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ORIGINAL ARTICLE

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Menatetrenone, a vitamin K_2 analog, ameliorates cytopenia in patients with refractory anemia of myelodysplastic syndrome

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Abstract Vitamin K_2 induces differentiation of leukemic cell lines and apoptosis of immature blasts in myelodysplastic syndrome (MDS). We recently reported a case of MDS-refractory anemia (MDS-RA) with trilineage hematologic response to oral administration of menatetrenone, a vitamin K_2 analog. To determine a possible role of this agent in treatment of MDS-RA, we conducted a prospective randomized trial assessing the safety and efficacy of menatetrenone. A total of 18 consecutive patients newly diagnosed with MDS-RA were randomized to receive either 45 mg of oral menatetrenone (group 1) or no menatetrenone (group 2). Administration of menatetrenone was well tolerated. Of the nine patients in group 1 (56%), five improved with menatetrenone treatment while only one (11%) of the group 2 patients improved. Three patients (33%) showed a major response in absolute neutrophil count (ANC), two (22%) showed a major response in hemoglobin concentration, and two of the nine (22%) showed a major response in platelet count. The ANC of group 1 patients rose after treatment, while that of group 2 patients decreased slightly at follow-up after 16 weeks (p=0.03). Significant improvement was also seen in final platelet count (p=0.01), but not in hemoglobin concentration. Given the absence of toxicity, menatetrenone can be recommended for all patients with MDS-RA.

Keywords Vitamin K_2 · Menatetrenone · Myelodysplastic syndrome · Refractory anemia

Introduction

Myelodysplastic syndrome (MDS) is a clonal disorder of hematopoiesis characterized by pancytopenia and bone marrow dysplasia [1]. The median age of MDS patients is between 60 and 70 years, and survival varies from 8 to 27 months [7]. Response to cytotoxic therapy is poor, and amelioration of symptoms by transfusions and antibiotics is the mainstay of treatment. High-dose chemotherapy produces remission in some patients with high-risk MDS but is associated with a high incidence of treatment-related death and an early relapse, resulting in no survival benefit [3, 4]. Therefore, establishment of new therapeutic strategies for MDS, particularly for elderly patients, is an important clinical issue.

Vitamin K_2 is a substrate essential for blood coagulation. This vitamin acts as a cofactor for vitamin K-dependent carboxylase in the carboxylation of coagulation factors [5]. It has previously been reported that vitamin K_2 has an inhibitory effect on the growth of hepatoma cells, although the mechanism of growth inhibition is not well understood [11]. Vitamin K_2 has also been shown to induce differentiation of myeloid leukemia cell lines such as HL-60 and U937 and to induce apoptosis of immature blasts in MDS [8, 12, 13].

We recently encountered a patient with refractory anemia of MDS (MDS-RA) who produced a trilineage hematologic response to oral administration of menatetrenone [10]. Thus, we expected that treatment with menatetrenone would stimulate hematopoiesis in other MDS-RA patients. To test this possibility, we conducted a prospective randomized trial assessing the safety and efficacy of menatetrenone.

Patients and methods

Patients

This study included patients newly diagnosed with MDS-RA according to the modified French-American-British classification criteria who had adequate liver and kidney function [1]. Exclusion criteria included prior history of chemotherapy or other therapy that could affect hematopoiesis, prior diagnosis of aplastic anemia or paroxysmal nocturnal hemoglobinuria, and the presence of severe uncontrolled infection or unexplained fever higher than 38°C. Eighteen consecutive patients meeting the above crite-

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ria were randomized for this study. No patients withdrew from the study.

Treatment protocol

Patients were randomized to receive either menatetrenone (group 1) or no menatetrenone (group 2). Over a period of 16 weeks menatetrenone was administered orally three times per day for a total daily dose of 45 mg daily. No treatments other than menatetrenone or blood cell transfusion were given to either group. The subjects were observed at our clinic every 2–4 weeks.

Response was evaluated at the completion of 16-week follow-up. Hematologic improvement was evaluated according to response criteria reported by Cheson et al. [2]. Informed consent was obtained from all patients prior to entering this study, and the ethical committee of our institution approved the study protocol.

Statistics

Statistical analysis was performed with two-tailed *t*-tests and chi-square analysis.

Results

Patient characteristics

This study was begun in June 1998, and results were analyzed in June 1999, after all 18 patients completed the 16-week treatment. Each group contained nine patients (Table 1). There was no significant difference in the mean age, sex ratio, and hematologic parameters at the time of randomization. In group 1, three patients had abnormal karyotypes: these included loss of chromosome Y in one, chromosome 20q- in one, and deletion of chromosome 20q11 plus addition of chromosome 7q11 in one. Likewise in group 2, three patients had cytogenetic abnormalities: loss of chromosome Y in two and addition of chromosome X in one.

