Brief Report

Reversal of bone marrow angiogenesis in chronic myeloid leukemia following imatinib mesylate (STI571) therapy

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Abstract

The effect of imatinib mesylate therapy on angiogenesis and myelofibrosis was investigated and compared with interferon (IFN) and hydroxyurea (HU) in 98 patients with newly diagnosed Ph+/BCR-ABL+ chronic myeloid leukemia in first chronic phase and no other pretreatment. By applying immunostaining (CD34) and morphometry a relationship between microvessel frequency and fiber density was detectable in initial bone marrow (BM) biopsies and sequential examinations after at least 8 months of therapy. First-line monotherapy with imatinib induced a significant reduction (normalization in comparison to controls) of microvessels and reticulin fibers. In most patients decrease in BM vascularity was associated with a complete cytogenetic response. A significant angiogenic effect was also observed after HU treatment, contrasting IFN administration or combination regimens (IFN+HU). In conclusion, our data support the angiogenic capacity of imatinib by normalization of vascularity. In contrast, hematological response following IFN treatment is independent from BM angiogenesis.

Key words: imatinib mesylate (STI571), angiogenesis, myelofibrosis, chronic myeloid leukemia, bone marrow biopsies
Introduction

Contrasting the wealth of data concerning efficacy of the tyrosine kinase inhibitor imatinib mesylate (STI571) in chronic myeloid leukemia (CML)\textsuperscript{1-6} scant knowledge exists about bone marrow (BM) findings. Preliminary results suggest a normalization of erythropoiesis accompanied by a marked reduction of granulocytes and megakaryocytes and a striking regression of fiber content.\textsuperscript{7,8} Concern has been expressed that cytoreductive pretreatment in the majority of these patients may have caused significant BM changes\textsuperscript{9} and therefore may obscure the specific imatinib-related effects. Persuasive evidence was provided that the microvascular endothelium within the hematopoietic microenvironment plays a pivotal role as gatekeeper controlling trafficking, differentiation, and homing of stem cells.\textsuperscript{10} Because imatinib selectively inhibits growth of primitive malignant progenitors,\textsuperscript{11} reports about increased vascularity in CML\textsuperscript{12,13} are of particular interest. Recent studies showed that levels of vascular endothelial growth factor (VEGF) expression were reduced in vitro by treatment with imatinib.\textsuperscript{14,15} In this context, preliminary data on BM biopsies confirmed the drug-related vascular effects.\textsuperscript{16} However, in this study the frequency of microvessels was determined by crude quantification without focus on architectural features of angiogenesis or long term changes of therapy. For this purpose, we examined the effect of imatinib induced on BM microvasculature and fiber content in patients without any pretreatment in comparison with other therapeutic regimens.

Study design

Patients

A multicenter evaluation of clinical records and BM biopsies was performed in 98 patients (median age 43 years) within first chronic phase of Ph\textsuperscript{+}/BCR-ABL\textsuperscript{+} CML following first-line therapy with imatinib, interferon-\textalpha\textsubscript{2}b (IFN), or hydroxyurea (HU). Eligibility included monotherapy for at least 8 months and a BM sample at diagnosis and at a (median) interval of 10-12 months. For 15 patients bone marrow examinations
at 2 years were available and 5 patients had sequential trephines after 3 years of therapy. In 14 patients imatinib was given at a dose of 400 to 600 mg per day, whereas 34 patients received IFN, 31 patients HU, and 19 patients a combination of IFN and HU. From the cohort of patients with first-line imatinib therapy 10 (71%) had a complete cytogenetic response, contrasting only 6 patients from the IFN group (18%) and 3 patients (16%) treated with IFN+HU. Finally, a control group of 25 patients without BM pathology was evaluated.

