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Phase I study of NKT-01

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Abstract A phase I study of NKT-01 (deoxyspergualin), which is a derivative of an antitumor antibiotic, spergualin, was performed by a cooperative study group. NKT-01 was given intravenously by 3-h infusion. The effect of single administration was studied prior to evaluation of daily administration for 5 consecutive days. In all, 5 and 33 patients with various malignancies, including leukemia, were entered into the trials of single and daily administration, respectively. In the single-administration study, all patients were evaluable and no clear adverse effect was observed at doses ranging from 20 to 320 mg/m^2. In the daily-administration study, 28 evaluable patients (16 men and 12 women; median age, 55.5 years) were treated with a daily dose of 20–500 mg/m^2. Toxicities such as myelosuppression, mild nausea/vomiting, anorexia, alopecia, tongue and perioral numbness, and hypotension were observed dose-dependently during or after the treatment. Grade 2 leukopenia, thrombocytopenia, and anemia were experienced at a dose of 500 mg/m^2. These usually recovered to normal values by approximately 3 weeks after treatment. A pharmacokinetic analysis of single administration revealed rapid plasma clearance, with mean half-lives for the α and β phases being 28 min and 6.9 h, respectively. Approximately 12% of the infused dose was excreted into the urine in unmetabolized form. The pharmacokinetic parameters obtained after 5-day administration were similar to those recorded after single administration. Concerning treatment response, a transient but significant reduction in the number of leukemic cells was observed in one patient with adult T-cell leukemia. In this study, perioral numbness, hypotension, and hematological toxicity were concluded to be dose-limiting, with the maximal acceptable dose being 500 mg/m^2. The recommended dose for a phase II study of NKT-01 against solid tumors was judged to be 400 mg/m^2 given daily by 3-h infusion for 5 days, every 3 weeks. In hematological malignancies, however, higher myelosuppressive schedules of administration should be investigated.

Key words NKT-01 · Deoxyspergualin · Phase I study · Pharmacokinetics

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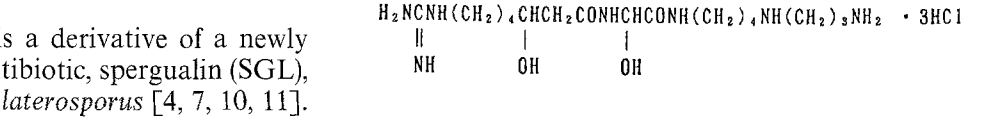
Introduction

NKT-01 (deoxyspergualin) is a derivative of a newly developed anti-neoplastic antibiotic, spergualin (SGL), that is produced by *Bacillus laterosporus* [4, 7, 10, 11]. Because SGL has a novel and interesting structure (Fig. 1) that is apparently different from those of previously known antineoplastic agents, extensive studies have been pursued on SGL derivatives so as to delineate the relationship between its structure and its activity [4, 11]. NKT-01 is a derivative that has shown strong anti-neoplastic activity in preclinical studies [11].

NKT-01 has exhibited excellent antitumor activity against a variety of experimental tumors, especially leukemias and a number of drug-resistant tumors [2, 3]. Its antitumor activity appears to be much stronger when it is given by daily consecutive administration or as a continuous infusion rather than by 1-day or one-shot therapy [8, 9]. These preclinical results encouraged us to proceed to a phase I clinical trial involving 14 institutions throughout Japan.

Spergualin:



NKT-01 (deoxyspergualin):

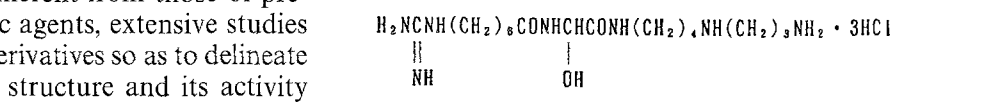


Fig. 1 Chemical structures of spergualin and NKT-01 (deoxyspergualin)

Patients and methods

All patients with malignant diseases were entered into this trial only when (1) the patients and/or their family gave consent for this study; (2) malignancy was confirmed histologically or cytologically; (3) the patients no longer has standard or appropriate treatment modalities but had a 4-week or longer life expectancy; (4) there was no potential toxicity that might have been induced by previous therapies, i.e., there had been at least a 2-week treatment-free interval since previous therapies; and (5) the patients met the inclusion criteria described below.

