Enhanced Secretion of Beta-Defensins in Endometrial Tissues and Epithelial Cells by Soy Isoflavones

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Objective: *This study aimed to investigate whether endometrial tissues and endometrial epithelial cells were capable of secreting beta-defensin (BD)-1 and -2, and soy isoflavones genistein or daidzein could promote these BD secretions. The effect of genistein on Cl^- secretion in correlation with the BD secretion was also examined.*
Material and Method: *Endometrial tissues or glandular epithelial cell monolayer were mounted in Ussing chamber for measurement of electrical parameters. The sample solutions from apical and basolateral compartments were collected before and after genistein or daidzein addition for the measurement of BD-1 and-2 levels by using ELISA technique.*
Results: *Endometrial tissues and epithelial cells constitutively secreted both BD-1 and -2 mostly at the apical compartment. Both genistein and daidzein induced BDs secretion which reached a peak at 5-15 min. The apical secretion of BDs was coincidence with increased Cl^- secretion induced by genistein.*
Conclusion: *Endometrial tissues and epithelial cells contributes to basal host defense against infection by secretion of BD-1 and -2, which could be enhanced by genistein or daidzein. This finding implies that these potent constituents of soy isoflavones may be useful to promote the innate immune function of human endometrium.*

Keywords: *Beta-defensin, Genistein, Daidzein, Endometrium, Cl^- secretion*

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The prevention of uterine infection is essential for successful human reproduction. The initial defense mediated by endometrium that occurs within approximately 48 h to clear the viable bacteria contamination during mating is associated with the presence of antimicrobial products[1]. Antimicrobial peptides are gene-encoded peptides that are key mediators of the immune system, of which β-defensins are the main family found at mucosal surface of a variety of epithelia[2,3].

β-defensins (BDs) are small cationic peptide of 3.5-6 kDa containing 29-42 amino acids with six cysteine residues that are conserved in three intramolecular disulphide bonds[2]. They offer a board range of antibacterial, antiviral and antifugal activities by directly disrupting the membrane surface to kill pathogens[4]. They also function as chemotractant and stimulate cytokine secretion from immune cells, leading to an interaction between the innate and the adaptive immune systems[5].

At present, four subtypes of human BDs, BD 1-4, have been characterized. Even though defensins are particularly contained in the microbicidal granules of polymorphonuclear leukocytes, widely distribution of BDs mRNA and proteins in the epithelium of the respiratory tract, gastrointestinal tract, and urogenital system have also been suggested[2,3]. Among the antimicrobial peptides defensin, only BD-1 and BD-2 have been detected in primary culture of human endometrial epithelial cells by immunohistochemistry study[6]. Moreover, mRNA and protein expression of BD-1, so-called epithelia defensin, are found highest in the glandular epithelial layers of female reproductive tract[7]. The secretion of BD-1 has been indicated to constitutively release with no intracellular storage[8]. However, production of BD-1 in the urogenital tract could be induced by appropriate stimuli, i.e. microbial components, and high concentration of BD-1 occurred near the site of secretion contributes to local antimicrobial defense[7]. By contrast, the BD-2 is inducible form, and its release is regulated by several factors including the presence of microorganism,

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cytokines and inflammatory response[9,10]. High BD-1 and BD-2 mRNA expression are found in the proliferative phase comparable to the secretory and menstrual phases of the menstrual cycle suggesting the regulation of BD synthesis by ovarian sex-steroids[8,11].

Isoflavones genistein and daidzein are the major biological active constituents rich in soybean. They perform a wide variety of beneficially pharmacological effects such as antioxidant, antiproliferative, differentiation, anticancer and antiangiogenesis[12]. Genistein and daidzein exert the immunoregulatory properties by increasing cytotoxic T-cell and NK cell activity[13], reducing inflammation[14], and increasing the phagocytic response and lymphocyte proportion[15]. In addition, genistein have been found to increase the mRNA expression of antimicrobial peptide cathelicidin in keratinocytes[16] and β-defensin 5 in Paneth cells[17], but suppress the expression of BD-2 induced by IL-1β in human corneal epithelial cells[18]. These data may indicate the regulatory role of soy isoflavones on endometrium innate immunity. Recently, genistein has been shown to stimulate the Cl^- secretion in endometrial epithelial cells through CFTR activation[19]. Therefore, the secretory action of genistein may crosstalk with or assist the release of luminal BD from glandular endometrial epithelia.

The aim of this study was to investigate whether endometrial tissues and endometrial epithelial cells were capable of the secretion of BD-1 and -2, and this secretion was modulated by the potent compound of soy isoflavones genistein or daidzein. Relevance between BD secretion and Cl^- secretion induced by genistein was also examined.

