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Therapeutic effects of tamoxifen on metabolic parameters and cytokines modulation in rat model of postmenopausal diabetic cardiovascular dysfunction: Role of classic estrogen receptors

Hossein Azizian[a,b], Mohammad Khaksari[c,*], Gholamreza Asadikaram[d], Gholamreza Sepehri[e], Hamid Najafipour[f]

[a] Department of Physiology, Faculty of Medicine, Kerman University of Medical Sciences, Kerman, Iran
[b] Department of Physiology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[c] Endocrinology and Metabolism Research, and Physiology Research Centers, Kerman University of Medical Sciences, Kerman, Iran
[d] Department of Biochemistry, and Metabolism & Endocrinology Research Center, Kerman University of Medical Sciences, Kerman, Iran
[e] Neuroscience Research Center, Institute of Neuropharmacology, Kerman Univerity of Medical Sciences, Kerman, Iran
[f] Physiology Research Center, Institute of Basic and Clinical Physiology Sciences, Kerman University of Medical Sciences, Kerman, Iran

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ABSTRACT

In postmenopausal women, the risk of diabetic cardiovascular disease drastically increases compared with that of premenopausal women. In the present study we surveyed the effects of Tamoxifen (TAM) and 17-β-estradiol (E2) on diabetic cardiovascular dysfunction. Female wistar rats were divided into six groups: sham-control, Diabetes, Ovariectomized (OVX) + Diabetes, OVX + Diabetes + Vehicle, OVX + Diabetes + E2, OVX + Diabetes + TAM. Type 2 diabetes was induced by High Fat Diet and low doses of STZ. E2 and TAM were administrated every four days for four weeks. Results show that, TAM or E2 reduces cardiac weight, atherogenic and cardiac risk indices. Mean arterial blood pressure (MABP) increased in diabetes group, while TAM and E2 prevented MABP increment. Also, fasting blood glucose was decreased by TAM and E2. Significant decrement in the level of IL-10 was observed in diabetes group and this effect was abolished by TAM and E2. Also, treatment with TAM and E2 resulted in improved inflammatory balance in favor of anti-inflammation. Although diabetes resulted in, increment of TC and LDL, TAM and E2 reduced lipids profile. Furthermore, treatment with TAM prevented the reduction of estrogen receptors (ERs) α and β protein levels, but its effect on the ERβ protein level was higher. Our results indicated that TAM protects against diabetic cardiovascular dysfunction and is a good candidate for E2 substitution.

1. Introduction

Tamoxifen (TAM) is a selective estrogen receptor modulator (SERM) that attaches to estrogen receptors (ERs) with high potency but it has different tissue effects than 17-β-estradiol (E2), as it acts as an agonist in a-one tissue and as an E2 antagonist in another tissue [1]. Epidemiological studies have shown that TAM in postmenopausal women significantly reduces cardiovascular morbidity such as myocardial damage [2], cardiac arrhythmias [3] and coronary artery atherosclerosis [4], without increasing any risks associated with E2 such as endometrial cancer [5]. On the other hand, the direct effects of SERMs, especially TAM, on cardiac cells is not known [6], although it has been reported that, reducing inflammation, oxidative stress, apoptosis [7] and improving dyslipidemia [8] are among possible TAM-regulated mechanisms reducing heart damages [3].

Diabetes mellitus is a chronic metabolic syndrome, which is associated with hyperglycemia and dyslipidemia [9]. Inflammation has also been suggested as a common pathological mediator for weight gain, diabetes and cardiovascular disease [10]. In fact, chronic inflammation is a common feature of diabetes and elevated levels of inflammatory biomarkers are associated with the higher incidence of diabetes and cardiovascular disease [11]. On the other hand, diabetes can affect the structure and function of the heart and lead to pathological events known as diabetic cardiomyopathy. Diabetes not only changes metabolic and inflammatory pathways in the myocardium, but also causes hypertrophy and fibrosis of myocytes [12].

Premenopausal women have been reported to have a lower incidence of left ventricular hypertrophy compared to men of similar age

* Corresponding author.
E-mail address: Mkhaksari@kmu.ac.ir (M. Khaksari).

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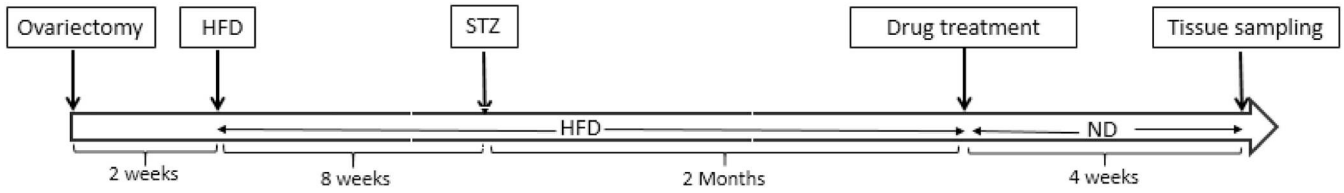

Fig. 1. Schematic diagram of experimental protocol. HFD: high fat diet, ND: normal diet, STZ: streptozotocin.

[13], and E2-based hormone therapy reduces hypertrophy in women after menopause [14]. Also, the loss of E2 in postmenopausal women is associated with the physiological changes and an increase in the inflammatory response and consequently an increase in the risk of several diseases, including cardiovascular disease [15]. Additionally, in postmenopausal women, increased risk of heart disease is parallel to increased insulin resistance and diabetes [16].

It has been shown that the anti-inflammatory effects of TAM are not limited to a specific tissue [17] and some of the physiological effects of TAM are independent of ERs [18]. It has also been shown that TAM regulates several markers involved in metabolic syndrome, including insulin secretion and body weight [19].

