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Thalidomide for the Treatment of Refractory Multiple Myeloma: Association of Plasma Concentrations of Thalidomide and Angiogenic Growth Factors with Clinical Outcome

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Recent reports showed that thalidomide has anti-angiogenic activity and is effective for the treatment of refractory multiple myeloma (MM). We examined the relationship between the clinical efficacy and adverse effects of thalidomide and the plasma concentrations of this drug as well as angiogenic growth factors in refractory MM. Ten out of twenty-four evaluable patients (42%) showed more than 25% reduction of M-protein, and eight (33%) achieved more than 50% reduction. These changes were associated with restoration of anemia and recovery of normal immunoglobulin level. Somnolence and headache, constipation, peripheral neuropathy and skin rash were frequently observed, but were well tolerated. However, grade 2–4 severe neutropenia was also observed in nine cases. These adverse effects other than neutropenia occurred more frequently in the patients with higher plasma concentrations of thalidomide (≥2.0 μg/ml at 12 h after the last administration) and were readily alleviated by dose reduction. In contrast, neutropenia developed regardless of the plasma concentration. Plasma concentrations of angiogenic growth factors were frequently elevated before treatment. After thalidomide treatment, these growth factor levels tend to decrease to near-normal ranges in responders but were still high in most non-responders. After thalidomide treatment, plasma vascular endothelial growth factor (VEGF) level was significantly reduced in responders (P=0.025), but not in non-responders (P=0.37). Reduction of plasma VEGF level might be an important indicator for anti-myeloma effect of thalidomide.

Key words: Thalidomide — Multiple myeloma — Fibroblast growth factor — Vascular endothelial growth factor — Neutropenia

Multiple myeloma (MM) is a hematological malignancy that affects terminally differentiated B-cells or plasma cells. Although MM runs an indolent clinical course at the early stage, MM becomes therapy-resistant and finally fatal within about 3 to 4 years.[1)] There has been some improvement in the management of MM, but conventional chemotherapy provides substantially no long-term survival.[2)] Several studies showed that high-dose chemotherapy combined with autologous hematopoietic stem cell transplantation offers prolonged overall survival compared with conventional chemotherapy.[3–6)] These treatment modalities could be applied for selected patients with MM, but it is still difficult to treat patients who relapse after stem cell transplantation or who become chemotherapy-resistant.

Thalidomide was first developed as a sedative/hypnotic drug in 1950s. Because of its teratogenicity, this drug was subsequently withdrawn from the market.[7)] In 1994, it was reported that thalidomide inhibits basic fibroblast growth factor (FGF-2)-induced angiogenesis, and it was suggested to be a candidate as a new anti-neoplastic agent.[8)] Clinical studies of thalidomide for various solid tumors have been widely conducted.[9–11)] Recently, it was also reported that bone marrow microvascular density was significantly increased in the patients with multiple myeloma, and thalidomide has been proposed to have anti-myeloma activity via an anti-angiogenic mechanism.[12–15)] We also found significant elevation of plasma concentrations of FGF-2 and vascular endothelial growth factor (VEGF) in patients with active MM.[16)] Recently, several reports have demonstrated the efficacy of thalidomide in 25–58% of refractory multiple myeloma with only minor complications such as somnolence, constipation, peripheral neuropathy or skin rash.[11, 17–20)] However, measurement of microvascular density of the bone marrow in MM failed to demonstrate a significant anti-angiogenic effect, and the precise mechanism of the anti-myeloma effect of this drug remains to be elucidated.[17)]

Here we report our experience on thalidomide for the treatment of refractory MM. In this study, we tried to cor-

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relate the clinical efficacy and toxicity of thalidomide with the plasma levels of angiogenic growth factors, as well as the drug. We demonstrated that plasma VEGF level decreased parallel to the clinical response to thalidomide, and the side effects except for neutropenia occurred more frequently in the patients with higher ($\geq$2.0 μg/ml) plasma concentration of the drug.

PATIENTS AND METHODS

Patients Total twenty-six patients with MM were included in this study and were treated with thalidomide at Keio University Hospital from November 1998 to March 2002. Since two patients received thalidomide treatment only for 4 days because of development of neutropenia, twenty-four patients were evaluated for efficacy of this drug. Mean follow-up time was 44 weeks, and mean treatment time was 19 weeks. Patients' eligibility was as follows: (1) patients whose MM relapsed after hematopoietic stem cell transplantation or those who were resistant to at least two different chemotherapeutic regimens. (2) At least 4 weeks elapsed since the last chemotherapy or radiotherapy. (3) Karnofsky performance status (KPS) was greater than 60. Women who might become pregnant during thalidomide treatment and also the spouses of such women were strictly excluded. Patients were also excluded if they had moderate to severe organ failure (specifically, if serum transaminase or γ-glutamyl transpeptidase (γ-GTP) level was higher than three times the upper limit of normal subjects, or if serum creatinine level was over 3.0 mg/dl), or if they showed severe neurological or psychological disturbance, or if they had cardiac failure or arrhythmia that needed medical interventions. Patients with active infections or positive for hepatitis B and C viruses, human immunodeficiency virus or human T-cell leukemia virus type I were also excluded. Written informed consents were obtained from all patients when they were enrolled in this study. This study was approved by the ethical committee of Keio University School of Medicine.

