

Thalidomide in multiple myeloma, myelodysplastic syndromes and histiocytosis. Analysis of clinical results and of surrogate angiogenesis markers

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Summary

Background: Thalidomide, as a single agent, has been recently found to induce a clinical response in one third of refractory or relapsed myeloma patients. Although it has been reported that thalidomide significantly inhibits angiogenesis, it is still unclear whether its clinical effect is mediated, at least in part, by its anti-angiogenic properties.

Patients and methods: We evaluated thalidomide as a single agent in myeloma, myelodysplastic syndromes (MDS) and histiocytosis, i.e. hematological diseases characterized by increased angiogenesis, and measured prospectively a number of surrogate angiogenesis markers.

Results: Clinical responses were observed in 7 of 17 mye-

loma and 2 of 5 MDS patients. The histiocytosis patient had a partial response. At the time of the best clinical response, plasma levels of angiogenic growth factors, vascular endothelial growth factor (VEGF) and basic-fibroblast growth factor (b-FGF), were significantly decreased, and flow cytometry indicated a decrease of activated endothelial cells in the bone marrow of responding MDS patients.

Conclusions: These observations confirm thalidomide efficacy in myeloma, suggest a possible use in MDS and histiocytosis and may contribute to the prediction of clinical response and to understanding the mechanism of thalidomide's action.

Key words: angiogenesis, histiocytosis, myelodysplastic syndromes, myeloma, thalidomide

Introduction

Thalidomide, developed in the 1950s as a sedative-hypnotic, was withdrawn in the 1960s after reports of teratogenicity associated with its use. Thereafter, thalidomide has been used for leprosy, discoid lupus erythematosus, aphthous ulcers in HIV syndromes and Behçet's disease [1]. D'Amato et al. [2] have reported that, in addition to already known actions (including effects on the expression of adhesion molecules, of cytokines such as TNF- α and IL-10, and modulation of cell-mediated immunity), thalidomide significantly inhibits angiogenesis. Vacca et al. [3] described relevant neovascularization in the bone marrow (BM) of myeloma patients, and Singhal et al. [4] reported that thalidomide, as a single agent, was able to induce a marked and durable response in approximately one third of 84 chemotherapy-refractory myeloma patients. Although two more reports have confirmed the clinical efficacy of thalidomide in small series of myeloma patients [5–6], it is still unclear whether this effect is mediated, at least in part, by its anti-angiogenic properties.

We have recently reported that angiogenesis is in-

creased in myelodysplastic syndromes (MDS), and that in this disease BM neovascularization is intermediate between healthy controls and acute myeloid leukemia [7]. Thus, we decided to evaluate thalidomide as a single agent in relapsed myeloma and MDS patients, and to measure a number of surrogate markers of angiogenesis to gain insight into the biological activity of this drug. Here we report an intention-to-treat analysis of 17 myeloma and five MDS patients enrolled in this study and of a patient suffering from histiocytosis, another disease characterized by increased angiogenesis.

Study design

The median age of the patients was 66 years (range 55–80). Of the myeloma patients enrolled, 3 of 17 relapsed after tandem high-dose chemotherapy, and 14 of 17 relapsed after (or were refractory to) at least two lines of conventional chemotherapy. MDS patients (two RA, three RAEB) failed to respond to cytokine-based therapies and were treated with supportive care, including red cell and platelet transfusions. The histiocytosis patient

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was in relapse after a previous velbe-steroid treatment. Thalidomide was administered orally at a starting dose of 100 mg/day. In the absence of side effects, the dose was increased every 2–4 weeks up to 3–400 mg/day. Singhal et al. [4] administered thalidomide in a single dose in the evening. Similarly to Juliusson et al. [6], and taking into account the 6 hours half-life of the drug [8], our patients were requested to fractionate the dose in at least two daily administrations. Patients were evaluated monthly for clinical response and every three months for plasma levels of angiogenic growth factors, vascular endothelial growth factor (VEGF) and basic-fibroblast growth factor (b-FGF), as we previously described [9]. In MDS patients, the frequency of resting and activated BM endothelial cells was evaluated by flow cytometry following a procedure that we previously validated in preclinical models of human hematopoietic malignancies [10], and in a clinical study enrolling lymphoma and breast cancer patients [11]. All procedures were in accordance with the ethical standards of the responsible committees on human experimentation and with the Helsinki Declaration of the World Medical Association

Results

Side effects were observed in 10 of 23 patients. Three patients had constipation, two had GI–2 neuropathy, two had dizziness, one had hypotension. In two patients the drug was discontinued for general or gastric intolerance. Patients without side effects were able to tolerate a higher dose of thalidomide. In fact, 7 of 23 patients failed to escalate the dose up to 3–400 mg/day because of the side effects. On the other hand, split daytime dosage did not increase somnolence, fatigue or other side effects. One patient reduced the dose to 50 mg/day after achieving a clinical response. Overall, the median thalidomide dose per day was 220 mg/day (range 50–400).

