ANTITUMOR SPECTRUM OF DEOXYSPERGUALIN AND ITS LACK OF CROSS-RESISTANCE TO OTHER ANTITUMOR AGENTS

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Antitumor activities of 15-deoxyspergualin (NKT-01), an analogue of spergualin (SGL), were examined in cultured tumor cells, transplantable murine tumors, and human tumor xenografts in nude mice. NKT-01 exhibited strong antitumor activity specifically against leukemias both *in vitro* and *in vivo*. Moreover, it also showed activity against AH66F hepatoma, M5076 fibrosarcoma and MH134 hepatoma. However, antitumor activity of NKT-01 against other non-leukemic tumors was marginal. Effective dose range of NKT-01 in sensitive tumors was so wide that the largest chemotherapeutic indexes were produced by NKT-01 in P388 and L1210 leukemias among 15 antitumor agents examined.

The efficacy of NKT-01 against doxorubicin- and cytosine arabinoside-resistant P388 leukemias was comparable to that against parental sensitive P388 leukemia. NKT-01 also retained activity against other P388 leukemia sublines resistant to cisplatin, 5-fluorouracil or nimustine, although the effect was slightly decreased. In addition, in the *in vitro* and *in vivo* experiments using NKT-01-resistant P388 and SGL-resistant L1210(IMC) leukemias, no cross-resistance was observed. Moreover, collateral sensitivity was observed especially to alkylating agents in animal study.

Spergualin (SGL) is an antitumor antibiotic produced by a strain of *Bacillus laterosporus*.[1] It has strong antitumor activity against transplantable tumors as reported previously.[2] Following determination of the structure of SGL,[3] synthesis of SGL[4] and its derivatives were performed.[5~8] Through these studies structure-activity relationships were revealed and 15-deoxyspergualin (NKT-01) was selected for its strongest antitumor activity against L1210 leukemia *in vivo*. In addition to the antitumor activity, NKT-01 was also shown to have an immunosuppressive activity in both *in vitro* and *in vivo* systems.[9,10] The clinical studies of NKT-01 as an antitumor drug and an immunosuppressant have been conducting.

In this paper, we report the details of the antitumor activity of NKT-01 against various murine and human tumor cells *in vitro* and *in vivo*. Moreover, to know the uniqueness of the action of the drug, we assess whether NKT-01 shows cross-resistance to other antitumor agents by using 5 strains of P388 leukemia cells resistant to other antitumor agents and 2 resistatnt tumor cells to SGLs.

Materials and Methods

Drugs

SGL and NKT-01 were prepared by Takara Shuzo Co., Ltd. (Ohtsu, Japan), and dissolved in physiological saline and stored in dark at 4°C before use. Other antitumor drugs were purchased from commercial sources.

Animals

DBA/2, C57BL/6 (Charles River Japan Corp., Atsugi, Japan), CDF_1, BDF_1, ICR (Shizuoka Laboratory Animal Center, Hamamatsu, Japan), C3H/He and BALB/c-nu/nu mice (Clea Japan Inc., Tokyo, Japan) and Donryu rats (Nippon Rat Co., Urawa, Japan) were used in experiments at an age of 6 to 7 weeks. Each treatment group was composed of at least 6 animals.

Tumors

We used 13 murine and 11 human tumor cell lines to examine the antitumor spectrum of NKT-01 *in vitro* and *in vivo*. Two strains of L1210 were used; one (L1210) was supplied by the Cancer Chemotherapy Center (Tokyo, Japan), which has been maintained in DBA/2 mice, and the other is its substrain, L1210 (IMC), maintained in CDF_1 mice at Institute of Microbial Chemistry (Tokyo, Japan).

P388 Leukemia resistant to NKT-01 (P388/NKT-01) and L1210(IMC) leukemia resistant to SGL (L1210(IMC)/SGL) were developed by the repetition of transplantations and daily treatment with 25.6 mg/kg/day of NKT-01 and 50 mg/kg/day of SGL *in vivo*, respectively. Other resistant P388 leukemia cells (to nimustine (ACNU), cisplatin (CDDP), doxorubicin (ADM), 5-fluorouracil (5-FU), and cytosine arabinoside (Ara-C)) were also established by serial *in vivo* treatment with subtoxic dose of each drug.

