NUTRITION RESEARCH 35 (2015) 774–783

Available online at www.sciencedirect.com

ScienceDirect

www.nrjournal.com

Original Research

Glutamic acid ameliorates estrogen deficiency–induced menopausal-like symptoms in ovariectomized mice

Na-Ra Han[a,1], Hee-Yun Kim[a,1], Woong Mo Yang[b], Hyun-Ja Jeong[c,*], Hyung-Min Kim[a,**]

[a] Department of Pharmacology, College of Korean Medicine, Kyung Hee University, Seoul, 130-701, Republic of Korea
[b] College of Korean Medicine and Institute of Korean Medicine, Kyung Hee University, Seoul, 130-701, Republic of Korea
[c] Department of Food Technology and Inflammatory Disease Research Center, Hoseo University, Asan, Chungcheongnam-do, 336-795, Republic of Korea

ARTICLE INFO

Article history:
Received 28 February 2015
Revised 15 May 2015
Accepted 9 June 2015

Keywords:
Glutamic acid
Microcomputed tomography
Alkaline phosphatase
Estrogen receptor-β
Estrogen response element
Extracellular signal-regulated kinase phosphorylation

ABSTRACT

Some amino acids are considered alternative therapies for improving menopausal symptoms. Glutamic acid (GA), which is abundant in meats, fish, and protein-rich plant foods, is known to be a neurotransmitter or precursor of γ-aminobutyric acid. Although it is unclear if GA functions in menopausal symptoms, we hypothesized that GA would attenuate estrogen deficiency–induced menopausal symptoms. The objective to test our hypothesis was to examine an estrogenic effect of GA in ovariectomized (OVX) mice, estrogen receptor (ER)–positive human osteoblast–like MG-63 cells, and ER-positive human breast cancer MCF-7 cells. The results demonstrated that administration with GA to mice suppressed body weight gain and vaginal atrophy when compared with the OVX mice. A microcomputed tomographic analysis of the trabecular bone showed increases in bone mineral density, trabecular number, and connectivity density as well as a significant decrease in total porosity of the OVX mice treated with GA. In addition, GA increased serum levels of alkaline phosphatase and estrogen compared with the OVX mice. Furthermore, GA induced proliferation and increased ER-β messenger RNA (mRNA) expression, estrogen response element (ERE) activity, extracellular signal–regulated kinase phosphorylation, and alkaline phosphatase activity in MG-63 cells. In MCF-7 cells, GA also increased proliferation, Ki-67 mRNA expression, ER-β mRNA expression, and ERE activity. Estrogen response element activity increased by GA was inhibited by an estrogen antagonist. Taken together, our data demonstrated that GA has estrogenic and osteogenic activities in OVX mice, MG-63 cells, and MCF-7 cells.

© 2015 Elsevier Inc. All rights reserved.

Abbreviations: μCT, microcomputed tomography; 3D, 3-dimensional; ALP, alkaline phosphatase; BMD, bone mineral density; BrdU, bromodeoxyuridine; Conn.D, connectivity density; DMSO, dimethyl sulfoxide; E_2, estrogen; ER, estrogen receptor; ERE, estrogen response element; ERK, extracellular signal–regulated kinase; FSH, follicle-stimulating hormone; GA, glutamic acid; HRT, hormone replacement therapy; mRNA, messenger RNA; OVX, ovariectomized; PCR, polymerase chain reaction; Tb.N, trabecular number.

* *Correspondence to:* H.J. Jeong. Tel.: +82 41 540 9681; fax: +82 41 542 9681.
** *Correspondence to:* H.M. Kim. Tel.: +82 2 961 9448; fax: +82 2 967 7707.
E-mail addresses: hjjeong@hoseo.edu (H.-J. Jeong), hmkim@khu.ac.kr (H.-M. Kim).
[1] Na-Ra Han and Hee-Yun Kim contributed equally to this report.

http://dx.doi.org/10.1016/j.nutres.2015.06.006
0271-5317/© 2015 Elsevier Inc. All rights reserved.

1. Introduction

Although menopause is a natural biological period during the female lifecycle, many women experience metabolic syndromes that include obesity or systemic skeletal diseases, such as osteoporosis, due to a deficiency of estrogen (E_2) during this period [1]. Estrogen is a key regulator of growth and function in tissues, such as the reproductive tract or skeletal system [2]. Osteoporosis is characterized by a systemic decrease in bone density and increases in the possibility of fragility fractures [3]. Hormone replacement therapy (HRT) is often conducted to alleviate these symptoms and to protect women against an E_2 deficiency. However, because HRT also increases the risk of breast cancer [4], menopausal women often seek complementary and alternative therapies for the symptoms [5].

