Comparison of the Cytotoxic Effects of Merocyanine-540 on Leukemic Cells and Normal Human Bone Marrow

Jens Atzpodien, Subhash C. Gulati, and Bayard D. Clarkson

ABSTRACT

Various chemical compounds have been described to induce photosensitization of tumor cells resulting in cell death. We studied the effect of merocyanine-540 (MC-540) on both leukemic and normal bone marrow (BM) cells. Acute promyelocytic leukemia (HL-60) and common acute lymphoblastic leukemia antigen-positive non-T, non-B acute lymphoblastic leukemia (Reh) cell lines were incubated with MC-540 and simultaneously exposed to white light. Normal human BM and mixtures of leukemic cells with BM cells were treated under similar conditions. At constant illumination rates of 50,000 lx, significant (at least 4 to 5 logs) tumor cell destruction was obtained with MC-540 concentrations of 20 μg/ml or more for HL-60, and 10 μg/ml or more for Reh cells. Incubation of BM under equivalent conditions preserved 18.0% of granulocyte-macrophage colony-forming units and 14.2% of erythroid burst-forming units. Similar results were obtained when tumor cells were mixed with irradiated BM and then treated with MC-540. In summary, cell photosensitization with MC-540 has a selective cytotoxic effect towards leukemic cells and therefore may be useful for purging tumor cells from autologous BM.

INTRODUCTION

It has frequently been suggested that proliferation of tumor cells may be accompanied by various cell membrane alterations affecting their transport mechanisms, enzymatic activities, antigen expression, and bioelectric membrane potentials (1, 2). With regard to the bioelectric properties of cell membranes, probes have been developed which are capable of indicating small changes in electrical potentials as they may occur, e.g., in the course of tumor cell development and proliferation (3, 4). Merocyanine dyes, such as the fluorescent compound MC-540, have been used as bioelectric membrane probes and are extensively studied for their staining properties (3-6). It has been found that MC-540 is a fluorescent probe for membranes which selectively stains leukemic cells and some immature hematopoietic cells (7). Normal BM cells and leukemic cells bind MC-540 to the outer part of their membrane (5, 7, 8); however, dye uptake depends on cell membrane polarization and is different in normal hematopoietic cells and leukemic cells (9-11). Increased uptake of membrane-bound MC-540 occurs upon light exposure, which causes photodynamic dye excitation that eventually results in cell damage and cell kill (7). The mechanism of this MC-540-mediated cytotoxicity is not totally understood; however, various models have been developed to compare the cytotoxicity of MC-540 against leukemic cells and normal hematopoietic progenitors (9, 12).

The experiments reported here were designed to test the in vitro dose-dependent photodynamic effect of MC-540 on acute promyelocytic and acute lymphoblastic leukemic cells in established cell lines. Similar experiments were performed to assess the in vitro sensitivity of normal BM CFU-GMs and BFU-ES towards MC-540.

MATERIALS AND METHODS

Reagents. Merocyanine-540 was obtained from Eastman Kodak (Rochester, NY). Stock solutions of the dye were prepared in 50% ethanol-water at 1 mg/ml and stored in the dark at 4°C. For each experiment, proper concentrations of the reagent were obtained by diluting the stock with phosphate-buffered saline.

Light Source. A 75-W GE filament light bulb (luminous flux of approximately 1170 lumen) was used as the source of white light. At a radial distance of 15 cm, clear polystyrene test tubes (15 mm diameter) containing 2 ml of cell suspension were placed in a circular array around the light bulb. As measured through polystyrene tubes, using a Gossen Luna-Pro photometer, illumination from the light source was constant at 50,000 lx. Temperature of light-exposed cell suspensions was measured inside all the test tubes and found to be 37.0 ± 0.2 (S.D.) °C.

Cells. The acute promyelocytic leukemia (HL-60) cell line (13, 14) and the common acute lymphoblastic leukemia antigen-positive non-T, non-B acute lymphoblastic leukemia (Reh) cell line (15) were chosen for the experiments. Cell lines were maintained in RPMI 1640, supplemented with 10% FCS (Hy Clone, Logan, UT)-1% penicillin-streptomycin-neomycin (Gibco, Chagnin Falls, OH)-1% L-glutamine, and kept at 37°C in a humidified atmosphere of 5% CO2 in air. Cells were maintained under exponential growth conditions, with viability ranging from 95 to 99%. Both HL-60 and Reh cell lines were found to be free of mycoplasma contamination.

Bone marrow specimens were obtained from normal healthy volunteers after written, informed consent. The aspirations were done under local anesthesia from the posterior iliac crest using plastic syringes with preservative-free heparin. All specimens were processed immediately after aspiration.

