Potent Antileukemic Activity of the Novel Agents Norsegoline and Dibezone

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ABSTRACT

We examined the effect of norsegoline, a natural marine product, and dibezone, a synthetic product, on the survival of human myeloid progenitor cells [colony-forming unit-cells (CFU-C)] from normal individuals and from 10 patients with Philadelphia-positive chronic myelogenous leukemia (CML) in chronic phase and blastic crisis. We compared their effect to the effect of IFN-α. Norsegoline, dibezone, and IFN-α inhibited the proliferation of CFU-C in a dose-dependent manner. The number of CFU-C from bone marrow (BM) of five CML patients in chronic phase exposed for 16 h to norsegoline (10⁻⁸–10⁻⁶ M), dibezone (10⁻⁸–10⁻⁶ M), and IFN-α (500 units/ml) was found to be statistically lower (P < 0.05) than the number of CFU-C derived from normal individuals. A 16-h drug exposure of CD34⁺ cells isolated from the peripheral blood of three CML patients in blastic crisis and from BM of two patients in chronic phase resulted in a marked inhibition in the ability of the cells to proliferate in liquid culture and a reduction in CFU-C content. Using the fluorescent in situ hybridization technique, we evaluated detection of the BCR/ABL fusion product in the CD34⁺ cells. All five patients were 100% Philadelphia positive at diagnosis. BCR/ABL translocations were detected in 94.6 ± 0.6% of cells following their growth in liquid culture for 7 days. Following exposure of CD34⁺ cells to norsegoline, dibezone, or IFN-α, BCR/ABL fusion signals could be detected in 73 ± 11%, 66.5 ± 4.7%, and 66.0 ± 2.5% of cells from BM and 72.3 ± 5%, 68.8 ± 7%, and 60.6 ± 6.8% of peripheral blood, respectively. Our data indicate that norsegoline and dibezone have in vitro an antileukemic effect against Philadelphia-positive cells and may be used in conjunction with currently available agents for ex vivo purging of BM and/or peripheral blood of CML patients in conjunction with autologous bone marrow transplantation.

INTRODUCTION

Norsegoline is a novel marine alkaloid derived from the marine tunicate Eudistoma sp. which initially displayed growth regulatory properties on mouse neuroblastoma cells and hamster fibroblasts (1). This agent has a fused tetracyclic pyrido[2,3,4-k]acridine ring system that was identified in some aromatic alkaloids isolated from diverse marine organisms including sponges, tunicates, and sea anemones (2). Reports concerning the biological activities of the aromatic alkaloids indicate that many of them are cytotoxic to a variety of cancer cell lines (2).

The analysis of marine organisms from a wide range of ocean habitats has shown that the marine environment is a rich source of many important pharmacological compounds including antineoplastic drugs (3–5). 1-β-D-arabinofuranosylcytosine, which is used clinically as an antineoplastic drug in acute myeloid leukemia (6, 7), was discovered as a result of research on arabinosyl nucleosides originally isolated from the Caribbean sponge Tethya crypta (5). Jasplamid, a naturally occurring cyclic peptide isolated from several marine sponges including the most recently investigated Hemiasterella minor (8), was found by Stingl et al. (9) and Senderowicz et al. (10) to demonstrate cytotoxicity to a number of tumor-derived cell lines including breast and prostatic cancers, whereas we have shown that Jasplamid inhibits self-renewal capacity of leukemic progenitors and induces immunophenotypic maturation of leukemic cell lines and of blasts from acute myelogenous leukemia patients (11).

Bioactive marine alkaloids are lead compounds for new drugs. In connection with our synthetic program of pyrido[2,3,4-k]acridines, we have prepared a series of relatedaza-heterocycles (12–15). One of these compounds was dibezone, dibenz[a,c]sa,6,7,8,9a-hexahydrophenazine, a condensation product of phenanthroquinone and trans-1,2-diaminocyclohexane.