Antitumor Experiments *In Vitro*

To compare the growth inhibitory activity of SGL and NKT-01, we used 14 kinds of tumors shown in Table 1, among which 3 mouse leukemia cells and 2 rat hepatoma cells were primary cultured and grown in suspension. The other tumor cell lines were maintained *in vitro* as monolayer culture.

All cells were cultured in either RPMI-1640 (Gibco Labs., Chagrin Falls, U.S.) or MEM medium (Flow Labs., McLean, U.S.) supplemented with 10% of human serum (Flow Labs.) and 60 μg/ml of kanamycin. Three mouse leukemia cell lines require addition of 5 μM of 2-mercaptoethanol, and MOLT-3, NALM-6 and Daudi cells were cultured in the presence of 1% of nonessential amino acid solution (Flow Labs.).

Growth rate was calculated from the cell number counted by Coulter Counter model ZBI before and after the exposure to drugs for a predetermined period. The growth inhibitory effect was assessed by comparing the growth rate of drug-treated cells and that of control, and then IC_{50} values were determined.

Table 1. Growth inhibitory effect of NKT-01 and SGL against cultured tumor cells.

Tumor	Origin	Incubation period (day)	IC_{50} (μg/ml)[a]		Ratio[b]
			NKT-01	SGL	
Mouse tumors:					
P388	Lymphocytic leukemia	3	0.0090	0.11	12
L1210	Lymphoid leukemia	3	0.0040	0.056	14
L1210(IMC)	Lymphoid leukemia	3	0.013	0.21	16
Lewis	Lung carcinoma	3	0.42	*ca.* 1	2.4
Rat tumors:					
AH66	Hepatoma	2	>100	>100	—
AH66F	Hepatoma	2	>100	>100	—
Human tumors:					
MOLT-3	Lymphatic leukemia	4	>100	>100	—
NALM-6	Lymphatic leukemia	4	>100	>100	—
K-562	Chronic myelocytic leukemia	4	0.66	5.4	8.2
J-111	Monocytic leukemia	5	0.24	2.5	10
U-937	Histiocytic lymphoma	4	0.34	6.3	19
Daudi	Burkitt lymphoma	5	5.0	24	4.8
HeLa S_3	Cervical tumor	3	32	40	1.3
KB	Nasopharynx tumor	3	0.11	0.36	3.3

[a] IC_{50}: Drug concentration required for 50% inhibition of cell growth.
[b] Ratio of IC_{50} of SGL to IC_{50} of NKT-01.

Table 2. Life-prolonging activity of NKT-01 against murine transplantable tumors.

Tumor	Origin	Host	Inoculum		Max T/C[a] (%)	Optimal dose (mg/kg/day)	Min ED[b] (mg/kg/day)	Index[c]
			Site	Size				
Leukemias:								
P388	Lymphocytic leukemia	CDF_1 ♀	ip	10^6	200	25.6	0.46	67
			sc		173	25.6	5.5	5.1
			iv		200	25.0	1.5	20
L1210	Lymphoid leukemia	CDF_1 ♀	ip	10^5	241	25.6	0.27	126
			sc		210	12.8	0.66	47
L1210(IMC)	Lymphoid leukemia	CDF_1 ♀	ip	10^5	>639(4/6)[d]	1.6	0.20	120
			sc		>604(4/6)	12.8	0.81	35
C1498	Myeloid leukemia	C57BL/6 ♂	ip	10^6	254	25.6	0.14	240
P815	Mastocytoma	DBA/2 ♀	ip	10^6	202	25.6	1.4	22
Non-leukemic tumors:								
M5076	Fibrosarcoma	C57BL/6 ♀	ip	10^6	165	12.8	6.9	3.9
			sc		158	25.6	20	1.4
B16	Melanoma	BDF_1 ♂	ip	5×10^5	102	25.6	—	—
Lewis	Lung carcinoma	BDF_1 ♂	ip	10^6	149	25.6	—	—
			iv	10^5	116	25.6	—	—
C26	Colon adenocarcinoma	CDF_1 ♀	iv	10^5	113	25.6	—	—
Ehrlich	Mammary carcinoma	ICR ♂	ip	10^6	108	6.4	—	—
MH134	Hepatoma	C3H/He ♂	ip	10^6	139	25.6	—	—
			sc		217	12.8	8.5	3.4
AH66	Hepatoma	Donryu ♀	ip	10^6	134	6.4	—	—
AH66F	Hepatoma	Donryu ♀	ip	10^6	>750(6/6)	0.2	0.005	780