Material and Method

Material

Genistein, daidzein, forskolin, insulin, non-essential amino acid and high purity grade salts were purchased from Sigma Chemical Co., (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), Dulbecco's phosphate buffer saline (DPBS), phenol red-free DMEM, fetal bovine serum (FBS), 0.05% trypsin-0.53 mM ethylenediaminetetraacetic acid (EDTA), penicillin-streptomycin were purchased from GIBCO BRL (Grand Island, NY).

Cell culture

The immortalized porcine endometrial epithelial cells (PEG cells) were provided by Professor Scott O'Grady, University of Minnesota. The PEG cells (passage 60-80) were cultured in DMEM containing glutamine supplemented with 5% FBS, 5 μg/ml insulin, 1% non-essential amino acid, 100 U/ml penicillin, 100 μg/ml streptomycin in 12 mm snapwell filters (Costar, Cambridge, MA, USA) and maintained at 37°C in humidified atmosphere of 95% air and 5% CO_2.

Uterine tissue preparation

Pig uterine horns obtained from the slaughter house were kept immediately in ice-cooled porcine Ringer's solution (130 mM NaCl, 6 mM KCl, 3 mM $CaCl_2$, 0.7 mM $MgCl_2$, 20 mM $NaHCO_3$, 0.3 mM NaH_2PO_4, 1.3 mM Na_2HPO_4, pH 7.4). After rinsing and removing out the connective tissues, the uterine horns were peeled off both serosal and myometrial layer and then mounted in Ussing chambers.

Ussing chamber experiment

The mucosal-submucosal tissues and cell monolayers were mounted in Ussing chambers where both apical and basolateral sides bathed with porcine Ringer's solutions at 37°C and bubbled with 95% O_2 and 5% CO_2 for measurement of electrical parameters and sample collection. The electrical parameters including transepithelial potential difference (PD) and short-circuit current (I_{sc}) were measured using the voltage-clamp amplifier (EVC-4000, World Precision Instruments) with $Ag/AgCl_2$ electrodes, and the data was connected to a PowerLab 2/26 converter and recorded with PC computer. The system calibration was performed before experiment. After mounting, the tissues and cell monolayers were equilibrated for at least 30 min to achieve a stable I_{sc}. The PD was measured before and after adding chemicals, and I_{sc} was continuously recorded during experiment. Positive I_{sc} reflects the movement of anions from the serosal to mucosal compartments or movement of cations from the mucosal to serosal compartments or a combination of both. After a stable baseline, genistein, daidzein (50 μM) or forskolin (10 μM) was added into the apical and basolateral solutions. These concentrations were chosen as they produced the maximal I_{sc} response of the PEG cells[19]. The samples from both apical and basolateral solutions were collected before (at time 0) and after adding compounds at 5, 10, 15, 30 and 60 min for BD measurement.

Measurement of β-defensins

The concentrations of BD-1 and -2 were measured using an enzyme-linked immunosorbent

assay (ELISA) test kit (Peprotech, NJ, USA). Briefly, each well of ELISA plate was coated with capture antibody for overnight. After blocking step, each well was incubated with BD standard solutions or samples for 2 h following with BD detection antibody incubation. Avidin-horseradish peroxidase (A-HRP) solution and substrate solution (3, 3', 5, 5'-tetramethylbenzidine; TMB) were used for color developing and stopped by 2 N sulfuric acid. An absorbance was read at 450 nm using an ELISA microplate reader (Tecan Sunrise™, Switzerland). Standard curve (15-1,000 pg/ml) was used to calculate BD concentrations.

Data analysis

All values are presented as mean ± standard error of mean (SEM), and n was the number of cell monolayers or tissues in each experiment. The statistical differences between control and experimental means were analyzed using ANOVA and post hoc test by Bonferroni' test (Prism 5.0, GraphPad Software, Inc., San Diego, CA, USA). A value of $p<0.05$ was considered statistically significant.

Results

Genistein or daidzein increased β-defensin 1-2 secretion in endometrial tissue

The bathing solutions from apical and basolateral sides of endometrial tissues mounted in Ussing chambers for 30 min were collected to determine the concentration of BD under basal condition (at time 0). As shown in Fig. 1, the BD-1 and -2 were detected in both apical (BD-1, 21.17±4.85 pg/ml; BD-2, 11.02±4.16 pg/ml, n = 14) and basolateral (BD-1, 6.25±2.5 pg/ml; BD-2, 5.03±2.27 pg/ml, n = 14) sides. Due to the BD secretion in the apical side was higher than in the basolateral side, only the apical secretion was focused for the next experiment.