The patients had to have WBC counts of more than 4,000/μl, platelet counts of more than 100,000/μl; a hemoglobin (Hb) level of more than 11 g/dl; SGOT and SGPT levels of less than 2 times the upper normal limits; a total bilirubin level of less than 3 mg/dl; a serum creatinine level of less than 1.5 mg/dl; no history of heart disease, with normal electrocardiogram findings; a performance status of 0–3 according to World Health Organization (WHO) criteria [12]; and an age of more than 15 years. Patients with hematological malignancies did not have to be in conformance with the criteria values for WBC counts, platelet counts, and Hb levels.

NKT-01 was dissolved in 500 ml of normal saline or 5% glucose in water and was given intravenously by a 3-h drip infusion once a day. The treatment schedule consisted of 1- or 5-day daily administration. A preliminary 1-day treatment study was started prior to the 5-day treatment study so as to analyze the safety and plasma concentration of the drug, because it had been reported that hypotension and suppression of respiratory function, depending on the peak plasma concentrations of the drug, induced acute death in dogs [5, 6].

The toxic dose low (TDL) of NKT-01 was 56.4 mg/m^2 in the canine toxicity test when the drug was given by 3-h intravenous infusion [6]. The starting dose for humans was decided to be one-third of the canine TDL, i.e., 20 mg/m^2. The dose for the 1-day treatment trial was increased from 20 mg/m^2 to 40, 80, 160, and, finally, 320 mg/m^2. For the 5-day treatment schedule, the daily dose was increased to 40, 80, 120, 180, 270, 400, and 500 mg/m^2 after analysis of the drug's toxicity and safety at each dose.

The patients who entered the 5-day treatment trial were closely observed for 4 weeks, with examination of objective and subjective factors as well as complete blood counts, blood chemistry profiles, and urinalysis being performed at least once a week, whereas the 1-day-trial patients were observed for at least 1 week.

For determination of the plasma levels of NKT-01, blood samples were collected in heparinized tubes at the following times: at 1 or 2 into the infusion and at the end of infusion as well as at 0.5, 1, 2, 6, and 21 or 24 h after the infusion for 1-day treatment and on the 1st and 5th days for 5-day treatment. Urine samples were also collected after the start of the infusion at the following intervals: at 0–6, 6–12, 12–24, and 24–48 h for 1-day treatment and at 24-h intervals for 5-day treatment. NKT-01 concentrations in plasma and urine were determined by reverse-phase high-performance liquid chromatography (HPLC) using a Shimadzu LC-6A HPLC system equipped with an SIL-6A autoinjector, an RS-535 fluorometric detector, and a C-R4A Chromatopac (Kyoto). Detection of NKT-01 was also carried out by postcolumn derivatization with orthophthaladehyde (Wako Pure Chemicals, Osaka).

Grading of toxicity was performed on the basis of WHO criteria [12]. Clinical response was evaluated for acute leukemias by the response criteria of the Cancer and Acute Leukemia Group, which has been described in detail by Ellison [1], and for solid tumors by WHO criteria [12].

The maximal acceptable dose (MAD) was determined when grade 2 or 3 toxicity in two of three patients or any grade 4 toxicity was encountered at a specific dose of NKT-01, or when patients developed unexpected toxicities that were considered to be dangerous by the safety committee of this study. The appropriate dose of the drug for phase II studies was to be determined as one dose level lower than the MAD (approximately 80% of the MAD).

Results

The patients' characteristics are shown in Table 1. A total of 5 patients for 1-day treatment and 33 patients for 5-day treatment were entered into this trial. Five patients were excluded from the 5-day treatment group; three had ineligible laboratory findings just before treatment, one was started on NKT-01 shortly after the cessation of previous treatment, and one was lost to follow-up. In this particular study, patients could be enrolled at different doses. One patient scheduled for a 1-day course of NKT-01 was treated three times (80, 160, and 320 mg/m^2), and two patients scheduled for 5-day administration received NKT-01 twice (40 and 180 mg/m^2) and four times (40, 80, 270, and 400 mg/m^2), respectively. Except for these patients, no

Table 1 Patients characteristics

	Number of patients	
	Single-day administration	5-consecutive-day administration
Entered	5	33
Evaluable	5	28
M:F	5:0	16:12
Median age (range)	84 (73–84) years	55.5 (22–74) years
Prior therapy: Yes	5	25
No	0	3
Diagnosis:		
Solid tumor		
Lung cancer	3	2
Gastric cancer	1	
Laryngeal cancer	1	
Colorectal cancer		3
Breast cancer		2
Esophageal cancer		1
Ovarian cancer		1
Cervical cancer		1
Hematological malignancy		
Acute leukemia		10
Adult T-cell leukemia (ATL)		5
Malignant lymphoma		2
Myeloma		1

other individual received repeated courses of NKT-01 during this study.