[a] Max T/C: Maximum value of T/C (%).
[b] Min ED: Minimum effective dose producing 150% of T/C.
[c] Index: Ratio of maximum- to minimum-dose producing T/C over 150%.
[d] Number in parentheses shows number of survivors 60 days after inoculation.

Antitumor Experiments using Transplantable Murine Tumors

In order to obtain antitumor spectrum of NKT-01 against transplantable tumors *in vivo*, we employed 13 tumors maintained by serial transplantation in ascitic or solid form in our laboratories.

Experimental host animals, inoculation sites and cell numbers used were summarized in Table 2. NKT-01 was injected ip daily for 9 days from the day after the inoculation. For other clinically using drugs, either single injection or 5 day administration schedule was chosen according to administration schedule dependency of each drug. Life-prolonging activity was expressed by T/C obtained by comparing median or mean survival time of animals in drug-treated group (T) and that in control one (C).

Antitumor Experiments using Human Tumor Xenografts

Four human leukemic tumors and MX-1 human mammary tumor were implanted sc with a trocar into athymic nude mice. NKT-01 was administered daily for 9 days after tumor volume reached about 300 mm^3. Tumor volume (V) was calculated as follows:

$$V = L \times W^2/2,$$

where L and W are length and width of tumor mass, respectively.

Results

Antitumor Effect of NKT-01 and SGL against Cultured Cells

The growth inhibitory effect of SGL and NKT-01 against 14 tumor cells is shown in Table 1 in terms of IC_{50} values. The growth inhibition curves of NKT-01 is presented in Figs. 1(A) and 1(B). Great difference in sensitivity of tumor cells was observed to NKT-01 as well as to SGL. The IC_{50} values of NKT-01 for mouse P388, L1210 and L1210(IMC) leukemia cells were around 0.01 μg/ml. In contrast, against rat AH66 and AH66F hepatomas, human MOLT-3 and NALM-6 leukemias, NKT-01 inhibited only about 20% of the growth even at the highest concentration of 100 μg/ml.

Although antitumor spectra of NKT-01 and SGL against these cultured cells were similar each other, NKT-01 was more potent than SGL in all tumors, the ratio of IC_{50} of SGL to NKT-01 being about ten in average.

As shown in Figs. 1(A) and 1(B), the growth inhibitory effect of NKT-01 was dependent very slightly on drug concentration and it tended to reach its plateau even at higher concentrations. The saturated level of the inhibitory effect in each tumor cells was parallel to IC_{50} value in general.