In the present study we evaluated the inhibitory effect of norsegoline and dibezone on the growth of myeloid progenitors from normal individuals and CML patients in chronic phase and in blastic crisis. The inhibitory effect was compared to the effect of IFN-α, a known inhibitor of CML proliferation which is currently in practice in CML (16). We found that marrow colony-forming units of CML patients in chronic phase were more sensitive to the drugs than normal marrow progenitors. Next, we proceeded and examined the effect of the drugs on CD34⁺ cells. All three compounds inhibited proliferation of...
CD34+ cells isolated from BM or peripheral blood of CML patients in chronic phase or in blastic crisis. Using the FISH technique we have shown that a marked decrease in the level of BCR/ABL fusion signals could be detected following exposure of CD34+ cells to norsegoline, dibezine, or IFN-α prior to their growth in liquid culture.

MATERIALS AND METHODS

Materials

The structures of norsegoline (M, 306) and dibezine (M, 286) are shown in Fig. 1. Norsegoline was isolated and purified from the Red Sea purple tunicate Eudistoma sp. as described previously (17). Synthesis of dibezine dibenzo[a,c]Sa,6,7,8,9,9a-hexahydrophenazine was as follows: phenanthrene-quinone (0.5 g) was refluxed with trans-1,2-diaminocyclohexane (0.3 g) in ethanol (20 ml) for 30 min to afford the condensation product in 85% yield. Yellow crystals were: melting point 173°C; mass spectra m/z 286 (100%) (C20H18N2), 285 (29), 186(49), 114(22), 97(46); 1H NMR (CDCl3) δ 8.25 (1H, d, J = 8 Hz), 7.42 (1H, t, J = 8 Hz), 7.00 (1H, d, J = 8 Hz), 7.12 (1H, s), 6.93 (1H, d, J = 8 Hz), 2.85 (1H, d), 2.50 (1H, d), 1.85 (1H, d), 1.68 (1H, d), 1.48 (1H, d); 13C NMR (CDCl3) 153.0 (s), 133.1 (s), 131.4 (d), 131.0 (s), 128.3 (d), 126.2 (d), 123.0 (d), 58.7 (d), 33.6 (s), 25.7 (t). A stock solution of 10−3 M norsegoline or dibezine was prepared in DMSO. The drugs were diluted prior to use with IMDM (GIBCO, Grand Island NY) to the appropriate concentration. Lyophilized recombinant INF-α (Hoffman LaRoche, Nutley, NJ) was dissolved in IMDM immediately prior to use. Human IL-3 produced in Escherichia coli was a generous gift from Genetics Institute, Inc. (Cambridge, MA). FBS was purchased from Hyclone Laboratories (Logan, UT). The mouse mAb anti-HPCA-2 (CD34; clone S9G12, IgG1) and the irrelevant, isotype-matched (IgG1) anti-rota virus mAb were purchased from Becton Dickinson (Rodemark, Germany). FITC-conjugated goat anti-mouse IgG was purchased from Bio-Yeda (Rehovot, Israel).

Patients

Ten CML patients (median age, 38 years; range, 10–63) were studied (six males and four females). Six patients were in chronic phase and four patients were in blastic crisis. BM cells obtained from CML patients in chronic phase and peripheral blood samples obtained from CML patients in blastic crisis were taken before the initiation of any treatment. In addition, human BM cells were obtained from three consenting individuals undergoing cardiac surgery who had no hematological disorders and served as normal controls. Marrow and peripheral blood specimens were obtained with informed consent.

Cell Preparation

Peripheral blood and marrow cells were aspirated in the presence of heparin to prevent clotting. The cells were then washed and resuspended in IMDM. Light-density (<1.077 g/cm3), nonadherent cells were obtained by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density centrifugation, and mononuclear cells were adhered to plastic tissue culture dishes in IMDM with 7.5% FBS. The nonadherent cells were removed after 60-min incubation at 37°C as described previously (18).