The results of 1-day treatment revealed only a slight decline of Hb levels, with no other significant toxicity being noted. Possible toxicities depending on the plasma concentration of the drug were not observed at doses of 20–320 mg/m^2.

Drug toxicities for 5-day administration are described below. The hematological toxicities encountered in patients with solid tumors are shown in Table 2. Leukopenia was observed in one patient treated at 80 mg/m^2 per day and in two patients receiving 500 mg/m^2 daily. Two of the three patients who received 500 mg/m^2 per day had grade 2 leukopenia. Also two patients receiving NKT-01 at 500 mg/m^2 per day experienced grade 1 or 2 thrombocytopenia. The nadir of leukopenia and thrombocytopenia was observed around 2 weeks after the commencement of therapy, with recovery occurring within 1 week thereafter. A reduction in Hb levels was seen in four patients, of whom three treated at 500 mg/m^2 per day experienced a significant decrease in Hb values (grade 1 toxicity in two patients and grade 2 toxicity in one individual). Hb levels also recovered within approximately 3 weeks of the start of treatment. Among 18 patients with hematological malignancies, one, seven and five patients were evaluable for hematological toxicity concerning leukopenia, thrombocytopenia, and anemia, respectively. Grade 1 leukopenia developed in one evaluable patient with adult T-cell leukemia (ATL) who received 400 mg/m^2 daily. No thrombocytopenia was observed among the seven evaluable patients. Grade 2 and grade 1 decreases in Hb values were observed in two patients receiving 180 and 400 mg/m^2 per day, respectively.

Table 2 Hematological toxicity of NKT-01

Daily dose (mg/m^2)	Number of patients	Decrease[a] in		
		WBC	Plt	Hb
20	1	0	0	0
40	0	0	0	0
80	1	1(1)[b]	0	1(1)
120	2	0	0	0
180	1	0	0	0
270	0	0	0	0
400	2	0	0	0
500	3	2(2)	2(1, 2)	3(1, 1, 2)

[a]Number of patients with abnormal hematological values
[b]Number in parenthesis shows the grade of toxicity according to WHO criteria [12]

The results of other laboratory examinations are shown in Table 3. Some patients showed abnormal liver or kidney functions, but they were usually of grade 1 as judged by WHO toxicity criteria. SGOT elevations were observed in one patient with acute leukemia; this patient received the test drug four times and experienced elevated SGOT values each time. Subjective and objective adverse effects, including less than grade 2 nausea/vomiting and hair loss, also occurred but

Table 3 Non-hematological toxicity of NKT-01

Daily dose (mg/m^2)	20	40	80	120	180	270	400	500	Total (%)
Number of patients	3	3	3	3	3	2	6	5	28
Numbness							2(1)[a]	2(1)	4[b] (14.3%)
Nausea/vomiting					1(1)	1(2)	1(1)	1(2)	4 (14.3%)
Alopecia						1(1)	1(1)	1(1)	3 (10.7%)
General fatigue					1				1 (3.6%)
Abdominal discomfort							1		1 (3.6%)
Fever				1(1)					1 (3.6%)
Hypotension								1[c]	1 (3.6%)
Bradycardia		1							1 (3.6%)
GOT elevation		1(2)[d]	1(1)[d]			1(1)[d]	1(1)[d]		4 (14.3%)
Creatinine elevation	1(1)								1 (3.6%)

[a]Number in parentheses shows the grade of toxicity according to the criteria defined by the WHO [12]
[b]Number of patinets with abnormal vlaues in the laboratory examination or abnormal symptoms
[c]20-mmHg reduction in systolic blood pressure
[d]Elevation of GOT was experienced each time when the same patient was repeatedly enrolled into these four dose levels

Table 4 Pharmacokinetic parameters of NKT-01 as determined in human plasma after repeated infusion of NKT-01[a]