Antitumor Effect against Transplantable Murine Tumors

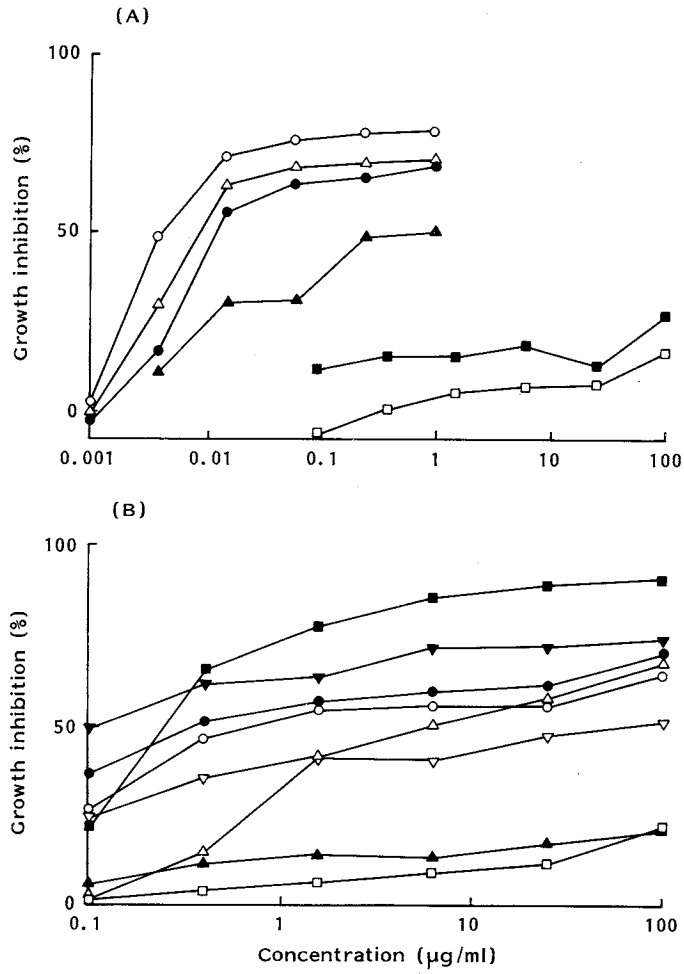
The life-prolonging effect of NKT-01 against 13 transplantable murine tumors are summarized in Table 2. NKT-01 showed strong antitumor activity against all the 5 leukemias tested (P388, L1210, L1210 (IMC), C1498 and P815) irrespective of the site of implantation. Among other non-leukemic tumors, NKT-01 exhibited potent activity against AH66F hepatoma, while its activity against M5076 fibrosarcoma, Lewis lung carcinoma and MH134 hepatoma was marginal, and it was ineffective against rest of the tumors.

The activities of NKT-01 against L1210(IMC) leukemia and AH66F hepatoma were extremely strong, and almost all animals survived at the optimal doses, which were rather lower as compared with its toxic dose. In other responsive tumors, T/C (%) values were around 200% and no long term survivors was obtained even at the optimal dose, which was just below the toxic dose.

As indicated by the chemotherapeutic index in Table 2, the effective dose ranges of NKT-01 for sensitive leukemias and AH66F hepatoma were very wide. The effectiveness of NKT-01 was compared with various types of clinically useful antitumor agents in P388 and L1210 leukemias, which are widely

Fig. 1. Growth inhibitory effect of NKT-01 against cultured murine (A) and human (B) tumor cell lines.

(A): ○ L1210 lymphoid leukemia, △ P388 lymphocytic leukemia, ● L1210(IMC) lymphoid leukemia, ▲ Lewis lung carcinoma, ■ AH66F hepatoma, □ AH66 hepatoma. (B): ■ J-111 monocytic leukemia, ▼ KB nasopharynx tumor, ● U-937 histiocytic lymphoma, ○ K-562 chronic myelocytic leukemia, ▽ HeLa S_3 cervical tumor, △ Daudi Burkitt lymphoma, ▲ MOLT-3 lymphatic leukemia, □ NALM-6 lymphatic leukemia.



used in evaluation of drug activity for their generally high sensitivity (Table 3). NKT-01 produced the largest chemotherapeutic indexes, estimated from the dose range over 150% of T/C, although its maximal T/C's were not remarkable. NKT-01 showed activity in 3-fold wider range than SGL did in these leukemias, similarly to other tumor systems (data not shown).

Antitumor Effect against Human Tumor Xenografts

The changes in relative tumor volume of 5 human tumor xenografts implanted sc into nude mice following 9 day treatment with NKT-01 were shown in Fig. 2. High dose of NKT-01 extremely inhibited the growth of J-111 monocytic leukemia, of which tumor volume remained the same as the initial volume over 30 days, even though treatment had been completed in 9 days. Although high dose of NKT-01 also showed inhibitory activity against other 3 leukemias and MX-1 mammary tumor, no significant effect was