Morphometry and evaluation

Representative BM trephine biopsies were fixed in formalin, decalcified and paraffin wax embedded. According with other investigators immunohistochemistry with CD34 was applied as the endothelial antigen of choice for a proper identification of microvessels. Staining reaction is very reliable with CD34, although few and dispersed progenitor cells are costained which are easily distinguishable from endothelial cells. A blinded double-crosschecked morphometric analysis was performed on biopsies with an artefact-free mean area of 15.4 ± 4.6 mm² by three independent investigators and further checked for accuracy by two others. Evaluation of the microvasculature regarded not only incidence of vessels in a certain section area, but also parameters reflecting more properly functional aspects of blood flow like luminal width and especially tortuosity and branching (shape factor, aspect ratio, maximal length, form factor). Following Gomori's silver impregnation density of fibers was determined by the line-intersection count method using an ocular grid. According to this technique amount of fibers was expressed as intersections (i). Quantification of these parameters was carried out at 500 x magnification by randomly selecting 50 fields of 3.77 x 10⁻² mm² in each specimen (total of 1.884 mm² BM area per biopsy). Reference to cellularity (hematopoietic area) was necessary to avoid the erroneous impression of a reduction in the quantity of corresponding vascular structures and fibers following a therapy-related expansion of adipose tissue or interstitial edema. The difference of each patients' pre- and posttreatment values was calculated with regard to vascular parameters as well as fiber density. Furthermore, the relative incidence of individual changes was evaluated for each treatment group.
Results and discussion

The mean pretreatment value of 126 ± 50 for microvessel density (MVD) in the 98 patients with CML at diagnosis (Fig. 1a) contrasted significantly the normal control group (76 ± 26). According to the strictly defined treatment modalities imatinib and HU exerted a relevant impact on BM vascularity by showing a reduction in the number of vessels (Fig. 1b,c). This conspicuous feature was easily demonstrable by morphometry. On the other hand, IFN or a combination treatment with HU (IFN+HU) failed to normalize the MVD. Changes in luminal width included a wide range of microvessel area (MVA) in the HU group and after treatment with imatinib. These findings were also associated with an increased vascular roundness and decreased tortuosity of microvessels (Fig. 1c). Corresponding with the enhanced MVD the pretreatment biopsies in all patients showed a mean fiber density of 41.0 ± 25.2 opposed to a lower value of the controls (16.5 ± 5.3). Decrease in MVD during imatinib and HU therapy was associated with a reversal of myelofibrosis (Fig. 1d,e), contrasting relevant findings after IFN or combination regimens. After 3 months of treatment with imatinib in all patients hematological remission was observed, compared to response rates of 76% and 54% within 6 months for the IFN and HU group. In most imatinib-treated patients (90%) occurrence of cytogenetic response was correlated with a significant reduction of BM vascularity. Accordingly, patients with complete molecular response revealed a normalization of the MVD. However, in the groups with IFN administration alone or in combination with HU, due to the small number of cytogenetic responses no significant correlation with the MVD was observed.

Extending previous studies on angiogenesis the MVD is not only significantly increased in CML,12,13 but also related to myelofibrosis. These changes following imatinib, but not IFN therapy have to be discussed by considering the complex interactions of various mediators involved in the remodeling and vascularity of the BM stroma.20,12,21 A significant regression of BM fibrosis together with a marked decrease in megakaryocytes was noted in the majority of patients receiving imatinib.7,8 Contrasting HU comparable changes were not detectable after IFN treatment.19 It has been convincingly
demonstrated that imatinib reduces the BCR-ABL-mediated secretion of VEGF which is mainly responsible for the angiogenic effects of this drug. The significant correlation between number of VEGF-positive BM cells and MVD was supported by a corresponding decline of VEGF plasma levels in patients with decreased vascularity. Furthermore, in most patients cytogenetic response was also associated with a reduction of BM vascularity in the imatinib group. Interestingly, despite the putative angiogenic effect of IFN, involution of MVD lagged behind the reduction of the neoplastic population. Comparable observations were made by other groups that investigated different hematological disorders. On the other hand, recent data obtained from cultured human endothelial cells produced evidence that HU downregulates endothelial gene expression. This pathomechanism might be responsible for the reduced MVD observed in our HU-treated cohort. Since imatinib also targets platelet derived growth factor receptor activity, normalization of vascular structures and fiber density may be also influenced by effects exerted on megakaryopoiesis by neutralizing its stimulating function on the BM stroma.

In conclusion, the normalization of BM vascularity detectable during first-line treatment with imatinib is in most patients associated with complete cytogenetic response contrasting IFN therapy.
Table 1. Morphometric evaluation of bone marrow angiogenesis in 98 patients with Ph\(^1\)/BCR-ABL \(^{+}\) CML showing individual absolute and relative changes (difference between pre- and posttreatment values) following first-line therapy in sequential biopsies.

