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The effect of clinically relevant doses of immunosuppressive drugs on human mesenchymal stem cells

Eliska Javorkova[a,b], Julie Vackova[a], Michaela Hajkova[a,b], Barbora Hermankova[a,b], Alena Zajicova[b], Vladimir Holan[a,b], Magdalena Krulova[a,b,*]

[a] Department of Cell Biology, Faculty of Science, Charles University, Vinicna 7, Prague 2, 128 44, Czech Republic
[b] Department of Transplantation Immunology, The Czech Academy of Sciences, Institute of Experimental Medicine, Videnska 1083, Prague 4, 142 20, Czech Republic

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ABSTRACT

Immunosuppressive drugs are used to suppress graft rejection after transplantation and for the treatment of various diseases. The main limitations of their use in clinical settings are severe side effects, therefore alternative approaches are desirable. In this respect, mesenchymal stem cells (MSCs) possess a regenerative and immunomodulatory capacity that has generated considerable interest for their use in cell-based therapy. Currently, MSCs are tested in many clinical trials, including the treatment of diseases which require simultaneous immunosuppressive treatment. Since the molecular targets of immunosuppressive drugs are also present in MSCs, we investigated whether immunosuppressive drugs interact with the activity of MSCs. Human MSCs isolated from the bone marrow (BM) or adipose tissue (AT) were cultured in the presence of clinical doses of five widely used immunosuppressive drugs (cyclosporine A, mycophenolate mofetil, rapamycin, prednisone and dexamethasone), and the influence of these drugs on several factors related to the immunosuppressive properties of MSCs, including the expression of immunomodulatory enzymes, various growth factors, cytokines, chemokines, adhesion molecules and proapoptotic ligands, was assessed. Glucocorticoids, especially dexamethasone, showed the most prominent effects on both types of MSCs and suppressed the expression of the majority of the factors that were tested. A significant increase of hepatocyte growth factor production in AT-MSCs and of indoleamine 2,3-dioxygenase expression in both types of MSCs were the only exceptions. In conclusion, clinically relevant doses of inhibitors of calcineurin, mTOR and IMPDH and glucocorticoids interfere with MSC functions, but do not restrain their immunosuppressive properties. These findings should be taken into account before preparing immunosuppressive strategies combining the use of immunosuppressive drugs and MSCs.

1. Introduction

Commonly used therapy of many disorders, including autoimmune diseases, is frequently insufficiently effective. There is thus a need for novel therapeutic approaches that increase the efficiency of current treatment protocols and will be well tolerated. In view of their unique biological properties, mesenchymal stem cells (MSCs) are now being investigated as a promising tool to increase the efficiency of the present immunosuppressive protocols.

MSCs represent a heterogenous population of cells that can be isolated from various tissues. They are characterized by an adherence to plastic surfaces and extensive differentiation potential. They express the surface markers CD44, CD73, CD90 and CD105, and lack CD11b, CD14, CD19, CD34 and CD45 [1]. MSCs closely interact with the local microenvironment and in the presence of factors produced during inflammation, including proinflammatory cytokines, ligands of toll-like receptors and hypoxia, they become activated and increase their regulatory and anti-inflammatory properties [2,3]. There is substantial

Abbreviations: AT, adipose tissue; BM, bone marrow; CsA, cyclosporine A; COX-2, cyclooxygenase-2; DEX, dexamethasone; DMEM, Dulbeccós modified Eaglés medium; DMSO, dimethyl sulfoxide; ELISA, enzyme-linked immunosorbent assay; FasL, Fas ligand; HGF, hepatocyte growth factor; IDO, indoleamine 2,3-dioxygenase; IFN, interferon; IL, interleukin; IMPDH, inosine monophosphate dehydrogenase; MCP-1, monocyte chemoattractant protein 1; MMF, mycophenolate mofetil; MSCs, mesenchymal stem cells; mTOR, mechanistic target of rapamycin; PBMCs, peripheral blood mononuclear cells; PBS, phosphate buffered saline; PD-L1, programmed death-ligand 1; PHA, phytohemagglutinin; PL, platelet lysate; PRED, prednisone; RAPA, rapamycin; SDF-1, stromal-derived factor 1; TGF, transforming growth factor; TSG-6, tumour necrosis factor-inducible gene 6; VEGF, vascular endothelial growth factor