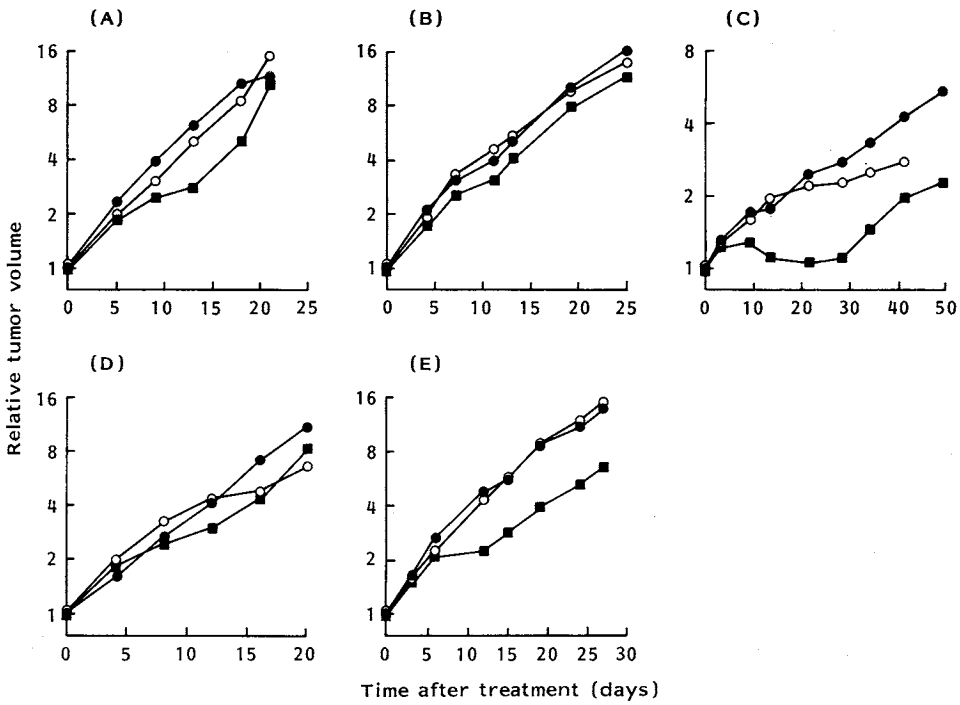
Table 3. Comparison of antitumor activity of NKT-01, SGL and clinically useful antitumor drugs against P388 and L1210 leukemias.

Drug	P388		L1210	
	Max T/C	Index[a]	Max T/C	Index
NKT-01	200	67	241	126
SGL	183	26	222	45
Cyclophosphamide	254	24	277	5.1
Carboquone	169	3.0	325	3.4
ACNU	255	11	325	15
CDDP	285	6.1	216	4.5
ADM	254	5.3	227	8.6
Aclarubicin	206	3.6	169	2.6
MMC	159	1.6	213	4.7
Peplomycin	121	0	133	0
5-FU	182	3.9	200	10
Ara-C	208	22	175	5.6
Methotrexate	182	4.4	192	*ca.* 10
VLB	195	6.0	n.t.	
Etoposide	225	35	361	36

[a] Index: Ratio of maximal- to minimal-dose producing T/C over 150%.
n.t.: Not tested.

Fig. 2. Changes in relative tumor volume of human tumor xenografts in nude mice after the start of treatment with NKT-01.

(A) HL-60 leukemia, (B) MB-2 myelocytic leukemia, (C) J-111 monocytic leukemia, (D) U-937 histiocytic lymphoma, (E) MX-1 mammary carcinoma. Fragment of human tumor was inoculated sc, and NKT-01 was administered daily for 9 days at the doses of (■) 25.6 mg/kg/day and (●) 6.4 mg/kg/day. ○: control.



observed at the lower dose.

Antitumor Activity of NKT-01 against P388 Leukemias Resistant to Other Antitumor Drugs

To know whether NKT-01 shows cross-resistance to other antitumor drugs, chemotherapeutic activities of NKT-01 against 5 resistant variants of P388 leukemia were compared with that against parental P388. As shown in Fig. 3, no difference in efficacy was observed against the sensitive parental cell line and its

Fig. 3. Antitumor activity of NKT-01 against parental and five drug-resistant P388 leukemias *in vivo*.

● P388, □ P388/Ara-C, △ P388/ADM, ■P388/CDDP, ▲ P388/5-FU, ○ P388/ACNU.

