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Modulation by NADPH oxidase of the chronic cardiovascular and autonomic interaction between cyclosporine and NSAIDs in female rats

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Diclofenac potassium (PubChem CID: 23667642)
Celecoxib (PubChem CID: 2662)
Fasudil hydrochloride (PubChem CID: 163751)
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ABSTRACT

Cyclosporine (CSA) and nonsteroidal antiinflammatory drugs (NSAIDs) are used together to manage arthritic disorders with an immune component. Previous reports showed contrasting effects for NSAIDs on CSA nephrotoxicity and acute elevations in blood pressure. Both effects were ameliorated or exaggerated after selective cyclooxygenase-2 (COX2) and nonselective COX inhibition, respectively. Here we investigated: (i) the interaction of CSA with NSAIDs possessing variable COX1/COX2 selectivities on hemodynamic, left ventricular (LV) and cardiac autonomic and histologic profiles, and (ii) role of NADPH-oxidase (NOX)/Rho-kinase (ROCK) pathway in the interaction. Female rats were pre-instrumented with femoral catheters and treated for 10 days with CSA (25 mg/kg/day), diclofenac (nonselective NSAIDs, 1 mg/kg/day), celecoxib (COX2 inhibitor, 10 mg/kg/day), or their combinations. CSA-mediated hypertension was maintained upon co-administration of either NSAID whereas the concomitant reductions in time- and frequency-domain indices of heart rate variability (HRV) were accentuated in presence of diclofenac but not celecoxib. The isovolumic relaxation time (Tau), a measure of diastolic function, was reduced by all regimens whereas LV contractility (dP/dt_{max}) remained unaffected. The CSA/diclofenac regimen, but not individual treatments, increased cardiac NOX2 expression and caused more cardiac structural damage. The inhibition of NOX by diphenyleneiodonium reversed CSA/diclofenac-evoked increases in MAP, decreases in HRV and Tau, cardiac structural damage, and increased NOX2 expression. No such effects were observed after ROCK inhibition by fasudil, despite concomitant decreases in NOX2 expression. In conclusion, CSA/diclofenac-treated female rats exhibit exacerbated hemodynamic, autonomic, LV, and histopathologic disturbances via ROCK-independent NOX2 upregulation.

1. Introduction

Inflammatory arthritis is a group of joint disorders that involve an immune component, e.g. rheumatoid arthritis, psoriatic arthritis, systemic lupus erythematosus, and ankylosing spondylitis. The management of these disorders involves the use of immunosuppressant drugs to dampen the immune response, together with NSAIDs. CSA, a calcineurin inhibitor with immunosuppressant properties (Matsuda and Koyasu, 2000), is employed as a disease modifying antirheumatic drug in severe inflammatory arthritis (Fraser et al., 2005; Gossec and Smolen, 2016). Its use in combination with NSAIDs was recognized to be associated with increased cardiovascular risk in epidemiological studies (Lee et al., 2016).

Despite its documented immunosuppressant effects, CSA was shown to negatively affect cardiac function by different mechanisms posing a serious challenge to its clinical use. CSA depresses cardiac functions through altered control of sarcoplasmic reticulum calcium (Janssen et al., 2000), reductions in stroke volume and cardiac output (Navarro et al., 1996), and left ventricular diastolic dysfunction (Therapondos et al., 2002), and impairment of autonomic and arterial baroreceptor control of cardiac activity (El-Mas et al., 2002). Many of the peripheral and central effects of CSA are attributed to increased oxidative stress (El-Mas et al., 2012). NOX2 is blamed for increased oxidative stress leading to CSA toxicity (Djamali et al., 2012). The consequent activation of ROCK (Borer et al., 2013) is also implicated in CSA toxicity (Park et al., 2011).

Although the hemodynamic profiling of individual NSAIDs is variable, a strong association exists among their long-term use, increased blood pressure, and cardiovascular risk (Sudano et al., 2012; Trelle et al., 2011). A review of the available safety data

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demonstrated that diclofenac use was associated with the highest cardiovascular risk among nonselective NSAIDs (Farkouh and Greenberg, 2009). The inhibition of COX2-mediated prostacyclin synthesis is proposed as the cause for the poor cardiovascular outcomes (Capone et al., 2010). In addition, prostaglandin F-2α, a product of COX activity, contributes to myocardial fibrosis in a manner that is dependent on ROCK activity (Ding et al., 2012) offering a potential for crosstalk between CSA- and NSAID/COX inhibition-mediated cardiotoxicity.

Thus, studying the mechanisms underlying the cardiovascular impairment due to the combined use of CSA and NSAIDs or selective COX2 inhibitors is prudent. Indeed, earlier studies showed that chronic use (> 2 weeks) of the CSA/NSAID combination led to marked reduction in renal function (Altman et al., 1992) and a two-fold increase of serum level of diclofenac (Kovarik et al., 1997) indicating a potential for a hemodynamic interaction between both drug classes. However, little information is available regarding the hemodynamic sequels of such an interaction, let alone the involved mechanisms. In particular, COX selectivity might play a role in the outcome of the interaction given previous observations of a divergent effect on renal function (El-Gowelli et al., 2014; Helmy et al., 2015) and the acute pressor effect of CSA (Ibrahim et al., 2017). In the present study, we intended to test whether the result of the interaction is dependent on COX selectivity. To this end, we examined the effect of CSA combination with diclofenac as a nonselective COX inhibitor (Kato et al., 2001), and celecoxib as a selective COX2 inhibitor (Kato et al., 2001). Furthermore, we assessed the cardiac protein expression of NOX2 and ROCK as possible mediators of CSA and NSAID cardiotoxicity. For the same reason, the effects of pharmacologic inhibition of NOX or ROCK by DPI and fasudil, respectively, on the observed alterations in hemodynamic parameters were investigated.