Daily dose (mg/m^2)	C_{max}(μg/ml)		AUC_{0-5} (μg h/ml)	
	Day 1	Day 5	Day 1	Day 5
20(2)[b]	0.38	0.45	0.98	0.97
40(2)	0.85	0.72	1.97	1.69
80(2)	1.57	1.16	3.97	2.58
120(3)	3.28 ± 1.85	2.67 ± 0.89	6.73 ± 2.79	5.44 ± 1.21
180(3)	5.51 ± 2.41	6.96(2)	11.51 ± 0.71	14.48(2)
270(2)	6.36	3.18	14.30	9.12
400(5)	6.11 ± 3.40	5.05 ± 2.23	18.32 ± 7.85(4)	14.02 ± 1.15(3)
500(4)	17.04 ± 6.31	13.42 ± 7.15(3)	43.64 ± 17.56	46.82(2)

Daily dose (mg/m^2)	Cl. (l/h)		$t_{1/2}\alpha$(min)		$t_{1/2}\beta$(h)	
	Day 1	Day 5	Day 1	Day 5	Day 1	Day 5
20(2)	34.53	31.49	29	28	–	–
40(2)	27.40	31.98	28	34	–	–
80(2)	28.16	44.88	29	37	–	–
120(3)	30.60 ± 9.03	36.35 ± 11.04	30 ± 7	24 ± 4	–	–
180(3)	21.15 ± 1.68	16.56(2)	18 ± 9	31 ± 21	8.9 ± 6.2	–
270(2)	24.25	38.47	16	24	4.8	–
400(5)	37.25 ± 16.85(4)	43.30 ± 5.44(3)	34 ± 15	53 ± 25	7.5 ± 5.1(4)	–
500(4)	19.86 ± 10.60	15.78(2)	37 ± 20(3)	54(2)	2.9(1)	–

[a]Data represent mean values ± SD
[b]The numbers of experiments conducted are given in parentheses

appeared to be dose-dependent. Four patients who received NKT-01 at a daily dose of more than 400 mg/m^2 experienced numbness around the mouth and at the tip of the tongue. Less frequently, general fatigue, fever, hypotension, and bradycardia were experienced by one patient each, these side effects were mild and transient.

The above mentioned unusual side effects, particularly numbness and hypotension, were also observed in patients who were excluded from the 5-day treatment group; numbness was experienced by two patients receiving 400 mg/m^2 per day or more, and one patient given 400 mg/m^2 daily experienced hypotension. These side effects usually appeared within 1–2 h of the start of the infusion and lasted for 2–4 h. No cumulative toxicity was observed in patients who received NKT-01 twice (40 and 180 mg/m^2 × 5 days; interval, 3 weeks) or four times (40, 80, 270, and 400 mg/m^2 × 5 days; intervals, 4–6 weeks).

In all, 9 patients with hematological malignancies and 14 patients with solid tumors were evaluable for tumor response. One patient with ATL had a transient

decline in leukemic cells after each 5-day treatment with NKT-01 at 40 and 180 mg/m^2 per day.

A pharmacokinetics study was performed in 5 patients with 1-day treatment and in 23 patients with 5-day treatment schedules. The study with 1-day treatment revealed a dose-dependent increase in the plasma level of NKT-01 and a rapid decline in the plasma concentration after the cessation of administration. The pharmacokinetic parameters of NKT-01 given on the 5-day treatment schedule are shown in Table 4. The areas under the plasma concentration-time curve (AUCs) found for unmetabolized NKT-01 on days 1 and 5 increased in an almost linear fashion along with dose escalations up to 400 mg/m^2 per day, whereas those determined at 500 mg/m^2 per day were much higher than expected as compared with those obtained at lower doses (Fig. 2). The plasma concentration reached the peak level at the end of the infusion in almost all cases and disappeared biphasically thereafter. The mean values obtained for plasma half-lives in the α and β phases on day 1 were 28 min and 6.9 h, respectively.

The pharmacokinetic parameters recorded on days 1 and 5 appeared to be similar. The 24-h cumulative urinary excretion of unmetabolized NKT-01 ranged from 12.0% to 12.5% of the delivered dose from day 1 through day 5 (Table 5).

Numbness and hypotension appeared in relation to the rise and fall of the plasma concentration of NKT-01. In five of seven patients who experienced these adverse effects, plasma levels of NKT-01 were examined. The peak levels recorded on the day the side effects appeared were 2.92, 4.17, and 10.34 μg/ml and 16.28 and 25.75 μg/ml as determined at doses of 400 and 500 mg/m^2 per day, respectively. In the case of patients without symptoms, the levels observed on day 1 were 6.64 and 9.01 μg/ml and 10.69 and 15.44 μg/ml as determined at doses of 400 and 500 mg/m^2 per day, respectively. The patient who had a plasma peak level of 25.75 μg/ml experienced much stronger numbness around the mouth and at the tip of tongue as well as in the extremities for up to 6 h after the cessation of drug administration.

