International Immunopharmacology 44 (2017) 137–142

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Investigations on the immunosuppressive activity of derivatives of mycophenolic acid in immature dendritic cells

Dorota Iwaszkiewicz-Grzes [a,b], Grzegorz Cholewinski [a,*], Agata Kot-Wasik [c], Piotr Trzonkowski [b], Krystyna Dzierzbicka [a]

[a] Department of Organic Chemistry, Gdansk University of Technology, ul. G. Narutowicza 11/12, 80-233 Gdansk, Poland
[b] Department of Clinical Immunology and Transplantology, Medical University of Gdansk, ul. Debinki 7, 80-211 Gdansk, Poland
[c] Department of Analytical Chemistry, Gdansk University of Technology, ul. G. Narutowicza 11/12, 80-233 Gdansk, Poland

ARTICLE INFO

Article history:
Received 30 September 2016
Received in revised form 9 December 2016
Accepted 8 January 2017
Available online 14 January 2017

Keywords:
Mycophenolic acid
Amino acids
Acridines
Acridones
Dendritic cells
Immunosuppressants

ABSTRACT

The main activity of mycophenolic acid **1** (MPA) and its analogs is the inhibition of proliferation of T cells. Here, we hypothesized that MPA and its conjugates inhibits also the activity of antigen-presenting cells (APC) including dendritic cells (DCs). We tested the effect of novel amino acid derivatives of MPA and conjugates of MPA with acridines/acridones on DCs by flow cytometry, ELISA and MLR assay. Both acridines/acridone derivatives could inhibit the maturation of DC, as shown by the decreased expression of B7 family receptors. It was confirmed in the mixed leucocyte reaction (MLR), in which T cells challenged with DCs pretreated with the analogs showed decreased proliferation and reduced cytokine secretion. The most interesting activity in this series of studies, that is, the suppression of CD86 receptor expression, decreased cytokine production and suppressed mixed leucocyte reaction, exhibited (mycophenoyl-*N*-3-propyl)-9-acridone-4-carboxamide ester **5a** and (mycophenoyl-*N*-5-pentyl)-9-acridone-4-carboxamide ester **5b**. These compounds reduced also the secretion of IL-2 and IL-15. In addition, they increased secretion of suppressive IL-10. Equally promising results were obtained for the *N*-mycophenoyl-D-glutamic acid **4b**, which previously gave the highest value of selectivity. Acridone derivatives of MPA are therefore good immunosuppressive drug candidates for further testing.

1. Introduction

The first successful renal transplantation was performed between identical twins and therefore there was no need for immunosuppression [1]. Since that time solid organ transplantation has been rapidly evolving as a life-saving therapeutic intervention that greatly contributes to a better quality of life in organ recipients. Genetic donor-recipient match is still the best guarantee of uneventful post-transplant follow up while appropriate immunosuppression is the most common strategy to keep the transplanted organ in good condition [2]. Unfortunately allograft rejection is still a major cause of graft loss in the first year posttransplant [3].

The inhibition of lymphocyte proliferation is one of the best maneuvers used in immunosuppression therapies. Mycophenolic acid (MPA) **1** is an uncompetitive inhibitor of inosine-5′-monophosphate dehydrogenase (IMPDH), the crucial enzyme in *de novo* purine nucleotide biosynthesis. There are two clinically approved derivatives of mycophenolic acid **1** (MPA, Fig. 1) – mycophenolate mofetil **2** (2-morpholinoethyl, MMF, CellCept (Roche), Fig. 1) and mycophenolate sodium **3** (MPS, Myfortic (Novartis), Fig. 1). MMF **2** is a prodrug metabolised to active form (MPA **1**) and first was used in early 1990s as an immunosuppressive drug. Both compounds, MMF **2** and MPS **3**, are used in the prevention of allograft rejection and treatment of autoimmune diseases [3–8].