Treatment schedule During treatment with thalidomide, patients were not allowed to receive any chemotherapeutic agents including steroids and radiotherapy. Only supportive therapies including blood transfusion, granulocyte-colony stimulating factor (G-CSF), supplemental γ-globulin and pamidronate disodium were concomitantly used. Thalidomide was supplied by Sociedade Farmaceutic Brasifa Ltda. (Rio de Janeiro, Brazil) or Penn Pharmaceutical Service (Tredegar, UK) in 100-mg tablets. Two hundred milligrams was administered as an initial dose at night to all patients, and if serious adverse effects were not observed for 1 week, the dose was increased to 400 mg and continued as a maintenance dose. If dose escalation was difficult because of adverse effects, patients continued to receive the lower dose of thalidomide. When no response was obtained after at least 4 weeks of treatment or when disease progression was observed after obtaining an initial response, a treatment plan other than thalidomide was considered.

Assessment of response Response to thalidomide was evaluated by using the modified Gore's criteria.[21] All responses and stable disease had to be maintained for at least 4 weeks to be counted as a response. The definition of responses was as follows. Complete remission (CR); (1) disappearance of monoclonal spike and urinary Bence-Jones Protein confirmed at two different time points 2 weeks apart and/or (2) disappearance of plasmacytoma and (3) less than 5% plasma cells in bone marrow and (4) no progression of bone lesion and (5) normalization of anemia and hypercalcemia. Partial remission (PR); (1) more than 25% decrease of serum M-protein level or daily urinary Bence-Jones Protein which was sustained for a month or more and (2) decrease of bone marrow plasma cells and (3) no progression of plasmacytoma and lytic bone lesion, and (4) normalization of serum calcium level. If the decrease of serum M-protein level or daily urinary Bence-Jones Protein was more than 75%, the cases were regarded as PR1; if more than 50%, PR2; and if more than 25%, PR3. No change (NC); (1) less than 25% decrease of M-protein level and (2) no progression of plasmacytoma and bone lesion. Progression of disease (PD); (1) increase of M-protein or bone marrow plasma cells or (2) progression of plasmacytoma or bone lesion. Patients who obtained CR or PR were considered as responders, and those with NC or PD were considered as non-responders. In responders, increase in serum or urine M-protein by more than 25% above the minimum value on at least two occasions was considered as a relapse.

Assessment of adverse effects Every patient was admitted to Keio University Hospital or visited our outpatient clinic at least every month, and complete blood cell counts, serum transaminase, serum creatinine, serum calcium, C-reactive protein (CRP), M-protein level, and β2 microglobulin (β2M) were measured. Patients' symptoms were also monitored. Severity of adverse effects was graded according to the National Cancer Institute-common toxicity criteria. The dose of thalidomide was adjusted between 100 mg to 400 mg according to the adverse effects as follows. If grade 2 non-hematologic toxicity occurred, the dose was reduced by one-half. Then, if the toxicity was not improved by dose reduction, the drug was withheld. If white blood cell count was below 2000/μl or neutrophil count was less than 1000/μl, the dose of thalidomide was reduced by one-half, and G-CSF was used if necessary. If white blood cell count was below 1000/μl or neutrophil count was less than 500/μl, thalidomide was discontinued. If any other grade 3 or higher hematological toxicity was observed, the drug was withheld. Thalidomide can be restarted at 50% of the original maintenance

dose when non-hematologic toxicity is alleviated to grade 1 or when white blood cell count recovers to the pretreatment level.

Plasma concentration of thalidomide Venous blood samples were collected in tubes containing EDTA and were centrifuged (2000*g*, 10 min) within 2 h after blood sampling and stored at −80°C until assayed. Plasma samples were analyzed within 1 week after sampling. The concentration of thalidomide was analyzed according to the HPLC method.[22] Chromatographic conditions were as follows: The HPLC unit (Shimadzu LC-VP) consisted of a photodiode-array detector set at 224 nm. Separation was performed at 40°C on a 5 μm ODS column (Shinmeiwa Chemical Industries, Tokyo, 150 mm×4.6 mm ID). The mobile phase consisted of (methanol/acetonitrile=4:1)/water=28:72 and the flow rate was 1.5 ml/min. Methyl *p*-hydroxybenzoate dissolved in 1 *N* hydrochloric acid (HCl) was used as an internal standard. Plasma (500 ml) was combined with acidified methyl *p*-hydroxybenzoate and then extracted with 5 ml of chloroform. The separated chloroform layer was evaporated under reduced pressure and the samples were reconstituted with 100 μl of methanol. A calibration curve was constructed using thalidomide, based on the internal standard peak area ratio. The lower limit of detection was 0.1 μg/ml. According to our previous analyses, the pharmacokinetics of a single dose of 200 mg and 400 mg thalidomide in patients with multiple myeloma fitted a one-compartment model (Yamaguchi *et al.*, data in preparation). The peak concentration appeared at 4−6 h, and the concentration stabilized during 10−14 h after each dose. Since thalidomide was given nightly to all patients, plasma concentration was determined using a plasma sample taken in the next morning at 12±2 h after the last thalidomide administration. Plasma samples in the fifth week of therapy were used for studying the correlation with effects and side effects.

