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Original article

The selective estrogen receptor modulators (SERMs) raloxifene and tamoxifen improve ANP levels and decrease nuclear translocation of NF-kB in estrogen-deficient rats

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ABSTRACT

Background: The selective estrogen receptor modulators (SERMs) raloxifene and tamoxifen are used for the treatment of osteoporosis and cancer, respectively, in women. The impairment of both the Atrial Natriuretic Peptide (ANP) cell signaling system and the translocation of nuclear factor-kappa B (NF-kB) to the cell nucleus are associated with detrimental cardiovascular effects and inflammation. The effects of SERMs on these parameters in the cardiac tissue of estrogen-deficient rats has not been reported.
Methods: We investigated the effects of raloxifene and tamoxifen on ANP signaling, p65 NF-kB nuclear translocation, cardiac histology and contractility. Female rats were divided into five groups: control (SHAM), ovariectomized (OVX), OVX-treated 17-β-estradiol (E), OVX-treated raloxifene (RLX) and OVX-treated tamoxifen (TAM). The treatments started 21 days after ovariectomy and continued for 14 days.
Results: Ovariectomy reduced ANP mRNA in the left atrium (LA), decreased the content of ANP protein in the LA and in plasma, and increased the level of p65 NF-kB nuclear translocation in the left ventricle. Both 17-β-estradiol and SERMs were able to reverse these alterations, which were induced by the estrogen deficient state. The hemodynamic and cardiac structural parameters analyzed in the present work were not modified by the interventions.
Conclusions: Our study demonstrates, for the first time, the additional benefits of raloxifene and tamoxifen in an estrogen-deficient state. These include the normalization of plasmatic and cardiac ANP levels and cardiac p65 NF-kB translocation. Therefore, these treatments promote cardiovascular protection and may contribute to the prevention of cardiac dysfunction observed long-term in postmenopausal women.

Introduction

Atrial natriuretic peptide (ANP) is a polypeptide hormone secreted by cardiac muscle cells and is a very important regulator of cardiac homeostasis. Impairment of ANP function is associated with cardiovascular disorders [1]. The biological effects of ANP are mediated by the binding of this peptide to natriuretic peptide receptors (NPRs). Three types of NPR are known: NPR-A, NPR-B and NPR-C. NPR-A has guanylate cyclase activity, and it regulates the majority of the biological effects of ANP [2]. In mammals, cardiac muscle cells of the heart atria produce and secrete the polypeptide hormone ANP in a regulated manner [3]. Atrial expression of both ANP and B-type natriuretic peptide (BNP) is up-regulated during chronic hemodynamic overload as a protective mechanism [4].

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Moreover, ANP can counter-regulate the functions of renin-angiotensin, vasopressin, and the sympathetic nervous system, all of which participate in the pathophysiology of cardiovascular diseases [5]. In fact, therapies that are capable of normalizing ANP levels may contribute to protection against cardiovascular diseases [6]. The neurohormonal response to the failing myocardium is directly or indirectly linked to inflammation [7].

Nuclear factor-kB (NF-kB) is a family of transcription factors that has been implicated in the regulation of expression of inflammatory genes, including tumor necrosis factor alpha (TNF-α) and other cytokines. The inflammatory response itself is usually dependent on the activity of NF-kB [8]. Prolonged activation of NF-kB appears to be involved in impaired cardiovascular function [9]. However, NF-kB can have either protective or detrimental effects due its signaling complexity. Its activation is generally transient and while the short activation might be beneficial, the long activation usually promotes chronic inflammation and organism injury [10]. NF-kB has a dual role in context-dependent control of apoptosis leading to either cell proliferation, differentiation, and survival or, in a different way, programed cell death [11]. Moreover, the crosstalk between estrogen receptors (ERs) and NF-kB pathway contributes to the development of endocrine therapy resistance in breast cancers [11].

Selective estrogen receptor modulators (SERMs) are compounds that interact with ERs in target tissues and can act as agonists or antagonists [12]. Regarding the cardiovascular effects, raloxifene and tamoxifen have been reported to not only have cardioprotective effects in specific groups, but also to contribute to the healthy endothelial function and vasorelaxation. Additionaly, raloxifene and tamoxifen can decrease inflammatory cytokines, serum homocysteine levels, and C-reactive protein [13,14]. However, despite several beneficial effects of SERMs, some side effects are reported. Tamoxifen can cause hot flashes, night sweats, depression, forgetfulness, sleep alterations, weight gain, vaginal and sexual disturbance and, in some cases, endometrial hyperplasia, endometrial cancer or venous thromboembolic disease [15]. Raloxifen has also been related to venous thromboembolism [16].

