Brief report

Farnesyltransferase inhibitor tipifarnib (R115777) preferentially inhibits in vitro autonomous erythropoiesis of polycythemia vera patient cells

Jérôme Larghero, Nathalie Gervais, Bruno Cassinat, Jean-Didier Rain, Marie-Hélène Schlageter, Rose Ann Padua, Christine Chomienne, and Philippe Rousselot

Polycythemia vera (PV) is an acquired myeloproliferative disorder with primary expansion of the red cell mass leading to an increased risk of thrombosis and less frequently to myelofibrosis and secondary acute leukemia. Standard therapies include cytoreduction with either phlebotomy or chemotherapeutic agents and antithrombotic drugs. Because long-term exposure to cytotoxic chemotherapy may increase the risk of acute transformation, new therapeutic options are needed. Tipifarnib is a nonpeptidomimetic inhibitor of farnesyl transferase that was developed as a potential inhibitor of *RAS* signaling. In the present study we report that tipifarnib used at pharmacologically achievable concentrations strongly inhibits the erythroid burst-forming unit (BFU-E) autonomous growth that characterizes patients with PV. Moreover, at low tipifarnib concentrations (0.15 μM), the inhibitory effect was preferentially observed in PV BFU-E progenitors and not in normal BFU-E progenitors and was not rescued by erythropoietin (EPO). Thus tipifarnib may specifically target PV stem cells and may be of clinical interest in the treatment of patients with PV. (Blood. 2005;105: 3743-3745)

Introduction

Polycythemia vera (PV) is a chronic myeloproliferative disorder characterized by an increased production of mature red cells. Despite the fact that the molecular mechanism in PV is still unknown, hypersensitivity of PV hematopoietic progenitors to cytokines and growth factors including erythropoietin (EPO), granulocyte-macrophage colony-stimulating factor, interleukin-3, insulin-like growth factor, or stem cell factor has been demonstrated.[1] Thus, spontaneous erythroid burst-forming unit (BFU-E) growth represents one of the major features of in vitro PV studies. PV as well as essential thrombocythemia (ET) may progress, with time, to blastic transformation[2] but genetic alterations associated with disease progression have not been extensively studied. Although *P53* and *N*- and *K-Ras* mutations have been described in a small subset of patients with PV in blastic transformation, disruptions of these genes appear to be extremely rare in nontransformed PV patients.[3-5]

Farnesyl transferase inhibitors (FTIs) have emerged as promising new targeted therapies for malignant diseases. FTIs inhibit farnesylation of low–molecular-weight guanosine triphosphatases. Among these, RAS proteins require farnesylation to induce activation and oncogenic transformation. Farnesylated proteins other than *RAS* are also targeted by FTIs. It has been demonstrated that FTI-mediated tumor growth inhibition does not always correlate with *Ras* mutation status,[6] and other pathways such as rhodamine bright, PI3K/Akt/BAD (phosphatidylinositol 3–kinase/beta-alanyl-alpha-ketoglutarate transaminase/Bcl-XL/Bcl-2–association death promoter), or centromere-associated proteins E and F are potential targets for FTIs.[7] Interestingly, FTIs abrogate p38 mitogen-activated protein kinase (MAPK) activation,[8] shown to be up-regulated in the majority of patients with PV.[9] Tipifarnib is a nonpeptidomimetic inhibitor of farnesyl transferase with demonstrated activity in human tumor cell lines with either wild-type or mutant *Ras*.[10,11] In humans, tipifarnib has so far demonstrated clinical activity in advanced cancer, myelodysplasia, acute myelogenous leukemia, and multiple myeloma.[12-15]

In this study, we show that tipifarnib inhibits spontaneous erythroid progenitor growth from patients with PV and may be a novel therapeutic approach in polycythemia vera.

Study design

Patients

Thirteen consecutive patients with PV were included in the study after informed consent was provided. The diagnosis of PV was established according to the Polycythemia Vera Study Group diagnostic criteria. None of the patients had entered the blastic phase of the disease. Peripheral blood was collected during routine biologic control at diagnosis. The study was approved by the PV Nord group.

Reagents

The FTI tipifarnib was kindly provided by Janssen and preserved protected from air. Recombinant human EPO was purchased from Roche (Basel, Switzerland) and used at a final concentration of 2 U/mL.

From the Laboratoire de Biologie Cellulaire Hématopoïétique, Institut d'Hématologie and Service de Médecine Nucléaire, Hôpital Saint-Louis, Paris, France; Service Clinique des Maladies du Sang, Hôpital Saint-Louis, Paris, France; and Unité de Thérapie Cellulaire, Hôpital Saint-Louis, Paris, France.