* Corresponding author at: Faculty of Science, Charles University, Vinicna 7, Prague 2, 128 44, Czech Republic.
E-mail addresses: javorkova@biomed.cas.cz (E. Javorkova), julie.vackova@natur.cuni.cz (J. Vackova), michaela.hajkova@natur.cuni.cz (M. Hajkova), barbora.hermankova@biomed.cas.cz (B. Hermankova), zajicova@biomed.cas.cz (A. Zajicova), holan@biomed.cas.cz (V. Holan), krulova@natur.cuni.cz, magdalena.krulova@natur.cuni.cz (M. Krulova).

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evidence that the beneficial effects of MSCs can be attributed to their capacity to produce a variety of bioactive molecules, including cytokines, chemokines, immunomodulatory enzymes, growth and angiogenic factors and extracellular matrix remodelers [4–6].

It has been described that MSCs attenuate a harmful immune response and are effective in the treatment of several immune-related disorders [7–10]. The ability of MSCs to modulate pathological responses is mediated by both paracrine factors and contact-dependent mechanisms [11]. Similarly, the migration of MSCs into the injured tissues is regulated by a complex interaction of specific receptors and their ligands expressed by both MSCs and the damaged endothelium [12–14].

For therapeutic application in clinical settings, autologous or allogeneic MSCs are used depending on availability and suitability of the cell source. The main obstacle in performing for allogeneic cell transplantation may be a deleterious immune response, and therefore various immunosuppressive drugs are frequently used. It has been documented that the suppressive action mediated by MSCs is further increased in the presence of immunosuppressive drugs, and such combined treatment resulted in a significant prolongation of graft survival [15–17].

These findings have made MSCs an attractive tool for the suppression/attenuation of harmful immune reactions or rejection after transplantation. In this respect, a combination of MSCs and immunosuppressive drugs offers a perspective strategy to reduce the dosage of drugs and thus decrease their severe side effects.

In a previous study, we demonstrated that MSCs attenuate the adverse effects of several immunosuppressive drugs on T-cell subpopulations in a mouse model [18]. In this study, we assessed the effect of widely used immunosuppressive drugs which have different mechanisms of action on the spectrum of secreted factors, enzymes and surface molecules responsible for the therapeutic effects of MSCs. Tested drugs included calcineurin inhibitor – cyclosporine A (CsA), mechanistic target of rapamycin (mTOR) inhibitor – rapamycin (RAPA), inosine monophosphate dehydrogenase (IMPDH) inhibitor – mycophenolate mofetil (MMF) and two glucocorticoids – dexamethasone (DEX) and prednisone (PRED). MSCs isolated from different tissues share many phenotypical and functional characteristics, yet they may differ in some important features. Therefore, we studied MSCs isolated from bone marrow (BM-MSCs) and from adipose tissue (AT-MSCs), both of which represent the main sources of these cells for therapy. To mimic inflammatory conditions, interferon-γ (IFN-γ)-treated MSCs were also tested. The results can serve as a basis for potential new strategies in the clinical use of MSCs.

2. Material and methods

2.1. Preparation of peripheral blood mononuclear cells (PBMCs)

PBMCs were isolated from buffy coat obtained from the peripheral blood of a healthy donor. A mixture of buffy coat and phosphate buffered saline (PBS) was layered on Ficoll Paque Plus (GE Healthcare, Chicago, IL) and centrifuged (400*g* for 35 min) according to the manufactureŕs instructions. A layer with PBMCs was collected and washed three times in Dulbeccós modified Eaglés medium (DMEM, PAA Laboratories, Pasching, Austria), supplemented with 10% of fetal bovine serum (FBS, Gibco, Waltham, MA), antibiotics (100 μg/mL of streptomycin and 100 U/mL of penicillin) and 10 mM HEPES buffer (hereafter referred to as complete DMEM supplemented with FBS). PBMCs were stored in liquid nitrogen at a concentration of 14×10^6 cells/mL in 1.6 mL of complete DMEM, supplemented with FBS and 10% of dimethyl sulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO).