Plasma concentrations of growth factors Plasma samples were collected immediately after blood sampling as described in "Plasma concentration of thalidomide." Plasma concentrations of FGF-2, VEGF, hepatocyte growth factor (HGF), tumor necrosis factor-α (TNF-α) and interleukin 8 (IL-8) were determined using a sandwich enzyme-linked immunosorbent assay (ELISA) technique according to the manufacturer's instructions (R&D Research Corp., Minneapolis, MN). All time points were performed in triplicate and the absolute values in each ELISA plate were adjusted to a standard curve using FGF-2, VEGF, HGF and TNF-α recombinant protein as the control. The plasma concentrations of normal subjects for FGF-2 were 2.19±1.87 (1.0−7.67) pg/ml, for VEGF 17.7±5.4 (15.6−38.3) pg/ml and for TNF-α 3.71±2.02 (1.5−12.0) pg/ml. Those for HGF and IL-8 were all below 0.3 ng/ml and below 10 pg/ml, respectively.[16] In all patients, plasma concentrations were measured before and 4 weeks after the start of thalidomide. Additional sampling and measurement were conducted in several patients.

Statistical analysis Improvement of anemia and difference of plasma concentrations of thalidomide between responders and non-responders were evaluated by means of the Mann-Whitney *U* test. Association between clinical characteristics and response to thalidomide was examined by the Fisher exact test and by the χ^2 test. Reduction of plasma concentrations of growth factors by thalidomide was evaluated by the Wilcoxon test. The software package Stat-View 5.0 (SAS Institute, Inc., Cary, NC) was used for statistical analysis. *P* values <0.05 were considered as statistically significant.

RESULTS

Anti-myeloma effect of thalidomide The patients' profile and the effect of thalidomide are shown in Table I. All patients were in stage III,[23] and eleven cases had been treated with high-dose chemotherapy combined with hematopoietic stem cell transplantation, including two cases with allogeneic transplantation, prior to thalidomide treatment. Although none of the patients achieved a CR, ten out of twenty-four (42%) showed a PR3, more than 50% reduction of M-protein (PR2) was observed in eight cases (33%), and more than 75% reduction (PR1) was obtained in five (21%). NC or PD was observed in the other fourteen cases. Decrease of bone marrow plasma cells was also confirmed in six responders, suggesting that a reduction of M-protein level was induced by the antitumor effect of thalidomide, not simply inhibition of secretion of M-protein from myeloma cells. The time course of M-protein levels in responders is shown in Fig. 1. A significant decrease of M-protein was seen within the first 4 weeks of thalidomide treatment in all responders. Thus, responsiveness to this drug can be evaluated after a 4-week observation period. At the time of writing, mean treatment time and mean duration of effect in responders were 33 and 24 weeks, respectively. Six responders relapsed during treatment with thalidomide and became resistant to this drug.

The daily dose of thalidomide given to each patient is also shown in Table I. Fourteen patients received 400 mg of thalidomide, and six showed more than 25% decrease of M-protein (Table I). Although ten patients received a lower dose, 100 mg or 200 mg, because of adverse effects, four showed a more than 25% reduction of M-protein. None of the clinical parameters including age, sex, anemia, β2M, serum calcium, C-reactive protein, immunoglobulin subtype and prior therapy with stem cell transplantation was significantly associated with the response to thalidomide. In this study, five patients possessed unfavorable cytogenetic abnormalities including deletion of chromosome 13 or 17 and aberration of 11q,

Table I. Effect of Thalidomide

Case		Stage	Type	Prior HSCT	Dose (mg)	Response	M-protein level		BM plasma cell %		Hb level (g/dl)	
							Before (g/dl)	After (g/dl) (% reduction)	Before	After	Before	After
R1	45M	IIIA	IgG (k)	+	400	PR1	11.90	1.77 (−85)	87	2	6.7	9.2
R2	57F	IIIB	BJP (l)	−	400	PR1	6.18*	0.56 (−90)	70	NE	7.6	6.7 (3 w)
R3	57F	IIIA	IgG (k)	−	400	PR2	10.40	3.01 (−70)	63	6	8.1	10.8
R4	55F	IIIA	IgA (l)	−	400	PR2	4.01	1.07 (−58)	20	19	8.1	8.7
R5	53M	IIIA	BJP (l)	−	400	PR1	7.24*	<0.1 (−95)	34	NE	12.9	14.0
R6	63F	IIIA	IgG (k)	−	100	PR2	4.45	1.49 (−67)	30	8	8.4	10.4
R7	59M	IIIA	IgA (k)	−	200	PR3	5.87	3.53 (−40)	35	19	10.0	10.0
R8	52M	IIIB	IgG (l)	+	200	PR3	2.06	1.46 (−30)	2	ND	7.8	7.4 (3 w)
R9	64F	IIIA	IgG (k)	+	400	PR1	7.29	1.95 (−76)	1	ND	7.8	8.1 (4 w)
R10	64M	IIIB	BJP (k)	−	200	PR1	10.67*	1.18 (−89)	92	8	7.7	8.4
N1	57M	IIIA	IgA (l)	−	400	PD	6.28	7.27	39	56	9.9	11.6
N2	55F	IIIA	IgG (k)	+	400	PD	2.85	5.20	11	21	11.4	10.2
N3	70F	IIIA	IgD (l)	−	400	PD	4.65	4.71	18	32	10.7	10.7
N4	44M	IIIA	IgA (k)	+	400	PD	4.89	6.32	4	4	8.3	7.1 (4 w)
N5	47M	IIIA	IgD (l)	+	400	PD	0.50	1.82	ND	ND	12.7	9.9
N6	48M	IIIA	IgG (l)	+	400	PD	1.87	2.13	17	NE	14.9	13.2
N7	70F	IIIA	IgG (k)	−	100	PD	5.01	6.13	18	23	8.4	9.6
N8	41M	IIIA	IgG (k)	+	200	PD	4.12	4.91	2	NE	10.0	10.1
N9	38M	IIIA	IgG (l)	+	200	PD	3.21	4.61	12	17	11.0	8.8
N10	52M	IIIA	IgG (k)	+	200	PD	8.94	9.83	15	ND	8.3	7.2 (2 w)
N11	59F	IIIA	BJP (k)	+	400	NC	1.48*	1.86	9	ND	11.0	10.2
N12	56M	IIIA	IgG (l)	−	200	NC	4.02	3.62	34	ND	9.9	11.2
N13	51M	IIIB	BJP (k)	−	200	PD	13.56*	10.96	52	ND	8.4	7.4 (4 w)
N14	56F	IIIA	BJP (k)	−	400	NC	1.18*	1.01	ND	ND	10.8	10.3