Purification of CD34+ Cells

The purification of CD34+ cells was performed as described previously (19). Briefly, BM and peripheral blood of the CML patients were separated on Ficoll-Hypaque. Mononuclear cells (25 × 106) were incubated in 4 ml 0.5% Sandoglobulin (Sandoz, Basel, Switzerland) for 15 min at room temperature. The cells were then layered onto a T-25 MicroCELLector cell culture flask (AIS, Menlo Park, CA) that was bound to soybean agglutinin for 1 h at room temperature. The resulting nonadherent soybean agglutinin cell population was consequently layered onto a T-25 MicroCELLector flask bound to anti-CD34 mAb. Following an 1-h incubation at room temperature, the supernatant was decanted, the flask was gently washed, and the adherent CD34+ cell population was dislodged in PBS/10% FBS by hitting the side of the flask. These cells were >85% CD34+ according to fluorescence-activated cell sorting analysis.

Exposure of Nonadherent Mononuclear BM Cells to Norsegoline, Dibezine, or IFN-α and Agar Cultures

Nonadherent mononuclear BM cells from CML patients in chronic phase or normal individuals were brought to a concentration of 1 × 106 cells/ml in IMDM containing 15% heat-inactivated FBS, 2 mM glutamine, and antibiotics. The cells were exposed to norsegoline (10−6 to 10−8 M), dibezine (10−12 to 10−10 M), and IFN-α (500 and 1000 units/ml; control cells were incubated in medium) for 4 or 16 h, after which time the cells were washed twice in PBS and cultured in agar as described previously (18). Briefly, 2 × 105 cells in 1 ml IMDM containing 15% FBS, 15 units/ml IL-3 (complete medium), and 0.3% agar were added to 35-mm Petri dishes. Cultures were incubated at 37°C in a humidified atmosphere of 5% CO2 in air. Myeloid colonies (CFU-C) containing more than 40 cells were counted on day 10 using an inverted microscope.

Exposure of CD34+ Cells to Norsegoline, Dibezine, or IFN-α and Secondary Plating

The purified CD34+ cells were suspended at a concentration of 1 × 106 cells/ml in IMDM containing 15% FBS. Norsegoline (10−6 M), dibezine (10−6 M), or IFN-α (500 U/ml) were added, and the cells were incubated for 16 h at 37°C in a 5% CO2/95% air atmosphere. Control CD34+ cells were precultured with medium. Following incubation the cells were washed twice with PBS, resuspended in IMDM, and 3 × 104 cells were plated in agar. For suspension culture following exposure of the CD34+ cells for 16 h to the drugs, as indicated above, 1 × 105...
cells were plated in flat-bottomed 24-well tissue culture plates (Lindbro; Flow). After incubation for 7 days in the presence of 15 units IL-3/ml in liquid culture at 37°C in 5% CO₂/95% air atmosphere, the cells were collected, counted, and replated in agar culture at a concentration of 3 × 10⁵ or 2 × 10⁵ cells/ml (for chronic phase and blastic crisis, respectively) in the presence of 15 units/ml IL-3. Colonies that gave rise to 40 or more cells were counted on day 10 as described above.

Flow Cytometry

Cells (5 × 10⁵) were incubated with anti-CD34 mAb or irrelevant isotype control mAb. Cells were incubated at 4°C for 30 min, washed twice with PBS, and 20 μl FITC Ab were added. The cells were incubated for an additional 30 min at 4°C, and fluorescence was measured on a fluorescence-activated cell sorting 440 fluorocytometer (Becton Dickinson, San Jose, CA).

FISH Analysis

Probes. The v-abl probe was a 1740-bp genomic HindIII fragment. The bcr probe was a 6.5-kb genomic BamHI fragment. Both probes were inserted into the blue script vector by standard cloning methods (20). Plasmids were labeled by biotin 14-dUTP and digoxigenin 11-dUTP (abl and bcr, respectively) using a nick translation system (BRL) as recommended by the manufacturer.