Photodynamic Drug Effect Assays. HL-60 cells, Reh cells, and Ficoll-Hypaque-separated mononuclear cells from normal BM were incubated with different concentrations of MC-540 at 37°C and simultaneously exposed to white light at 50,000 lx for 1 h. A total of ten million cells were treated at a final concentration of 5 × 104 cells/ml in phosphate-buffered saline containing 0.25% albumin. In mixing experiments, tumor cells were incubated together with equal numbers of irradiated (4500 rads) BM cells. During the incubation period of 1 h, cells were kept in suspension by vortexing the test tubes gently every 5 min. After incubation, the cells were washed twice with RPMI 1640 before use in the assays described below.

For all tumor cell assays, the liquid culture system was chosen to evaluate MC-540-mediated cytotoxicity at multiple regular postincubation intervals, since exact data on posttreatment growth kinetics could not be obtained from clonal assays. In tumor cell assays and mixed tumor cell-BM assays, cells, after incubation, were resuspended in 15 ml of fresh media (RPMI 1640 supplemented with 10% FCS-1% penicillin-streptomycin-neomycin-1% L-glutamine) and kept for long-term culture in a humidified atmosphere of 5% CO2 in air. Daily cell counts and viability analyses (trypan blue exclusion) were performed. Cells were considered to be undetectable when a 10-fold concentrate showed no viable cells in 0.8 mm (i.e., both four-square areas) of the 0.1 mm improved Neubauer hemacytometer. Medium in each flask was changed every 4 days.

Bone marrow cells after incubation and two washes were resuspended...
MC-540 CYTOTOXICITY

RESULTS

The experiments described above allowed us to evaluate the *in vitro* differential sensitivity of HL-60 cells, Reh cells, and normal hematopoietic progenitors towards MC-540-mediated photosensitization.

Photodynamic Cytotoxicity of MC-540 against HL-60 and Reh. Both HL-60 and Reh cells (10 x 10^6) were incubated with up to MC-540, 25 µg/ml and exposed to light (50,000 lx) for 1 h. Both cell lines could be reduced to an undetectable number with MC-540 doses ranging from 10 to 25 µg/ml. MC-540 concentrations of 20 µg/ml or more for HL-60 and 10 µg/ml or more for Reh resulted in a tumor cell reduction of 4 to 5 logs at 72 h (Fig. 1). Following simultaneous exposure to MC-540 and white light, no regrowth of cells from either line was observed within 20 days, while control cells grew exponentially (see below). At lower MC-540 dosages (i.e., less than 20 µg/ml for HL-60 and below 10 µg/ml for Reh), cell numbers were still found to be markedly reduced following drug exposure; however, the remaining cells were able to regrow and reach numbers comparable to untreated control cells. In all experiments reported here, the maximum *in vitro* cytotoxic effect of MC-540-mediated photosensitization was observed within 1 to 24 h after exposure of cells. Cytotoxic effects from illumination-related hyperthermia could be ruled out. No significant difference in cell growth of controls was found when cells that had been exposed to light only in the absence of MC-540 were compared to cells that were incubated with MC-540 in the dark at 37°C and to cells that had been incubated at 37°C without dye and light exposure (Table 1).

Cytotoxic Effects of MC-540 on HL-60 and Reh Cells in Presence of Normal Bone Marrow. Similar cytotoxicity towards HL-60 or Reh cells was observed when incubation with MC-540 was carried out in the presence of irradiated BM cells (5 x 10^6). Under the same conditions of light exposure as before, a MC-540 concentration of 25 µg/ml was found to reduce cell numbers below the limit of accurate detection, while control cells (which were not exposed to the photosensitizing drug) were able to grow in logarithmic patterns (Fig. 2). To assess the extent to which the observed cell reduction could be attributed to a decrease in the number of viable BM cells following irradiation, BM cells (5 x 10^6) were treated alone with MC-540, and their cell counts were followed simultaneously with drug-treated mixtures of tumor cells and normal irradiated BM. Maximum reduction in both irradiated marrow cells and mixtures of irradiated BMs and tumor cells was observed within 92 h after drug exposure of cells. In all cell counts performed, no significant difference was found between cell mixtures (5 x 10^6 leukemic and 5 x 10^6 irradiated marrow cells) and 5 x 10^6 BM cells alone, indicating that any detectable cell population in the mixing studies reported must be attributed to the remaining number of normal marrow cells in the assay. In all mixing experiments performed with MC-540 concentrations of...
Table 1 Effect of different doses of MC-540 and/or light on tumor cells (HL-60 and Reh)

Cells (10 x 10^6) were incubated with different doses of MC-540 and either simultaneously exposed to white light (50,000 lx) or kept in the dark (0 lx) at 37°C for 1 h and washed twice with RPMI-1640 prior to long-term culture.

<table>
<thead>
<tr>
<th>MC-540 (µg/ml)</th>
<th>Illumination (lx)</th>
<th>Viable cells x 10^6 at different intervals after exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h</td>
<td>24 h</td>
</tr>
<tr>
<td>HL-60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>50,000</td>
<td>10.73*</td>
</tr>
<tr>
<td>10</td>
<td>50,000</td>
<td>9.63</td>
</tr>
<tr>
<td>20</td>
<td>50,000</td>
<td>10.27</td>
</tr>
<tr>
<td>Reh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>50,000</td>
<td>10.31</td>
</tr>
<tr>
<td>10</td>
<td>50,000</td>
<td>10.05</td>
</tr>
<tr>
<td>20</td>
<td>50,000</td>
<td>10.25</td>
</tr>
</tbody>
</table>

* Mean, calculated from three or more experiments.