Of the 17 myeloma patients enrolled, five (29%) had a > 50% decrease of the monoclonal Ig, two had a 26%–50% reduction, five had a 1%–25% reduction, three had progressive disease (PD), two discontinued the drug within the first 30 days. In responding patients, median time to response was 36 days. In this limited group of patients, a clear evaluation of dose-response relationship was not possible. We observed durable clinical responses in some patients who achieved a response at 3–400 mg/day and reduced the drug dosage to 1–200 mg/day to avoid side effects. Apart from two patients who discontinued the drug for side effects, other patients discontinued because of progressive disease. Interestingly, the patient who achieved the best clinical response (normalization of Ig levels with 50 mg/day thalidomide for more than one year) was the one with highest baseline VEGF and b-FGF levels. Of the seven patients with a > 25% decrease in monoclonal Ig, three had PD (median time to progression 14 months) after a median follow-up of 16 months.

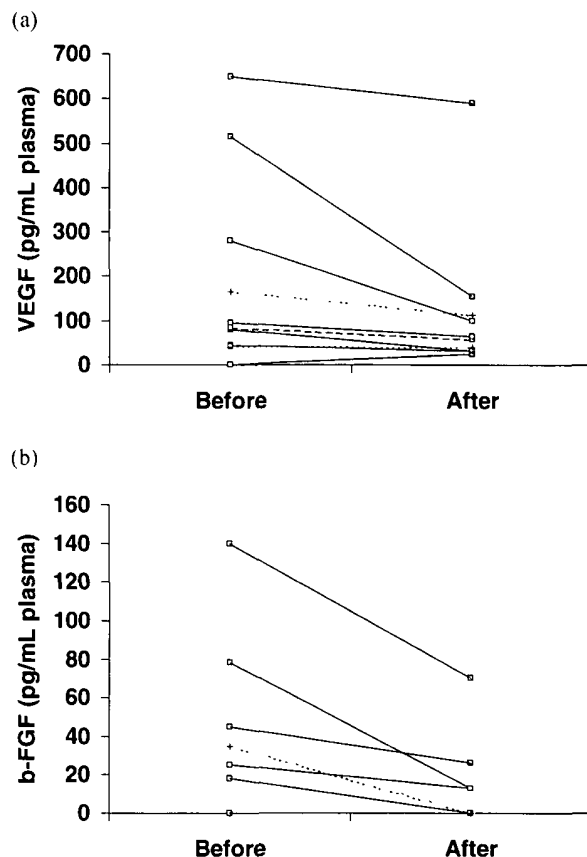


Figure 1. Plasma levels of (a) VEGF and (b) b-FGF before thalidomide administration and at the time of best clinical response in myeloma (solid lines), MDS (small dot lines) and histiocytosis (large dot line) patients. $P = 0.020$ and 0.027 by Wilcoxon-matched pair test for VEGF and b-FGF, respectively.

Of the five MDS patients, two had a clinical response as defined by a recent consensus report [12]. A 64 year-old male RAEB patient became transfusion-independent, and his BM blasts were reduced from 20% to 5%. Time to progression was four months. A 70 year-old female RA patient had increased red cell, white cell and platelet count, and transfusion interval increased from 15 to 60 days. Time to progression was seven months. The 63 year-old female histiocytosis patient had a significant decrease of cutaneous lesions and pain, and time to progression was six months.

As shown in Figure 1, at the time of the best response both VEGF and b-FGF were significantly decreased when compared to pre-treatment values ($P = 0.020$ and 0.027 by Wilcoxon matched pair test for VEGF and b-FGF, respectively). As reported in Figure 2, flow cytometry indicated a striking decrease of activated endothelial cells (CD45⁻, CD14⁻, CD31⁺, CD105⁺) in the BM of responding MDS patients.

Discussion

Our data confirm that thalidomide, as a single agent, is active in a significant proportion of refractory myeloma

