Biochemical and Biophysical Research Communications xxx (2018) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Dietary omega-3 and -6 fatty acids affect the expression of prostaglandin E2 synthesis enzymes and receptors in mice uteri during the window of pre-implantation

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ARTICLE INFO

Article history:
Received 14 July 2018
Accepted 22 July 2018
Available online xxx

Keywords:
Embryo implantation
Prostaglandin
Diet
Fatty acids
Pregnancy

ABSTRACT

Considering possible effects of poly-unsaturated fatty acids (PUFA) on embryo implantation more likely through PGs, we investigated effects of dietary omega-3 and -6 PUFA on prostaglandin E2 (PGE2) signaling in mice uterus during pre-implantation period. The mRNA expressions of microsomal- and cytosolic- PGE synthase (mPGES and cPGES) and protein expressions of PGE receptor 2 and 4 (EP2 and EP4) were evaluated in uterus tissues of control as well as omega 3 and omega 6 received mice at days 1–5 of pregnancy. Expression of cPGES gene was not significantly different between groups but the mPGES expression on days 4 and 5 of pregnancy in supplemented groups was higher than controls. Omega-3 significantly decreased EP2 levels on days 3 and 4, while omega-6 caused an increase on days 3–5 of pregnancy. The levels of EP4 were significantly higher in the omega-6 group than other groups on days 4 and 5 of pregnancy. Also the implantation rate was higher in omega -6 compared to omega-3 group ($p = 0.006$). Moreover, there were significant correlations between implantation rate with expression levels of mPGES and EP2. Our results showed negative and positive effects of respectively dietary omega-3 and -6 PUFA on PGE2 signaling and implantation rate.

1. Introduction

The food habits have changed in favor of poly unsaturated fatty acids (PUFA), especially among the population of developed countries, although there is still some ambiguity regarding the consumption ratio between mega-3 (ω3) to omega-6 (ω6) [1]. Successful embryo implantation guarantees the outcome of pregnancy, and so, in order to increase the endometrial receptivity, appropriate changes in the endometrium are required [2–5]. Previous studies have demonstrated the influence of PUFA on embryo implantation and pregnancy outcome [6,7]. PUFA is believed to enforce potential influences on pregnancy via a wide variety of mechanisms (reviewed by Wathes et al. [8]) such as regulation of the prostaglandins (PGs) pathway [9]. PGs, especially the 2-series, play an essential role in embryo implantation [10]. The crucial roles of PGE2 in endometrial vascular permeability, blastocyst spacing, implantation, and decidualization [11], possibly through the PGE receptor 2 and 4 (EP2 and EP4), have been emphasized in the past [12,13]. Moreover, it has been demonstrated that reduced embryo adhesion, following the administration of PG inhibitors, could be restored by the addition of the PGE2 or the EP2 agonists [14].

The PGE2 is derived from PUFA through a certain synthesis pathway that contains various key enzymes such as cPGES, and mPGES [15]. Coyne et al. [9] have reported that dietary PUFA exerts luteotrophic effects in bovine endometrium via an increasing expression of mPGES. Previous studies have shown the inhibitory effect of docosahexaenoic acid (DHA) on PGE2 secretion in human decidual cells [16], bovine [17], and rat uteri [18].

Considering the aforementioned reports, we investigated that if

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https://doi.org/10.1016/j.bbrc.2018.07.109
0006-291X/

the ω3 and ω6 supplementation could affect PGE2 signaling in the uterine tissue of mice during the window of pre-implantation, and consequently, affect the implantation rate.

2. Materials and methods

2.1. Animals and supplementation

Sixty female and 20 male adult albino NMRI mice varying weights of 20.5 ± 3.4 were prepared in the RAZI Institute of Iran. All the mice were kept under controlled conditions (temperature 25 ± 2 °C, 60–70% humidity with 12:12 h light and dark cycles), diet, and water, ad-libitum. The experimental procedures were in accordance with the National Institutes of Health guide for the care and use of Laboratory animals and were approved by the Animal Ethical Committee of the Tabriz University of Medical Sciences (Permit Number: 5/46139). After one week of adaptation, the females were randomly divided into three groups of 20 mice each; normal (fed standard pellets), omega-3 (fed standard pellets + 10% w/w of omega-3), and omega-6 (fed standard pellets + 10% w/w of omega-6). The omega-3 (ω3) supplement was provided as fish oil from Danna Pharma Co. (Tabriz, Iran) and for the omega-6 (ω6), soybean oil was used (Italy). After three weeks of supplementation, three female mice of each group were kept with a male mouse in a separated cage overnight in order to simulate natural mating. Observation of a vaginal plug and spermatozoa in the vaginal smear was considered as Day 1 of pregnancy. The female mice were killed between 09:00–10:00 h of Days 1–5 of pregnancy, and the uterine tissues were collected (four mice for each day in any group). After carefully washing the samples, they were frozen in liquid nitrogen and stored at −70 °C for subsequent measurements. The implantation site counts were evaluated by injecting of 0.1 ml of 1% Chicago blue (Sigma–Aldrich, USA) in the saline via a tail vein according to the described method (Fig. 1) [19].