Glutamic acid (GA) clearly plays a role in the biosynthesis of arginine, which is an essential amino acid [6]. An arginine diet decreases hot flashes and endothelial dysfunction in postmenopausal women [7]. A deficiency of amino acids, including GA, in menopause contributes to metabolic and cardiovascular risks [8]. Osteocalcin functions as a regulator of bone mineral maturation via vitamin K–dependent GA carboxylation [9]. Estrogen modulates cognitive functions by raising GA-induced intracellular calcium, and HRT can maintain cognitive functions during menopause [10]. Porcine placenta, which is a reservoir of a large number of amino acids including GA, enhances neuroprotection and cognition in postmenopausal women [11,12].

The predominant biological effects of E_2 are induced through intracellular E_2 receptors (ERs), ER-α and ER-β[13]. The biological function of the ER is mediated through the ability of ER to regulate the expression of genes containing an E_2 response element (ERE) sequence in their promoter [14]. Estrogen-ER complexes binding to an ERE regulate gene transcription and subsequent tissue responses, such as cell proliferation [2]. Cell survival and proliferation are mediated principally through extracellular signal–regulated kinase (ERK) MAPK pathways [15]. Alkaline phosphatase (ALP) activity, which is a marker of osteoblast differentiation and bone formation [16], is up-regulated via the activation of ERK signaling [3]. The analysis of bone cell–specific markers, such as ALP, is frequently used to characterize osteoblasts [17].

We hypothesized that dietary GA would lessen E_2 deficiency–induced menopausal symptoms. To investigate this hypothesis, we attempted to clarify the mechanism of E_2-like activity of GA on menopausal-like symptoms in a mouse model of E_2 loss. The approach was to investigate the effect of GA on menopausal-like symptoms in ovariectomized (OVX) mice that present loss of ovary functions [18]. We also examined if GA would have an E_2-like function such as proliferation and ALP activity using ER-positive human osteoblast–like MG-63 cells and ER-positive human breast cancer MCF-7 cells.

2. Methods and materials

2.1. *Glutamic acid and E_2 preparation*

Glutamic acid (no. G1251, minimum 99% pure; Sigma Chemical Co, St Louis, MO, USA) was dissolved in distilled water and prepared at a dose of 10 mg/kg, based on previous reports [19,20]. Estrogen (no. E8875; Sigma Chemical Co) was dissolved in 1% dimethyl sulfoxide (DMSO) and prepared at a dose of 100 nmol/L, based on a previous report [21]. Genistein (no. G6649; Sigma Chemical Co) and fulvestrant (no. I4409; Sigma Chemical Co) were dissolved in DMSO and prepared at a dose of 1 μmol/L, respectively, according to previous reports [22,23].

2.2. *Animal study design*

Female mice (7-week-old Balb/c) were purchased from Dae-Han Experimental Animal Center (Eumsung, Republic of Korea). The mice were acclimatized for 2 weeks to local vivarium conditions. Ovariectomy was conducted, as described previously [24]. Briefly, mice were anesthetized with a combination of Zoletil and Rompun, and their ovaries were bilaterally removed. The mice in the sham-operated group were anesthetized, laparotomized, and sutured but leaving their ovaries. After 3 weeks of recovery from their ovariectomies, the mice were divided into 4 groups (n = 5 per group): sham, OVX, OVX administered orally with GA using an oral sonde (10 mg/kg per day), and OVX administered orally with E_2 using an oral sonde (100 nmol/L per day). Glutamic acid or E_2 was given to OVX mice consecutively for 8 weeks, based on previous reports [25,26]. The body weights of all groups were measured once a week until the last day of administration. After the mice were euthanized by cervical dislocation, blood samples and tissue specimens were collected. All animal experiments were conducted according to guidelines approved by the institutional animal care committee of Kyung Hee University (KHUASP (SE)-14-024).

2.3. *Methylene blue staining*

The dissected vaginas were fixed with formaldehyde. The vaginas were embedded in paraffin and cut into 4-μm-thick sections. The sections were stained with 1% methylene blue for 45 minutes, according to a previous procedure [27].

2.4. *Microcomputed tomography*

Microcomputed tomographic (μCT) scans were performed on fixed tibia using a high-resolution μCT scanner. Trabecular bone parameters were determined at approximately 0.4 to 0.9 mm from the growth plate. Volumetric analysis was completed using the associated software applications, as described previously [28,29]. Reconstruction was carried out using Sky Scan Nrecon software (Sky Scan, Ltd, Kartuizersweg, Kontich, Belgium). The x-ray source was set at 75 kV and 100 μA. Four hundred projections were acquired over an angular range of 180°. The image slices were reconstructed using cone-beam reconstruction software, based on the Feldkamp algorithm (Dataviewer; Sky Scan, Ltd., Kartuizersweg, Kontich, Belgium). The trabecular bone was extracted by drawing ellipsoid contours with CT analyzer software. Three-dimensional (3D) parameters were analyzed from a Marching cubes-type model with a rendered surface. To analyze the 3D parameters, the entire bone was scanned, and 600 slices were placed through the former area. After this, trabecular number (Tb.N), connectivity density (Conn.D), and total porosity were determined.

Download English Version:

https://daneshyari.com/en/article/5904420

Download Persian Version:

https://daneshyari.com/article/5904420

Daneshyari.com