Imatinib mesylate inhibits autonomous erythropoiesis in patients with polycythemia vera in vitro

Short title: Imatinib mesylate inhibits autonomous erythropoiesis

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Summary

The overproduction of red blood cells in patients with Polycythemia Vera (PV) is reflected in vitro by the formation of erythroid-burst forming units (BFU-E) in the absence of exogenous erythropoietin. In contrast to other myeloproliferative disorders, the molecular mechanism of PV is unknown and no specific chromosomal abnormality has been described. We speculated that imatinib mesylate may reverse the pathological overproduction of red cells by inhibition of autonomous erythropoiesis. In the present study, imatinib mesylate was found to either block or strongly inhibit autonomous BFU-E formation in vitro in all patients tested. Moreover, autonomous BFU-E growth was also markedly reduced by exposure of PV cells to imatinib mesylate prior to cultivation in semisolid medium. The profound effect of imatinib mesylate on autonomous erythropoiesis suggests the involvement of an yet unidentified kinase in the pathogenesis of PV and should provide the rationale for a forthcoming clinical trial.

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Introduction

Polycythemia vera (PV) is a clonal disorder of the multipotential hematopoietic progenitor cell. The disease is characterized by increased red blood cells and elevated numbers of granulocytes and platelets are not uncommon. Two distinct populations of erythroid progenitor cells in PV marrow have been described, indicating the coexistence of a malignant and a nonmalignant population of hematopoietic progenitors.1 Erythroid progenitors of the malignant clone can form hemoglobinized colonies (burst forming units-erythroid, BFU-E) in the absence of exogenous erythropoietin (EPO) in vitro.2 Such spontaneous BFU-E growth is never observed in normal individuals and, thus, represents a highly characteristic feature in PV.3-5 EPO-independent BFU-E growth may either be due to the secretion of growth stimuli by the malignant clone or to the release of growth factors from accessory cells to hypersensitive PV-precursors.6-9 In this respect we were able to demonstrate that the cytokine-synthesis inhibiting molecule interleukin-10 is able to profoundly inhibit the spontaneous formation of erythroid colonies in PV by suppression of endogenous GM-CSF production.10 Although the mechanism for autonomous erythropoiesis remains unknown, mutations that involve tyrosine kinases may deregulate normal signaling pathways and render them constitutively activated without the requirement of the physiological stimulus. A growing number of oncogenes known to code for tyrosine kinases, including Bcr-Abl, c-Kit, EGF-R, PDGF-R, and Tel-Abl are involved in the pathogenesis of various malignancies.11 Recently, a rearrangement of the platelet-derived growth factor receptor beta (PDGF-RB) gene with TEL (ETV6), usually caused by a t(5;12)(q33;p13) translocation, in some cases of chronic myeloproliferative disease have been described.12,13 Although not known yet, similar mechanisms may cause growth alteration of a single aberrant pluripotent hematopoietic progenitor cell in PV thus resulting in excess production of mature red cells.

Imatinib mesylate (STI571, Gleevec) is a potent inhibitor of Bcr-Abl tyrosine kinase activity, and has recently been approved for the treatment of chronic myeloid leukemia (CML).14 This small molecule is a competitive antagonist of the ATP-binding site of Bcr-Abl tyrosine kinase domain and reverses the growth advantage of immature and mature malignant progenitor cells in CML.15 Imatinib mesylate does not exclusively inhibit Bcr-Abl, but also other tyrosine kinases encoded by proto-
oncogenes such as c-kit, and PDGF-R. Recently, Apperley et al described the successful treatment of four cases of MPDs with imatinib mesylate. In these patients the causative molecular abnormality was likely to be a rearrangement of the PDGF-RB gene. We show here, that in PV imatinib mesylate is a potent inhibitor of autonomous erythropoiesis in vitro. In contrast, imatinib mesylate exerts only minor effects on cytokine-stimulated colony growth derived from PV patients and normal controls.