The main activity of MPA and its analogs is the inhibition of proliferation of T cells, which stops these cells from allograft rejection [9]. Nevertheless, there are some reports suggesting that the drug inhibits also the activity of antigen-presenting cells (APC) [10]. It may be a special importance in transplantation as alloantigens presented by antigen-presenting cells (APC) trigger T cell alloresponse and graft rejection. The most important APC in the induction of such an immune response are dendritic cells (DCs), notably myeloid subset of DC (moDC), which are mainly considered as involved in organ rejection [11,12]. If it is true, MPA might be an agent that not only stops the proliferation of allosensitized lymphocytes, but also inhibits the allosensitization itself. Some changes to the particle structure might further enhance the latter activity without deteriorating the former one.

Hence, there were designed MPA immunosuppressive derivatives combining antiproliferative and anti-antigen-presentation activities, including amino acids derivatives of MPA and acridone/acridine analogs of MPA [13,14]. We evaluated their cytotoxic (IC_{50} - half maximal inhibitory concentration) in colorimetric test MTT (3-(4,5-dimethylthiazol-

* Corresponding author.
E-mail address: grzchole@pg.gda.pl (G. Cholewinski).

http://dx.doi.org/10.1016/j.intimp.2017.01.011
1567-5769/

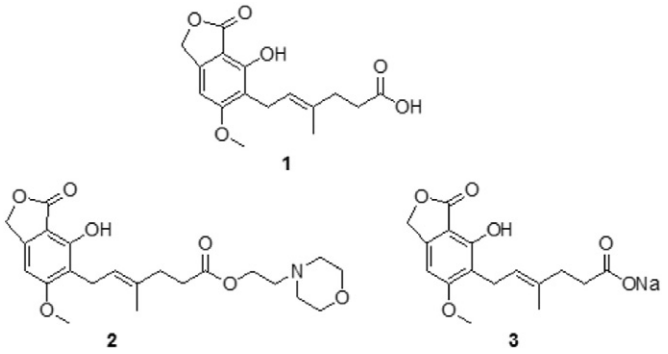

Fig. 1. Structure of MPA **1**, MMF **2** and MPS **3**.

2-yl)-2,5 diphenyltetrazolium bromide) and antiproliferative activity (EC_{50} - half maximal effective concentration) in proliferation test with ^{3}H-thymidine incorporation (^{3}H-TdR) (for an example of the diagram see Fig. S-1 in the electronic Supplementary information), influence on inosine-5′-monophosphate dehydrogenase (IMPDH) performed in absence or presence of guanosine monophosphate (GMP) and accounted selectivity index (SI). According to these studies, there were selected the most promising compounds for consecutive tests. Methyl ester *N*-mycophenoyl-L-phenylalanine **4a**, *N*-mycophenoyl-D-glutamic acid **4b**, *N*-mycophenoyl-L-leucine **4c**, (mycophenoyl-*N*-3-propyl)-9-acridone-4-carboxamide ester **5a**, (mycophenoyl-*N*-5-pentyl)-9-acridone-4-carboxamide ester **5b** and (mycophenoyl-*N*-3-propyl)-acridine-4-carboxamide ester **6a**, (mycophenoyl-*N*-5-pentyl)-acridine-4-carboxamide ester **6b**, (mycophenoyl-*N*-6-hexyl)-acridine-4-carboxamide ester **6c** (Fig. 2) were considered for *in vitro* examinations as potential immunosuppressive agents inhibiting antigen-presentation by myeloid DCs.

2. Materials andmethods

2.1. MPA analogs

Tested analogs of MPA **4a–c**, **5a,b** and **6a–c** (Fig. 2) were obtained in accordance with general procedure for the preparation amino acid derivatives of MPA **4a–c** described by Iwaszkiewicz-Grzes et al. [13] and ester derivatives of MPA and acridines **5a,b** or acridones **6a–c** described by Cholewinski et al. [14]. Both publications include complete identification of compounds and their characteristic data [13,14]. For clarity, the most promising analogs, such a **4b**, **5a**, and **5b**, are presented in main figures, while results from all analogs are shown in electronic Supplementary information.