Slide Preparation. Fresh CD34⁺ cells obtained from BM and peripheral blood of CML patients at time 0 and cells grown in liquid culture following pretreatment with the drugs were suspended in hypotonic medium (KCl, 0.075 M) and were fixed five times in methanol acetic acid (3:1; Refs. 21 and 22). Air-dried slides were incubated at 60°C overnight. Just before hybridization, slides were treated with RNase (100 μl/ml; 100 μg/ml) in 2× SSC at 70°C for 60 min, washed (three times), dehydrated in an ethanol series at 23°C, and air dried. The slides were then digested with proteinase K (100 pg/ml) for 8 min at 37°C and dehydrated in an ethanol series. Air-dried slides were denatured for 5 min at 70°C in 2× SSC, 70% formamide, 50 mM NaH₂PO₄ (pH 7.0), chilled in 70% ethanol at 0°C, further dehydrated in ethanol, and air dried (22).

Hybridization and Washing. Equal volumes of prepared probes were mixed: 30 μl/slide were added and hybridized overnight at 37°C in a moist dark chamber. Avidin Texas Red (Vector Laboratories, Burlingame, CA) diluted 1:1000 was added and incubated for 30 min at 37°C. The slides were again incubated for 30 min at 37°C with biotinylated goat antiavidin (Vector) diluted 1:100 (amplification step). After the amplification, washing and blocking steps were repeated. Both biotin and digoxigenin signals were amplified simultaneously with mouse antidigoxigenin (Sigma) diluted 1:500 and avidin Texas Red. After the washing and blocking steps were repeated, the digoxigenin signal was fluorescently labeled with rabbit anti-mouse FITC (Sigma) diluted 1:1000, and the biotin signal was further amplified with diluted biotinylated goat antiavidin. The washing and blocking steps were repeated and both signals were further amplified with goat anti-rabbit FITC diluted 1:1000 and avidin Texas Red.

The DNA was stained for 3 min with 4,6-diamidino-2-phenylindole (Sigma) in McIlvane’s buffer (100 μl/slide at 750 ng/ml) and mounted with antifade medium consisting of 2% (w/v) 1,4-diazobicyclo(2, 2, 2)-octane, 9 parts glycerol, 1 part 0.2 M Tris-HCl (pH 7.5), and 0.2% Na₂. Microscopy was performed using an Olympus BHS microscope with fluorescence equipment and filter for simultaneous viewing of FITC and Texas Red (Imagene Optics). One hundred fifty to 300 cells in interphase were analyzed. The slides were blindly scored. Hybridization with the BCR and ABL plasmids yielded small, but distinct signals in interphase nuclei. In normal interphase nuclei, two green and two red fluorescent signals were seen resulting from the hybridization of the BCR and ABL genes to chromosomes 22 and 9. The signals were randomly distributed throughout the nucleus. In contrast, “abnormal cells” exhibited interphase nuclei that were red-green resulting from the colocalization of the BCR-ABL gene fusion, in addition to one red and one green normal fluorescent signal.

Statistical Analyses

Statistical analyses were performed using Student’s t test.

RESULTS

Comparison of the Effects of Norsegoline, Dibezone, and IFN-α on CFU-C Growth from BM of Normal Individuals.

The cloning efficiency of nonadherent BM cells isolated from normal individuals incubated in the presence of 15 units/ml IL-3 was 55 ± 7.5 CFU-C per 1 × 10⁵ cells. No change in the number of CFU-C was observed when cells were preincubated in medium alone for 4 or 16 h before plating in agar. Fig. 2 indicates that exposure of the cells to norsegoline, dibezone, or IFN-α inhibited the proliferation of CFU-C in a dose-dependent manner. Similar inhibition effects were observed following incubation of the cells with the above-mentioned drugs for 4 h or 16 h (Fig. 2). The remaining experiments were performed following preincubation of the cells with the drugs for 16 h.

Effect of Norsegoline, Dibezone, and IFN-α on CFU-C Growth from BM of Normal Individuals versus CML Patients.

The cloning efficiency of nonadherent BM cells isolated from CML patients in the chronic phase in the presence of IL-3 was 76 ± 27/1 × 10⁵ cells (n = 5). A 16-h exposure of myeloid progenitors from CML patients and normal individuals to norsegoline, dibezone, and INF-α in liquid culture inhibited the subsequent proliferation of CFU-C in a dose-dependent fashion (Fig. 3).

