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Inhibitory effects of merocyanine 540-mediated photodynamic therapy on cellular immune functions: A role in the prophylaxis of graft-versus-host disease?

Donald L. Traul, Fritz Sieber *

Department of Pediatrics, Medical College of Wisconsin, Milwaukee, WI 53226, USA

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ABSTRACT

Merocyanine 540-mediated photodynamic therapy (MC540-PDT) has been used in clinical trials for the purging of autologous hematopoietic stem cell grafts. When the same combinations of dye and light were applied to human peripheral blood lymphocytes, a broad range of T- and B-cell functions were impaired, prompting speculations about a potential role of MC540-PDT in the prophylaxis of graft-versus-host disease (GVHD). We here report on the effects of MC540-PDT on in vitro functions of murine lymphocytes as well as a preliminary evaluation of MC540-PDT for the prevention of GVHD in murine models of allogeneic bone marrow transplantation. Mixed lymphocyte reactions, proliferative responses to lectins, interleukin-2 and lipopolysaccharide, T-cell-mediated lysis, and NK activity were all inhibited by moderate doses of MC540-PDT. Whether MC540-PDT reduced the incidence and/or the severity of GVHD in murine models of allogeneic hematopoietic stem cell transplantation depended on the composition of the mismatched grafts and the intensity of the preparative regimen. MC540-PDT was only beneficial (i.e. reduced the incidence and/or severity of GVHD) when the spleen cell content of grafts was low and/or the radiation dose of the preparative regimen was not myeloablative, and, therefore, may have encouraged mixed chimerism.

1. Introduction

The introduction of T-cell depletion in allogeneic hematopoietic stem cell transplantation has reduced the incidence and severity of acute and chronic graft-versus-host disease (GVHD) and has encouraged a greater utilization of grafts from unrelated and mismatched donors (reviewed by [1–3]). Despite these and other advances, GVHD remains a major challenge in hematopoietic stem cell transplantation, as many recipients of T-cell-depleted allogeneic grafts still experience acute and/or chronic GVHD. Furthermore, some of the gains achieved by T-cell depletion are offset by higher relapse rates (loss of graft-versus-tumor effect) as well as higher incidences of graft failures, post-transplant lymphoproliferative disorders, and prolonged immunodeficiencies [4–9]. These unintended consequences of T-cell depletion have been explained by inadequate depletions of donor cells that cause GVHD, inadequate depletions of donor cells that inhibit engraftment and immune reconstitution, excessive depletions of hematopoietic stem and progenitor cells, excessive depletions of cells that support hematopoietic engraftment, excessive depletions of cells that provide protective immunity after the transplant, and/or excessive depletions of cells that are responsible for graft-versus-tumor effects. This situation has motivated many transplant centers to explore alternative methods of T-cell depletion. Reports of successful applications of phototherapy (light therapy) and photochemotherapy (light therapy in the presence of photosensitizing agents) in the treatment of GVHD and autoimmune diseases, as well as the prevention of allosensitization and graft rejection [10–15] have prompted investigations into possible roles of phototherapy and photochemotherapy in the prevention of GVHD [16–18].

Immunological studies performed as part of a phase I/II clinical trial of merocyanine 540-mediated photodynamic therapy (MC540-mediated PDT) for the extracorporeal purging of autologous bone marrow grafts from leukemia and lymphoma patients [19] showed that combinations of dye and light that did not prevent hematopoietic reconstitution after marrow-ablative therapy inhibited a broad range of human B- and T-cell functions [20], prompting speculations about a potential role of MC540-mediated PDT in the prophylaxis of GVHD in allogeneic hematopoietic stem cell transplantation. We have now extended these investigations to murine lymphocytes and to several murine models of allogeneic bone marrow transplantation.

2. Materials and Methods

2.1. Materials

MC540 (5-[(3-sulfopropyl-2(3H)-benzoxazolylidine)-2-butenylidene]-1,2-dibutyl-2-thiobarbituric acid) (Fig. 1) was from Kodak (Rochester,

* Corresponding author at: Department of Pediatrics, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226, USA.
E-mail addresses: dltraul@msn.com (D.L. Traul), fsieber@mcw.edu (F. Sieber).