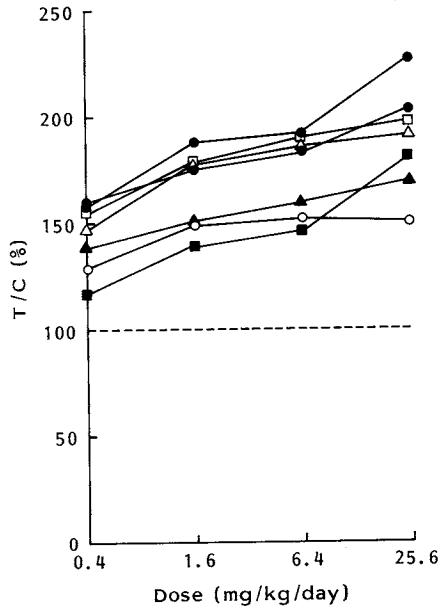


Table 4. Sensitivity of parental and NKT-01-resistant P388 leukemia cells to various antitumor drugs *in vitro*.

Drug	IC_{50} (μg/ml)[a]		Ratio[b]
	P388	P388/NKT-01	
NKT-01	0.027	4.9	180
SGL	0.25	>10	>40
L-PAM	0.14	0.035	0.25
ACNU	1.3	0.80	0.62
CDDP	0.028	0.012	0.43
ADM	0.0054	0.0062	1.1
MMC	0.012	0.0048	0.40
PEP	2.2	1.6	0.73
5-FU	0.054	0.043	0.80
Ara-C	0.0015	0.0012	0.80
MTX	0.0017	0.0018	1.1
VCR	0.0018	0.0016	0.89
Etoposide	0.036	0.032	0.89

Cells were treated with each drug for 3 days.

[a] IC_{50}: Drug concentration inhibiting 50% of cell growth.

[b] Ratio of IC_{50} in P388/NKT-01 to IC_{50} in parental P388.

Table 5. Sensitivity of parental and resistant P388 and L1210(IMC) leukemias to various antitumor drugs *in vivo*.

Drug	Dose schedule (mg/kg/day)	T/C (%)		T/C (%)	
		P388	P388/NKT-01	L1210(IMC)	L1210(IMC)/SGL
NKT-01	25.6×9	202	105	367 (2/5)[a]	170
CYC	50.0×1	207 (1/5)	554 (3/5)	134	154
ACNU	7.5×1	177	180	162	194
CDDP	1.5×5	207	594 (4/5)	169	214
ADM	5.0×1	197	233 (1/5)	149	406 (1/5)
MMC	6.0×1	187	556 (5/5)	163	170
5-FU	20.0×5	190	181	241	258
Ara-C	200.0×5	217	204	235	222 (1/5)
MTX	1.3×5	167	158	214	439 (1/5)
VCR	0.5×5	217	282	201	128
ET	2.0×5	187	193	383	664 (3/5)

[a] Number in parentheses shows number of survivors 60 days after inoculation.

ADM- or Ara-C-resistant sublines. Although the T/C (%) values obtained in P388/CDDP, P388/5-FU and P388/ACNU were lower than that obtained in sensitive P388 leukemia, NKT-01 retained the activity with maximal T/C (%) of 150% to 180%.

Sensitivity of NKT-01-resistant P388 and SGL-resistant L1210(IMC) Leukemias to Other Antitumor Drugs

To confirm the lack of cross-resistance of NKT-01 to other drugs, NKT-01-resistant P388 leukemia was established and sensitivity to various antitumor drugs was determined *in vitro*. As shown in Table 4, the resistant cell line was 180-fold resistant to NKT-01 and also resistant to SGL. No cross-resistance to other types of drugs was seen at all. In addition, P388/NKT-01 showed slight collateral sensitivity to alkylating agents such as melphalan (L-PAM), ACNU, CDDP and mitomycin C (MMC).

Furthermore, *in vivo* sensitivities of P388/NKT-01 and L1210(IMC)/SGL leukemias to various antitumor drugs were examined. As shown in Table 5, activities of drugs against resistant tumors were not inferior to those against sensitive tumors except that vincristine (VCR) showed cross-resistance to NKT-01 in L1210(IMC) leukemia (but not in P388/NKT-01). Moreover, although 5-FU and Ara-C showed the same activities in both pairs of tumors, other drugs including VCR exhibited higher activity against both or one of the two resistant tumors than each parental counterpart.

