Available online at www.sciencedirect.com
ScienceDirect
Nutrition Research 26 (2006) 585–590
NUTRITION RESEARCH
www.elsevier.com/locate/nutres

Ginsenosides promote meiotic maturation of mouse oocytes in cumulus-oocyte complexes involving increased expression of nitric oxide synthase

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Received 20 July 2006; revised 10 September 2006; accepted 15 September 2006

Abstract

Ginseng has long been used in Asia because of its beneficial effects on human health. Ginsenosides are the primary biologically active components in ginseng. The aim of the present study was to determine the role of ginsenosides on meiotic maturation of murine oocytes to better understand the effects of ginsenosides on reproductive health. Cumulus-oocyte complexes (COCs) and denuded oocytes in germinal vesicle stage were isolated from equine chorionic gonadotropin-primed immature mice and were cultured for 24 hours in 10% fetal calf serum medium, which was supplemented with 4 mmol hypoxanthine to maintain meiotic arrest. Ginsenosides (1.0-10 μg/mL) overcame the hypoxanthine-maintained meiotic arrest. In addition, ginsenosides-stimulated meiotic maturation in COCs was assessed by increased germinal vesicle breakdown and first polar body extrusion. No effect of ginsenosides was observed on denuded oocytes. In addition, ginsenosides (1.0 μg/mL) could synergize with follicle-stimulating hormone (0.1 IU/mL) to promote oocyte meiotic maturation in COCs. Tamoxifen (an estrogen receptor α antagonist) did not influence the ginsenosides-induced meiotic maturation, suggesting the effect was not mediated by estrogenic action. However, a nitric oxide (NO) synthase inhibitor, N^{ω}-nitro-L-arginine methyl ester (L-NAME, 1 mmol), reduced the ginsenosides-induced oocyte maturation in COCs. Immunocytochemical analysis demonstrated that ginsenosides enhanced expression of inducible nitric oxide synthase (iNOS) in the cumulus cells. L-NAME reduced the ginsenoside-induced increase in iNOS immunoreaction. Our results indicate that ginsenosides might stimulate meiotic maturation of mouse oocytes through a paracrine pathway involving the NO/iNOS system.

Keywords: Mouse; Ginsenosides; Oocyte maturation; Cumulus-oocyte complex; Nitric oxide; Nitric oxide synthase

1. Introduction

Ginseng (*Panax ginseng*), a traditional medicinal herb in Asia, has been widely studied for its beneficial effects on human health. The multiple biological actions of ginseng are generally attributed to ginsenosides (ginseng saponins), the primary active ingredient of ginseng. Ginsenosides are saponins, having a 4-ring, steroid-like structure with sugar moieties attached. More than 30 different ginsenosides have been isolated from ginseng.

Ginsenosides exert varying effects on a myriad of cells and tissues including pharmacological responses on the central nervous, cardiovascular, endocrine, and immune systems [1]. However, the mechanism of action for the health effects of ginsenosides remains unclear. Lee et al [2] reported that ginsenosides regulated lipoprotein metabolism through peroxisome proliferator–activated receptor α. Ginsenosides may directly block capsaicin-activated channels,

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doi:10.1016/j.nutres.2006.09.011

resulting in attenuation of the signals in rat sensory neurons [3]. They may prevent the development of opioid tolerance in the central nervous system [4] and protect scopolamine-induced memory impairment [5]. Ginsenosides blocked the nicotinic Ach receptors or the receptor-operated Na^+ channels; inhibited Na^+ influx through the channels, consequently reducing both Ca^{2+} influx and catecholamine secretion in bovine adrenal chromaffin cells [6]; and inhibited voltage-dependent Ca^{2+} channels in sensory neurons as well as in chromaffin cells [7]. The dexamethasone-induced down-regulation of glucocorticoid receptor may be partially reversed by ginsenosides [8]. The ginsenoside monomer Rg1 possessed estrogen-like activity [9]. Ginsenosides stimulated endogenous production of nitric oxide (NO) in the kidney [10] and enhanced NO production by induction of inducible nitric oxide synthase (iNOS) in addition to its direct effect of increasing intracellular Ca^{2+} in endothelial cells [11]. These studies indicate that ginsenosides may regulate cell functions through different mechanisms, among them, an important function, is to regulate cellular permeability and partition of nutrients, thus affecting diverse cell functions.