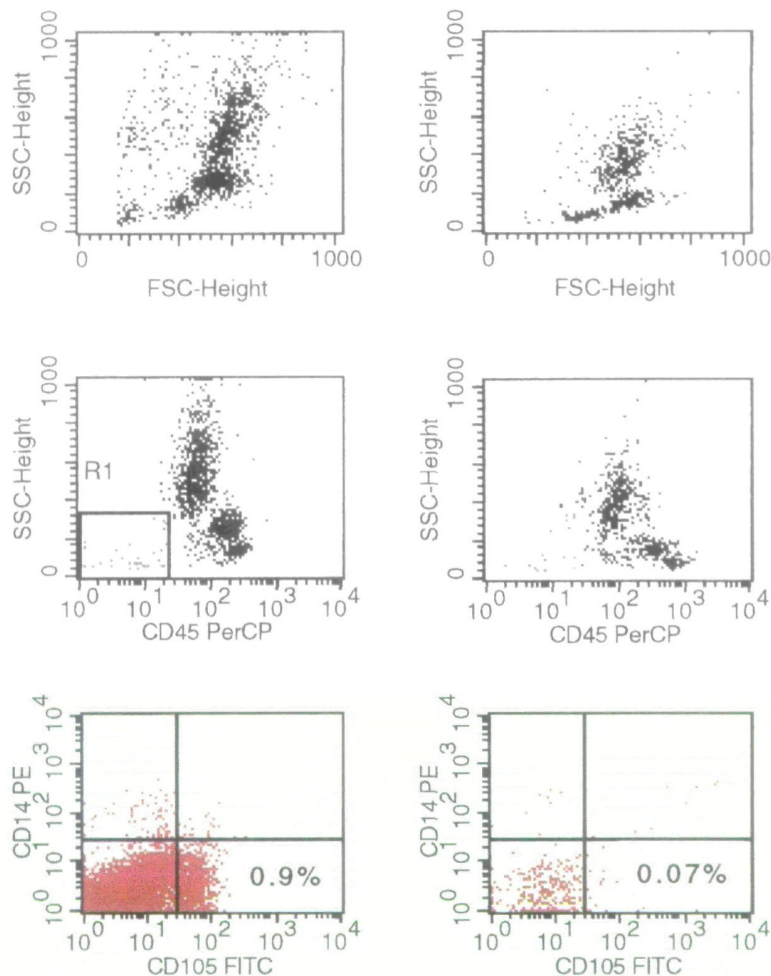


Figure 2. Representative dot plots of the frequency of bone marrow-activated endothelial cells evaluated by flow cytometry in a MDS patient before (panels on the left) and after (panels on the right) thalidomide. Upper panels show the side and the forward scatter of the bone marrow cells. A significant increase of large blasts is observed in the left panel (before thalidomide), whereas a normal distribution is observed after therapy. Middle panels show the gate used to exclude CD45+ hematopoietic cells. Panels on the bottom show the frequency of activated endothelial cells (CD45-, CD14-, CD31+, CD105+). A ten fold reduction of activated endothelial cells and return to normal values was observed after thalidomide treatment.

patients, and indicate a promising clinical activity of single-agent thalidomide in both MDS and histiocytosis, two hematological malignancies that (similarly to myeloma) are associated with relevant angiogenesis. Along this line, we observed that circulating angiogenic growth factors VEGF and b-FGF, as well as BM activated endothelial cells, are significantly decreased at the time of best clinical response. It should be noted that, unlike serum VEGF, plasma VEGF is not influenced by VEGF released from platelets [9, 13]. Thus, fluctuations in the platelet count of myeloma and, particularly, MDS patients did not bias our measurements of circulating VEGF.

In myeloma [14] and MDS [7], VEGF and b-FGF are involved in paracrine loops generated by stroma, endothelial and neoplastic cells. Thus, our findings support the hypothesis that the action of thalidomide might be due (at least in part) to the inhibition of cytokine-signaling between stroma, endothelial and neoplastic cells [15]. Sezer et al. [16] recently reported increased VEGF and b-FGF levels in myeloma patients compared to

controls. VEGF and b-FGF were found to decrease in patients responding to chemotherapy and not in patients who did not achieve a remission. Accordingly, the decrease of VEGF and b-FGF observed in the present study in myeloma patients at the time of the best response to thalidomide might also reflect a decrease in tumor load.

Another relevant issue is whether high levels of VEGF, b-FGF and/or BM-activated endothelial cells may predict a clinical response to thalidomide. Although our series of patients is too small to draw definitive conclusions, it should be noted that myeloma patients showing higher VEGF and b-FGF levels had clinical responses, and that the two MDS patients with higher levels of BM-activated endothelial cells responded to thalidomide. Singhal et al. [4] reported a decrease in BM microvessel density (MVD) in some myeloma patients responding to thalidomide, but differences in MVD between responding and non-responding patients were not statistically significant. Our flow cytometry assay generates quantitative data on the frequency of endo-

thelial cells, while MVD indicates the frequency of blood vessels. In animal models of human myeloid and lymphoid malignancies, tumor engraftment potential, speed of engraftment and the frequency of apoptotic tumor cells correlated better with the frequency of endothelial cells enumerated by flow cytometry than with MVD evaluation [10]. We are currently enrolling more myeloma and MDS patients and evaluating different biological parameters to further elucidate the role of surrogate angiogenesis markers as prognostic and/or predictive factors.

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