Submitted July 30, 2004; accepted December 30, 2004. Prepublished online as *Blood* First Edition Paper, January 4, 2005; DOI 10.1182/blood-2004-07-2949.

Supported by Janssen Research Foundation.

Reprints: Philippe Rousselot, Service Clinique des Maladies du Sang, Hôpital Saint-Louis, 1 avenue Claude Vellefaux, 75475 Paris cedex 10; e-mail: philippe.rousselot@chu-stlouis.fr.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 U.S.C. section 1734.

Cell preparation and semisolid clonogenic assay

Fresh peripheral blood mononuclear cells (PBMCs) from 13 patients with PV and bone marrow mononuclear cells (BMMCs) from 5 healthy donors were isolated by Ficoll-Hypaque gradient centrifugation. Positive selection of $CD34^+$ cells was performed in normal BMMCs from 3 donors by means of a magnetically activated cell sorting CD34 isolation kit (Miltenyi Biotec, Bergish Gladbach, Germany). For clonogenic assay, PV PBMCs were counted and seeded in semisolid medium (StemCell Technologies, Vancouver, BC, Canada) at a concentration of 5×10^5 mononuclear cells/mL in 35-mm Petri dishes, in the presence or absence of tipifarnib at a concentration of 10^{-9} to 10^{-6} M. Percentages of inhibition were expressed as the ratio between the number of colonies observed in control and in tipifarnib-treated samples. As a control for the nonspecific activity of tipifarnib, BMMCs and $CD34^+$ cells from healthy individuals (n = 5) were studied in the same conditions (BMMCs, 10^5 cells/mL; $CD34^+$ cells, 10^3 cells/mL). Spontaneous colony growth was assessed in the absence of exogenous cytokine in the culture medium. Ability of EPO to rescue PV PBMC colony growth inhibition by tipifarnib was performed by adding EPO (2 U/mL) to the culture medium in the presence or absence of tipifarnib. Results were expressed as the ratio between the number of colonies observed in EPO-treated control and in tipifarnib + EPO–treated samples. Cultures were incubated at 37°C and 5% CO_2 in air for 14 days, at which time colonies were visualized and scored with an inverted microscope, which allows the distinction between granulocyte macrophage colony-forming units (CFU-GMs), CFU-Gs, CFU-Ms, and BFU-E/CFU-Es. Analyses were performed in triplicate.

Statistical analysis

A Student *t* test was used for BFU-E number comparisons. A value of *P* less than .05 was used to define statistical significance.

Results and discussion

For all patients with PV tested, spontaneous growth of erythroid colonies was observed in the absence of EPO as expected. The median BFU-E number was 36 per 5×10^5 mononuclear cells (range, 15-130 per 5×10^5 mononuclear cells). No spontaneous BFU-E growth could be detected in PBMCs or $CD34^+$ cells from healthy donors (n = 5) tested in the same period. EPO induced a 4-fold increase of the median BFU-E number of PV PBMCs (median, 120 per 5×10^5 mononuclear cells; range, 55-230 per 5×10^5 mononuclear cells; $P < .001$). Tipifarnib inhibition of spontaneous BFU-E growth was assessed at 3 different concentrations: 15 nM, 150 nM, and 1.5 μM. As shown in Figure 1, tipifarnib-mediated inhibition was dose dependent with a mean percentage of inhibition of 41% ± 24% (n = 13 at 15 nM), 65% ± 19% (n = 12 at 150 nM), and 82% ± 9% (n = 8 at 1.5 μM). Of note, these concentrations can be easily achieved in patients with a daily dose ranging from 25 mg to 300 mg.[16]

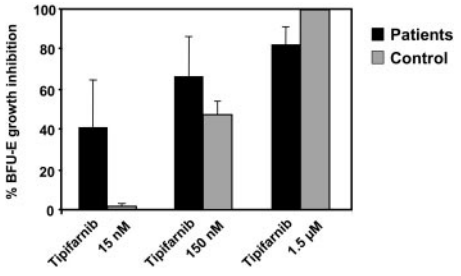

Figure 1. Dose-dependent effect of tipifarnib on spontaneous BFU-E growth. Peripheral blood mononuclear cells (5×10^5/mL) from patients with PV (■) were plated for 14 days in the presence of various concentrations of tipifarnib (15 nM to 1.5 μM) without addition of EPO in the medium and were BFU-E numbered. Results are given as mean ± SD of triplicate BFU-Es. Controls (▨) are BFU-Es obtained from bone marrow mononuclear cells of healthy donors cultured in the presence of EPO 2 IU/mL and tipifarnib (15 nM to 1.5 μM).