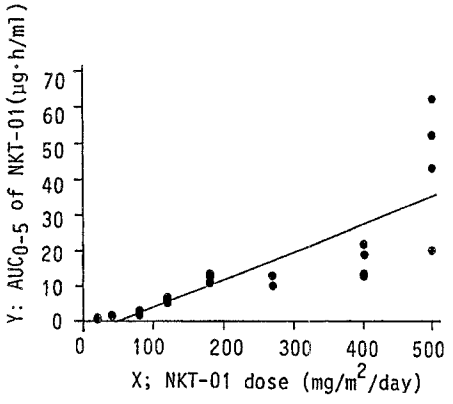


Fig. 2 Relationship between the NKT-01 dose and the plasma AUC_{0-5h} measured on day 1

Discussion

There was no apparent adverse effect with single administration of NKT-01, whereas 5-day treatment gave rise to anorexia, nausea and vomiting, paresthesia, hypotension, hematological toxicities, and liver and kidney dysfunction. Hematological toxicities were experienced depending on the delivered dose. At 500 mg/m^2 per day, grade 2 toxicities of leukopenia, thrombocytopenia, and reduction in Hb levels were experienced, although they were reversible and returned to pretreatment levels within about 3 weeks after the treatment. The incidence of the development of liver and kidney dysfunction was very low, and the degree of abnormality was also very mild.

Table 5 Cumulative excretion rate of intact NKT-01 to total dose as determined in human urine after repeated infusion for 5 days[a]

Daily dose[b] (mg/m^2)	Collecting period(day) 1[c]	2[c]	3[c]	4[c]	5[c]	6	7
20(3)	4.5	5.4	5.3	7.4	7.2	7.2	7.9(2)
40(2)	14.2	15.0	15.2	15.6	21.2(1)	21.2(1)	–
80(2)	8.8	16.0	14.7	14.2	14.8	14.9	14.9
120(3)	17.3	13.4	13.8	13.5	13.0	14.3(2)	14.3(2)
180(3)	11.4	10.9	10.5	10.6	10.5	10.6	10.7(1)
270(1)	5.1	7.4	9.5	9.4	–	–	–
400(4)	11.3	11.5	11.4	11.0	10.3	10.4	10.2(3)
500(2)	27.6	22.4	18.6	17.5	16.0	20.9(1)	20.9(1)
Mean	12.5	12.4	12.0	12.1	12.0	12.2	12.4
SD	8.2	6.5	4.9	4.5	4.5	4.8	4.6
	(20)	(20)	(20)	(20)	(18)	(16)	(11)

[a]Data represent mean values
[b]The numbers of experiments conducted are given in parentheses
[c]NKT-01 was infused on these days

Numbness around the mouth and at the tip of tongue as well as hypotension were observed at daily doses of 400 and 500 mg/m^2. The results suggest that numbness and hypotension seem to occur dependently on drug concentrations in the blood, although there were significant differences in drug concentrations among individuals in relation to complications. In preclinical studies, NKT-01 also induced hypotension in dogs at 0.3 mg/kg given by bolus injection [5], and a dog that received 20 mg/kg per hour (about 400 mg/m^2 per hour) by a 3-h intravenous infusion died of respiratory insufficiency at 2 h after the start of the infusion [6], when the plasma concentration reached 66.3 μg/ml (K. Yamashita, personal communication).

In conclusion, grade 2 leukopenia was observed in two of three patients who received 500 mg/m^2 per day, and unexpected toxicities of paresthesia and hypotension occurred at daily doses of 400 and 500 mg/m^2. As judged from the canine experiments described above, these unexpected toxicities might have been aggravated when the doses were further escalated. These results and prospects indicate that the MAD for 5-day consecutive administration is 500 mg/m^2 per day. The dose-limiting factors should be considered to be myelosuppression, including leukopenia and thrombocytopenia; paresthesia, especially perioral numbness; and hypotension. The dose and treatment schedule for a phase II study of NKT-01 against solid tumors are recommended to be 400 mg/m^2 given daily for 5 days, every 3 weeks, whereas hematological malignancies should probably receive a more myelosuppressive treatment schedule. Further studies are warranted to investigate the tumor response to the antineoplastic agent NKT-01.

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