HSCT, hematopoietic stem cell transplantation; NE, not evaluable; ND, not done; PR, partial response; NC, no change; PD, progression of disease. PR1, PR2 and PR3 indicate more than 75%, 50% and 25% reduction of serum or urine M-protein level, respectively. M-protein level of responders showed maximun reduction in responders and concentrations at cessation of therapy in non-responders. * showed daily urine M-protein level, g/day. Hemoglobin level after threatment was evaluated at 8 weeks of therapy otherwise indicated.

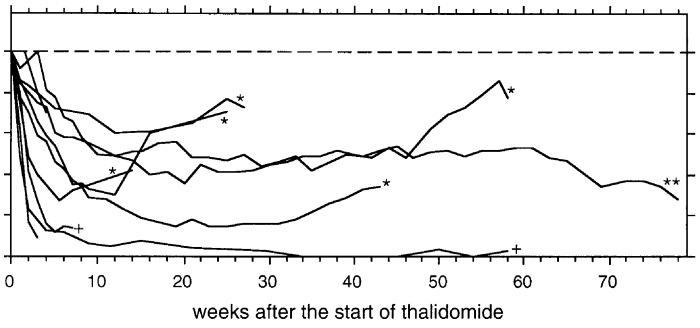

Fig. 1. Percent decrease of M-protein level over time in ten responders. + indicates that thalidomide treatment continued. * indicates relapsed cases. ** Although the M-protein level of this case was low, bone marrow plasma cells were significantly increased, and this case was considered to be relapsed.

and two of them showed good response.[24] These unfavorable cytogenetic alterations do not appear to predict a poor response to thalidomide.

Reductions in M-protein and bone marrow plasma cells were also associated with an increase in hemoglobin levels and recovery of normal immunoglobulin levels. Mean increase of hemoglobin concentration at 8 weeks of therapy was 1.4 g/dl in responders (range from 0 to 2.7 g/dl), which was significantly better than in non-responders (mean −0.44 g/dl, range from −2.8 to 1.7 g/dl, P=0.011 by Student's t test) (Table I). Suppression of normal γ-globulin level was also alleviated in four responders.

Adverse effects of thalidomide Adverse effects observed during the treatment with thalidomide are summarized in Table II. There was no death due to the direct toxicity of thalidomide. Except for neutropenia, grade 2 toxicity was observed only in three patients as mild muscle weakness in two cases and skin rash in one, which were immediately

Table II. Adverse Effects and Plasma Concentration of Thalidomide

Adverse effect	All cases (n=26)	≥2.0 μg/ml (n=11)	<2.0 μg/ml (n=9)
	No. of patients (%)	No. of patients (%)	No. of patients (%)
Somnolence	19 (73)	10 (91)	7 (77)
Peripheral neuropathy	13 (50)	10 (91)	2 (22)
Constipation	11 (42)	8 (73)	2 (22)
Skin rash	9 (35)	5 (45)	3 (33)
Neutropenia (<50% pretreatment level)	9 (35)	3 (27)	4 (44)
Dry mouth	7 (27)	4 (36)	2 (22)
Headache	7 (27)	4 (36)	2 (22)

In 26 patients, plasma samples were available from 20 cases, and the concentrations at 12±2 h after the last dose were examined. Only adverse effects observed in more than five cases are shown.