Materials and Methods
Patients. Thirteen patients with PV as established by the diagnostic criteria of the Polycythemia Vera Study group were studied. Patients were treated by phlebotomy at the time of study and none of them had previously received cytostatic drugs or radioactive phosphorus. PB was collected from routine clinical controls after obtaining informed consent. Bone marrow was drawn from one patient who underwent bone marrow biopsy of the iliac crest at diagnosis.

Reagents: Imatinib mesylate was a kind gift of Novartis pharmaceutical Cooperation (Basel, Switzerland). Recombinant human interleukin (rhIL)-3 (Novartis, Basel, Switzerland), rhGM-CSF (R&D Systems, Minneapolis, USA), and rhEPO was purchased from Roche (Basel, Switzerland).

Colony assay. PBMCs (1x10^5 /mL) from PV patients (n = 13) or normal donors (n = 5) were cultured in semisolid medium either in the presence or absence of imatinib mesylate (0.01 to 10 µM). Autonomous colony growth was assessed in the absence of exogenous cytokines. Stimulated colony growth was investigated in the presence of EPO (2 U/mL), GM-CSF (10 ng/ml), and IL-3 (10 U/mL). Colonies containing more than 100 hemoglobinized cells were counted as BFU-E.

Results and Discussion

PBMCs of all PV patients enrolled in this study spontaneously formed erythroid colonies in the absence of exogenous EPO. As shown in Table 1, imatinib mesylate completely blocked autonomous BFU-E growth in 7 of 12 patients tested and
suppressed burst formation by at least 62% in the remaining five PV patients. The median BFU-E number was 8.5 (range, 2 to 126) /10^5 PBMCs in cultures without imatinib mesylate compared to 0 (range, 0 to 48) BFU-E /10^5 PBMCs in cultures containing 10 μM imatinib mesylate (p = 0.002). Spontaneous BFU-E formation was also completely suppressed in one patient from whom BMMCs could be obtained for in vitro studies (Table 1; Pat. E.P.). The amount of suppression was dose-dependent and, importantly, occurred at concentrations of imatinib mesylate that are also achieved in patients (Figure 1; Pat. S.E.). Thus, imatinib mesylate at a concentration of 1 μM suppressed autonomous BFU-E growth with a mean inhibition of 73% (n = 7, range of inhibition, 51 to 100%, p = 0.004).

The effect of imatinib mesylate on PV precursor cell growth was also assessed in the presence of the growth promoting molecules IL-3, GM-CSF and EPO. The median stimulated BFU-E number was 81.5 (range, 16 to 302) /10^5 PBMCs in methylcellulose cultures without imatinib mesylate and 53.5 (range, 13 to 137) in the presence of imatinib mesylate, respectively (p > 0.05). The minor suppression of erythroid colony formation in the presence of exogenous cytokines may be explained by a coexistence of both malignant and nonmalignant populations of hematopoietic progenitors. The addition of exogenous cytokines may thus enhance the outgrowth of normal BFU-E while PV progenitors are suppressed by imatinib mesylate. Alternatively, pharmacological doses of exogenous growth stimulators, as used in this study, may at least in part overcome imatinib mesylate induced inhibition of PV progenitors. It is known from the literature that imatinib mesylate has no or only minor effects on normal hematopoiesis in vitro. In agreement with these data, we observed no relevant inhibition of cytokine-stimulated colony growth from normal PBMCs by imatinib mesylate in concentrations up to 10 μM (data not shown).

We next investigated the effect of preincubation of PV cells with imatinib mesylate at a concentration of 10 μM. Following pretreatment with imatinib mesylate for 24 to 96 hours, cells were washed and autonomous BFU-E growth was subsequently assessed in semisolid medium. Exposure to the drug resulted in a time dependent inhibition of autonomous BFU-E growth. Interestingly, culturing with imatinib mesylate for 24 and 48 hours, respectively, did not sufficiently inhibit spontaneous burst formation. In contrast, a prolonged exposure up to 96 hours led to a 59% reduction of BFU E growth as compared to untreated cells (data not shown). Cell numbers after precultivation did not differ between culture flasks and there was no evidence of
increased cell death as evaluated by morphology and trypan blue exclusion. Therefore, it is unlikely that the observed effects on autonomous BFU-E growth were due to increased proliferation in the untreated cell cultures or, alternatively, by a toxic effect of imatinib mesylate on treated cells. It is therefore tempting to speculate that the drug specifically inhibits erythroid progenitor cells of the malignant clone.