Interestingly, NF-kB also can mediate cardiac hypertrophy under disruption of guanylyl cyclase/NPRA signaling [17,18]. Furthermore, previously published reports have demonstrated an anti-inflammatory role for ANP by showing that ANP-induced cyclic GMP signaling could suppress the release TNF-α in vitro [19,20] and in vivo [21,22] through inhibition of NF-kB activity as well as ANP can inhibit the NF-kB in THP-1 cells [23]. On the other hand, estrogen has been shown to have a profound influence in the ANP system, even though only few studies have investigated the effects of hormone replacement therapy (HRT) on the ANP system [24,25]. To our knowledge, there are no data available regarding the chronic effects of raloxifene and tamoxifen on the natriuretic peptide system in animal models of estrogen-deficiency. This is the first study to examine the effects of these SERMs on both the ANP system and NF-kB function in the hearts of estrogen-deficient animals.

In clinical practice, raloxifene is used for the treatment and prevention of post-menopausal osteoporosis [26], while tamoxifen is the most widely used anti-estrogen for the management of breast cancer [27,28]. Because of their potential long-term usage by patients, it is important to identify the effects of SERMs on cardiac function. We hypothesize that treatment with SERMs may be able to reverse negative alterations in cardiac tissue induced by estrogen-deficiency. Accordingly, the aim of this study was to investigate the effects of raloxifene and tamoxifen on the ANP system and p65 NF-kB translocation in cardiac tissue in ovariectomized rats, along with relevant hemodynamic and morphometric parameters.

Materials and methods

Experimental animals

These investigations were conducted in accordance with the biomedical research guidelines for the care and use of laboratory animals and were approved by Ethics Committee of the university where the research was conducted (no. 012/2008). Eight-week-old female Wistar rats were housed in groups with a 12-h (light)–12-h (dark) cycle – 25 °C. Standard rat chow and tap water were available *ad libitum*. Five groups (n = 5–8/group) were studied: control (SHAM); ovariectomized (OVX); OVX treated with 17-β-estradiol (E: 0.5 μg/kg/day; Sigma Chemical Co., St. Louis, MO, USA); OVX treated with raloxifene (RLX: 2.0 mg/kg/day; Eli Lilly, Indianapolis, IN, USA); and OVX treated with tamoxifen (TAM; 1.0 mg/kg/day; Sandoz, Cambé, PR, Brazil). The Study design in graphical representation is shown in Fig. 1.

Ovariectomy and treatments

Female rats were anesthetized with an intraperitoneal injection (*ip*) of ketamine/xylazine (70/10 mg/kg) and underwent bilateral ovariectomy as previously described [24]. The female SHAM group only underwent an incision. Twenty-one days after surgery, the ovariectomized female rats were subcutaneously given 17β-estradiol diluted in peanut oil while the SERMs were pulverized, dissolved in water and administrated by gavage. The SHAM and ovariectomized groups received only vehicle. These treatments lasted 14 days.

Estrous cycle phase determination

Daily vaginal smears were obtained from each SHAM rat between 8:00 and 10:00 a.m. as previously described to confirm that their oestrous cycles. The rats with normal oestrous cycles were sacrificed during the proestrus phase [29].

Measurements hemodynamic

In the end of treatment period, rats were anesthetized with ketamine/xylazine (70/10 mg/kg, *ip*) and left ventricular (LV) function was assessed as previously described [30]. Right common carotid artery was catheterized with a polyethylene catheter (P50) and connected to a pressure transducer (TRI 21, Letica Scientific Instruments, Spain) and then to a digital system (Powerlab/4SP ML750, ADInstruments, Australia). Parameters were measured as LV +dP/dT, which is the maximum rate of ventricular pressure increase or the peak positive value of the first derivative of the LV pressure, as well as the rate of pressure decay (-dP/dT). The signal was expressed in mmHg/s. Following this procedure, the catheter was withdrawn from the LV and the arterial pressure was measured again and if a decrease in the diastolic blood pressure was observed the animal was not computed. Data were analyzed using the LabChart software 7.

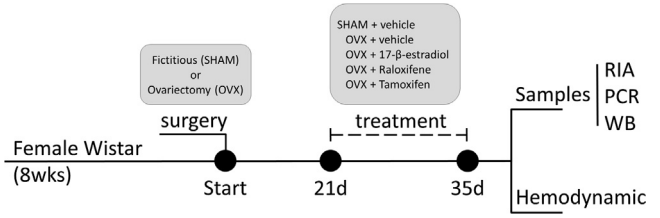

Fig. 1. Study design in graphical representation. The timeline shows the days (d) from the surgery procedure to final experiments, including the radioimmunoassay (RIA), polymerase chain reaction (PCR) and Western Blot (WB) analysis.