TGIR/number of evaluated tumors, [b] the number of tumors showing tumor remission (indicated as "+" in Table 2)/number of tumors evaluated. In both cases, the evaluation as effective was negated by one or more mice deaths.

Table 4. Comparative study of effects of SPM VIII and 5'-DFUR on human tumor xenografts.

Tumor	SPM VIII, 12 mg/kg/day × 8			5'-DFUR, 185 mg/kg/day × 20		
	TGIR[a]	Remission[b]	No. of dead mice[c]	TGIR	Remission	No. of dead mice
SC-9	99**[,d]	+	−	< 50	−	−
COL-1	94**	+	−	23	−	−

Tumor fragments were implanted sc into nude mice. When the tumor volume reached 100~300 mm^3, 12 mg/kg/day of SPM VIII was given iv 8 times at 3- or 4-day intervals. 185 mg/kg/day of 5'-DFUR was given po 20 times (5 times/week). From the start of drug injection, the relative tumor volume was measured once or twice a week for 6 weeks.

[a] Maximum TGIR through the experimental period; ** $p < 0.01$ compared to control.

[b] +, Minimum mean relative tumor volume in the experimental period was less than 1; −, 1 or more.

[c] −, All mice survived through the experimental period.

[d] Underlined values were evaluated as "Effective" according to the criteria described in "Materials and Methods".

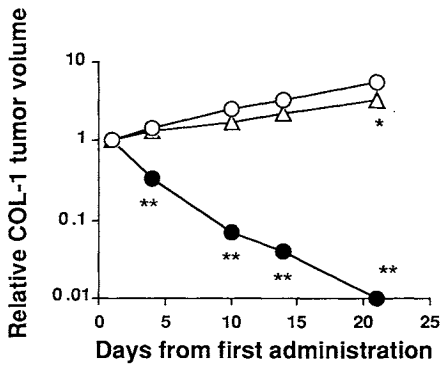
relative tumor volume reached approximately 1/100 of the start volume. In contrast, MMC had no effect on reduction of the tumor volume. In accordance with the regression of tumor, the body weight of mice treated with SPM VIII rapidly increased (Fig. 3).

Discussion

In this study we found that: 1) the antitumor spectrum of SPM VIII showed a different pattern from that of MMC, 2) the overall response rates of stomach, breast, lung, colon and esophageal cancers were approximately equal to those of MMC, 3) the response rates of colon cancers to SPM VIII evaluated by both TGIR and remission were higher than those of MMC and 4) SPM VIII had a strong effect especially on SC-9 and COL-1, being superior to those of MMC and 5'-DFUR. SPM VIII showed wide therapeutic indices in both tumors (8 in SC-9[3] and 4 in COL-1, data not shown). Among these findings, the antitumor activity of SPM VIII against colon cancers is notable because the response rates of colon cancers to antitumor drugs are low in the human tumor xenograft model[12] and also in clinical chemotherapy[13]. COL-1, which was most sensitive to SPM VIII among the tested cancers, is reported to be resistant to anticancer drugs including doxorubicin, cisplatin and fluorouracil[14]. Therefore, it is suggested that KRN5500 is different from other drugs in both mode of action and antitumor spectrum.

In the evaluation of antitumor effect, we emphasized the ability of tested compounds to reduce the tumor mass in addition to TGIR because the antitumor activities of drugs against solid tumors are usually

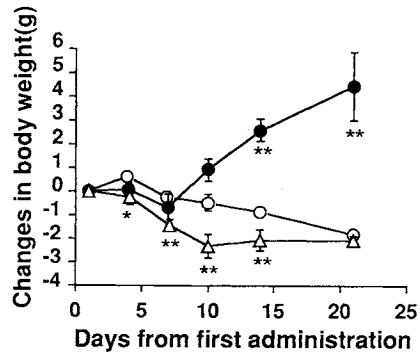
Fig. 2. Changes in relative tumor volume of COL-1 after chemotherapy by SPM VIII or MMC.



Chemotherapy was started when the tumor (COL-1) volume reached 100~300 mm^3 after about 3 weeks from the day of implantation. 6 mg/kg/day of SPM VIII was given iv from days 1~5, or 6.7 mg/kg of MMC was given iv on day 1. From the start of administration, the relative tumor volume was measured once or twice a week. Open circles, closed circles and open triangles indicate the means of five determinations of the untreated control group, SPM VIII treated group and MMC treated group, respectively.

*$p<0.05$, **$p<0.01$ compared to control group.

Fig. 3. Changes in body weight of COL-1 bearing nude mice after the start of chemotherapy by SPM VIII or MMC.