Toxicity

Administration of menatetrenone was well tolerated; all nine patients in group 1 completed the 16-week treatment without toxicity.

Response

When hematologic effects of menatetrenone were evaluated according to response criteria [2] 16 weeks after initiation of treatment, five of the nine (56%) patients in group 1 improved, while one (11%) of the group 2 patients improved (p=0.046, Table 2). There was a bilineage response in two and single lineage response in three patients, one of whom had a cytogenetic abnormality (missing Y). Trilineage response was not seen. Three patients (33%) showed a major response in ANC, two (22%) showed a major response in hemoglobin concentration, and two (22%) of the nine showed a major response in platelet count. During the study period, none of the patients in either group progressed to RA with excess of blasts (RAEB) or to acute leukemia. Table 3 shows characteristics of patients showing hematologic improvement in response to menatetrenone. Figure 1 illustrates the clinical course of a patient who exhibited trilineage hematologic improvement with erythroid and platelet responses according to response criteria [2] following initiation of menatetrenone. The patient, an 81-year-old Japanese man, was diagnosed with MDS-RA in October 1998. Results of cytogenetic analysis of his marrow cells at diagnosis of MDS-RA were normal. Gradual improvements in anemia, thrombocytopenia, and neutropenia were observed as early as 4 weeks after administration of menatetrenone. With menatetrenone treatment alone, the patient has remained transfusion-

Table 1 Patient characteristics

Characteristic	Group 1	Group 2
No. of patients (male:female)	9 (5:4)	9 (4:5)
Mean age (years) (range)	75 (51–86)	69 (48–84)
ANC ($\times 10^3/\mu l$)	1.9 (1.0–3.1)	1.6 (0.8–2.5)
Platelet count ($\times 10^3/\mu l$)	116 (67–178)	98 (13–157)
Hemoglobin (g/dl)	10.0 (6.8–13.8)	10.2 (6.8–14.3)

Table 2 Hematologic effect of menatetrenone evaluated at 16 weeks after treatment

	Group 1 No.	Group 2 No.	*P*
Hematologic improvement	5	1	0.046
Trilineage response	0	0	
Bilineage response	2	0	
Single lineage response	3	1	
Erythroid response	2 (2 major)	1 (1 minor)	0.5
Platelet response	2 (2 major)	0	0.1
Neutrophil response	3 (3 major)	0	0.1

Table 3 Characteristics of patients showing hematological improvement in response to menatetrenone

Case	Age	Sex	Bone marrow data at diagnosis of MDS-RA			Hematologic response		
			Cellularity	% of blast cells	Dysplasia	Erythroid	Neutrophil	Platelet
1	72	Male	Hypercellular	0.8	Trilineage	Absent	Major	Absent
2	51	Male	Normocellular	0.4	Trilineage	Absent	Absent	Major
3	83	Female	Hypercellular	2.4	Trilineage	Absent	Major	Absent
4	86	Female	Hypercellular	2.8	Trilineage	Major	Major	Absent
5	81	Male	Normocellular	0.8	Trilineage	Major	Absent	Major

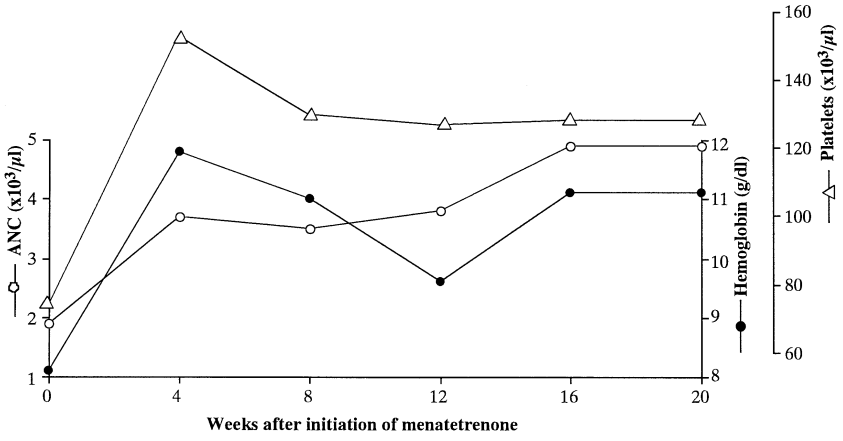

Fig. 1 Hematologic effects of menatetrenone in an 81-year-old man with MDS-RA. Trilineage hematologic recovery occurred following initiation of menatetrenone. ANC (*open circles*), hemoglobin (*solid circles*), platelet counts (*triangles*)