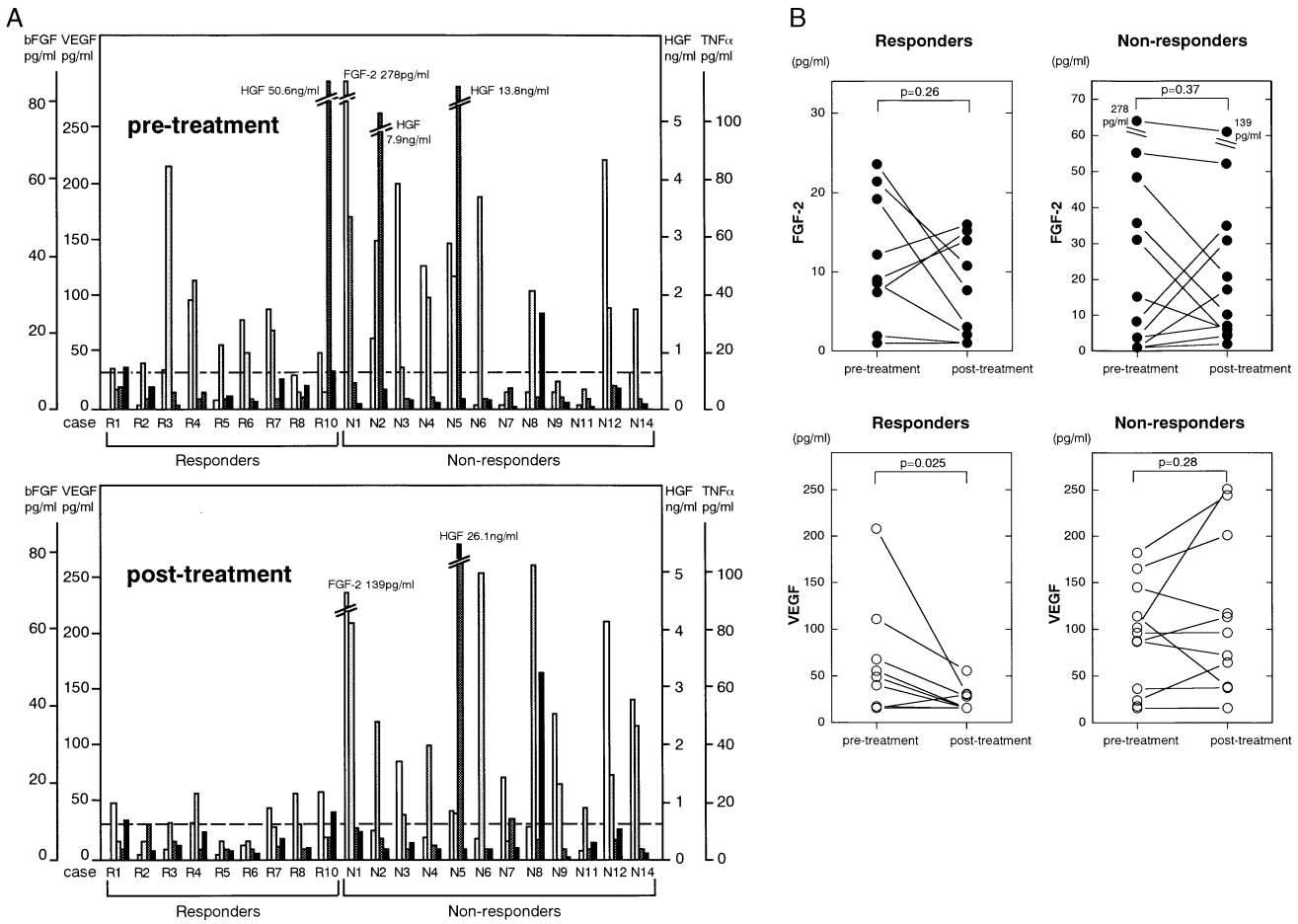

Fig. 2. Change of plasma growth factor levels by thalidomide treatment. A. Growth factor levels before and after treatment. Dashed lines indicate the highest values of control subjects. ☐ FGF-2, ▒ VEGF, ▓ HGF and ■ TNF-α. B. Decrease of FGF-2 and VEGF concentrations after treatment was evaluated by use of the Wilcoxon test.

improved by the transient cessation or dose reduction of thalidomide. The remaining adverse effects other than granulocytopenia were all grade 1 and well tolerated. Although hematologic toxicity has been reported as a rare adverse effect of thalidomide, severe neutropenia including six cases of grade 4, two cases of grade 3 and one case of grade 2 toxicity was unexpectedly observed in nine patients (Table II).[17] G-CSF was used in seven cases, and the neutrophil counts readily recovered in four cases. However, in three cases, neutropenia progressed despite of the use of G-CSF when thalidomide was continued, and cessation of thalidomide was necessary for the recovery of neutrophil counts in these three cases. Progression of concomitant thrombocytopenia and anemia was observed in four cases. Other less frequent adverse effects, which are not described in Table II, include low-grade fever in three cases, finger tremor in three, mild elevation of liver transaminase in one, dyspnea in one, lymphocytopenia in one, depression in one and mild myalgia with elevation of serum creatinine phosphokinase (CPK) in one, and all were grade 1 toxicity.

Association of the occurrence of adverse effects with plasma concentrations of thalidomide at 12 h after the last administration was examined (Table II). Plasma samples obtained in the fifth week of therapy were available in twenty patients, who were divided into two groups based on the plasma concentrations ($\geq$2.0 μg/ml and $<$2.0 μg/ml at 12 h after the last administration). As shown in Table II, somnolence was observed in most patients. Peripheral neuropathy and constipation and skin rash were observed predominantly in the higher concentration group. However, neutropenia developed regardless of plasma concentration of this drug.