<table>
<thead>
<tr>
<th></th>
<th>IMATINIB</th>
<th>IFN</th>
<th>HU</th>
<th>IFN + HU</th>
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</thead>
<tbody>
<tr>
<td><strong>No. of patients</strong></td>
<td>14</td>
<td>34</td>
<td>31</td>
<td>19</td>
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<tr>
<td><strong>Median biopsy intervals</strong></td>
<td>12</td>
<td>10</td>
<td>16</td>
<td>11</td>
</tr>
<tr>
<td><strong>Absolute changes during therapy</strong> (mean ± SD)</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Fiber density (i x 10(^2))</td>
<td>- 13.5 ± 13.4 (a)</td>
<td>+ 30.7 ± 34.0</td>
<td>+ 0.1 ± 27.1</td>
<td>+ 31.1 ± 53.4</td>
</tr>
<tr>
<td>(per mm(^5) hematopoiesis)</td>
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<tr>
<td>Microvessel density (MVD)</td>
<td>- 32.7 ± 52.5 (a)</td>
<td>+ 17.8 ± 52.2</td>
<td>- 2.6 ± 51.9</td>
<td>+ 17.4 ± 62.0</td>
</tr>
<tr>
<td>(per mm(^5) hematopoiesis)</td>
<td></td>
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<tr>
<td>Microvessel area (MVA)</td>
<td>+ 48.6 ± 204.7 (a)</td>
<td>- 0.4 ± 78.9</td>
<td>+ 31.7 ± 144</td>
<td>+ 7.8 ± 138.8</td>
</tr>
<tr>
<td>(µm(^2))</td>
<td></td>
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<tr>
<td>Tortuosity of microvessels</td>
<td>+ 0.8 ± 9.8</td>
<td>- 0.1 ± 2.4</td>
<td>+ 0.5 ± 3.7</td>
<td>- 0.6 ± 3.6</td>
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<tr>
<td>(maximal length in µm)</td>
<td></td>
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<tr>
<td>Aspect ratio</td>
<td>+ 0.17 ± 0.87</td>
<td>- 0.14 ± 0.43</td>
<td>- 0.67 ± 0.52</td>
<td>- 0.47 ± 0.47</td>
</tr>
<tr>
<td>Form factor</td>
<td>- 0.18 ± 0.06</td>
<td>- 0.02 ± 0.04</td>
<td>+ 0.01 ± 0.05</td>
<td>+ 0.04 ± 0.06</td>
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<tr>
<td>(circular deviation)</td>
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<td><strong>Relative changes during therapy</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Fiber density decrease / stable increase</td>
<td>78.6 % (a)</td>
<td>23.4 %</td>
<td>50.3 %</td>
<td>30.4 %</td>
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<tr>
<td>Fiber density increase</td>
<td>21.4 %</td>
<td>76.6 %</td>
<td>49.7 %</td>
<td>69.6 %</td>
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<tr>
<td>Microvessel density (MVD)</td>
<td>78.6 % (a)</td>
<td>37.5 %</td>
<td>55.2 %</td>
<td>33.3 %</td>
</tr>
<tr>
<td>decrease / stable increase</td>
<td>21.4 %</td>
<td>62.5 %</td>
<td>44.8 %</td>
<td>66.7 %</td>
</tr>
</tbody>
</table>

\(a\) Imatinib versus IFN \(p < 0.01\) (Mann-Whitney U-test)
References


Caption to figure 1

Bone marrow angiogenesis and myelofibrosis after first-line imatinib therapy of chronic phase Ph+/BCR-ABL+ CML: (a) the pretreatment bone marrow biopsies reveal a significantly increased number of microvessels with enhanced tortuosity and branching. (b,c) following imatinib treatment a remarkable reduction of vascularity (b) accompanied by increased vascular roundness and decreased tortuosity of microvessels (c) is observable. These findings are generally associated with a reduction of the fiber content and occurrence of cytogenetic response (d - pretreatment biopsy, e - 8 months after imatinib treatment)

a-c CD34 immunostaining
d,e Gomori silver impregnation
a,b,d  x180; c,e x380
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