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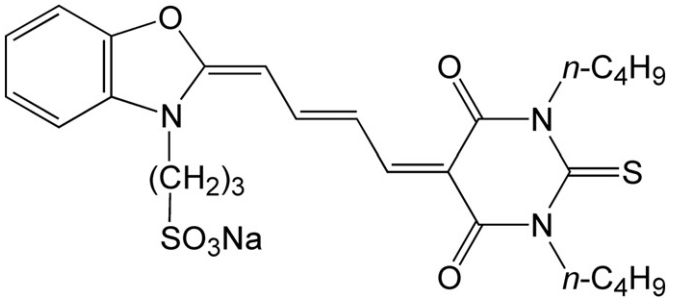

Fig. 1. Structure of Merocyanine 540.

NY), fetal bovine serum from Irvine Scientific (Santa Ana, CA), N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES) from Research Organics (Cleveland, OH), Tris(hydroxymethyl)-aminomethane (Tris) from Boehringer Mannheim Biochemicals (Indianapolis, IN), anti-Thy 1.2 monoclonal antibody and Low-Tox-M® rabbit complement from Cedarlane (Hornby, Ontario, Canada), phytohemagglutinin HA 15 (PHA) from Wellcome Diagnostics (Dartford, England), lipopolysaccharide (LPS; E. coli) from Difco (Detroit, MI), recombinant human interleukin-2 (IL-2) from Amgen Biologicals, (Thousand Oaks, CA), tritiated thymidine (thymidine [methyl-^{3}H]-; 2 Ci mmol^{-1}) from New England Nuclear (Boston, MA), and ^{51}Cr (sodium chromate; 250–500 mCi mg^{-1} Cr) from Amersham (Arlington Heights, IL). All other reagents were from Sigma Chemical Co. (St. Louis, MO).

2.2. Animals and Cells

Female B6D2F1/J (C57BL/6J × DBA/2J) (H-2$^{b/d}$), C57BL/6J (H-2^{b}), DBA/2J (H-2^{d}), LP/J (H-2^{bc}), AKR/J (H-2^{k}), and B10.BR/SgSnJ (H-2^{k}) mice (6–9 weeks old) were obtained from Jackson Laboratories (Bar Harbor, ME). Immunosuppressed animals were housed in autoclaved polycarbonate cages (≤5 animals per cage) on laminar flow racks in a facility that was fully accredited by the American Association for the Accreditation of Laboratory Animal Care. Autoclaved chow and acidified sterile water were provided ad libitum. YAC-1 lymphoma cells (H-2^{a}) (ATCC TIB 160) and P815 mastocytoma cells (H-2^{d}) (ATCC TIB 64) were from the American Type Culture Collection (Rockville, MD). Spleen cells were prepared as described by Mishell and Shiigi [21] and depleted of red cells by briefly suspending them in a hypotonic Tris–NH_4Cl buffer. In vitro proliferation experiments were typically conducted with pooled spleen cells from 2–4 animals.

2.3. Transplantation Experiments

Unless indicated otherwise, recipient mice received 11 Gy (single dose) of total body irradiation from an attenuated ^{137}Cs source (Shepard Mark I; 89.6 R min^{-1}; JL Shepard, San Fernando, CA) followed by the intravenous injection of spleen cells (2×10^7 or 5×10^7) or a mixture of bone marrow cells (10^7) and spleen cells (5×10^5 to 5×10^7). It is common practice to use spleen cells or mixtures of bone marrow and spleen cells in murine models of allogeneic bone marrow transplantation, as grafts consisting of bone marrow cells only would not elicit a significant graft-versus-host response [22]. However, there is no consensus as to which ratio of bone marrow-to-spleen cells most closely mimics a clinical allograft. We, therefore, included a series of experiments that explored how MC540-mediated PDT affected GVHD caused by allografts with different bone marrow-to-spleen cell ratios. The total number of bone marrow cells was chosen to insure hematopoietic reconstitution after marrow-ablative total body irradiation (TBI) even when grafts had been subjected to MC540-PDT prior to infusion into hosts [23]. The standard number of spleen cells was chosen to provoke robust (often lethal) graft-versus-host disease. Both the numbers of bone marrow cells and the numbers of spleen cell used for this study were identical or similar to the numbers used by other investigators [22].