2.2. Gene expression analysis

Under sterile conditions, the total RNA was extracted (miRCURY™ RNA Isolation Kit, Exiqon, Denmark). The samples were treated with DNase I to avoid genomic DNA contamination and the NG dART RT kit (Eurex, Poland) was applied to synthesize cDNA from the RNA. For the expression analysis following primers were used: cPGES forward 5′-ATGGAGCAGATGATGATTC-3′ and cPGES reverse 5′-GGTTAGAGGAGGCAAGTA-3′; mPGES forward 5′-GTGAGAAGGACTGAGATC-3′ and mPGES reverse 5′-ACTAATGATGACAGAGGAG-3′; and GAPDH forward 5′-GCGACTTCAACAGCAACTC-3′ and GAPDH reverse 5′-GCCGTATTCATTGTCATACCAG-3′. We used the MIC real-time PCR detection system (Bio Molecular Systems, Australia) and the SYBR Green kit (Eurex, Poland) for real-time PCR assay. Triplicate assays with following program were conducted: 10 min of initial denaturation at 95 °C, up to 40 cycles of 10 s in 95 °C for denaturation, 20 s in optimized annealing temperature, and 20 s in 72 °C as extension temperature. The melting curves were evaluated for product verification, and considering that the amplification efficiencies of the target and reference were approximately equal, the ΔΔCT calculation method was used to obtain the relative quantities [20].

2.3. Western blot analysis

For lysing the uterine samples ice cold RIPA Buffer (Sigma–Aldrich, USA), containing protease inhibitors (cOmplete™ Protease Inhibitor Cocktail, Roche, Germany) were used. After centrifugation, the protein concentration was evaluated in the supernatants (Pierce TM BCA protein assay kit, Thermo Fisher Scientific, USA). The samples were electrophoresed at equal concentration of protein (50 mg/lane) in 10% w/v sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using the Mini-PROTEAN Tetra Cell system (Bio-Rad, USA). After transferring the protein bands form gel to a methanol-preactivated polyvinylidene fluoride (PVDF) membrane (Roche, Germany), the membrane non-specific binding sites were blocked for one hour while gently shaking using dried non-fat milk 3% w/v in TBS plus 0.1% tween-20. Based on the target protein, the membrane was incubated overnight with the anti-prostaglandin E receptor (EP2) antibody diluted at a ratio of 1:700 (ab124419, abcam, USA) or the anti-prostaglandin E receptor (EP4) antibody diluted at a ratio of 1:1000 (ab93486, abcam, USA) at 4 °C. The β-actin was applied as reference using the anti-beta actin antibody (ab103548, abcam, USA). After washing the membrane incubated for one hour at 4 °C with a secondary antibody (anti-rabbit IgG-horseradish peroxidase, A6154, Sigma-Aldrich, USA) diluted at a ratio of 1:5000. The membrane was again washed and the bands were visualized using the Clarity™ Western ECL Substrate (Bio-Rad, USA). In order to identify the protein bands' molecular weight markers, (Thermo Scientific™, USA) was used. The densities of EP2, EP4, and β-actin were determined using the Image J software package and the relative density of each target protein was calculated to β-Actin.

2.4. Statistical analysis

The normal distribution of data was confirmed by the Kolmogorov–Smirnov test. The One-Way ANOVA Test following Tukey's Post Hoc Test was conducted to compare the data among groups as well as among different pregnancy days in each group. The Pearson Correlation Test was used to investigate the possible

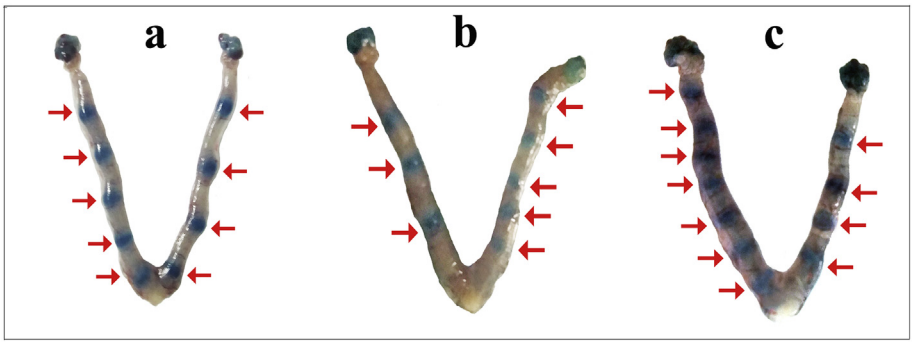

Fig. 1. Embryo implantation sites on the uterine horns of (a) control (b) omega-3 and (c) omega-6 groups at the fifth day of pregnancy; the red arrows indicate implantation. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Please cite this article in press as: M. Shahnazi, et al., Dietary omega-3 and -6 fatty acids affect the expression of prostaglandin E2 synthesis enzymes and receptors in mice uteri during the window of pre-implantation, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.07.109