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Cyclosporine A enhances gluconeogenesis while sirolimus impairs insulin signaling in peripheral tissues after 3 weeks of treatment

P.C. Lopes [a], A. Fuhrmann [a], F. Carvalho [a], J. Sereno [b,c], M.R. Santos [a], M.J. Pereira [d], J.W. Eriksson [d], F. Reis [b], E. Carvalho [a,e,*]

[a] Center for Neuroscience and Cell Biology, University of Coimbra, 3000 Coimbra, Portugal
[b] Laboratory of Pharmacology & Experimental Therapeutics, IBILI, Medicine Faculty, University of Coimbra, 3000-548 Coimbra, Portugal
[c] Institute for Nuclear Sciences Applied to Heath—ICNAS, University of Coimbra, Coimbra, Portugal
[d] Department of Medical Sciences, Uppsala University, 75185 Uppsala, Sweden
[e] The Portuguese Diabetes Association (APDP), 1250 203 Lisbon, Portugal

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ABSTRACT

Cyclosporine A (CsA) and sirolimus (SRL) are immunosuppressive agents (IA) associated with new-onset diabetes after transplantation (NODAT). This study aims to evaluate the effects of 3-weeks of treatment with either CsA (5 mg/kg BW/day) or SRL (1 mg/kg BW/day) on insulin signaling and expression of markers involved in glucose metabolism in insulin-sensitive tissues, in Wistar rats.

Although no differences were observed in fasting glucose, insulin or C-peptide levels, both treated groups displayed an impaired glucose excursion during both glucose and insulin tolerance tests. These results suggest glucose intolerance and insulin resistance.

An increase in glucose-6-phosphatase protein levels (68%, $p < 0.05$) and in protein-tyrosine phosphatase 1B (163%, $p < 0.05$), a negative regulator of insulin was observed in the CsA-treated group in the liver, indicating enhanced gluconeogenesis and increased insulin resistance. On the other hand, glucokinase protein levels were decreased in the SRL group (35%, $p < 0.05$) compared to vehicle, suggesting a decrease in glucose disposal. SRL treatment also reduced peroxisome proliferator-activated receptor γ coactivator 1 alpha protein expression in muscle (~50%, $p < 0.05$), while no further protein alterations were observed in muscle and perirenal adipose tissue nor with the CsA treatment. Moreover, the phosphorylation of key proteins of the insulin signaling cascade was suppressed in the SRL group, but was unchanged by the CsA treatment.

Taken together, these data suggest that CsA treatment enhances gluconeogenic factors in liver, while SRL treatment impairs insulin signaling in peripheral tissues, which can contribute to the development of insulin resistance and NODAT associated with immunosuppressive therapy.

Abbreviations: Akt, protein kinase B; AS160, protein kinase B substrate of 160 kDa; CsA, cyclosporine A; FKBP12, FK506-binding protein (12-kD); FOX, forkhead box; GK, glucokinase; GLUT, glucose transporter; G6P, glucose-6 phosphate; G6Pase, glucose-6-phosphatase; IR, insulin receptor; IRS, insulin receptor substrate; mTOR, mammalian target of rapamycin; NODAT, new onset diabetes after transplantation; p70S6K, p70 ribosomal S6 kinase; PDK1, phosphoinositide-dependent kinase 1; PEPCK, phosphoenolpyruvate carboykinase; PI3K, phosphatidylinositol 3-kinase; PGC1-α, peroxisome proliferator-activated receptor-coactivator; PTP1B, protein-tyrosine phosphatase 1B; SRL, sirolimus.

* Corresponding author.
E-mail addresses: patriciacmmlopes@gmail.com (P.C. Lopes), lia2211@hotmail.it (A. Fuhrmann), fabcarvalho27@gmail.com (F. Carvalho), jose6sereno@hotmail.com (J. Sereno), martarebelosantos@gmail.com (M.R. Santos), maria.pereira@medsci.uu.se (M.J. Pereira), jan.eriksson@medsci.uu.se (J.W. Eriksson), freis@fmed.uc.pt (F. Reis), ecarvalh@cnc.uc.pt (E. Carvalho).

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1. Introduction

Immunosuppressive therapy is used in the treatment of autoimmune diseases and after organ transplantation, to promote tolerance to allografts [1]. Two of the main immunosuppressive agents are cyclosporine A (CsA) and sirolimus (SRL). CsA is a peptide of fungal origin that forms a complex with its intracellular receptor, cyclophilin A, an important intracellular acceptor protein with peptidyl-prolyl *cis–trans* isomerases (PPIase) activity [2]. Consequently, the drug-immunophilin complex binds to and inhibits the serine-phosphatase activity of calcineurin required for T-cell activation. Prevention of the calcineurin-mediated dephosphorylation of the transcription nuclear factor of activated T-cells, blocks its translocation to the nucleus. Interleukin (IL)-2 production is inhibited and, consequently also the proliferation and differentiation of T-cells [1,3]. On the other hands, SRL, an antifungal macrolide, binds to the 12-kD FK506-binding protein (FKBP12) and this complex inhibits the target of rapamycin (TOR) Ser/Thr kinase. As mTOR regulates mRNA translation initiation and progression from the G1 to S phase of the cell cycle, its inhibition prevents T-cell proliferation [4].