The number of marrow colony-forming units of CML patients in culture were found to be statistically lower than those of normal individuals when exposed to norsegoline at concentrations of 10⁻⁶ to 10⁻⁸ M (P < 0.05) to dibezone at 10⁻⁶ to 10⁻⁸ M and to IFN-α at 500 units/ml (P < 0.05; Fig. 3).

In Vitro Growth of CD34⁺ Cells Isolated from CML Patients following Treatment with Norsegoline, Dibezone, and IFN-α.

CD34⁺ cells were isolated from the peripheral blood of three CML patients in blastic crisis and from BM of two CML patients in the chronic phase. The efficiency of extraction of CD34⁺ cells was 3.1 ± 1% (mean ± SE, n = 5). The selected cells were incubated for 16 h in the presence of norsegoline [10⁻⁶ M], dibezone [10⁻³ M], IFN-α (500 units/ml, or medium alone (control cells)]. Following incubation, the cells were washed, resuspended in fresh medium, and plated in

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aggar (primary CFU-C, Table 1). For suspension cultures the cells were grown in medium containing 15 units/ml IL-3 for an additional 7 days, after which the cells were collected and counted. Cells from aliquots were replated in agar until day 10 with 15 units/ml IL-3, and the number of CFU-C was determined.

As shown in Table 1, norsegoline (10^{-6} M) and dibezine (10^{-6} M) significantly inhibited primary colony formation by CD34^{+} cells. In suspension culture, the total cell number of the untreated cells increased following stimulation with IL-3 in comparison to the number of cells at day 0 (3.4- and 2-fold increase for peripheral blood and BM, respectively). A 16-h exposure to the drugs led to a variable decrease in the number of surviving cells and a decrease in the number of CFU-C.

**FISH Analysis of CD34^{+} Cells from CML Patients following Their Exposure to Norsegoline, Dibezine, and IFN-α.** We evaluated detection of the BCR/ABL fusion product in CD34^{+} cells isolated from the peripheral blood of three CML patients in blastic crisis and from the BM of two patients in the chronic phase following exposure of the cells for 16 h to norsegoline (10^{-6} M), dibezine (10^{-6} M), IFN-α (500 units/ml), or medium alone, after which the cells were grown in liquid culture in the presence of 15 units/ml IL-3 for 7 days. All five patients were 100% Philadelphia positive at diagnosis. BCR/ABL translocations were detected by FISH in 94.6 ± 0.6% of the untreated cells following 7 days in liquid culture, indicating that there is no decline in the percentage of Philadelphia-positive cells in culture as detected by FISH. A significant decrease in the BCR/ABL signal detection was observed in the progenitor cells from all five patients examined following their exposure to dibezine, and IFN-α. Our data demonstrate (Table 1) that ex-norsegoline (10^{-6} M), dibezine (10^{-6} M), or IFN-α (500 units/ml). As indicated in Fig. 4 the levels of BCR/ABL fusion signals
of normal individuals and BM and peripheral blood of CML are in correlate with those of Tabpaz. In our findings we have found that a 16-h exposure of myeloid progenitors from CML patients to 500 (16, 23-29). In the present study we have found that a 16-h proliferative effects of norsegoline and dibezine than progenitors. Exposure of CD34 cells (for 16 h) to the drugs resulted in an inhibition in the ability of the cells to proliferate in the presence of the cells to proliferate in the presence inhibition in the ability exposure of myeloid progenitors from CML patients to 500 (16, 23-29). In the present study we have found that a 16-h proliferative effects of norsegoline and dibezine than progenitors. These results are in contrast to Dowding et al. (31) who found no difference in the sensitivity of CFU-C to IFN-α between normal and CML patients. In our study we examined the sensitivity of CD34+ cells isolated from the peripheral blood and BM of CML patients in BLASTIC CRISIS and CHRONIC PHASE, respectively.