Plasma angiogenic growth factor level The relationship between efficacy of thalidomide and serial plasma concentrations of angiogenic growth factors was examined. As shown in Fig. 2A, FGF-2 and VEGF levels were frequently elevated before treatment. However, significant elevation of HGF and TNF-α before treatment was observed only in three and one cases, respectively (Fig. 2A). No significant elevation of IL-8 level was observed (data not shown). After treatment with thalidomide, the concentrations of these four growth factors become near-normal in responders while plasma growth factor levels were still significantly elevated in most non-responders (Fig. 2A). The correlation of the reduction of FGF-2 and VEGF levels with clinical response was evaluated (Fig. 2B). Non-parametric analysis demonstrated that VEGF was significantly decreased after thalidomide treatment in responders (P=0.025), but not in non-responders (P=0.37). Plasma concentrations of angiogenic growth factors at relapse were available in three responders, R3, R4 and R7. Plasma VEGF levels decreased with the effect of thalidomide, but increased at the time of recurrence of disease activity. Namely, VEGF levels in R3 were 250 pg/ml before treatment, 55 pg/ml during response and 240 pg/ml at relapse. In R4, they were 110 pg/ml, 55 pg/ml and 90 pg/ml, respectively. In R7, they were 70 pg/ml, 30 pg/ml and 55 pg/ml, respectively.

DISCUSSION

MM is exclusively a fatal disease, and its progression can not be controlled by any conventional approach in terminal or therapy-resistant cases. A recent report on the use of thalidomide for MM indicated a significant decrease of M-protein level in 25–58% of refractory MMs.[17–20] We administered 100–400 mg of thalidomide to twenty-six Japanese MMs including eleven post-transplant relapsed cases. Ten out of twenty-four evaluable patients (42%) showed a partial response to thalidomide, and six cases (33%) achieved more than 50% reduction of paraprotein level (Table I). Efficacy of relatively low-dose thalidomide (100–400 mg per day) in our study is as good as that in other studies using much higher doses. A previous report also indicated efficacy of low-dose thalidomide against a certain population of refractory MM.[25]

We have examined the plasma concentrations of thalidomide. However, no significant difference of plasma level of thalidomide between responders and non-responders was observed. Further studies with larger numbers of patients are needed to elucidate the relationship of efficacy with the dose. However, it should be noted that four cases, R6, R7, R8 and R10, showed an excellent response to low-dose thalidomide (100 mg or 200 mg), and the plasma concentrations of thalidomide were below 1.5 μg/ml. These results suggested that response to thalidomide is not simply determined by the plasma level of this drug, and the plasma concentration of this drug necessary for antitumor activity seems to be different in each patient, probably due to variation of the biological characteristics of the tumor cells.

Adverse effects were seen in all patients. Those adverse effects, except for neutropenia, were well tolerated. In this study, we monitored the plasma concentrations of thalidomide and the frequency of adverse effects (Table II). As described in "Results," peripheral neuropathy, skin rash and constipation were more frequent in the higher plasma concentration group. We also experienced neutropenia, including six cases of grade 4 toxicity, which has not been emphasized as an important side effect in previous reports.[17–20] Unlike other side effects, neutropenia was observed frequently in the patients receiving only 100–200 mg, in whom the plasma concentration of this drug were lower. These patients showed significantly lower platelet counts before treatment than the cases without neutropenia, probably due to deterioration of normal hematopoiesis by previous intensive therapy or by prolif-

eration of myeloma cells in the bone marrow (Hattori *et al.*, submitted). Our observations also showed that thalidomide inhibited the growth of colony-forming cells of normal individuals (Hattori *et al.*, submitted). In addition, pancytopenia occurred in four cases. Thus, reduced function of bone marrow hematopoiesis is an important predisposing factor for neutropenia, and therefore, special precautions such as transient cessation of this drug or combined use of G-CSF are necessary for those patients. Neutropenia was observed in five responders and in two non-responders. In two other patients, response to thalidomide could not be evaluated because this drug was discontinued within 1 week due to sudden progression of neutropenia. Thus, association of this side effect with response to thalidomide is not clear. Recently, deep venous thrombosis was reported during thalidomide treatment.[26] However, this adverse effect was not observed in our study.

The exact role of anti-myeloma effects of thalidomide is not known. We and others have observed that addition of therapeutic concentrations of thalidomide to the culture medium did not significantly inhibit the growth of myeloma cell lines or primary bone marrow myeloma cells.[27] It was also reported that a synthetic thalidomide analogue induced apoptosis directly in myeloma cells.[27] This suggests that unknown natural metabolites of thalidomide may exert a direct cytopathic effect on myeloma cells. On the other hand, our data indicated that thalidomide reduced plasma concentrations of angiogenic growth factors. As shown in Fig. 1A, the patients with MM frequently showed elevation of plasma concentrations of a series of growth factors. Previously we also reported that elevated plasma concentrations of FGF-2 and VEGF were observed in 55% of myeloma patients, and correlated with disease activity.[16] This growth factor paradigm may play an important role in the progression of myeloma cells, and reduction of plasma concentration of growth factors by thalidomide may induce tumor regression. Our results indicate that plasma VEGF level may correlate with clinical response to thalidomide in responders (Fig. 2, B and C). Recently, it was reported that a high plasma FGF-2 level was associated with response to thalidomide.[28] In our study, however, this tendency was not observed. Since VEGF is a potent angiogenic growth factor, bone marrow microvascular density was also evaluated before and after thalidomide treatment. However, our observation showed that the decrease of microvascular density after the treatment with thalidomide was not significant even in responders (Du *et al.*, data in preparation), and we failed to obtain clear evidence for an anti-angiogenic effect of thalidomide in MM, although such an effect was described in another study.[16] Further study is needed to elucidate the biological significance of reduction of growth factor level by thalidomide.