Discussion

In the present studies, NKT-01 exhibited antitumor activity against leukemias both *in vitro* and *in vivo* rather than against non-leukemic tumors. Especially, in the experiments using transplantable tumors, NKT-01 showed strong activity against all the 5 leukemias tested. These results suggested that NKT-01 is active against leukemic tumors. This results was supported by the reported antileukemic activities of NKT-01 to the growth of *N*-butyl-*N*-nitrosourea-induced rat leukemias.[11]

The effective dose range of NKT-01 in sensitive tumors was the widest among representative antitumor agents used in the clinic. It means that NKT-01 has a good selective toxicity and that its activity does not strongly depend on injected dose intensity. This was correspondent with the slight dependency of the growth inhibitory activity on the concentration observed *in vitro* experiments. We used human serum in these *in vitro* experiments in place of usually used bovine serum, because NKT-01 is known to be oxidized by amine oxidase present in bovine serum at high concentration.[12] Although thus oxidized molecules show cytocidal activity in a strongly concentration-dependent manner, KURAMOCHI *et al.* suggested this additional activity is not responsible for the activity observed *in vivo*.[13] Our results concerning the weak dependency on concentration observed in both *in vitro* and *in vivo* systems supported their conclusion. In addition, by using human serum in the *in vitro* studies, good correlation of sensitivity of tumors to NKT-01 was obtained between *in vitro* cell culture and *in vivo* animal model; 3 mouse leukemias showed higher sensitivity than Lewis lung carcinoma, and J-111 showed higher sensitivity than U-937 both *in vitro* and *in vivo* experiments.

However, NKT-01 showed remarkable activity against L1210(IMC) leukemia and AH66F hepatoma *in vivo* especially at lower doses, although L1210(IMC) was less sensitive *in vitro* compared with L1210 and P388 leukemias, and AH66F was insensitive at all. This seemed to be due to host-mediated immunological effect induced by the lower doses of NKT-01. UMEZAWA *et al.* demonstrated that low dose of SGL could induce specific cytotoxic T-lymphocytes (CTL) to L1210(IMC) cells, and that the induced CTL reject the second inoculation of L1210(IMC) up to 10^6 cells in SGL-cured mice.[14,15] We obtained similar results in NKT-01-treated mice cured from L1210(IMC) as well. The rats cured from AH66F by treatment with NKT-01 also rejected the second inoculation of 10^7 of AH66F hepatoma cells (data not shown). Induction of specific CTL to L1210(IMC) or AH66F cells by NKT-01 might result in the high sensitivity to NKT-01 observed *in vivo*.

NKT-01 was shown to be lacking of cross-resistance to other antitumor agents by using 5 substrains of P388 leukemias resistant to ACNU, CDDP, ADM, 5-FU and Ara-C. Essentially consistent results were

reported by HARRISON *et al.*[16] We further carried out the experiments using P388/NKT-01 and L1210 (IMC)/SGL both *in vitro* and *in vivo* and confirmed the lack of cross-resistance. From these results, it is likely that NKT-01 exhibits its efficacy by different mechanism(s) from other antitumor drugs examined in this study. Furthermore, collateral sensitivity to NKT-01 was observed in these resistant cells especially *in vivo*. Therefore, NKT-01 could be expected to be useful in the second or salvage therapy for some recurrent tumors as well as in primary therapy for leukemias.