Table 4 Changes in hematologic parameters during the study period

ANC	Median (×10³/μl) (range)		
Weeks after treatment	0	16	P
Group 1	1.9 (1.0–3.1)	2.2 (1.1–7.4)	0.22
Group 2	1.4 (0.8–2.5)	1.4 (0.4–2.2)	0.17
P	0.38	0.04	
Hemoglobin	Median (g/dl) (range)		
Weeks after treatment	0	16	P
Group 1	8.9 (6.8–13.8)	10.6 (5.5–13.1)	0.46
Group 2	10.1 (6.8–14.3)	10.7 (6.2–14.0)	0.50
P	0.86	0.86	
Platelets	Median (×10³/μl) (range)		
Weeks after treatment	0	16	P
Group 1	97 (67–178)	128 (69–192)	0.01
Group 2	110 (13–157)	79 (5–152)	0.03
P	0.40	0.01	

free and has maintained a hemoglobin concentration of over 10 g/dl for 2 years.

Table 4 shows changes in hematologic parameters during the study period. The mean increase in ANC of group 1 was 162% of the mean of the pretreatment level. The difference in ANC between the two groups was statistically significant at 16 weeks (p=0.009). The ANC of group 1 patients started to rise as early as 4 weeks after the start of treatment and remained significantly higher than that of group 2 patients throughout the 16-week treatment period (data not shown). Although the platelet count rose considerably in two patients in the menatetrenone group, menatetrenone improved thrombocytopenia in group 1 patients as a whole. At the completion of the study, there was no difference in hemoglobin concentration between the two groups. Similarly, over the course of this study, there was no difference in absolute reticulocyte count between the two groups (data not shown). Likewise, this was true for other parameters such as the necessity for red cell transfusion, absolute count of lymphocytes, monocytes, eosinophils, and basophils, and the level of lactate dehydrogenase.

Discussion

Although several differentiation-inducing agents such as vitamin D_3, vitamin A, retinoic acid, and interferon have been administered to MDS patients in hopes of improving pancytopenia, the outcomes of such clinical trials have been unsatisfactory [14]. Here we demonstrate that menatetrenone is effective in ameliorating neutropenia and thrombocytopenia in patients with MDS-RA. In the menatetrenone group, five of nine (56%) patients showed hematological improvement at week 16 of the study, while only one (11%) of the nine patients in the control group improved. In addition, cytopenia in three of the remaining four patients in the menatetrenone group was unchanged, but in five of the remaining eight patients in the control group, cytopenia deteriorated after the study (data not shown). This fact suggests that menatetrenone could prevent hematologic deterioration in the MDS patients.

There are several possible mechanisms by which menatetrenone causes hematological improvement in MDS-RA patients. Sakai et al. [8] reported that vitamin K_2 can induce in vitro differentiation of several myeloid leukemic cell lines. Menatetrenone may have induced differentiation of defective stem cells in MDS-RA patients and accelerated production of mature blood cells. The finding that signs of hematologic improvement appeared without an initial worsening of pancytopenia after initiation of menatetrenone therapy supports this hypothesis.

The second possibility is that menatetrenone may have improved hematopoiesis by causing the apoptotic elimination of abnormal clones that had presumably inhibited hematopoiesis by normal clones. A recent in vitro study demonstrated that vitamin K_2 selectively induces apoptosis of immature blasts from MDS patients [12]. A survey conducted in Japan showed that administration of menatetrenone reduced the percentage of blasts in bone marrow in 8 (73%) of 11 patients with RAEB in transformation [6]. Menatetrenone also reduced blasts in 6 (50%) of 12 patients with acute myeloid leukemia (AML) that evolved from MDS, although 10 of the 14

successfully treated patients concomitantly received other agents such as prednisolone, ubenimex, vitamin D_3, ascorbic acid, and cytarabine ocfosfate.

To date, all five patients responding to menatetrenone remain in good hematologic condition after 33–38 months solely with menatetrenone treatment. No signs of toxi-city or progression to acute leukemia have been observed. Vitamin K_2 is a naturally occurring substance and has very low toxicity [8]. Since menatetrenone, a vitamin K_2 analog, has been used to treat patients with osteoporosis for a long time in Japan, its safety has already been established [9]. Menatetrenone may produce better results if administered in combination with interferon or retinoic acid [8]. Therefore, further clinical trials of menatetrenone treatment for MDS are warranted.

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