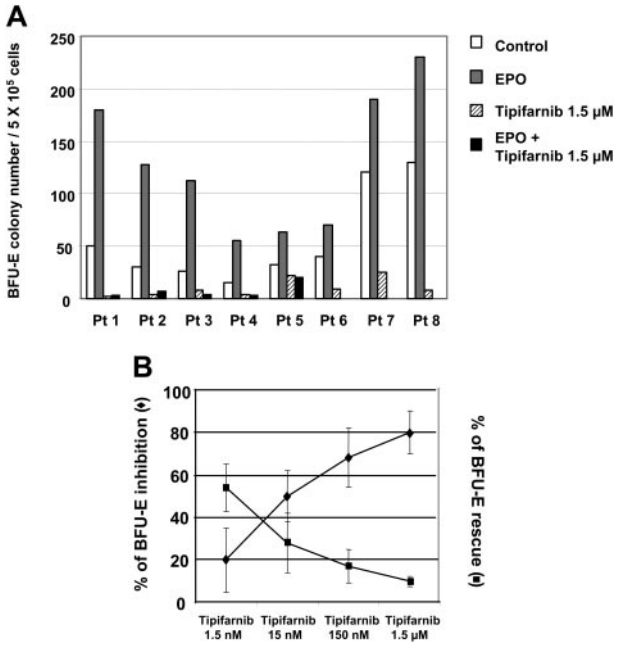

Figure 2. EPO does not relieve tipifarnib BFU-E growth inhibition. (A) BFU-E colony number in patients with PV (n = 8) at 1.5 μM tipifarnib concentration in the absence or presence of EPO. □ indicates control; ▨, EPO; ▨, tipifarnib; and ■, EPO + tipifarnib. Pt indicates patient number. (B) ◆ indicates dose-dependent BFU-E growth inhibition: ratio of BFU-E colony growth assessed in PV samples between medium alone and tipifarnib-treated samples (n = 8). ■ indicates EPO rescue of tipifarnib BFU-E growth inhibition: ratio of BFU-E colony growth between EPO- and tipifarnib + EPO–treated samples (n = 8). BFU-E colonies were counted at day 14 with each experiment assessed in triplicate. Error bars indicate 95% confidence intervals.

Tipifarnib-erythroid progenitor growth inhibition in patients with PV was next assessed in the presence of EPO. Mean number of BFU-Es was 128.5 ± 53 (n = 13) in the presence of EPO alone. In EPO-treated samples, addition of tipifarnib 1.5 μM drastically reduced BFU-E number in all patients to a mean value of 4.5 ± 2 (Figure 2A). Interestingly, presence of EPO did not rescue BFU-E growth inhibition. Inhibition persisted up to a 15-nM concentration of tipifarnib. Partial rescue was only observed at 1.5 nM of tipifarnib (Figure 2B). Thus, an inverse dose-response relationship was observed between inhibition of BFU-E colony formation and efficacy of rescue by EPO, suggesting that tipifarnib is able to interfere with EPO and that there may be other growth factor signaling pathways in PV precursor cells.

In order to assess the specificity of tipifarnib-induced growth arrest in BFU-Es from patients with PV, normal bone marrow purified mononuclear cells from healthy donors were incubated with identical concentrations of the drug in the presence of EPO 2 U/mL (n = 3). While tipifarnib equally inhibits BFU-E growth of normal progenitors, a 10-fold higher concentration is required to obtain the same inhibitory effect observed in PV erythroid progenitors (Figure 1). Furthermore, tipifarnib concentrations below 150 nM did not reduce normal CFU-GM or CFU-G colony numbers (n = 3; data not shown), indicating a specific effect of tipifarnib on PV BFU-Es.

Our results indicate that tipifarnib might be useful in the treatment of patients with PV. We found a differential effect of the drug on malignant and nonmalignant cells in the presence of EPO at tipifarnib doses below 150 nM. These observations suggest that

PV erythroid precursor cells might be more sensitive to tipifarnib than normal progenitor cells. The target of tipifarnib in PV remains unknown. As p38 MAPK up-regulation has been shown in patients with PV,[9] tipifarnib may either target upstream or downstream p38 MAPK signaling pathways and result in PV cell growth arrest. Imatinib mesylate, which targets tyrosine kinases encoded by oncogenes such as *Bcr-Abl, c-Kit,* and *PDGF-R,* reduces PV progenitor spontaneous growth but unlike tipifarnib fails to inhibit the BFU-E proliferation in the presence of hematopoietic growth factors.[17]

Conventional treatment of PV relies on myelosuppressive drugs such as hydroxyurea or pipobroman, which unfortunately may increase the risk of leukemia. Clinical trials with other drugs such as anagrelide or interferon alpha are currently being evaluated with the aim to propose alternative therapies. Nevertheless, as these compounds are not well tolerated and do not appear as effective as conventional treatments to achieve rapid and sustained long-term remission, other approaches should be tested.

