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Is steroid resistance related to multidrug resistance-I (MDR-I) in rheumatoid arthritis?

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Abstract

Both healthy ageing and rheumatoid arthritis (RA) are frequently associated with acquired steroid resistance. Here, we investigated the potential involvement of steroid resistance with multidrug resistance (MDR) and explored the impact of pathological ageing on lymphocyte sensitivity to glucocorticoids. Seventy-four RA patients and 26 healthy controls took part in this study. Peripheral blood mononuclear cells were isolated and T-cell sensitivity to glucocorticoids was measured *in vitro*. The functional activity of P-glycoprotein was analyzed by flow cytometry and *ABCB1/MDR-1* gene polymorphisms were assessed in peripheral lymphocytes. Patients and controls had similar sensitivities to glucocorticoids. Only controls presented age-related immunological changes, including reduced T-cell proliferation and relative resistance to corticosterone. Patients had a higher percentage (72%) of lymphocytes actively extruding rhodamine 123 ($Rh123^{dim}$) than controls (60%) in spite of similar P-glycoprotein activity. A higher percentage of $Rh123^{dim}$+ lymphocytes was observed in patients who were more resistant to dexamethasone *in vitro*. The distribution of *ABCB1* genotypes in RA patients did not differ significantly from that in controls and were not associated to steroid sensitiveness or disease activity. These data suggest that peripheral lymphocytes of arthritic patients are fully responsive to GCs *in vitro* in spite of displaying higher MDR activity.

Keywords: Rheumatoid arthritis; Glucocorticoids; P-glycoprotein; Lymphocyte proliferation; Polymorphisms; *ABCB1/MDR1*

1. Introduction

The treatment of rheumatoid arthritis (RA) includes anti-inflammatory drugs, immunosuppressors, anti-malarics and cytokine inhibitors [1]. Of special note, the glucocorticoids (GCs) have been commonly used as powerful anti-inflammatory drugs for the treatment of RA, asthma, systemic lupus erythematosus, leukemias, lymphomas and transplant rejection [2,3]. However,

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chronic GC treatment is associated with acquired steroid resistance [4,5]. There is some data suggesting that RA patients develop GC resistance, requiring greater drug amounts and/or extended period of treatment [4,5]. However, there is considerable debate over this finding since others have found cells with increased sensitivity to steroids in RA [6]. These discrepancies could be explained by disease-modifying factors including age and severity of disease [7]. Indeed, we have recently described that healthy ageing is associated with significant T-cell resistance to GC treatment [8]. However, the impact of the pathological ageing on this GC immunoregulation is largely unknown. This information can be useful for a more individual-oriented therapy [9].

To date, it is unknown to what extent the steroid resistance is associated with resistance to other drugs in RA. Typical multidrug resistance (MDR) is characterized by the overexpression of the *ABCB1* gene, which encodes for a transmembrane protein P-glycoprotein (P-gp). This protein is expressed on lymphocytes (but not macrophages) and functions as an efflux pump to decrease the intracellular accumulation of a variety of lipophilic drugs, including chloroquine, immunosuppressors and GCs [10,11]. Since lymphocytes are not involved in detoxification or hormone secretion, the physiological role of P-gp in these cells is still obscure. Little is known about the possible role of P-gp in inflammatory and autoimmune diseases that require therapy with drugs actively extruded by this transporter. There is some evidence that increased P-gp activity in lymphocytes from systemic lupus erythematosus [12] and RA [13] might influence disease outcome or steroid requirements for disease control. Some genetic polymorphisms of the *ABCB1* gene have been described and are associated with disposition of P-gp substrates in humans [14,15]. However, the association of the polymorphisms *ABCB1* gene and expression and function of P-gp is largely unknown.

In this study, we investigated (i) the potential involvement of steroid resistance with MDR function and genetic polymorphisms and (ii) to further explore the age-related effects on lymphocyte sensitivity to GCs in peripheral blood lymphocytes.

2. Materials and methods

2.1. Subjects

Seventy-four patients with RA (mean age 53.04±1.79) were recruited from the Rheumatology Unit (São Lucas Hospital, PUCRS, Porto Alegre, Brazil). The diagnosis of RA was made according to the criteria of the American College of Rheumatology [16]. Patients were subgrouped accordingly to the following age ranges: young adults (20–40 years; $n=47$) and elderly (>60 years; $n=27$). Twenty-four had active disease and 50 were considered in clinical remission. Active disease was defined as six or more swollen joints and at least two of the following: nine joints tender to pressure, morning stiffness of at least 1 h duration, and an erythrocyte sedimentation rate of a least 20 mm/h. In addition, 26 age-matched healthy control subjects (20–79 years; mean age 45.08±3.32) also took part in this study and included health care workers, undergraduates and local community dwellers. Both patients and controls were of similar ethnicities. Exclusion criteria included infections, heart disease, under nourishment, anemia, leucopenia, neoplasias, major depression, HIV, thyroiditis and diabetes. Written informed consent was obtained from all subjects according to the Declaration of Helsinki. The study protocol was approved by both scientific and ethics committees (PUCRS, Porto Alegre, Brazil).

2.2. Collection of peripheral blood and isolation of mononuclear cells

Twenty milliliters of peripheral blood was collected by venepuncture in the morning (between 9 and 10 h) and samples stored into lithium-heparin tubes prior to analyses. Samples were always collected at the same time of day to minimize circadian variations. Peripheral blood mononuclear cells (PBMCs) were isolated by centrifugation over a Ficoll-Hypaque (Sigma) gradient (900×*g*, 30 min). Cells were counted by means of microscopy (100×) and viability always exceeded 95%, as judged from their ability to exclude Trypan blue (Sigma).

2.3. Cell cultures and steroid sensitivity assays

PBMCs were cultured in flat bottomed 96-well microplates in a final concentration of 1.5×10^5 cells/well in complete culture medium (i.e. supplemented with gentamicin 0.5%, glutamine 1%, fungizone 0.1%, HEPES 1% and heat-inactivated fetal calf serum 10%, all from Sigma) for 96 h at 37 °C in 5% CO_2 atmosphere. Stimulation by the selective T-cell mitogen phytohemagglutinin (PHA 2, 1% and 0.5%; Gibco, USA) was performed in triplicates (100 μL/well). In non-stimulated cultures (PHA 0), mitogen was substituted by culture medium. To assess *in vitro* T-cell sensitivity to steroids, DEX (a synthetic GC receptor agonist) and corticosterone (CORT, binds to both GR and mineralocorticoid receptors) were added in duplicates (10^{-9} to 10^{-4} M; all from Sigma) to mitogen-stimulated (PHA 1%) lymphocyte cultures. Data are presented as percentage of basal proliferation, where 100% (basal) represents cultures of PHA 1% without steroids.

2.4. Cell proliferation/viability assay

The cell proliferation/viability responses were determined by a modified colorimetric assay [17,18]. In the last 4 h of culture, 100 μL of the supernatant was gently discarded and