References

1) TAKEUCHI, T.; H. IINUMA, S. KUNIMOTO, T. MASUDA, M. ISHIZUKA, M. TAKEUCHI, M. HAMADA, H. NAGANAWA, S. KONDO & H. UMEZAWA: A new antitumor antibiotic, spergualin: Isolation and antitumor activity. J. Antibiotics 34: 1619~1621, 1981
2) NISHIKAWA, K.; C. SHIBASAKI, K. TAKAHASHI, T. NAKAMURA, T. TAKEUCHI & H. UMEZAWA: Antitumor activity of spergualin, a novel antitumor antibiotic. J. Antibiotics 39: 1461~1466, 1986
3) UMEZAWA, H.; S. KONDO, H. IINUMA, S. KUNIMOTO, Y. IKEDA, H. IWASAWA, D. IKEDA & T. TAKEUCHI: Structure of an antitumor antibiotic, spergualin. J. Antibiotics 34: 1622~1624, 1981
4) KONDO, S.; H. IWASAWA, D. IKEDA, Y. UMEDA, Y. IKEDA, H. IINUMA & H. UMEZAWA: The total synthesis of spergualin, an antitumor antibiotic. J. Antibiotics 34: 1625~1627, 1981
5) IWASAWA, H.; S. KONDO, D. IKEDA, T. TAKEUCHI & H. UMEZAWA: Synthesis of (−)-15-deoxyspergualin and (−)-spergualin-15-phosphate. J. Antibiotics 35: 1665~1669, 1982
6) UMEDA, Y.; M. MORIGUCHI, H. KURODA, T. NAKAMURA, H. IINUMA, T. TAKEUCHI & H. UMEZAWA: Synthesis and antitumor activity of spergualin analogues. I. Chemical modification of 7-guanidino-3-hydroxyacyl moiety. J. Antibiotics 38: 886~898, 1985
7) UMEDA, Y.; M. MORIGUCHI, H. KURODA, T. NAKAMURA, A. FUJII, H. IINUMA, T. TAKEUCHI & H. UMEZAWA: Synthesis and antitumor activity of spergualin analogues. II. Chemical modification of the spermidine moiety. J. Antibiotics 40: 1303~1315, 1987
8) UMEDA, Y.; M. MORIGUCHI, K. IKAI, H. KURODA, T. NAKAMURA, A. FUJII, T. TAKEUCHI & H. UMEZAWA: Synthesis and antitumor activity of spergualin analogues. III. Novel method for synthesis of optically active 15-deoxyspergualin and 15-deoxy-11-*O*-methylspergualin. J. Antibiotics 40: 1316~1324, 1987
9) NEMOTO, K.; M. HAYASHI, F. ABE, T. NAKAMURA, M. ISHIZUKA & H. UMEZAWA: Immunosuppressive activities of 15-deoxyspergualin in animals. J. Antibiotics 40: 561~562, 1987
10) SUZUKI, S.; M. KANASHIRO & H. AMEMIYA: Effect of a new immunosuppressant, 15-deoxyspergualin, on heterotopic rat heart transplantation, in comparison with cyclosporine. Transplantation 44: 483~487, 1987
11) ITO, J.; T. YAMASHITA, K. TAKAHASHI, H. HORINISHI, T. NAKAMURA, T. TAKEUCHI & H. UMEZAWA: Anti-leukemic activity of 15-deoxyspergualin against *N*-butyl-*N*-nitrosourea-induced autochthonous rat leukemia. J. Antibiotics 39: 1488~1490, 1986
12) KUNIMOTO, S.; K. MIURA, H. IINUMA, T. TAKEUCHI & H. UMEZAWA: Cytotoxicity of spergualin and amine oxidase activity in medium. J. Antibiotics 38: 899~903, 1985
13) KURAMOCHI, H.; M. HIRATSUKA, S. NAGAMINE, K. TAKAHASHI, T. NAKAMURA, T. TAKEUCHI & H. UMEZAWA: The antiproliferative action of deoxyspergualin is different from that induced by amine oxidase. J. Antibiotics 41: 234~238, 1988
14) UMEZAWA, H.; K. NISHIKAWA, C. SHIBASAKI, K. TAKAHASHI, T. NAKAMURA & T. TAKEUCHI: Involvement of cytotoxic T-lymphocytes in the antitumor activity, of spergualin against L1210 cells. Cancer Res. 47: 3062~3065, 1987
15) ISHIZUKA, M.; T. MASUDA, S. MIZUTANI, M. OSONO, H. KUMAGAI, T. TAKEUCHI & H. UMEZAWA: Induction of antitumor resistance to mouse leukemia L1210 by spergualins. J. Antibiotics 39: 1736~1743, 1986
16) HARRISON, S. D., Jr.; R. W. BROCKMAN, M. W. TRADER, W. R. LASTER, Jr. & D. P. GRISWOLD, Jr.: Cross-resistance of drug-resistant murine leukemias to deoxyspergualin (NSC 356894) *in vivo*. Invest. New Drugs 5: 345~351, 1987