2. Materials and methods

2.1. Animal studies

All experiments were conducted according to a protocol approved by institutional Animal Care and Use Committee and in accordance with standard guidelines on the care and use of the laboratory rat. Female Wistar rats (200–230 g) were obtained from the Faculty of Pharmacy Animal Facility, Alexandria University, Egypt. Animals were housed in plastic cages on wood-chip bedding, provided with water and standard rodent chow (16% protein, Tanta oil and Soap Co., Tanta, Egypt) ad libitum, and exposed to a 12 h dark/light cycle. Postoperatively, rats were monitored every 3–4 h during daytime for signs of weight loss, cyanosis, or illness. Over 90% of operated rats suffered no complications or unexpected death outcomes at any time prior to the experimental endpoint. Criteria for euthanasia included weight loss, infection, or general weakness. At the conclusion of experiments or in the rare incidence of severe illness, rats were euthanized with an overdose of thiopental (100 mg/kg i.v).

2.2. Drug regimens

We studied the hemodynamic and cardiac autonomic and structural effects of individual and combined chronic administration of CSA, diclofenac, and celecoxib. Six groups of female rats (n=6–8 each) were used and treated subcutaneously with one of the following regimens for 10 consecutive days: (i) vehicle (Olive oil + DMSO), (ii) CSA (25 mg/kg/day) (Oriji and Keiser, 1998), (iii) celecoxib (10 mg/kg/day) (El-Gowelli et al., 2014; El-Mas et al., 2015), (iv) diclofenac (1 mg/kg/day) (Martinez et al., 2005), (v) celecoxib + CSA, (vi) diclofenac + CSA. To investigate the role of the NADPH/ROCK oxidative pathway in the exaggerated hemodynamic and cardiac structural effects induced by the combined CSA/diclofenac regimen, two additional groups of CSA/diclofenac-treated female rats were used and treated simultaneously with i.p. fasudil (ROCK inhibitor, 3 mg/kg/day) (Wesselman et al., 2004) or diphenyleneiodonium (DPI, NADPH-oxidase inhibitor, 0.75 mg/kg/day) (Wang and Pang, 1993) for 10 days. Working solutions of all drugs used were prepared so that the maximum volume of DMSO injected per day would not exceed 0.2 ml, which was injected to the control group. Preliminary experiments showed that this volume did not affect the measured hemodynamic parameters when compared to a daily injection of an equivalent volume of normal saline. For all rats, intravascular cannulation and subsequent hemodynamic, autonomic, and histopathologic measurements were performed as described below.

2.3. Intravascular cannulation

Eight days after starting drug treatments, femoral artery and vein cannulation was performed as described previously (El-Mas and Abdel-Rahman, 1997b, 1999). Briefly, rats were anesthetized with thiopental (50 mg/kg, i.p.). Polyethylene Catheters (consisting of 5-cm PE-10 tubing tightly bonded to 15-cm PE-50 tubing) were placed in the abdominal aorta and vena cava by insertion through the femoral artery and vein, for measurement of blood pressure (BP) and intravenous (i.v.) administration of drugs, respectively. The catheters were subcutaneously tunneled and exteriorized at the back of the neck between the scapulae. Finally, the catheters were flushed with heparin (0.2 ml, 100 U/ml) and plugged by stainless steel pins. Incisions were closed by surgical sutures and swabbed with povidone iodine solution. Operated rats received an intramuscular injection of 60,000 U of penicillin G benzathine in an aqueous suspension and were housed in separate cages. Experiments recording blood pressure and hemodynamic parameters were conducted on freely-moving conscious rats two days following the surgery. On the day of the experiment (i.e. 2 days after intravascular cannulation), the arterial catheter was connected to a BP transducer (model P23XL; Astro-Med, West Warwick, RI.) that was attached through MLAC11 Grass adapter cable to a computerized data acquisition system with LabChart-7 pro software (Power Lab 4/35, model ML866/P; AD Instruments Pty Ltd., Castle Hill, Australia). The heart rate (HR) was computed from BP waveforms and displayed on another channel of the recording system.

After an initial 45 min equilibration period, mean arterial pressure (MAP) and HR were measured. The isovolumic relaxation time constant (Tau), which represents the exponential decay of the ventricular pressure during isovolumic relaxation (Matsubara et al., 1995), and the maximum rate of rise of BP waves ($+dP/dt_{max}$), index of LV contractility (el-Mas and Abdel-Rahman, 1997a), were computed by LabChart-7 pro software. Time and frequency domain analyses were also determined as described below.

2.4. Time-domain analysis of heart rate variability (HRV)

Two time-domain measures of the cardiac autonomic activity were employed, the standard deviation of beat-to-beat intervals (SDNN) and the root mean square of successive beat-to-beat differences in R-R interval durations (rMSSD) (El-Mas and Abdel-Rahman, 2005; Omar and El-Mas, 2004). The RR intervals were computed as the reciprocal of HR in ms. SDNN is comparable to the total power of the spectrum of RR variability, which measures the overall autonomic balance of the heart. rMSSD is largely validated as a measure of the parasympathetic input to the heart and, therefore, correlates with the high frequency power of the spectrum (Stein et al., 1994). Each variable was estimated as a five-minute average through an area of a stable recording pattern.

2.5. Frequency-domain analysis of HRV

Spectral hemodynamic fluctuations, quantitative indices of cardiac autonomic control (Stein et al., 1994) were used to reflect changes in sympathetic and vagal outflows. HRV was analyzed in the frequency