2.2. Human blood samples

Human buffy coats were obtained from anonymous healthy donors from the Regional Blood Bank in Gdańsk (RCKiK).

2.3. Isolation of peripheral blood mononuclear cells (PBMC)

The heparinized blood was diluted with sterile phosphate-buffered saline (PBS, pH 7.4 W/O CAMQ USA PLASTIC, Life Technologies Polska, Poland) (1.5/1 *v*/v). PBMC were separated by density-gradient centrifugation (2500 rpm by 25 min) in Ficolle-Paque Premium (VWR International, Poland). PBMC, as a dense white band above Ficolle-Paque, were collected in 50 ml tube (BD Falcon Tubes PP, Diag-Med, Poland). The cells were washed twice (1500 rpm by 10 min) with sterile PBS and then resuspended with RPMI-1640 medium (Immuniq, PAA, Poland) (10^{8} cells/10 ml). The cells were counted using Türk's solution (Merck Millipore, Poland) in Fuchs-Rosenthal counting chamber.

2.4. Isolation of monocytes from PBMC

Monocytes were prepared from PBMC by a 2 h adherence step at 37 °C (5% CO_2, 98% H_2O) in RPMI-1640 medium (Immuniq, PAA, Poland) supplemented with 10% FBS (Fetal Bovine Serum USA origin, Heat Inactivated, sterile-filtered, suitable for cell culture, Sigma-Aldrich, Poland) and P/S (Penicillin-Streptomycin Sterile-Filtered, Sigma-Aldrich, Poland). Non-adherent cells were removed by extensive washing with sterile PBS warmed to temperature 37 °C. The remaining adherent cells were kept on ice for 20 min and immediately subjected to the moDCs and counted.

2.5. Generation of dendritic cells

DCs were generated by previously described protocols, with some modifications [15]. Adherent monocytes were cultured for 7 days in GMP-DC medium (GMP Serum-free Dendritic Cell Medium (DC), CellGenix Technologie Transfer, Germany) containing 50 ng/ml granulocyte-macrophage colony-stimulating factor (GM-CSF, R&D systems, Biokom, Poland) and 100 ng/ml interleukin-4 (IL-4, R&D systems,

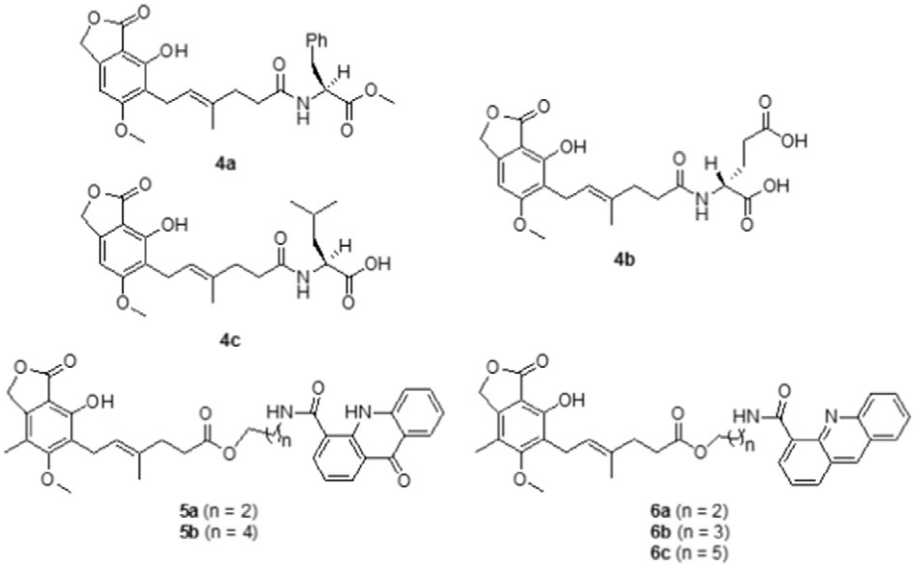

Fig. 2. Structures of selected analogs of MPA **4a–c**, **5a,b** and **6a–c**.