In summary, this study demonstrates significant and dose dependent in vitro effects of imatinib mesylate on spontaneous burst formation of erythroid progenitors in patients with PV. Recently, R.T. Silver reported the results of imatinib mesylate treatment in seven patients with PV.21 These patients experienced a suppression of erythropoiesis as evidenced by the cessation of the need for phlebotomy. Regardless of the mechanism involved, those encouraging clinical findings and the results of our in vitro study should form the basis of a larger clinical trial for imatinib mesylate treatment in patients with PV.
References


Table 1. In vitro effect of imatinib mesylate on autonomous BFU-E growth in PV patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Source</th>
<th>Autonomous BFU-E ± SD / dish*</th>
<th>Percent of inhibition</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control 10 µM</td>
<td>Imatinib mesylate 10 µM</td>
<td></td>
</tr>
<tr>
<td>S.C.</td>
<td>PB</td>
<td>126 ± 24.6</td>
<td>48 ± 5.6</td>
<td>62</td>
</tr>
<tr>
<td>S.A.</td>
<td>PB</td>
<td>2 ± 0.6</td>
<td>0 ± 0</td>
<td>100</td>
</tr>
<tr>
<td>E.P.</td>
<td>BM</td>
<td>19 ± 8</td>
<td>0 ± 0</td>
<td>100</td>
</tr>
<tr>
<td>R.C.</td>
<td>PB</td>
<td>8 ± 1.5</td>
<td>0 ± 0</td>
<td>100</td>
</tr>
<tr>
<td>W.W.</td>
<td>PB</td>
<td>8 ± 3</td>
<td>0 ± 0</td>
<td>100</td>
</tr>
<tr>
<td>N.J.</td>
<td>PB</td>
<td>31 ± 8</td>
<td>2 ± 1</td>
<td>94</td>
</tr>
<tr>
<td>G.J.</td>
<td>PB</td>
<td>54 ± 6.1</td>
<td>5 ± 1</td>
<td>91</td>
</tr>
<tr>
<td>W.A.</td>
<td>PB</td>
<td>7 ± 2</td>
<td>0 ± 0.3</td>
<td>100</td>
</tr>
<tr>
<td>F.J.</td>
<td>PB</td>
<td>4 ± 2.5</td>
<td>0 ± 0</td>
<td>100</td>
</tr>
<tr>
<td>I.E.</td>
<td>PB</td>
<td>9 ± 0.6</td>
<td>0 ± 0</td>
<td>100</td>
</tr>
<tr>
<td>K.K.</td>
<td>PB</td>
<td>4 ± 1.1</td>
<td>0 ± 0.3</td>
<td>100</td>
</tr>
<tr>
<td>R.M.</td>
<td>PB</td>
<td>21 ± 2</td>
<td>1 ± 0.5</td>
<td>95</td>
</tr>
<tr>
<td>S.E.</td>
<td>PB</td>
<td>103 ± 13</td>
<td>9 ± 3</td>
<td>91</td>
</tr>
</tbody>
</table>

* mean number of erythroid bursts / 10^5 mononuclear cells ± SD from triplicates

Abbreviations: BFU-E, burst forming unit-erythroid; PV, polycythemia vera; SD, standard deviation; PB, peripheral blood; BM, bone marrow;
**Figure legend**

**Figure 1. Dose-dependent effect of imatinib mesylate on autonomous BFU-E formation.** PB-MNCs (1x10^5 /mL) from four patients with PV were cultured in semi-solid medium together with graded amounts of imatinib mesylate (0.01 to 10 µM) in the absence of exogenous growth factors. Autonomous BFU-E formation was assessed after a culture period of 14 days. The mean BFU-E numbers of triplicates (+ standard deviation) are given from one representative experiment (Pat. S.E.).
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