Vascular Pharmacology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Vascular Pharmacology

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

Baroreflex sensitivity and central hemodynamics after omega-3 polyunsaturated fatty acids supplementation in an animal model of menopause

Pasquale Losurdo [a,*], Andrea Grillo [a], Emiliano Panizon [a], Michela Zanetti [b], Moreno Bardelli [a], Gianni Biolo [b], Bruno Fabris [a], Renzo Carretta [a]

[a] U.C.O. Medicina Clinica, Department of Medicine, Surgery and Health Sciences, University of Trieste, Ospedale di Cattinara, Strada di Fiume 447, 34149 Trieste, Italy
[b] U.C.O. Clinica Medica Generale e Terapia Medica, Department of Medicine, Surgery and Health Sciences, University of Trieste, Ospedale di Cattinara, Strada di Fiume 447, 34149 Trieste, Italy

ARTICLE INFO

Article history:
Received 20 July 2014
Received in revised form 19 December 2014
Accepted 21 December 2014
Available online xxxx

Keywords:
Menopause
Omega-3 polyunsaturated fatty acids
Ovariectomized rats
Baroreflex
Arterial stiffness
Cardiovascular disease

ABSTRACT

Objective: Baroreflex sensitivity (BRS) and central arterial function are significantly worsened after menopausal transition. This study tested the hypothesis that administration of n-3 polyunsaturated fatty acids (n-3 PUFA) might contribute to prevent these adverse changes in the vascular function of ovariectomized rats, an animal model of experimental menopause.
Methods: We randomized 30 female Wistar rats, 2 months old, into 3 groups: control (CTRL), sham surgery, normal diet; ovariectomized with normal diet (OVX) and ovariectomized with n-3 PUFA supplementation by daily gavage (0.8 g/kg/d) (OVX + O3). Two months after surgery, BRS was calculated as the bradycardic response to phenylephrine-induced blood pressure increase, while large artery function was estimated by the graphical analysis of the aortic pressure wave (diastolic to systolic pressure-time integral ratio, DTI/STI).
Results: Ovariectomy caused a significant decrease in BRS (CTRL: 6.23 ± 0.68 ms/mm Hg; OVX: 2.85 ± 0.75; $p < 0.001$). n-3 PUFA supplementation prevented part of the decline of BRS caused by surgical menopause (OVX + O3: 4.75 ± 0.53; $p < 0.01$ vs OVX). In animals treated with n-3 PUFA, the central arterial pressure profile did not show the changes in DTI/STI ratio seen in OVX (OVX: 3.31 ± 1.72; OVX + O3: 3.83 ± 1.52; $p < 0.01$).
Conclusions: In an experimental model of menopause, treatment with n-3 PUFA normalized central hemodynamics and prevented the decrease in BRS, associated with the reduction of compliance of the arterial wall. These findings suggest a therapeutic benefit of n-3 PUFA supplementation in the prevention of postmenopausal vascular disease.

1. Introduction

Cardiovascular diseases remain the leading cause of death among women worldwide [1].

Although there is an increasing concern about the effects of treatments in gender subgroups [2], recent data suggest that clinical evidences for cardiovascular care are still affected by the underrepresentation of women [3]. The gender inequality in patients enrolled in randomized clinical trials [4] and in animals involved in experimental studies [5], strongly limits the progress to a truly personalized medicine, and there is an urgent need of sex specific biomedical research to avoid this longstanding gender bias.

Epidemiologic studies suggest that the risk for a cardiovascular event is low before menopause, but rises sharply after menopausal transition [6]. This phenomenon could be explained by considering estrogen activity, which may abrogate age-related vascular remodeling in premenopausal women [7]. The changes occurring in cardiovascular system after menopause can be closely reproduced in animal models. In female rats subjected to ovarian hormone deprivation, compared to control rats, several alterations were seen both in cardiovascular function and structure [8].

