Antileukemic Effects of Deferoxamine on Human Myeloid Leukemia Cell Lines

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ABSTRACT

Deferoxamine (DFO) possesses antiproliferative activity against mitogen-stimulated lymphocytes, several tumor cell lines, and human leukemia and neuroblastoma cells. We have investigated its effects on the human myeloid leukemia lines HL-60, HEL, and U-937. In suspension culture, DFO causes a dose-dependent inhibition of proliferation of each cell line, with maximal inhibition observed at concentrations greater than 20 μM. These effects were prevented by cotreatment with iron salts and were at least partially reversible by removal of DFO from the culture system or addition of iron before 48 h of DFO exposure. Similar results were obtained in methylcellulose cultures of leukemic cells, with complete abolition of cell aggregates at day 7 in concentrations of 20 μM DFO or higher. DFO treatment caused a dose- and time-related decrease in DNA synthesis as measured by [3H]thymidine uptake, which was also reversed by treatment with iron salts. DFO caused slight reduction in RNA synthesis and did not affect protein synthesis. DFO caused significant antiproliferative effects on three myeloid leukemia cell lines, associated with inhibition of DNA synthesis, with in vitro effects observed at concentrations attainable in vivo. Evaluation of the antileukemic properties of DFO should continue.

INTRODUCTION

The iron chelator DFO inhibits DNA synthesis in normal human lymphocytes and thereby prevents their proliferation in response to mitogenic stimulation (1-4). DFO also possesses antitumor activity against leukemia and neuroblastoma cell lines and against malignant cells from patients with acute lymphoid leukemia, acute myeloid leukemia, and neuroblastoma (5-9). Additionally, an infant with refractory acute lymphoid leukemia responded briefly to DFO given with 1-β-D-arabinofuranosyl cytosine (10). For the most part, these antiproliferative and cytotoxic effects of DFO are prevented or reversed by concomitant treatment with stoichiometric amounts of iron salts. We have evaluated the effects of DFO on the human myeloid leukemia cell lines HL-60, HEL, and U-937. Our results demonstrate that DFO exerts a time- and dose-dependent, iron-reversible, antiproliferative effect on these cell lines, which is associated with an inhibition of DNA synthesis but not RNA or protein synthesis.

MATERIALS AND METHODS

Cell Lines. HL-60 (human promyelocytic leukemia) and U-937 (human histiocytic lymphoma) lines were obtained from American Type Culture Collection (Rockville, MD). HEL (human erythroleukemia) cells were a kind gift from Dr. Thalia Papayannopaulou, University of Washington. All cells were maintained in RPMI (RPMI 1640; GIBCO, Grand Island, NY; 10% heat-inactivated fetal calf serum, GIBCO; penicillin, 50 units/ml; and streptomycin, 20 mg/ml) at 37°C in 5% CO₂, split weekly, and fed twice weekly. Experiments were performed with cells in the logarithmic phase of growth.

Antiproliferative Effects of DFO. Cells from each cell line were cultured in 16-mm wells in triplicate at a concentration of 1 x 10⁵ cells/ml TCM. Deferoxamine mesylate (CIBA, Summit, NJ), with or without stoichiometric amounts of ferric chloride (Sigma, St. Louis, MO), was added as indicated in “Results.” DFO and FeCl₃ were dissolved in sterile H₂O and then diluted to 10⁻² M stock solution in RPMI. Viable cells, determined by trypan blue exclusion, were counted daily for 5 days. In some experiments, after variable periods of exposure to DFO, the cells were washed twice in Hanks’ balanced salt solution to remove DFO or FeCl₃ and was added to the DFO-treated cells, as described in “Results.”

Effect of DFO on Growth in Methylcellulose. Leukemic cells (1 x 10⁵) were added to 1.0 ml of 0.9% methylcellulose-containing 20% medium (Gibco), 15% fetal calf serum, and 10% human leukocyte-conditioned medium, with or without DFO and FeCl₃, as described in “Results.” Cell aggregates (>20 cells) were counted at 7 and 14 days. In some experiments, cells were treated with DFO in suspension culture for 24-72 h, washed twice in Hanks’ balanced salt solution, and then cultured in methylcellulose as described above, with or without supplemental FeCl₃.

DNA, RNA, and Protein Synthesis. Following 12- to 72-h treatment with various amounts of DFO, 100 μl of cell suspension (1 x 10⁴ viable cells/ml) were plated in 96-well microtiter plates. Cells were then pulsed with 1 μCi of [³H]thymidine (DNA synthesis), [³H]uridine (RNA synthesis), or [³⁵S]methionine (protein synthesis) (all from New England Nuclear, Boston, MA), for 4 h. Cells were harvested by an M-12 cell harvester (Brandel, Gaithersburg, MD), collected on filter paper, and dried. Uptake of radioisotope was measured by liquid scintillation, and results were expressed as the mean of triplicate values for each experimental point minus the mean value for three blood bank wells of TCM without cells.