In summary, we demonstrated the clinical efficacy of thalidomide for the treatment of refractory multiple myeloma. However, special care is needed to identify adverse effects, including severe granulocytopenia. We suggested that the reduction of plasma concentrations of angiogenic growth factors, especially VEGF, correlates with the clinical response to thalidomide.

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REFERENCES

1) Alexanian, R. and Dimopoulos, M. The treatment of multiple myeloma. *N. Engl. J. Med.*, **330**, 484–489 (1994).
2) Wada, M., Mizoguchi, H., Kuriya, S., Taguchi, H., Kawamura, T., Maekawa, I., Shimazaki, C., Sato, Y., Niho, Y., Miyazaki, T., Shibata, A., Kitani, T., Hamajima, N. and Ohno, R. and Japanese Co-operative Study Group for the Treatment of Multiple Myeloma. Induction therapy consisting of alternating cycles of ranimustine, vincristine, melphalan, dexamethasone and interferon (ROAD-IN) and a randomized comparison of interferon maintenance in multiple myeloma: a co-operative study in Japan. *Br. J. Haematol.*, **109**, 805–814 (2000).
3) Harousseau, J. L., Attal, M., Divine, M., Marit, G., Leblond, V., Stoppa, A. M., Bourhis, J. H., Caillot, D., Boasson, M., Abgrall, J. F., Facon, T., Linassier, C., Cahn, J. Y., Lamy, T., Troussard, X., Gratecos, N., Pignon, B., Auzanneau, G. and Bataille, R. Autologous stem cell transplantation after first remission induction treatment in multiple myeloma: a report of the French registry on autologous transplantation in multiple myeloma. *Blood*, **85**, 3077–3085 (1995).
4) Attal, M., Harousseau, J. L., Stoppa, A. M., Sotto, J. J., Fuzibet, J. G., Rossi, J. F., Casassus, Ph., Maisonneuve, H., Facon, T., Ifrash, N., Payen, C. and Bataille, R. for the Intergroupe Francaise du Myeloma. A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. *N. Engl. J. Med.*, **335**, 91–97 (1996).