In the experiment in Fig. 2, each nude mouse was weighed once or twice a week. Open circles, closed circles and open triangles indicate the weight of the untreated control group, SPM VIII treated group and MMC treated group, respectively. Each point and vertical bars show the means of five mice weights without the tumor weight and standard error.

*$p<0.05$, **$p<0.01$ compared to control group.

evaluated by the degree of tumor reduction in the clinical stage. The ability of SPM VIII to reduce the tumor mass demonstrated in this study is a notable characteristic as a potential chemotherapeutic agent.

As structural similar compounds, septacidin and its analogues are reported. They have antitumor effects on murine adenocarcinoma CA 755 and P388 murine leukemia[15-17]. But therapeutic effects of them on human tumors have not been reported and they have not been in clinical use yet.

Although the study of the mode of action of SPM VIII is still in progress, SPM VIII, which has a unique structure and a marked antitumor activity against human tumor xenografts, might be a novel antitumor compound effective for human cancers including cancer of the digestive organs.

Acknowledgments

This study was supported in parts by a grant from the Ministry of Health and Welfare, Japan.

References

1) HAYAKAWA, Y.; M. NAKAGAWA, H. KAWAI, K. TANABE, H. NAKAYAMA, A. SHIMAZU, H. SETO & N. OTAKE: Studies on the differentiation inducers of myeloid leukemic cells. III. Spicamycin, a new inducer of differentiation of HL-60 human promyelocytic leukemia cells. J. Antibiotics 36: 934~937, 1983

2) HAYAKAWA, Y.; M. NAKAGAWA, H. KAWAI, K. TANABE, H. NAKAYAMA, A. SHIMAZU, H. SETO & N. OTAKE: Spicamycin, a new differentiation inducer of mouse myeloid leukemia cells (M1) and human promyelocytic leukemia cells (HL-60). Agric. Biol. Chem. 49: 2685~2691, 1985

3) KAMISHOHARA, M.; H. KAWAI, A. ODAGAWA, T. ISOE, J. MOCHIZUKI, T. UCHIDA, Y. HAYAKAWA, H. SETO, T. TSURUO & N. OTAKE: Structure-antitumor activity relationship of semi-synthetic spicamycin analogues. J. antibiotics 46: 1439~1446, 1993

4) BERGER, D. P.; H. H. FIEBIG, B. R. WINTERHALTER, E. WALLBRECHER & H. HENSS: Preclinical phase II study of Ifosfamide in human tumour xenografts *in vivo*. Cancer Chemother. Pharmacol. 26: S7~S11, 1990

5) FUJITA, M.; F. FUJITA, K. SHIMOZUMA, Y. SAKAMOTO, T. KUSUYAMA, M. USUGANE, H. ORIGASA & T. TAGUCHI: Application to chemosensitivity test of human tumor-nude mouse system and subrenal capsule assay system. Jpn.