RESULTS

Antiproliferative Effects of DFO. Each leukemic cell line proliferated rapidly in suspension culture, growing from 1 x 10⁵ cells/ml to confluence in 5-7 days. DFO exerted a potent dose-related antiproliferative effect on each line, with inhibition observed at doses as low as 10 μM (Table 1). At 20 μM DFO, the number of cells at day 5 was only 20-40% of that in control cultures, and at 50 μM DFO, only 12-20% of control. Although all three cell lines were inhibited by DFO, HL-60 was somewhat less sensitive than the others. The antiproliferative effects of DFO were abrogated at least partially by cotreatment with stoichiometric amounts of FeCl₃.

Although reductions in proliferation were observed as early as 24 h after treatment with 20 or 50 μM DFO, major inhibition was observed only after 48 h, after which only minimal further proliferation occurred (Fig. 1).

Exposure Time for DFO Effects. In some experiments, cells were treated with 20 or 50 μM DFO for various periods, after which stoichiometric amounts of FeCl₃ were added to the culture. Exposure to all concentrations of DFO (even 50 μM) for 12-24 h, followed by iron rescue, did not significantly inhibit 5-day cell counts (Table 2), and partial recovery of proliferative capacity was observed when FeCl₃ was added as late as 48 h. At some point between 48 and 72 h, however, the major effects of DFO were not reversible. Additionally, when DFO was washed from cultures up to 36 h after initial exposure and the cells were replated in TCM, day 5 cell counts were only minimally reduced (data not shown).

Growth in Methylcellulose. Cells from each line proliferated readily in 0.9% methylcellulose cultures, with aggregates of 20
cells or more becoming apparent within 4–5 days. Even with an initial concentration of cells of 0.5 × 10^6/ml and no added conditioning medium, control cells grew to confluence by day 14 so that colony or cluster enumeration was impossible at that point. DFO treatment greatly reduced the numbers of aggregates at day 7, even at low doses (Table 3). By day 14, all cells in DFO-treated cultures of 10 μM or more were dead. The antileukemic effects were reversed by cotreatment with iron or by addition of iron to the methylcellulose culture within 48 h (data not shown). In some experiments, leukemic cells were treated with 20 or 50 μM DFO for 24, 48, or 72 h in suspension culture, then washed twice, and cultured in methylcellulose, as described (1 × 10^6 viable cells/ml). With up to 48-h exposure, no decrease in 7-day aggregate counts was observed (Table 4). At 72 h, however, a dose-related reduction occurred, compared to controls, even though equal numbers of viable cells were used in treated and control cultures.

**DNA, RNA, and Protein Synthesis.** Levels of DNA, RNA, and protein synthesis were measured indirectly by quantitating [3H]thymidine, [3H]uridine, and [35S]methionine incorporation, respectively, with and without treatment with various doses of DFO. DFO caused a dose- and time-related decrease in [3H] thymidine uptake in each cell line (Table 5). DFO at 10 μM caused moderate decreases in uptake, while 20 and 50 μM treatment led to major reductions in cpm. These effects were observed initially at 36 h and were maximal after 48 h of DFO exposure. Cotreatment with stoichiometric FeCl₃ prevented the inhibition of DNA synthesis caused by DFO. Furthermore, addition of FeCl₃ within 24 h of DFO treatment prevented the
DEFEROXAMINE EFFECTS

Table 6  Effect of FeCl₃ addition on DNA synthesis in DFO-treated HL-60 cells

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>FeCl₃</th>
<th>Control</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>135,420</td>
<td>141,010</td>
</tr>
<tr>
<td>12</td>
<td>125,210</td>
<td>39,320</td>
</tr>
<tr>
<td>24</td>
<td>138,300</td>
<td>23,740</td>
</tr>
<tr>
<td>48</td>
<td>28,600</td>
<td>10,200</td>
</tr>
<tr>
<td>72</td>
<td>9,400</td>
<td>6,100</td>
</tr>
</tbody>
</table>

Table 7  Effect of DFO on DNA, RNA, and protein synthesis, as assessed by quantitation of [³²P]thymidine, [³H]uridine, and [¹⁵S]methionine uptake, respectively

<table>
<thead>
<tr>
<th>Cells</th>
<th>Uptake (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL-60</td>
<td>148,126</td>
</tr>
<tr>
<td></td>
<td>33,830</td>
</tr>
<tr>
<td></td>
<td>23</td>
</tr>
<tr>
<td>HEL</td>
<td>139,743</td>
</tr>
<tr>
<td></td>
<td>60,417</td>
</tr>
<tr>
<td></td>
<td>43</td>
</tr>
<tr>
<td>U-937</td>
<td>136,507</td>
</tr>
<tr>
<td></td>
<td>43,340</td>
</tr>
<tr>
<td></td>
<td>32</td>
</tr>
</tbody>
</table>

* Results are not statistically significantly different from controls in three experiments.