It is well known that E2 exerts its effects by binding to ERs. There are three types of ERs including ERα, ERβ (classic), and G-protein coupled receptor 30 (GPR30) (Nonclassic) [20]. ERs are expressed in animal and human hearts [21,22] and are regulated by the levels of E2 [23]. However, the role of E2 and TAM in regulating the expression of these receptors in the cardiovascular function and in normal and diseased human hearts is still largely unknown [24].

Although there are many studies regarding the effects of TAM on cardiovascular disorders in postmenopausal women, very few studies have been done so far to investigate the effects of TAM on cardiovascular function in diabetes. Since TAM acts as a SERM without increasing the risk of endometrial cancer and also it possesses anti-inflammatory properties, so the present study was conducted to investigate the possible protective effects of TAM on cardiac inflammatory cytokines in type 2 diabetes (T2D) in female rats.

2. Marerials and methods

2.1. Experimental animals

Adult female Wistar rats (200–250 g body weight) were housed in temperature and humidity controlled animal house with a 12-h light/dark cycle and free access to food and water. All procedures were approved by the ethical committee (Permission No: 95/105KA) of Kerman University of Medical Sciences, Kerman, Iran, in accordance with the National Guide for the Care and Use of Laboratory Animals.

2.2. Surgical procedure of ovariectomy

Animals were anesthetized with a mixture of ketamine/xylazine (80/10 mg/kg intraperitonealy (i.p.) and the ventral mid-lumbar area was shaved bilaterally. A 2-cm incision was made through the skin and the muscle wall. After that both of the ovaries were identified, the ovarian arteries were ligated and they were extracted. Finally, 1–2 ml of normal saline was poured into the abdomen and the skin and muscle were then sutured separately using 4-O sterile suture. All experimental animals were ovariectomized two weeks before the experiment [25].

2.3. Experimental protocol

T2D was induced as described by Reed et al. [26]. The experimental groups of female rats were given a high fat diet (HFD), modified (365 g/kg standard chow, sheep fat 310 g/kg, casein 250 g/kg, cholesterol 10 g/kg, vitamin and mineral mix 60 g/kg, DL-methionine 3 g/kg, yeast powder 1 g/kg, and NaCl 1 g/kg). After 8 weeks of dietary manipulation, animals were injected a single dose of streptozotocin (STZ) (30 mg/kg). After 72 h, blood was collected from the tail and rats with a fasting blood glucose range ≥ 300 mg/dL (11.1 mM) were considered diabetic and included in the study. Rats in the normal group received standard animal chow. The animals were divided into six groups of six animals in each group. Groups composed 1: sham-control; 2: diabetes (Dia); 3: Ovariectomized (OVX) + Dia; 4: OVX + Dia + Vehicle (Veh); 5: OVX + Dia + E2 (1 mg/kg) [27]; 6: OVX + Dia + TAM (5 mg/kg) [28]. E2 and TAM were administered through i.p injection every four days for four weeks after two months of induction of T2D. Also, animals were placed on the normal diet during four weeks of treatment [29]. The schematic diagram of experimental protocol is shown in Fig. 1. Body weight and daily food and water consumption were recorded during the experiment. STZ was dissolved in 0.1 M citrate buffer (pH 4.4). E2 and TAM were dissolved in DMSO before administration. STZ and Dimethyl sulfoxide (DMSO) were purchased from Sigma (St. Louis, MO, USA). Ketamine and xylazine were purchased from Alfasan Inc., Utrecht, Netherland. E2 and TAM were obtained from Aburaihan Pharmaceutical (Tehran, Iran).

2.4. Assessment of cardiovascular function

After 4 weeks of drug administration, the left common carotid artery was cannulated with a PE-50 polyethylene catheter connected to a pressure transducer (MLT0380) and Power Lab Recording System (AD Instruments, Australia) for continuous monitoring of heart rate and mean arterial blood pressure (MABP) for 30 min. At the end, blood samples were collected from the left ventricle under deep anesthesia, the hearts were removed and rinsed with cold saline and then weighed. The degree of cardiac hypertrophy was assessed by the ratio of heart weight to body weight (mg/g), called cardiac weight index (CWI) [30]. Atherogenic index [31], and the cardiac risk indices [32] were calculated using the following formulas:

$$\text{Atherogenic index} = [(\text{total cholesterol} - \text{HDL cholesterol}) / \text{HDL cholesterol}]$$

$$\text{Cardiovascular risk index I (CRI} - \text{I)} = \text{Total cholesterol/HDL cholesterol}$$

$$\text{Cardiovascular risk index II (CRI} - \text{II)} = \text{LDL cholesterol/HDL cholesterol}$$

2.5. Blood sampling and biochemical analysis

Plasma insulin was assayed by ELISA Kit (Hangzhou, Eastbiopharm). Fasting blood glucose (FBG), total cholesterol (TC), triglyceride (TG) and high-density lipoprotein (HDL) levels were measured using commercial kits (Pars Azmoon, Iran). The LDL cholesterol was calculated using the following formula: LDL cholesterol = total cholesterol – [HDL cholesterol + (triglyceride / 5)] [31]. We used the homeostasis model assessment (HOMA) [33] to assess insulin resistance {HOMA-IR = [fasting glucose (mmol/L) × fasting insulin (μU/mL) / 22.5]} and pancreatic β-cell function {HOMA β-cell = [20 × fasting insulin (μU/mL)] / [fasting glucose (mmol/L) – 3.5]}. Also, Quantitative Insulin Sensitivity Check Index (QUICKI) was calculated using