All grafts were injected in 0.5 ml HEPES-buffered (10 mM, pH 7.4) alpha-medium supplemented with 5% fetal bovine serum. Unless indicated otherwise, treatment groups and control groups consisted of 10 animals each. Where indicated, allogeneic grafts were treated either with anti-Thy 1.2 antibody and complement or with MC540 and light prior to injection. Radiation controls received an infusion of HEPES-buffered alpha-medium containing 5% fetal bovine serum but no cells. Animals were monitored for ≥100 days for survival and obvious signs of GVHD (loss of body weight, dermatitis on tails and ears, chronic diarrhea). All animal experiments were conducted under protocols approved by the Institutional Animal Care and Use Committee.

2.4. T-cell Depletion

T-cell depletions by complement-mediated immune lysis were performed as described by Korngold and Sprent [24]. In brief, mixtures of bone marrow and spleen cells were suspended at a density of 2×10^7 cells ml^{-1} in HEPES-buffered alpha-medium supplemented with 5% fetal bovine serum and anti-Thy 1.2 monoclonal antibody (diluted 1:500) and incubated on ice for 60 min. The cells were pelleted by low-speed centrifugation and resuspended in a 1:10 dilution of rabbit complement, incubated at 37 °C for 60 min, washed once, and then resuspended in the original volume of HEPES-buffered alpha-medium supplemented with 5% fetal bovine serum.

2.5. MC540-sensitized Reactions

The MC540-sensitized photoirradiation of bone marrow cells, spleen cells, or mixtures of marrow and spleen cells was performed as described previously [20,23,25]. In brief, cells were suspended at a density of 10^7 cells ml^{-1} in HEPES-buffered alpha-medium supplemented with 12% fetal bovine serum. Dye was added from a 1-mg ml^{-1} stock solution in 50% ethanol to a final concentration of 15 μg ml^{-1}. Clear polystyrene tubes (15 ml; Corning Glass Works, Corning, NY) containing the cell suspension were mounted on a Plexiglass disk that rotated at approximately 30 rpm between two banks of tubular fluorescent lights (5 lights per bank; F20T12.CW; General Electric, Cleveland, OH) and irradiated for up to 90 min. The fluence rate at the sample site was 35 W m^{-2} as determined by a United Detector Technology (Hawthorne, CA) power meter model S351A equipped with detector model 262 and radiometric filter number 1158. The reaction was terminated by transferring the tubes to the dark and by washing the cells once with HEPES-buffered alpha-medium supplemented with 5% fetal bovine serum. Cells that were exposed to dye in the dark, to light in the absence of dye, or to neither dye nor light served as controls. The highest doses of PDT used in this study were identical to the ones used previously for the ex vivo purging of murine bone marrow grafts contaminated with tumor cells [23,25,26]. They would have reduced the concentration of leukemia and lymphoma cells by ≥4 orders of magnitude while preserving enough pluripotent hematopoietic stem cells to insure hematopoietic reconstitution after marrow-ablative TBI.

2.6. Proliferation Assays

MC540-treated or non-treated control cells were suspended at a density of 5×10^6 cells ml^{-1} in alpha-medium supplemented with 10% heat-inactivated fetal bovine serum and distributed in 0.1-ml aliquots into flat-bottom 96-well plates (Flow Laboratories, McLean, VA). Concanavalin A (ConA), PHA, LPS and IL-2 were dissolved in the same medium and added to the cells to final concentrations of 2.5 μg ml^{-1}, 25 μg ml^{-1}, 5 μg ml^{-1}, and 25 U ml^{-1}, respectively (pilot experiments had indicated that these concentrations were optimal for our application) and a final volume of 0.2 ml. Control wells received 0.1 ml of mitogen-free medium. After 48 h at 37 °C in a humidified atmosphere