Only a few studies have evaluated the action of ginsenosides on reproduction. Liu and Zhang [12] reported that ginsenosides stimulated proliferation of chicken germ cells via a protein kinase C–mediated pathway. Ginsenosides ameliorated ovarian dysfunction caused by excessive stimulation with pregnant mare serum gonadotropin in immature rats [13]. In contrast, ginsenosides exerted direct teratogenic effects on rat embryos [14]. The effect of ginsenosides on oocyte maturation remains unknown. During meiotic maturation, oocytes acquire the competence to resume meiosis, undergo germinal vesicle breakdown (GVBD), and develop to metaphase II. In most mammalian species, oocytes are arrested at the diplotene stage of the first meiotic division shortly before ovulation. Resumption of meiosis is induced by the preovulatory surge of luteinizing hormone in vivo. However, fully grown oocytes in the germinal vesicle (GV) stage undergo spontaneous maturation after liberation from the ovarian follicles in vitro [15]. The mechanism by which oocytes acquired competence to resume meiosis during maturation has not yet been understood. A previous study showed that the follicular inhibitory substance, hypoxanthine, inhibited mouse oocytes from resuming meiosis during in vitro maturation [16]. Therefore, hypoxanthine-induced in vitro meiotic arrest provides a system to study meiotic resumption of mammalian oocytes. Several studies reported that follicle-stimulating hormone (FSH) could overcome hypoxanthine inhibition and induce oocyte maturation in isolated cumulus-oocyte complexes (COCs) in vitro [16].

In the present study, we evaluated the effect of ginsenosides on oocyte meiotic maturation to advance understanding of ginsenosides actions and potential effects of ginsenosides on human health. In addition, we investigated the underlying mechanism of ginsenosides effect on oocyte maturation.

2. Methods and materials

2.1. Animals

Immature ICR female mice (obtained from the Center of Laboratory Animals, Zhejiang University, China), weighing 16 to 18 g, were kept at 20 to 22°C with a 12-hour light/dark photoperiod and were given free access to feed and water. Mice were injected with 5 units of equine chorionic gonadotropin to induce multiple follicular development. Animal use was approved by the Committee of Zhejiang University for Animal Research.

2.2. Collection and culture of oocytes

Mice were killed by cervical dislocation 46 to 48 hours after equine chorionic gonadotropin injection. Ovaries were transferred to M199 culture medium (BRL, USA), supplemented with 4 mmol hypoxanthine, 20 mmol HEPES, 5 mmol $NaHCO_3$, 0.23 mmol pyruvate, 2 mmol glutamine, 10% fetal calf serum (Hyclone), 100 IU/mL penicillin, and 100 μg/mL streptomycin. This medium was assigned as hypoxanthine medium.

Oocytes were isolated in hypoxanthine medium by puncturing antral follicles with a 26-gauge needle under a stereomicroscope. The COCs (GV stage) were collected. Denuded oocytes were obtained by gentle, repeated aspiration and flushing of the COCs by mouth pipet. Oocytes were washed 3 times in fresh medium and transferred to the test medium. All oocytes were cultured at 37°C and 100% humidity with 5% carbon dioxide in air for 24 hours in 24-well plates (Nunc, Denmark). Each well contained 500 μL of medium. Each experiment was repeated at least 3 times.

2.3. Treatment of oocytes with chemicals

Hypoxanthine medium was used as the control medium. At the beginning of culture, oocytes were treated with ginsenosides (0-10 μg/mL; Kangfulai Health Protection Co, China) alone and in combination with FSH (0.1 IU/mL; Ningbo Hormone Co, China). In a second experiment, oocytes were challenged with tamoxifen (1 μg/mL; Sigma) and N^{ω}-nitro-L-arginine methyl ester (L-NAME, 1 mmol; Sigma) to study the mechanism of ginsenosides-stimulated meiotic maturation. Tamoxifen was dissolved in ethanol. The final concentration of ethanol in the medium was 0.1% or less. Control oocytes received the vehicle only.

2.4. Examination of oocytes

At the end of culture, oocytes were evaluated for GV status and presence or absence of the first polar body (PB1) with an IX70 phase contrast microscope (Olympus, Japan). Images were captured with a digital video camera (Pixera Pro 150ES) connected to a computer. The percentage of