Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical Pharmacology 70 (2005) 1518–1526
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Effective prevention of lethal acute graft-versus-host disease by combined immunosuppressive therapy with prodigiosin and cyclosporine A

Sang-Bae Han [1], Chang Woo Lee [1], Yeo Dae Yoon, Jong Soon Kang, Ki Hoon Lee, Won Kee Yoon, Young Kook Kim, Kiho Lee, Song-Kyu Park, Hwan Mook Kim *

Korea Research Institute of Bioscience and Biotechnology (KRIBB), 52 Oundong, Yusong, Taejon 305-333, South Korea

Received 5 July 2005; accepted 29 August 2005

Abstract

Prodigiosin (PDG), a bacterial metabolite, is a known T cell-specific immunosuppressant. Here, we compared its inhibitory potency and mode of action with cyclosporine A (CsA) in a mouse model. PDG efficiently inhibited T cell proliferation with an IC_{50} of 3.37 ng/ml, a similar dose to that of CsA (IC_{50} of 2.71 ng/ml). PDG inhibited only IL-2Rα expression, but not IL-2 expression, whereas CsA inhibited both. Exogenously added IL-2 reversed the suppressive activity of CsA, but not that of PDG. Moreover, although both PDG and CsA markedly reduced mortality rates in lethal acute graft-versus-host disease (GVHD), the combined treatment was more effective than either drug alone. These results demonstrate that PDG and CsA have similar inhibitory potencies, but different modes of action, and suggest that PDG has potential use as a supplementary immunosuppressant in combination with CsA for the treatment of GVHD.

Keywords: Prodigiosin; Cyclosporin A; Graft-versus-host disease

1. Introduction

Acute graft-versus-host disease (GVHD) is a major cause of morbidity and mortality in patients undergoing allogeneic bone marrow transplantation (BMT) [1]. Acute GVHD begins when a donor's Th1 cells recognize alloantigens presented by host antigen presenting cells (APCs) via interactions of T cell receptor-major histocompatibility, CD28/B7 and CD40/CD40L [2]. The resulting activated Th1 cells secret IL-2 and IFN-γ, which stimulate macrophages and cytotoxic T cells to ultimately produce effector molecules, such as TNF-α, nitric oxide, free radicals, granzyme and perforin, which cause tissue destruction and GVHD. Thus, T cells play a prominent role in the pathogenesis of GVHD.

The interactions between IL-2 and its receptors critically regulate the magnitude and duration of T cell activation [3]. IL-2 exerts multiple biological functions by binding to high-affinity receptors (IL-2R) composed of α, β and common γ chain subunits [4]. Undetectable on resting T cells, α chain expression is triggered by antigen, a stimulus that can be mimicked by Concanavalin A (Con A) or by anti-T cell receptor antibodies. Moreover, IL-2Rα expression is an essential determinant of the acquisition by a cell of full IL-2 responsiveness. Recent intensive studies on the biochemical process of IL-2/IL-2R signal transduction have targeted this pathway for potential pharmacological interventions capable of altering the progression of a broad range of T cell-mediated diseases, including GVHD. Moreover, the blockade of IL-2 transcription by cyclosporin A (CsA) and IL-2-driven signaling by rapamycin have been demonstrated to dampen immunological responsiveness [5,6].

During the past decade, the prodigiosin family has been suggested to be a source of reference compounds for a growing family of drugs with potential therapeutic benefits. Members of this family include prodigiosin (PDG), cycloprodigiosin hydrochloride, undecylprodigiosin and metacycloprodigiosin [7]. They, which contain a

* Corresponding author. Tel.: +82 42 860 4660; fax: +82 42 860 4605.
E-mail address: hwanmook@kribb.re.kr (H.M. Kim).
[1] Contributed equally to this work.

0006-2952/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2005.08.017

methoxypyrrole ring, have several biological activities, e.g., as antibacterials, antimycotics, antimalarials and as anticancer agents [7–12]. In addition, many studies imply that they have strong immunosuppressive activity [13–17]. PDG is also known to selectively suppress the immune functions of T cells, but not those of B cells or macrophages. In addition, it has been reported that PDG has a unique mode of action, namely, that it blocks IL-2Rα expression but not IL-2 expression. In contrast, CsA, which is the most well-known T cell-specific immunosuppressant, inhibits IL-2 production by blocking NF-AT activation [18].

Combination immunosuppressive therapies are used to achieve a maximum therapeutic effect whilst minimizing attendant toxicities [15,19]. One prerequisite for the combined use of different drugs is that they should have different modes of action and unrelated toxicities. However, so far no comparative study has been performed on PDG and CsA. Therefore, in the present study, we compared the inhibitory potencies and modes of action of PDG and CsA and further examined the combined therapeutic effects of PDG and CsA on GVHD.