Norsegoline and dibezine inhibited colony formation in a concentration-dependent manner. Precursor cells from patients in the chronic phase of CML were more sensitive to the antiproliferative effects of norsegoline and dibezine than progenitors from normal individuals. IFN-α was shown to be therapeutically active against CML and to induce karyotypic remissions (16, 23-29). In the present study we have found that a 16-h exposure of myeloid progenitors from CML patients to 500 units/ml IFN-α resulted in enhanced inhibition of CFU-C growth as compared to that of normal individuals. Our findings are in correlate with those of Talpaz et al. (25) who have shown that treatment of CML patients with IFN-α resulted in a progressive decline of the BM-CFU-C and with McGlave et al. (30) who have shown that IFN-γ suppressed hematopoietic progenitors of CML patients to a greater extent than normal progenitors. These results are in contrast to Dowding et al. (31) and Williams et al. (32) who found no difference in the sensitivity of CFU-C to IFN-α from patients with BM and normal individuals.

The growth of CD34+ cells from CML patients in liquid and semisolid cultures following treatment with norsegoline, dibezine, and IFN-α is shown in Table 1. The table shows the number of CFU-C recovered from BM and peripheral blood of CML patients in three different conditions: none, norsegoline (10⁻⁸ M), and dibezine (10⁻⁶ M). The percentage of inhibition of CFU-C formation is also shown. The data are presented in a table format with the following columns: stage of CML disease, treatment, no. of colonies, % inhibition, no. of cells recovered (×10⁵), and % inhibition. The table is as follows:

<table>
<thead>
<tr>
<th>Stage of CML disease</th>
<th>Treatment</th>
<th>No. of colonies</th>
<th>% Inhibition</th>
<th>No. of cells recovered (×10⁵)</th>
<th>% Inhibition</th>
<th>No. of CFU-C recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blastic crisis</td>
<td>None</td>
<td>61.0 ± 4.2</td>
<td>0</td>
<td>6.8 ± 1.0</td>
<td>0</td>
<td>126.0 ± 8.9</td>
</tr>
<tr>
<td></td>
<td>Norsegoline (10⁻⁸ M)</td>
<td>40.2 ± 8.2</td>
<td>34</td>
<td>5.1 ± 6.1</td>
<td>25.0</td>
<td>58.3 ± 3.7</td>
</tr>
<tr>
<td></td>
<td>Dibezine (10⁻⁶ M)</td>
<td>37.1 ± 4.2</td>
<td>39</td>
<td>4.3 ± 0.7</td>
<td>37.6</td>
<td>77.0 ± 7.6</td>
</tr>
<tr>
<td></td>
<td>IFN-α (500 units/ml)</td>
<td>47.0 ± 1.8</td>
<td>23</td>
<td>5.2 ± 0.9</td>
<td>24.0</td>
<td>64.5 ± 8.0</td>
</tr>
<tr>
<td>Chronic phase</td>
<td>None</td>
<td>82.0 ± 7.5</td>
<td>0</td>
<td>4.0 ± 0.01</td>
<td>0</td>
<td>388 ± 72.0</td>
</tr>
<tr>
<td></td>
<td>Norsegoline (10⁻⁸ M)</td>
<td>57.2 ± 7.8</td>
<td>30</td>
<td>3.2 ± 2.6</td>
<td>18.5</td>
<td>252 ± 6.0</td>
</tr>
<tr>
<td></td>
<td>Dibezine (10⁻⁶ M)</td>
<td>62.3 ± 10.3</td>
<td>24</td>
<td>2.9 ± 0.8</td>
<td>26.6</td>
<td>167 ± 9.0</td>
</tr>
<tr>
<td></td>
<td>IFN-α (500 units/ml)</td>
<td>68.0 ± 8.0</td>
<td>17</td>
<td>3.2 ± 1.3</td>
<td>20</td>
<td>287 ± 6.5</td>
</tr>
</tbody>
</table>

* Values represent mean ±SE of experiments performed in duplicates (n = 3 and 2 for blastic crisis and chronic phase, respectively).
* CD34+ cells (1 × 10⁶ cells/ml) were incubated with the above-mentioned drugs for 16 h. Following incubation the cells were washed, resuspended in IMDM, and 3 × 10⁴ cells/ml were plated in agar. Colonies were counted on day 10.
* CD34+ cells (1 × 10⁶ cells/ml) were incubated with the above-mentioned drugs for 16 h. Following incubation the cells were washed, resuspended in complete medium, and 1 × 10⁴ cells/ml were plated in 24-well tissue culture plates. After incubation for 7 days in liquid culture, cells were collected and counted.