Loss of estrogen activity accelerates many processes involved in vascular aging, including smooth muscular cells proliferation [9] and endothelial dysfunction [10]. Increase in arterial stiffness [11], decline in flow-mediated dilation [12] and changes in the autonomic vascular control [13] occur during the early phases of menopause. Baroreflex sensitivity (BRS), the ability to buffer the blood pressure response to pressor or depressor stimuli, is considered an established tool for the assessment of cardiovascular autonomic function. A depressed BRS has been found in menopause both in humans and in animal models [4,9],

* Corresponding author at: U.C.O. Medicina Clinica — Department of Medicine, Surgery and Health Sciences, Ospedale di Cattinara, Strada di Fiume 447, 34149 Trieste, Italy. Tel.: +39 3209313940; fax: +39 040 3994912.
E-mail address: losurdopa@gmail.com (P. Losurdo).

http://dx.doi.org/10.1016/j.vph.2014.12.005
1537-1891/

and is linked to increased cardiovascular mortality [14,15]. However, there are few data in literature concerning the effects of pharmacologic strategies to prevent vascular hemodynamic and autonomic changes induced by ovarian hormones deprivation.

Omega-3 polyunsaturated fatty acids (n-3 PUFA) have been widely studied for their multiple benefits on cardiovascular health, but in the clinical setting their role for cardiovascular prevention is still under discussion [16]. A number of experimental studies and clinical trials have proved their ability to improve hemodynamics by reducing arterial stiffness [17]. Previous studies have also suggested the ability of n-3 PUFA to prevent cardiac arrhythmia and to modulate autonomic nervous system [18], while effects of n-3 PUFA on BRS in humans are not univocal.

In a recent study from our group [19], we demonstrated the ability of n-3 PUFA to prevent the increase of arterial stiffness in ovariectomized rats, a validated animal model of experimental menopause. The purpose of the present study was to determine the effects of n-3 PUFA supplementation on BRS and on the aortic pressure wave, as a marker of the large artery elasticity, in the same experimental setting.

2. Methods

2.1. Animals

Experiments were performed on female Wistar-Kyoto rats, 2 months old, initially weighing 140 to 170 g, fed with a standard rat chow (Harlan 2018, 3.4 cal/g, macronutrients composition: crude protein 18.6%, fat 6.2%, crude fiber 3.5%. Supplied by Harlan, Italy), for at least two weeks. The animals were kept in temperature-controlled facilities on a 12-hours light/dark cycle. Animals were then randomly assigned to three experimental groups: a) control group of sham operated rats receiving normal diet (CTRL group), b) ovariectomy group receiving normal diet (OVX group), c) ovariectomy group receiving normal diet with the addition of daily gavages administration of a mixture of eicosapentaenoic acids (EPA) and docosahexaenoic acids (DHA) (OVX + O3 group). Animals were sacrificed 2 months after surgical procedure.

10 animals from each of the three experimental groups were randomly selected for the following experimental phase, while other 30 randomly selected animals were included in a different experiment (data previously published [20]). All experiments were performed according to the guidelines and protocols approved by the European Union (EU Council 86/609; D.L. 27.01.1992, no. 116) and by the Animal Research Ethics Committee of the University of Trieste, Italy.

2.2. Diets

Diets were prepared weekly and stored to prevent degradation, and food was provided and removed daily. To standardize study protocol CTR and OVX rats received an equal volume of normal saline solution by gavage per day. A commercially available pharmaceutical preparation was used for n-3 PUFA treatment (EPA and DHA mixture, 0.9 to 1.5 ratio, EPA + DHA content not inferior to 85%, Eskim, SigmaTau, Italy). The manufacturing process of the drug fulfilled the good manufacturing practice standards, and the production was optimized to improve the stability of the compound. The average content of EPA and DHA was, respectively, 450 ± 50 and 395 ± 55 mg/g. A daily dose of 0.8 g/kg/d was administered to rats in the OVX + O3 group, corresponding to a 0.65 g/kg/d of purified active compound (EPA and DHA). As mentioned in our previous experiment [20], this dose of n-3 PUFA was capable to increase omega-3 index in red cell membranes of about 50%. The study dose corresponded to a human equivalent dose of about 7 g/d. Saline solution (0.9% NaCl) was manufactured by Diaco (Italy).