J. Cancer Chemother. 13: 1227~1234, 1986
6) INOUE, K.; S. FUJIMOTO & M. OGAWA: Correlation between antitumor effects against human mammary carcinoma (MX-1) in nude mice and clinical efficacy of human breast cancer in thirty-four antitumor agents. J. Jpn. Soc. Cancer Ther. 18: 13~20, 1983
7) NOSO, Y.; K. NIIMI, M. NISHIYAMA, N. HIRABAYASHI, T. TOGE, M. NIIMOTO & T. HATTORI: Clinical studies on a new screening assay for anticancer agents using nude mice and isotopic evaluation. Cancer Res. 47: 6418~6422, 1987
8) FIEBIG, H. H.: Comparison of tumor response in nude mice and in patients: *In* Human Tumour Xenografts in Anticancer Drug Development. *Ed.*, B. WINOGRAD *et al.*, pp. 25~30, Springer-Verlag, Berlin, Germany, 1988
9) INABA, M.; T. TASHIRO, T. KOBAYASHI, S. FUJIMOTO, Y. SAKURAI, K. MARUO, Y. OHNISHI, Y. UEYAMA & T. NOMURA: Evaluation of response rates to various antitumor agents of human gastric tumors implanted in nude mouse. Jpn. J. Cancer Res. (Gann) 77: 190~196, 1986
10) GIOVANELLA, B. C.; J. S. STEHLIN & L. J. WILLIAMS: Heterotransplantation of human malignant tumors in "nude" thymusless mice. II. Malignant tumors induced by injection of cell cultures derived from human solid tumors. J. Nat. Cancer Inst. 52: 921~930, 1974
11) FUJITA, F.; M. FUJITA & T. TAGUCHI: Effects of 5'-deoxy-5-fluorouridine on human gastrointestinal and breast cancers xenografted to nude mice. Jpn. J. Cancer Chemother. 11: 1635~1643, 1984
12) KUBOTA, T.; K. ISHIBIKI & O. ABE: The clinical usefulness of human xenografts in nude mice: *In* Prediction of Response to Cancer Therapy. *Ed.*, T. C. HALL, pp. 213~225, Alan R. Liss Inc., New York, 1988
13) TOMINAGA, T.: Chemotherapy for advanced and recurrent colorectal cancer. Jpn. J. Cancer Chemother. 13: 2298~2306, 1986
14) NOMURA, T.; Y. SAKURAI & M. INABA: Nude mice and evaluations of anticancer drugs. pp. 86~90, Kanishobo, Tokyo, 1991
15) DUTCHER, J. D.; M. H. VON SALTZA & F. E. PANSY: Septacidin, a new antitumor and antifungal antibiotic produced by *Streptomyces fimbriatus*. Antimicrob. Agents Chemother. 1963: 83~88, 1964
16) ASZALOS, A.; P. LEMANSKI, B. BERK & J. D. DUTCHER: Septacidin analogues. Antimicrob. Agents Chemother. 1965: 845~849, 1966
17) ACTON, E. M.; K. J. RYAN & A. E. LUETZOW: Antitumor septacidin analogues. J. Med. Chem. 20: 1362~1371, 1977
18) INABA, M.; T. TASHIRO, T. KOBAYASHI, Y. SAKURAI, K. MARUO, Y. OHNISHI, Y. UEYAMA & T. NOMURA: Responsiveness of human gastric tumors implanted in nude mice to clinically equivalent doses of various antitumor agents. Jpn. J. Cancer Res. 79: 517~522, 1988
19) KUBOTA, T.; M. NAKADA, K. TSUYUKI, T. INABA, F. ASANUMA, K. ISHIBIKI & O. ABE: Cell kinetics and chemosensitivity of human carcinomas serially transplanted into nude mice. Jpn. J. Cancer Res. 77: 502~507, 1986
20) INABA, M.: T. KOBAYASHI, T. TASHIRO, Y. SAKURAI, K. MARUO, Y. OHNISHI, Y. UEYAMA & T. NOMURA: Evaluation of antiumor activity in a human beast tumor/nude mouse model with a special emphasis on treatment dose. Cancer 64: 1577~1582, 1989
21) TASHIRO, T.; M. INABA, T. KOBAYASHI, Y. SAKURAI, K. MARUO, Y. OHNISHI, Y. UEYAMA & T. NOMURA: Responsiveness of human lung cancer/nude mouse to antitumor agents in a model using clinically equivalent doses. Cancer Chemother. Pharmacol. 24: 187~192, 1989
22) SEMPLE, T. U.; L. A. QUINN, L. K. WOODS & G. E. MOORE: Tumor and lymphoid cell lines from a patient with carcinoma of the colon for a cytotoxicity model. Cancer Res. 38: 1345~1355, 1978
23) TOM, B. H.; L. P. RUTZKY, M. M. JAKSTYS, R. OYASU, C. KAYE & B. KAHAN: Human colonic adenocarcinoma cells. I. Establishment and description of a new line. In Vitro 12: 180~191, 1976
24) DREWINKO, B.; M. M. ROMSDAHL, L. Y. YANG, M. J. AHEARN & J. M. TRUJILLO: Establishment of a human carcinoembryonic antigen-producing colon adenocarcinoma cell line. Cancer Res. 36: 467~475, 1976
25) FOGH, J. & G. TREMPE: New human tumor cell lines. *In* Human Tumor Cells *In Vitro*. *Ed.*, J. FOGH, pp. 115~141, Plenum Press, New York, N.Y., 1975
26) NOGUCHI, P.; R. WALLAGE, J. JOHNSON, E. M. EARLEY, S. O'BRIEN, S. FERRONE, M. A. PELLEGRINO, J. MILSTIEN, C. NEEDY, W. BROWNE & J. PETRICCIANI: Characterization of WiDr: a human colon carcinoma cell line. In Vitro 401~408: 1979
27) TIBBETTS, L. M.; M. Y. CHU, J. C. HAGER, D. L. DEXTER & P. GALABRESI: Chemotherapy of cell-line-derived human colon carcinomas in mice immunosuppressed with antithymocyte serum. Cancer 40: 2651~2659, 1977