Fig. 2. Effect of MC-540 on leukemia cell lines in mixtures with normal human marrow. Irradiated bone marrow cells alone (A, 5 x 10^6 cells; MC-540, 25 µg/ml) and mixtures of HL-60 cells (5 x 10^6 cells; MC-540: 0, 25 µg/ml) or Reh cells (5 x 10^6 cells; MC-540: 0, 25 µg/ml) with irradiated marrow cells (5 x 10^6 cells) were incubated with MC-540 at 37°C and simultaneously exposed to white light at 50,000 lx for 1 h, then washed twice with RPMI 1640 prior to long-term culture. At a drug concentration of 25 µg/ml, no regrowth of cells was observed after 20 days.

25 µg/ml, no regrowth of cells was observed over a 20-day period of long-term culture.

MC-540-mediated Photosensitization of Normal Hematopoietic Progenitor Cells. As compared to both HL-60 and Reh cells, normal hematopoietic progenitor cells were found to be less sensitive towards MC-540-mediated photodynamic cytotoxicity. At drug concentrations and illumination rates (25 µg/ml; 50,000 lx) sufficient to induce a tumor cell reduction of at least 4 to 5 logs, CFU-GMs and BFU-Es were inhibited by 82.0 and 85.8%, respectively (Fig. 3). Similar results were obtained with MC-540 dosages of 15 µg/ml, where 20.9% of CFU-GMs and 15.8% of BFU-Es were spared. Early erythroid progenitor cells appeared to be slightly more sensitive to MC-540 than did normal granulocyte-macrophage progenitors.

DISCUSSION

The data presented in this paper show MC-540 to be effective against malignant myeloid and lymphoid cell lines with minimal toxicity towards normal hematopoietic progenitor cells. These findings are in agreement with earlier reports indicating a preferential sensitivity of leukemic cells towards MC-540-mediated photosensitization (8–12). Though currently under further investigation, the basic mechanism of the selective cytotoxicity of MC-540 against neoplastic cells remains unclear. Both leukemia cells and human marrow progenitors are known to bind MC-540 on the cell surface (5, 7); however, it appears that either the electrical properties (6, 7) or the lipid composition (18) of neoplastic cell membranes allow MC-540 to preferentially penetrate and subsequently damage these cells. MC-540-incorporation has been shown to be substantially (4- to 5-fold) greater in leukemic cells than in normal human bone marrow mononuclear cells (7). These findings corroborate the results presented in this study.

We exploited the preferential sensitivity of two leukemic cell lines (HL-60 and Reh) towards MC-540 in order to evaluate this compound’s usefulness for the ex vivo purging of neoplastic cells from human bone marrow. The mixing studies and progenitor cell assays reported here strongly suggest that MC-540 doses of 25 µg/ml may be sufficient to induce at least 4 to 5 logs of tumor cell reduction in both acute myelogenous leukemia (HL-60) and acute lymphocytic leukemia (Reh) cell lines while sparing around 14 and 18% of normal human BM BFU-E and CFU-GM, respectively. Since common acute lymphoblastic leukemia antigen-positive Reh cells were found to be highly sensitive towards MC-540-mediated cytotoxicity, our data primarily indicate the usefulness of MC-540 for purging acute lymphoblastic leukemia cells; however, differences (e.g., in cell growth, drug clearance, and cell-killing mechanism) between the ex vivo purging of leukemia-contaminated BM and bone marrow progenitors may provide alternative therapeutic strategies.
experimental in vitro treatment of tumor cell lines mixed into normal human marrow may exist and may limit the final evaluation of these data.

Currently, a wide variety of different bone marrow purging techniques are under intensive investigation, including the use of cytotoxic drugs, antibodies, and differentiating agents as well as physical separation methods (19-27). Unlike most of these in vitro purging techniques, MC-540-mediated cell photosensitization may be equally useful in different types of malignancies presenting with BM involvement. As compared to the photo-toxic effects of MC-540, conventional chemotherapeutic agents have less selective toxicity for tumor cells compared to normal hematopoietic progenitors (19, 26, 27). Given this fact, it may be especially worthwhile to further explore the use of MC-540 for purging bone marrow of residual leukemic and other neo-plastic cells.

ACKNOWLEDGMENTS

The authors would like to thank Judy Reid and Lana Borzak for their excellent secretarial assistance.

REFERENCES

Comparison of the Cytotoxic Effects of Merocyanine-540 on Leukemic Cells and Normal Human Bone Marrow

Jens Atzpodien, Subhash C. Gulati and Bayard D. Clarkson


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/46/10/4892

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.