Inhibition of DNA synthesis at 48 h (Table 6). However, when DFO exposure persisted for 48 h or longer, no increase in [³H]thymidine uptake occurred following FeCl₃ addition. DFO treatment caused a slight reduction in [³H]uridine uptake at 50 μM but no significant reduction in [¹⁵S]methionine uptake (Table 7).

DISCUSSION

Dferoxamine inhibits the proliferation of three human myeloid leukemia cell lines, HL-60, HEL, and U-937. Although slight inhibition occurred at concentrations of DFO less than 10 μM, maximal inhibition was observed at 20 μM and greater concentrations. Initial effects on proliferation were observed after 24 h of continuous exposure to DFO and became maximal after 48 h. The antiproliferative effects of DFO were completely reversible, up to 24 h, by addition of stoichiometric amounts of FeCl₃ or by removal of washing of DFO from the culture medium and were partially reversible after 48 h.

Additionally, DFO inhibited the growth of myeloid leukemia cell lines in methycellulose cultures, with marked reduction in 7-day cell aggregate counts observed at 10 μM and higher concentrations. The effects were also reversible by addition of iron salts to methycellulose cultures. However, exposure to DFO (20 or 50 μM) for 72 h in suspension culture later led to a reduction of proliferative capacity on methycellulose, even in cells which remained viable and were no longer exposed to DFO.

DFO also caused a dose- and time-dependent reduction in DNA synthesis, as measured by [³H]thymidine uptake. These effects were also reversible if DFO was washed from the culture or if stoichiometric FeCl₃ was added by 24 h of DFO exposure. DFO had minimal effects on RNA synthesis and no effect on protein synthesis, as measured by [³H]uridine and [¹⁵S]methionine uptake, respectively.

Previously, DFO was found to inhibit cell division and DNA synthesis slightly and increase transferrin binding in K-562 cells (5). Additionally, blockade of iron uptake by suramin, used in combination with the lipophilic chelator pyridoxal isonicotinoyl hydrazine, led to an iron-reversible inhibition of cellular proliferation and DNA synthesis in U-937 but not K-562 cells (6). Our results extend these observations to include the effects of DFO on proliferation in suspension culture and semisolid media and on DNA, RNA, and protein synthesis in three distinct myeloid cell lines, HL-60 (promyelocytic), HEL (erythroleukemic), and U-937 (monocytoid). Similar results have been observed in DFO-treated peripheral blood lymphocytes (1–4) and neuroblastoma cell lines (7), as well as in cells from patients with neuroblastoma and acute leukemia (8, 9).

Blatt and Stitely (7) reported antineuroblastoma activity of DFO on the CHP126 and CHP100 human neuroblastoma cell lines, which was observed at concentrations of 15 μM or greater and which was prevented by pretreatment with ferric citrate. Additionally, we have recently reported cytotoxic and antiproliferative activity of DFO against bone marrow neuroblastoma cells and cells from patients with acute leukemia (8, 9). Thus, DFO seems to exert an antiproliferative and/or cytotoxic effect against multiple cell types at doses of 15–20 μM or greater. This effect is prevented or reversed by iron supplementation and is associated with inhibition of DNA synthesis. Additionally, normal human cells are only minimally inhibited by DFO, indicating antitumor selectivity (7, 11).

The antiproliferative effect of DFO may lead to further preclinical and clinical evaluation. DFO has been used extensively for over two decades as an iron chelator in patients requiring chronic RBC transfusions, without significant toxicity (12). Recently, Allain et al. (13) described a method for measurement of DFO levels and demonstrated that healthy, non-iron-overloaded controls develop peak plasma levels in the range of 15 μmol/liter following a single i.m. injection of 10 mg/kg body weight. Thus, plasma levels associated with in vitro antitumor response in our report and others should be attainable with easily tolerated i.v. doses. In fact, Estrov et al. (10) reported treating an infant with refractory leukemia with DFO, 10 mg/kg/h, by i.v. infusion 12–24 h a day for 6 days, in combination with low doses of 1-β-D-arabinofuranosyl cytosine. This regimen led to a partial remission, with clearing of peripheral blasts and in vivo differentiation to a myeloid phenotype. More recently, Wolf et al. (14) reported that mice with L1210 lymphocytic leukemia that was treated with DFO had prolonged survival compared to untreated animals.

In summary, we have studied the effects of the iron chelator DFO on three human myeloid leukemia cell lines, HL-60, HEL, and U-937. DFO inhibited proliferation of each cell line in both suspension and methycellulose culture systems, with initial effects observed at a concentration of 10 μM. DFO also caused a dose-related decrease in DNA synthesis, as determined by [³H]thymidine uptake. The antiproliferative effect of DFO on myeloid cells appears to be related to iron deprivation. These studies may provide a basis for further preclinical and clinical evaluation of DFO as an antileukemic agent.

ACKNOWLEDGMENTS

The authors wish to thank Rai Smith for typing the manuscript.
REFERENCES

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