Although these immunosuppressive agents are very effective in their function, they are also responsible for the development of metabolic complications, linked to higher rates of cardiovascular disease and infections, which are the major causes of morbidity and mortality after transplantation [5–7]. One of the complications is NODAT, usually manifested in the first few months post-transplantation and varying according to the type of immunosuppressive agent, their different combinations and patient demographics [8]. NODAT is reported in 2.5 to 40% of patients that underwent renal, liver, heart or lung transplant [9]. Similar to type 2 diabetes, NODAT has been associated with impairment in glucose tolerance, insulin secretion and dysfunctional hepatic gluconeogenesis [10]. Insulin directly regulates gluconeogenesis, however in insulin resistance states it does not properly suppresses gluconeogenesis in the liver, leading to enhanced activation of forkhead box-containing transcription factors of the FOXO subfamily, promoting increased transcription of glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK), rate-limiting enzymes in hepatic glycogenolysis and gluconeogenesis, respectively [11,12]. Moreover, according to Ropelle et al. [13] the physical interaction of peroxisome proliferator-activated receptor γ coactivator 1 (PGC-1α) and FOXO1 promote an important signal transduction pathway responsible for the synthesis of glucose by the liver. Furthermore, PGC-1α expression is a tissue-specific regulatory marker activated in diabetic states, as well as the fasted state. It is perhaps responsible for increased hepatic glucose production and consequently hyperglycemia [13,14], making it a marker of interest together with its downstream targets.

On the other hand, insulin participates in many physiological processes, particularly important in maintaining glucose homeostasis. After a meal, glucose increases in circulation, stimulating the secretion of C-peptide and insulin, which inhibit glycogenolysis and gluconeogenesis, promoting at the same time glycogen synthesis and glucose uptake. Insulin binds to its cell surface receptor (IR), activating its intrinsic tyrosine kinase activity and leading to receptor auto-phosphorylation, which in turn leads to the phosphorylation of insulin receptor substrates proteins (IRS-1–IRS-4). As a result, several downstream signaling pathways are activated, including the p85 regulatory subunit of PI3-kinase and protein kinase B (Akt/PKB). This last step activates pyruvate dehydrogenase kinase 1 (PDK1) and protein kinase C (PKC), leading to the translocation of the muscle and fat specific glucose transporter (GLUT)4 from intracellular vesicles to the plasma membrane [15]. Alterations in these signaling pathways may affect glycemia and lead to unwanted metabolic consequences like diabetes and dyslipidemia [15]. Although CsA and SRL have been linked with NODAT, the underlying mechanisms are still not completely understood. SRL has been shown to improve insulin-stimulated glucose uptake and Akt/PKB phosphorylation in L-6 muscle cells, 3T3-L1 cells and in differentiated adipocytes [16–18], while other studies have shown reduced glucose uptake [19] and Akt/PKB phosphorylation in human mature adipocytes [20]. On the other hand, while immunosuppressive agents like CsA have been involved in the inhibition of the phosphorylation of the IR, it has not been associated with alterations in the expression or phosphorylation of proximal insulin signaling cascade proteins (Pereira et al., unpublished data), [21]. Therefore, there is still a lack of consensus regarding the underlying mechanism for NODAT caused by both CsA and SRL.

Recently, we and others have reported that treatments with either CsA or/and SRL leads to metabolic alterations in liver, muscle and adipose tissue and possibly contribute to the development of dyslipidemia and insulin resistance associated with immunosuppressive therapy; however, no insulin signaling studies have been performed to unravel the underlying mechanisms in these tissues [5,19,22–24]. Therefore, the main aim of this in vivo study is to understand how these immunosuppressive agents affect gluconeogenesis and insulin signaling in liver, muscle and adipose tissue after 3 weeks of treatment, in a rodent model.

2. Materials and methods

2.1. Chemicals

CsA (Sandimmune Neoral®) was supplied by Novartis Pharma (Lisbon, Portugal) and SRL (Rapamune®) by Wyett Europe Ltd (Berkshire, United Kingdom) through the Pfizer Laboratories Lda (Lisbon, Portugal). Human insulin, Actrapid was kindly provided by NovoNordisk A/S (Lisbon, Portugal). Ketamine (Ketalar®, Parke-Davis) was purchase from Pfizer Labs, while chlorpromazine (Largatil®, Rhône-Poulenc Rorer) was from Vitória labs (Amadora, Portugal). The High Capacity cDNA Reverse Transcription kit was obtained from Applied Biosystems (Forest City, CA, USA) and the RNeasy® MiniKit and the QIAzol® Lysis Reagent from QIAGEN Sciences (Germantown, MD, USA). Diethyl pyrocarbonate (DEPC) was acquired from AppliChem (Darmstadt, Germany). Methanol, isopropanol and chloroform were obtained from Merck (Darmstadt, Germany). PCR primers were designed by us, using Vector NTI Advanced 10 Software (Life technologies, Grand Island, NY, USA) and were synthesized by Integrated DNA Technologies, Inc. (IDT, Coralville, IA, USA).

2.2. Animals and treatments

Male Wistar rats, weighing ~300 g, 10 weeks old, were obtained from Charles River Lab. Inc. (Barcelona, Spain). Animal studies were conducted using protocols approved by to the National and European Community Council Directives on Animal Care. The animals were housed in a light-controlled 12 h dark/light cycles and were given standard laboratory chow (IPM-R20, Letica, Barcelona, Spain) and free access to tap water. Body weight was monitored every week [19].

Animals were randomly divided into three groups (n = 16 per group): Vehicle (orange juice); CsA–5 mg/kg body weight (BW)/day of Sandimmune Neoral® and SRL—1 mg/kg BW/day of Rapamune®. The agents were diluted in orange juice as is the usual procedure in the clinic for the patients [25]. The use of a diluted form of orange juice was applied to vehicle and CsA and SRL-treated rats eliminating or highly minimizing any possible effect in glucose metabolism. Doses were chosen to have blood