2. Materials and methods

2.1. Materials

Female C57BL/6 (H-2^b) and BALB/c (H-2^d) mice were purchased from Daehan Biolink Co. Ltd. (Chungbuk, Korea) and maintained under specific pathogen free conditions until required. The experimental procedures used in this study were approved by the KRIBB Animal Experimentation Ethics Committee. PDG (molecular weight 323) was prepared from the culture broth of *Serratia marcescens* [14,20]. Con A and CsA were purchased from Sigma (St. Louis, MO), and mouse recombinant IL-2 was purchased from R & D Systems Inc. (Minneapolis, MN).

2.2. Lymphoproliferation assay

Spleen cells were obtained from specific pathogen free C57BL/6 mice (female, 6–7 weeks) and were freed of red blood cells by lysis buffer treatment. Splenic B cells were isolated by negative depletion by using biotinylated antibodies to CD4, CD8, GR-1 and CD11c (BD Pharmingen) and Dynabeads M-280 Streptoavidin (Dynal Inc., Oslo, Norway), as previously described [21,22]. Splenic T cells were isolated by negative depletion using biotinylated antibodies to B220, GR-1 and CD11c. Purity was typically >90%. Cells were cultured in RPMI 1640 medium (Gibco BRL, Grand Island, NY) supplemented with 10% fetal calf serum (Hyclone, Logan, UT), 2 mM L-glutamine and 50 μM 2-mercaptoethanol (Sigma). T cells were activated with Con A (1 μg/ml) or plate-bound anti-CD3 (10 μg/ml) plus soluble anti-CD28 antibodies (10 μg/ml). B cells were activated with LPS (1 μg/ml) or anti-IgM antibody (10 μg/ml) [23,24]. PDG and CsA were treated at concentrations ranging from 3 to 30 ng/ml. Cells were pulsed with ^{3}H-thymidine (113 Ci/nmol, NEN, Boston, MA) at a concentration of 1 μCi/well for the last 18 h and harvested on day 3 using an automated cell harvester (Inotech, Dottikon, Switzerland). The amount of ^{3}H-thymidine incorporated into cells was measured using a Wallac Microbeta scintillation counter (Wallac, Turku, Finland).

2.3. Cytokine gene expression

Splenic T cells were stimulated with 1 μg/ml of Con A for 24 h and total RNA was extracted using an Ultraspec II RNA isolation Kit (Biotech Lab. Inc., Houston, TX). Reverse transcription-polymerase chain reaction (RT-PCR) was used to examine cytokine gene expressional changes, as described previously [14]. The primer sequences used were as follows: IL-2, sense, 5′-CTT GCC CAA GCA GGC CAC AG-3′, antisense, 5′-GAG CCT TAT GTG TTG TAA GC-3′; IFN-γ, sense, 5′-AGC GGC TGA CTG AAC TCA GAT TGT AG-3′, antisense, 5′-GTC ACA GTT TTC AGC TGT ATA GGG-3′; IL-2Rα, sense, 5′-AAC AAC TGC AAT GAC GGT GA-3′, antisense, 5′-GCC CTC TCT CCC ATT AAA GC-3′; TNF-α, sense, 5′-CCT GTA GCC CAC GTC GTA GC-3′, antisense, 5′-TTG ACC TCA GCG CTG AGT TG-3′; IL-1β, sense, 5′-TGC AGA GTT CCC CAA CTG GTA CAT C-3′, antisense, 5′-GTG CTG CCT AAT GTC CCC TTG AAT C-3′; β-actin, sense, 5′-TGG AAT CCT GTG GCA TCC ATG AAAC-3′, antisense, 5′-TAA AAC GCA GCT CAG TAA CAG TCCG-3′. After analyzing band areas using an image analysis system (Multi-Analyst, Bio-Rad, CA), target mRNA expression levels were calculated as relative ratios versus β-actin [25].

2.4. Lethal acute GVHD

Recipient BALB/c mice received 10 Gy of total body irradiation from a Soft M-150 WE (Softex, Tokyo, Japan) ^{60}Co source at a rate of 0.5 Gy/min [2]. One day after irradiation, the donor mice (C57BL/6 for allogeneic and BALB/c for syngeneic transplantation) were killed by cervical dislocation. Femora were excised aseptically and bone marrow (BM) cells were removed from the femoral shafts by inserting a 25-gauge needle into proximal ends. Total splenic cells were freed of red blood cells by lysis buffer treatment. Irradiated BALB/c mice received a single injection of 0.25 ml of Phosphate Buffered Saline (PBS) containing 1×10^7 BM cells and 5×10^7 spleen cells through a tail vein. BALB/c mice were administered 1 mg/kg of PDG and/or CsA intraperitoneally for 13 days from the day of transplantation.

2.5. Collagen-induced arthritis (CIA)

Male DBA/1 mice were purchased from Charles River Japan Inc. (Yokohama, Japan). Bovine type II collagen was