5) Barlogie, B., Jagannath, S., Vesole, D. H., Naucke, S., Cheson, B., Mattox, S., Bracy, D., Salmon, S., Jacobson, J., Crowley, J. and Tricot, G. Superiority of tandem autologous transplantation over standard therapy for previously untreated multiple myeloma. *Blood*, **89**, 789–793 (1997).
6) Barlogie, B., Jagannath, S., Desikan, K. R., Mattox, S., Vesole, D., Siegel, D., Tricot, G., Munshi, N., Fassas, A., Singhal, S., Mehta, J., Anaissie, E., Dhodapkar, D., Naucke, S., Cromer, J., Sawyer, J., Epstein, J., Spoon, D., Ayers, D., Cheson, B. and Crowley, J. Total therapy with tandem transplants for newly diagnosed multiple myeloma. *Blood*, **93**, 55–65 (1999).
7) Tseng, S., Pak, G., Washenik, K., Pomeranz, M. K. and Shupak, J. L. Rediscovering thalidomide: a review of its mechanism of action, side effects, and potential uses. *J. Am. Acad. Dermatol.*, **35**, 969–979 (1996).
8) D'Amato, R. J., Loughnan, M. S., Flynn, E. and Folkman, J. Thalidomide is an inhibitor of angiogenesis. *Proc. Natl. Acad. Sci. USA*, **91**, 4082–4085 (1994).
9) Baidas, S. M., Winer, E. P., Fleming, G. F., Harris, L., Pluda, J. M., Crawford, J. G., Yamauchi, H., Isaacs, C., Hanfelt, J., Tefft, M., Flockhart, D., Johnson, M. D., Hawkins, M. J., Lippman, M. E. and Hayes, D. F. Phase II evaluation of thalidomide in patients with metastatic breast cancer. *J. Clin. Oncol.*, **18**, 2710–2717 (2000).
10) Fine, H. A., Figg, W. D., Jaeckle, K., Wen, P. Y., Kyritsis, A. P., Loeffler, J. S., Levin, V. A., Black, P. M., Kaplan, R., Pluda, J. M. and Yung, W. K. A. Phase II trial of the antiangiogenic agent thalidomide in patients with recurrent high-grade glioma. *J. Clin. Oncol.*, **18**, 708–715 (2000).
11) Gutheil, J. and Finucane, D. Thalidomide therapy in refractory solid tumour patients. *Br. J. Haematol.*, **110**, 754 (2000).
12) Vacca, A., Ribiatti, D., Roncali, L., Ranieri, G., Serio, G., Silvestris, F. and Dommacco, F. Bone marrow angiogenesis and progression in multiple myeloma. *Br. J. Haematol.*, **87**, 503–508 (1994).
13) Vacca, A., Ribiatti, D., Roncali, L. and Dommacco, F. Angiogenesis in B cell lymphoproliferative diseases. Biological and clinical studies. *Leuk. Lymphoma*, **20**, 27–38 (1995).
14) Vacca, A., Loreto, M. D., Ribiatti, D., Stefano, R. D., Gadaleta-Caldarola, G., Lodice, G., Caloro, D. and Dammacco, F. Bone marrow of patients with active myeloma: angiogenesis and plasma cell adhesion molecules LFA-1, VLA-4, LAM-1, and CD44. *Am. J. Hematol.*, **50**, 9–14 (1995).
15) Vacca, A., Ribiatti, D., Presta, M., Minischetti, M., Iurlaro, M., Ria, R., Albini, A., Bussolino, F. and Dommacco, F. Bone marrow neovascularization, plasma cell angiogenic potential, and matrix metalloproteinase-2 secretion parallel progression of human multiple myeloma. *Blood*, **93**, 3064–3073 (1999).
16) Sato, N., Hattori, Y., Du, W., Yamada, T., Kamata, T., Kakimoto, T., Okamoto, S., Kawamura, C., Kizaki, M., Shimada, N., Ote, Y., Hata, J. and Ikeda, Y. Elevated level of plasma basic fibroblast growth factor in multiple myeloma correlates with increased disease activity. *Jpn. J. Cancer Res.*, **93**, 459–466 (2002).
17) Singhal, S., Mehta, J., Desikan, R., Ayers, D., Roberson, P., Eddlemon, P., Munshi, N., Anaissie, E., Wilson, C., Dhodapkar, M., Zeldis, J. and Barlogie, B. Anti-tumor activity of thalidomide in refractory multiple myeloma. *N. Engl. J. Med.*, **341**, 1565–1571 (1999).
18) Alexanian, R. and Weber, D. Thalidomide for resistant and relapsing myeloma. *Semin. Hematol.*, **37**, 22–25 (2000).
19) Juliusson, G., Celsing, F., Turesson, I., Lenhoff, S., Adriansson, M. and Malm, C. Frequent good partial remissions from thalidomide including best response ever in patients with advanced refractory and relapsed myeloma. *Br. J. Haematol.*, **109**, 89–96 (2000).
20) Kneller, A., Raanani, P., Hardan, I., Avigdor, A., Levi, I., Berkowicz, M. and Ben-Bassat, I. Therapy with thalidomide in refractory multiple myeloma patients—the revival of an old drug. *Br. J. Haematol.*, **108**, 391–393 (2000).
21) Gore, M. E., Selby, P. J., Viner, C., Clark, P. I., Meldrum, M., Millar, B., Bell, J., Maitland, J. A., Milan, S., Judson, I. R., Zuiable, A., Tillyer, C., Slevin, M. and Malpas, J. S. Intensive treatment of multiple myeloma and criteria for complete remission. *Lancet*, **ii**, 879–881 (1989).
22) Boughton, B. J., Sheehan, T. M. T., Wood, J., O'Brien, D., Butler, M., Simpson, A. and Hale, K. A. High-performance liquid chromatographic assay of plasma thalidomide: stabilization of specimens and determination of a tentative therapeutic range for chronic graft-versus-host disease. *Ann. Clin. Biochem.*, **32**, 79–83 (1995).
23) Durie, B. G. M. and Salmon, S. E. A clinical staging system for multiple myeloma. Correlation of measured myeloma cell mass with presenting clinical features, response to treatment, and survival. *Cancer*, **36**, 842–854 (1975).
24) Schreiber, S., Ackermann, J., Obermair, A., Kaufmann, H., Urbauer, E., Aletaha, K., Gisslinger, H., Chott, A., Huber, H. and Drach, J. Multiple myeloma with deletion of chromosome 13q is characterized by increased bone marrow neovascularization. *Br. J. Haematol.*, **110**, 605–609 (2000).
25) Larkin, M. Low-dose thalidomide seems to be effective in multiple myeloma. *Lancet*, **354**, 925 (1999).
26) Osman, K., Comenzo, R. and Rajikumar, S. V. Deep vein thrombosis and thalidomide therapy for multiple myeloma. *N. Engl. J. Med.*, **344**, 1951–1952 (2001).
27) Hideshima, T., Chauhan, D., Shima, Y., Raje, N., Davies, F. E., Tai, Y. T., Treon, S. P., Lin, B., Schlossman, R. L., Richardson, P., Muller, G., Stirling, D. I. and Anderson, K. C. Thalidomide and its analogs overcome drug resistance of human multiple myeloma cells to conventional therapy. *Blood*, **96**, 2943–2950 (2000).
28) Neben, K., Moehler, T., Egerer, G., Kraemer, A., Hillengass, J., Benner, A., Ho, A. D. and Goldschmidt, H. High plasma basic fibroblast growth factor concentration is associated with response to thalidomide in progressive multiple myeloma. *Clin. Cancer Res.*, **7**, 2675–2681 (2001).