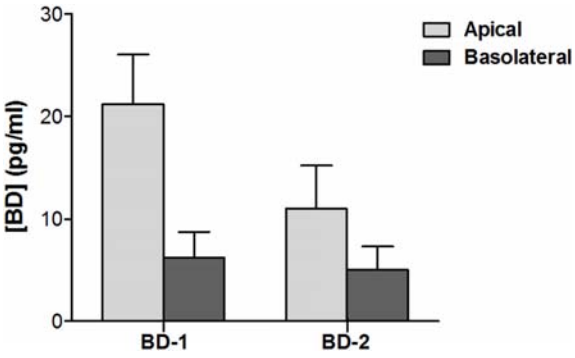

Fig. 1 Basal level of β-defensin (BD)-1 and -2 in apical or basolateral solutions bathing endometrial tissues. The sample solutions of endometrial tissues mounted in Ussing chambers for 30 min were collected for measurement of BD concentrations by ELISA. Values are mean ± SEM (n = 14).

Genistein or daidzein at 50 μM was added to either apical or basolateral solutions bathing the tissues to compare its effect on the BD-1 and -2 secretion. An apical application of genistein produced a peak of BD-1 secretion at 15 min and dropped at 30-60 min to a somewhat higher than basal level whereas daidzein showed a peak BD-1 at 5 min and decreased close to the basal level (Fig. 2A). Likewise, the BD-1 secretion was maximally detected at 10 min by genistein or 5 min by daidzein after basolateral application (Fig. 2B). In both application, genistein exhibited higher effect on BD-1 secretion than daidzein. Similarly, genistein added to the apical or basolateral sides also increased the BD-2 secretion. As shown in Fig. 3A, when added apically either daidzein or genistein maximally secreted BD-2 at 5 and 10 min, respectively. Moreover, the basolateral addition of genistein highly increased the BD-2 secretion at 5 min, whereas daidzein showed less effect on the BD-2 secretion which was first detected at 5 min and reached peak response at 15 min (Fig. 3B). However, DMSO had no effect on the BD secretion when added to either the apical or basolateral sides of the endometrial tissues.

Correlation of genistein on I_{sc} and β-defensin 2 secretion in the PEG cell

Since the present results revealed a greater effect of genistein on the BD secretion, we then determined whether genistein on able to secrete the BD-2 along with the change in I_{sc} in the PEG cell monolayers. Our previous study in the PEG cells has been shown that both genistein and forskolin, produced increases in I_{sc} with correspond to Cl^- secretion[19]. Forskolin, an activator of adenylate cyclase, is well-known for stimulation of Cl^- secretion through intracellular cAMP. In consistence with the previous study, addition of genistein (50 μM) or forskolin (10 μM) rapidly produced a maximal I_{sc} response within 2-3 min. After that the I_{sc} induced by genistein gradually declined to the level above baseline whereas the forskolin-stimulated I_{sc} was rapidly decreased and sustained at a higher level (Fig. 4A).

Under control condition, addition of DMSO into the apical or basolateral solutions had no effect on the BD-2 secretion. Addition of genistein induced the BD-2 secretion into both solutions with a higher level

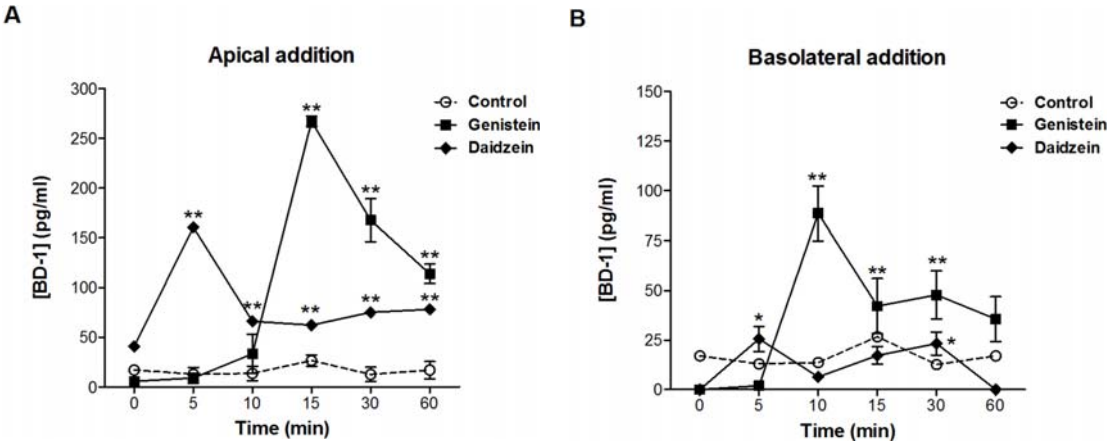

Fig. 2 Time course of β-defensin-1 (BD-1) level in the apical solution of endometrial tissues in response to apical (A) or basolateral (B) addition with genistein or daidzein. The apical solutions were collected at indicated time before and after addition with genistein, daidzein (50 μM) or DMSO (Control) for measurement of BD-1 concentration by ELISA. Values are mean ± SEM (n = 4-6). * $p<0.05$ and ** $p<0.01$ were significant difference from initial value at time 0 using ANOVA followed by Bonferroni's post-hoc.

