Pharmacological Reports 68 (2016) 201–205

Contents lists available at ScienceDirect

Pharmacological Reports

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

Original research article

Cyclosporine-A, but not tacrolimus significantly increases reactivity of vascular smooth muscle cells

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ARTICLE INFO

Article history:
Received 24 June 2015
Received in revised form 9 August 2015
Accepted 19 August 2015
Available online 5 September 2015

Keywords:
Cyclosporine A
Tacrolimus
Smooth muscle reactivity
Hypertension

ABSTRACT

Background: Application of cyclosporine-A (CsA) or tacrolimus is associated with numerous side effects. One of the main reasons for restricting usage of CsA is hypertension. In tacrolimus treated subjects the frequency of these phenomena is significantly lower.

The known molecular mechanism of action of tacrolimus and cyclosporine-A seems to be the same, thus we decided to compare modulatory effect of drugs on vascular smooth muscle contractility.

Methods: Experiments were performed on isolated and perfused tail artery of Wistar rats. Contraction force was measured by increased degree of perfusion pressure with a constant flow rate.

Results: Concentration–response curves for agonist in the presence CsA were significantly shifted to the left with increase in maximal responses. This effect was due to increased calcium influx from extracellular calcium stores whereas there were no significant changes in calcium influx in the presence of tacrolimus; concentration–response curve was comparable to controls.

Conclusion: Our results strongly support the idea that main difference between effects on smooth muscle contractility of calcineurin-dependent immunosuppressants: CsA and tacrolimus is related to the different level of extracellular calcium influx to the cytoplasm. The elucidation of these mechanisms may permit the identification of new therapeutic strategies against CsA-induced hypertension.

Introduction

Cyclosporine and tacrolimus are immunosuppressive drugs commonly used in therapy in patients after ograns transplantation [1]. Cyclosporine was isolated from fungus Tolypodadium *infatum* and introduced into clinical practice in the 1980s. Cyclosporine was the first drug of new generation of immunosuppressants and started the new era in transplantology. Tacrolimus was isolated in 1984 from a type of soil bacterium, *Streptomyces tsukubaensis*. The name tacrolimus is derived from 'Tsukuba macrolide immunosuppressant' [2].

Mechanism of action of cyclosporine and tacrolimus is related to decreasing production of proinflammatory IL-2 by inhibited lymphocytes. The mechanism of action of both drugs is related to creation of complexes lymphocyte's protein–cyclophilin and cyclosporine A (CsA) or tacrolimus. Secondary the level of lymphocytes activation is lower [3,4]. Mechanism of action of CsA and tacrolimus is similar but the risk in generation of the side effects is different. Treatment with CsA or tacrolimus may lead to side effects such as: hypertension, renal failure, hepatotoxicity, neurological [5]. One of the most common side effects, secondary restricting usage of CsA are nephrotoxicity and hypertension in up to 30% of patients. In tacrolimus treated subjects the risk of hypertension is about 10% [6].

Abbreviation: AVP, arg-vasopressin; CsA, cyclosporine A; E_A, effect of pharmacological stimulation; E_{max}, maximal effect, calculated as a percent of maximal response for controls; PHE, phenylephrine; RP, relative potency, calculated as ED50 for controls/ED50; TAC, tacrolimus.

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http://dx.doi.org/10.1016/j.pharep.2015.08.012
1734-1140/

Hypertension caused by immunosuppressants, may lead to organ complications especially cardiovascular remodeling, which is one the most important risk factors of heart failure, stroke and death. Treatment of hypertension should be based on clinical investigation including age, current classification of hypertension, presence of organ complication and additional risk factors [6,7].

The role of calcium ions in vascular smooth muscle tone

Activation of G protein coupled receptors such as α_1, V_1, ET_A and AT_1 will induce activation of cell membrane enzymes. The first activated enzyme is phospholipase C (PLC) catalyzing degradation of membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP_2) into two intracellular transmitters-diacylglycerol (DAG) and inositol triphosphate (IP_3) [8,9]. The last and most important enzyme is protein kinase C (PKC). Enzyme is activated by DAG, and stimulates the smooth muscle contraction by L-type calcium channels phosphorylation. Moreover PKC is intracellular calcium dependent. It means that in first step the influx from intracellular calcium store have to be started by IP_3 to activate PKC by DAG [10–14].

In one of our previous studies we analyzed the effect of CsA on vascular smooth muscle contraction. Our results suggested that CsA is able to directly stimulate PKC and in this way increase the concentration of calcium in cytoplasm. Moreover this effect can be reversed by the presence of calcium antagonists [15].

The known molecular mechanism of action of tacrolimus and CsA seems to be the same, thus we decided to compare modulatory effect of cyclosporine and tacrolimus on vascular smooth muscle contractility.

Materials and methods

Animals

The study was performed on isolated, perfunded arteries. Male Wistar rats were housed under a 12 h light/12 h dark cycle and had unlimited access to food and water. Rats weighing 250–350 g were pretreated with investigated drugs or placebo, anesthetized by intraperitoneal injection of 120 mg urethane per 1 kg of body mass, stunned and then sacrificed by cervical dislocation. The study protocol was approved by the Local Ethics Committee and all experiments were carried out in accordance with the United States NIH guidelines (Guide for the Care and Use of Laboratory Animals (1985), DHEW Publication No. (NIH) 85-23: Office of Science and Health Reports, DRR/NIH, Bethesda, MD, USA).