* CD34+ cells were treated with drugs and grown in liquid culture for 7 days as described in footnote . Following incubation, 3 × 10⁴ cells/ml (chronic phase) or 2 × 10⁵ cells/ml (blastic crisis) were plated in agar. Colonies were counted on day 10.

* p < 0.05

could be detected in 72.3 ± 5%, 68.8 ± 7%, and 60.6 ± 6.8% of the cells from the peripheral blood of patients in blastic crisis and 73.0 ± 11%, 66.5 ± 4.7%, and 66.6 ± 2.5% of the cells from BM of chronic phase patients, respectively.

**DISCUSSION**

In the present study we have investigated the effects of norsegoline, a novel aromatic alkaloid that was purified from the Red Sea purple tunicate Eudistoma sp., and dibezine, a synthetic product that was recently developed (by A. R and Y. K), on the proliferation of hematopoietic progenitor cells isolated from BM of normal individuals and BM and peripheral blood of CML patients in the chronic phase and in blastic crisis and compared it to the effect of IFN-α on these cells.

Norsegoline and dibezine inhibited colony formation in a concentration-dependent manner. Precursor cells from patients in the chronic phase of CML were more sensitive to the antiproliferative effects of norsegoline and dibezine than progenitors from normal individuals. IFN-α was shown to be therapeutically active against CML and to induce karyotypic remissions (16, 23-29). In the present study we have found that a 16-h exposure of myeloid progenitors from CML patients to 500 units/ml IFN-α resulted in enhanced inhibition of CFU-C growth as compared to that of normal individuals. Our findings are in correlate with those of Talpaz et al. (25) who have shown that treatment of CML patients with IFN-α resulted in a progressive decline of the BM-CFU-C and with McGlave et al. (30) who have shown that IFN-γ suppressed hematopoietic progenitors of CML patients to a greater extent than normal progenitors. These results are in contrast to Dowding et al. (31) and Williams et al. (32) who found no difference in the sensitivity of CFU-C to IFN-α from patients with BM and normal individuals.

In our study we examined the sensitivity of CD34+ cells isolated from the peripheral blood and BM of CML patients in blastic crisis and the chronic phase, respectively, to norsegoline, posure of CD34+ cells (for 16 h) to the drugs resulted in an inhibition in the ability of the cells to proliferate in the presence of the drugs.

Fig. 4 FISH analysis of CD34+ cells isolated from CML patients in blastic crisis (n = 3) and in the chronic phase (n = 2) following their exposure to norsegoline (10⁻⁸ M), dibezine (10⁻⁶ M), and IFN-α (500 units/ml). CD34+ cells were exposed for 16 h to the drugs and grown in liquid culture in the presence of 15 units/ml IL-3 for 7 days. Following incubation, FISH analysis was performed. One hundred fifty to 300 cells were analyzed from each of the five patients.
of IL-3, and that there was a reduction in CFU-C content. Using the FISH technique the data also suggest that lower levels of BCR/ABL fusion signals could be detected following exposure of CD34+ cells to nosogeline, dibezine, or IFN-α (Fig. 4). CML is a disease of the hematopoietic stem cells which generate an expanded pool of progenitors that undergo severe differentiation arrest during blast crisis. There is evidence that residual normal hematopoiesis persists during the disease stage (33–35), which could support autologous BM transplantation as an alternative therapy for CML following eradication of the Philadelphia-positive malignant clone. Indeed in several clinical trials, CML patients have been successfully transplanted with Philadelphia-negative autologous BM following long-term culture (36–39) or with Philadelphia-negative in vivo purged peripheral blood (40–42). The present data suggest that nosogeline and dibezine may serve as effective agents to be used in ex vivo purging of Philadelphia-positive cells from BM or peripheral blood of CML patients in conjunction with autologous BM transplantation.

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Potent antileukemic activity of the novel agents norsegoline and dibezone.

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