2.2. Preparation of human MSCs

AT-MSCs obtained from 4 and BM-MSCs from 6 healthy donors were used in experiments. The cells were obtained as excessive material collected primarily for therapeutic purposes. All donors signed informed consent for the use of excessive BM or AT for experimental research. BM-MSCs were cultured from nucleated cell fraction isolated from BM settled on gelofusine (B. Braun Melsungen, Melsungen, Germany). Nucleated cells were seeded at a concentration of 1×10^6 cells/mL in 10 mL of DMEM (PAA Laboratories), supplemented with 5% of human platelet lysate (PL, IKEM, Prague, Czech Republic), antibiotics (100 μg/mL of streptomycin and 100 U/mL of penicillin) and 10 mM HEPES buffer (hereafter referred to as complete DMEM supplemented with PL) in 75-cm^2 tissue culture flasks (TPP, Trasadingen, Switzerland). Adherent cells were cultured with regular exchange of medium and passaging using trypsin (Sigma-Aldrich), and in 3rd passage were harvested and frozen at a concentration of 1×10^6 cells/mL in complete DMEM supplemented with FCS and 10% of DMSO (Sigma-Aldrich) and stored in liquid nitrogen. AT-MSCs were isolated as described elsewhere [19]. For the experiments, stored MSCs were thawed and cultured in 75-cm^2 tissue culture flasks until confluence.

2.3. Culture of MSCs with immunosuppressive drugs

MSCs were cultured with immunosuppressive drugs (all purchased from Sigma-Aldrich) for 48 h in a volume of 3 mL of complete DMEM, supplemented with 10% of FBS. All immunosuppressive drugs were used at a final concentration of 0.5 μg/mL (only RAPA was used at a concentration 0.05 μg/mL). MSCs were cultured at a final concentration of 1×10^5 cells/mL, in 6-well tissue culture plates (Nunc, Roskilde, Denmark), unstimulated or stimulated with 10 ng/mL of human recombinant IFN-γ (PeproTech, Rocky Hill, NJ). Culture supernatants were collected after 48 h for cytokine concentration determination using enzyme-linked immunosorbent assay (ELISA). Cells were harvested into either 500 μL of TRI Reagent (Molecular Research Center, Cincinati, OH) for real-time PCR analysis or into PBS for flow cytometry analysis.

2.4. Culture of PBMCs with immunosuppressive drugs

For determination of proliferation of mitogen-stimulated lymphocytes, PBMCs were cultured in 96-well tissue culture plates (Nunc) at a final concentration of 0.6×10^6 cells/mL with immunosuppressive drugs, unstimulated or stimulated with 10 μg/mL of phytohemagglutinin (PHA, Pharmacia, New Jersey, NJ) in a volume of 200 μL of complete DMEM supplemented with 10% of FBS. The effects of all immunosuppressive drugs were tested at final concentrations of 50, 5, 0.5, 0.05 and 0.005 μg/mL. PBMC proliferation was determined on the basis of incorporation of ^{3}H-thymidne (1 mCi/well, PE Systems, Waltham, MA), added to the cultures for the last 6 h of the 72-h incubation period. For determination of IFN-γ production, PBMCs were cultured in 48-well tissue culture plates (Nunc) at a final concentration of 0.6 x 10^6 cells/mL with immunosuppressive drugs unstimulated or stimulated with 10 μg/mL of PHA (Pharmacia) in a volume of 500 μL of complete DMEM supplemented with 10% of FBS. The concentrations of immunosuppressive drugs were the same as those used in proliferation assay. Culture supernatants were collected after a 48-h period for determination of IFN-γ concentration using ELISA.

2.5. Flow cytometry characterization of MSCs

Untreated MSCs or MSCs treated with immunosuppressive drugs were harvested and stained for 30 min in PBS at 4 °C with the following monoclonal antibodies (all purchased from BioLegend, San Diego, CA): fluorescein isothiocyanate (FITC)-labeled anti-CD90 (clone 5E10), FITC-labeled anti-CD45 (clone HI30), allophycocyanin (APC)-labeled anti-CD44 (clone IM7), phycoerythrin (PE)-labeled anti-CD106 (clone STA), PE-labeled anti-Fas ligand (FasL, clone NOK-1), APC-labeled anti-programmed death-ligand 1 (PD-L1, clone 29E.2A3) and APC-labeled