2.3. Surgical ovariectomy

Bilateral ovariectomy and sham surgery were performed via a mid-abdominal route under Xylazine (10 mg/kg, intraperitoneal [IP] injection) and Tiletamine (40 mg/kg, IP) anesthesia. Anesthesia was assessed by complete absence of limb retraction upon painful stimulation. The fallopian tube was legated with absorbable suture, and the ovary was removed. This model of surgical menopause was validated in a previous experiment [20], which proved a significant decrease in serum estradiol levels after surgical bilateral ovariectomy, compared with sham-operated rats.

2.4. Hemodynamic measurements

After 2 months since the surgical procedure, 10 animals from each experimental (CTRL, OVX and OVX + O3) group were randomly selected for the hemodynamic measurements. The animals were anesthetized with Xylazine (5 mg/kg IP) and Tiletamine (10 mg/kg IP). Nylon cannulas were introduced into the left femoral arteries up to the aortic ostia. The anatomic locations of the tips of the cannula were checked by post-mortem dissection. Cannulas were connected to low-volume blood pressure (BP) transducers (STATHAM range −50 to +300 mm Hg), linked to a Coulbourn analog-to-digital convertor plus computer (Coulbourn Instruments; Lablink, Allentown, Pennsylvania). The frequency response of the cannula plus pressure transducer was minimum 100 Hz with 1% FS/24 Hz. Systolic and diastolic arterial BP and BP wave from the aortic ostia were recorded. The personnel who performed hemodynamic measures were blinded to study group assignment of each animal.

2.5. Determination of baroreflex

After a 15 minute wait for hemodynamic stabilization after invasive arterial cannulation, the basal pressure profile was recorded. The baroreflex sensitivity was performed by injecting a single dose of phenylephrine (100 μL of 0.01 g/mL solution) directly into the cannulated vessel. Phenylephrine, a pure alpha-agonist, was administered in order to rapidly increase the blood pressure of 15–30 mm Hg, and pressure wave recorded for at least 5 min. The pressure waves were analyzed in order to quantify changes in BP and in heart rate. The slope of the linear regression line between changes in systolic blood pressure and consequent lengthening of the RR interval is a quantitative measure of the baroreceptor control of heart rate. In the analysis, five c beats where the increase in blood pressure was followed by an increase in RR interval were considered for the quantitative measurement of BRS. BRS was calculated relating the changes in BP and in RR interval, as a mean index expressed as ms/mm Hg.

2.6. Pressure wave analysis

We performed a graphical analysis on aortic pressure waveform to calculate the time/pressure area under the pressure curves in systole and diastole. Only recordings of 10 consecutive heartbeats with stable BP values were used for the analysis. The systolic pressure–time integral (STI) and the diastolic pressure–time integral (DTI) were calculated as the mean area under the systolic and diastolic part of the waveform (Fig. 1). The relationship between diastolic and systolic pressure wave, considered a parameter evaluating central arterial function, was defined as the ratio between DTI and STI (DTI/STI). For the graphical analysis the Adobe Photoshop (Adobe Systems) v11.0 software package was used to calculate the area under the pressure curve.

2.7. Determination of blood glucose, plasma lipids, and omega-3 index

Levels of glucose, total cholesterol, triglycerides, HDL, Plasma 17β-estradiol, and membrane omega-3 index were obtained as referenced

Please cite this article as: Losurdo P, et al, Baroreflex sensitivity and central hemodynamics after omega-3 polyunsaturated fatty acids supplementation in an animal model of me..., Vascul. Pharmacol. (2015), http://dx.doi.org/10.1016/j.vph.2014.12.005