Clinical trials with tipifarnib have shown that the drug was well tolerated. In a phase 1 study in refractory and relapsed leukemic patients, drug-induced myelosuppression (absolute neutrophil count $< 0.1 \times 10^9$) occurred only at the 600-mg level and above.[18] Our results suggest that tipifarnib used at low doses (around 100 mg daily) could be safely investigated in patients with PV.

References

1. Green AR. Pathogenesis of polycythaemia vera. Lancet. 1996;347:844-845.
2. Cervantes F, Tassies D, Salgado C, Rovira M, Pereira A, Rozman C. Acute transformation in nonleukemic chronic myeloproliferative disorders: actuarial probability and main characteristics in a series of 218 patients. Acta Haematol. 1991;85: 124-127.
3. Janssen JW, Steenvoorden AC, Lyons J, et al. RAS gene mutations in acute and chronic myelocytic leukemias, chronic myeloproliferative disorders, and myelodysplastic syndromes. Proc Natl Acad Sci U S A. 1987;84:9228-9232.
4. Sakai H, Karasawa M, Okamoto K, et al. Leukemic transformation in three patients with polycythemia vera: analysis of the clinicopathological features and N-ras gene mutation. J Med. 1996; 27:183-191.
5. Tsurumi S, Nakamura Y, Maki K, et al. N-ras and p53 gene mutations in Japanese patients with myeloproliferative disorders. Am J Hematol. 2002;71:131-133.
6. Nagasu T, Yoshimatsu K, Rowell C, Lewis MD, Garcia AM. Inhibition of human tumor xenograft growth by treatment with the farnesyl transferase inhibitor B956. Cancer Res. 1995;55:5310-5314.
7. Sebti SM, Hamilton AD. Farnesyltransferase and geranylgeranyltransferase I inhibitors and cancer therapy: lessons from mechanism and bench-to-bedside translational studies. Oncogene. 2000; 19:6584-6593.
8. Vervenne WL, Bos CL, Rens LS, Peppelenbosch MP, Richel DJ. Farnesyl protein transferase inhibition interferes with activation of MAP kinase family members in human peripheral blood monocytes. Mol Med. 2002;8:857-862.
9. Pellagatti A, Vetrie D, Cordelia F, et al. Gene expression profiling in polycythemia vera using cDNA microarray technology. Cancer Res. 2003; 63:3940-3944.
10. End DW, Smets G, Todd AV, et al. Characterization of the antitumor effects of the selective farnesyl protein transferase inhibitor R115777 in vivo and in vitro. Cancer Res. 2001;61:131-137.
11. Smith V, Rowlands MG, Barrie E, Workman P, Kelland LR. Establishment and characterization of acquired resistance to the farnesyl protein transferase inhibitor R115777 in a human colon cancer cell line. Clin Cancer Res. 2002;8:2002-2009.
12. Lancet JE, Rosenblatt JD, Karp JE. Farnesyltransferase inhibitors and myeloid malignancies: phase I evidence of Zarnestra activity in high-risk leukemias. Semin Hematol. 2002;39:26-30.
13. Johnston SR, Hickish T, Ellis P, et al. Phase II study of the efficacy and tolerability of two dosing regimens of the farnesyl transferase inhibtor, R115777, in advanced breast cancer. J Clin Oncol. 2003;21:2492-2499.
14. Adjei AA, Croghan GA, Erlichman C, et al. A phase I trial of the farnesyl protein transferase inhibitor R115777 in combination with gemcitabine and cisplatin in patients with advanced cancer. Clin Cancer Res. 2003;9:2520-2526.
15. Alsina M, Fonseca R, Wilson EF, et al. Farnesyltransferase inhibitor tipifarnib is well tolerated, induces stabilization of disease and inhibits farnesylation and oncogenic/tumor survival pathways in patients with advanced multiple myeloma. Blood. 2004;103:3271-3277.
16. Zujewski J, Horak ID, Bol CJ, et al. Phase I and pharmacokinetic study of farnesyl protein transferase inhibitor R115777 in advanced cancer. J Clin Oncol. 2000;18:927-941.
17. Oehler L, Jaeger E, Eser A, et al. Imatinib mesylate inhibits autonomous erythropoiesis in patients with polycythemia vera in vitro. Blood. 2003;102:2240-2242.
18. Karp JE, Lancet JE, Kaufmann SH, et al. Clinical and biologic activity of the farnesyltransferase inhibitor R115777 in adults with refractory and relapsed acute leukemias: a phase 1 clinical-laboratory correlative trial. Blood. 2001;97:3361-3369.