Drugs and solutions

The study drugs (CsA and tacrolimus) or placebo (normal saline) were administered intraperitoneally once daily, for three days prior to the experiment. Similarly to previous studies the doses were: CsA (10 mg/kg) and tacrolimus (0.5 mg/kg). To confirm the efficacy of the administration of drugs in all animals, blood samples were taken and the assays were performed with the use of automatic analyser (Abbott ARCHITECT-fluorescence polarization immunoassay). The experiments were performed to determine the role of intracellular and extracellular calcium ions in contraction induced by PHE and AVP in control conditions and in CsA and tacrolimus pretreated arteries using two types of Krebs fluid: (1) FPSS—Ca^{2+}-free EGTA-Krebs with the following composition: NaCl (71.8 mM/l), KCl (4.7 mM/l), $MgSO_4$ (2.4 mM/l), $NaHCO_3$ (28.4 mM/l), KH_2PO_4 (1.2 mM/l), glucose (11.1 mM/l) with the addition of EGTA (30 μM/l); (2) PSS—fluid with Ca^{2+} EGTA-Krebs (normal) with the following composition: NaCl (71.8 mM/l), KCl (4.7 mM/l), $MgSO_4$ (2.4 mM/l), $NaHCO_3$ (28.4 mM/l), KH_2PO_4 (1.2 mM/l), $CaCl_2$ (1.7 mM/l), glucose (11.1 mM/l) with addition of EGTA (30 μM/l), after emptying the intracellular pool of calcium ions.

Study design and conduction

2.5 to 3.0 cm long segments of rat tail arteries were gently dissected from surrounding tissues, than proximal segment was cannulated and connected to the perfusion equipment. The arteries were placed in a 20-ml isolated organs bath filled with oxygenated Krebs solution at 37 °C. In the initial part of experiment, perfusion fluid flow was increased gradually up to 1 ml/min. The changes in continuously measured perfusate pressure in the experimental system were an exponent of arterial smooth muscle contractility. Investigations were performed on isolated organs bath system (TSZ-04, Experimetria Ltd. Budapest, Hungary). Perfusion pressure was measured on BPR-01 and BPR-02 transducers (Experimetria Ltd, Budapest, Hungary) connected with digital recorder Graphtec midi Logger GL820, Yokohama, Japan. Peristaltic pump was made by ZALIMP, Warszawa, Poland. Experiments were performed separately on control arteries and on arteries derived from rats pre-treated with CsA and tacrolimus [15,16].

Data analysis and statistical procedures

Classical pharmacometric van Rossum method was used to calculate concentration–response curves (CRCs) [17,18]. The maximal effect (E_{max}) of tissue stimulation was calculated as a percent of the maximal response for respective agonist. Half maximal effective dose (ED_{50}) was calculated using classical pharmacologic methods with pD_2 the negative logarithm of the ED_{50} [18–20]. We used the number of the CRC and E_{max} in all calculations estimating the statistical significance.

Data were presented as means ± SD. The Shapiro–Wilk test was used to determine normal distribution of the investigated variables. Statistical analysis was performed using the Newman–Keuls and ANOVA test for multiple comparisons of means. A two-sided difference was considered significant at $p < 0.05$.

Results

Treatment with CsA and tacrolimus

After 3 days of treatment with investigated drugs the concentration of the drug in therapeutic range were achieved for all animals. Mean concentration of tacrolimus was 12.4 ± 2.2 ng/ml and that for cyclosporine was 512.4 ± 14.6 ng/ml. This concentration corresponds with upper level of normal drug concentration during immunosuppressive treatment.

Effect of CsA and tacrolimus on the contractility of VSMC

In this part of the experiments, the reactivity of VSMC to PHE (10^{-9}–10^{-3} M/l), a preferential α_1-adrenoceptor agonist, and AVP (10^{-10}–10^{-4} M/l), a non-selective vasopressin receptor agonist in the control group and for arteries taken from CsA and tacrolimus pretreated rats was analyzed. ED_{50} values calculated for PHE and AVP were 6.95 ($\pm$0.86) × 10^{-8} M/l and 1.35 ($\pm$0.75) × 10^{-8} M/l, respectively. The CRCs obtained for PHE and AVP in the presence of CsA were shifted to the leftward with an increase in maximal responses (Figs. 1 and 2). Under these conditions ED_{50} values for PHE and AVP were 2.52 ($\pm$0.71) × 10^{-8} M/l ($p < 0.0001$) and 7.15 ($\pm$0.9) × 10^{-9} M/l ($p < 0.0001$), respectively. Pretreatment with tacrolimus did not shift CRCs for PHE and (Figs. 1 and 2). The maximal responses were similar to controls. Calculated ED_{50} values for PHE and AVP were 5.88 ($\pm$0.96) × 10^{-8} M/l (ns) and 1.14 ($\pm$0.82) × 10^{-8} M/l (ns), respectively and did not differ significantly from controls (Table 1).