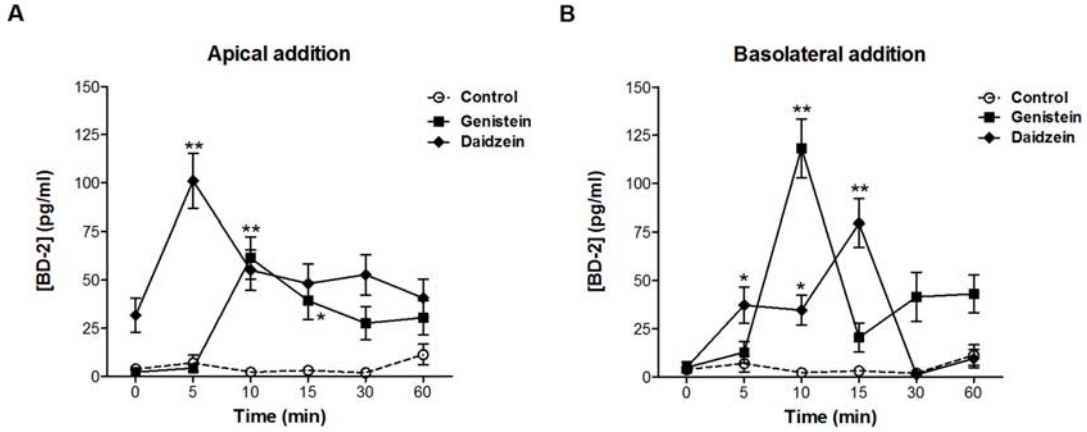

Fig. 3 Time course of β-defensin-2 (BD-2) level in the apical solution of endometrial tissues in response to apical (A) or basolateral (B) addition with genistein or daidzein. The apical solutions were collected at indicated time before and after addition with genistein, daidzein (50 μM) or DMSO (control) for measurement of BD-2 concentration by ELISA. Values are mean ± SEM (n = 4-6). * $p<0.05$ and ** $p<0.01$ were significant difference from initial value at time 0 using ANOVA followed by Bonferroni's post-hoc.

in the apical solution (Fig. 4B and C). The apical secretion was detected at 5 min, dropped to baseline, and subsequently increased at 30 min after genistein application. By contrast, the BD-2 secretion was slightly detected only in the apical solution at 5 min after forskolin addition (Fig. 4B).

Discussion

Although BD 1-2 have been identified and well characterized in female reproductive tract, the secretion and regulation of BD 1-2 in the endometrium are poorly reported. The present study showed that both porcine endometrial tissues and endometrial epithelial cells were able to secrete both BD-1 and -2 under basal condition. Higher concentration of BD-1 as compared to BD-2 being detected was consistent with its mRNA and protein found highest in the glandular epithelium of reproductive tract[7]. The finding that the basal level of BDs was greater in the apical than the basolateral solution was expected as the BDs are the peptides produced by epithelia at mucosal surfaces[2,3]. Moreover, the BDs secreted from

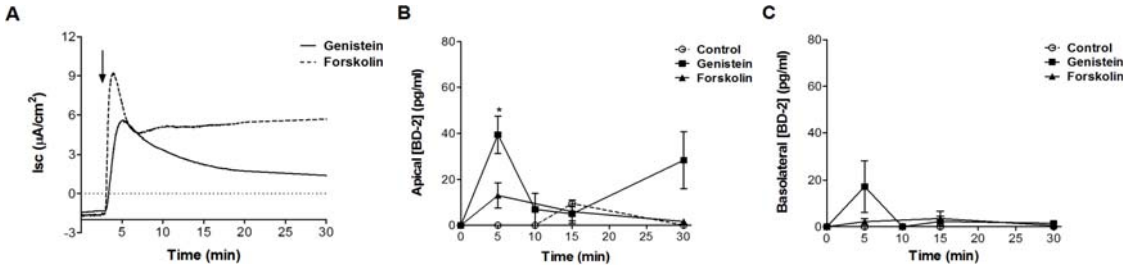

Fig. 4 Time course of I_{sc} change (A) in relationship with β-defensin-2 (BD-2) level in the apical solution (B) or basolateral solution (C) of PEG cell monolayer in response to genistein (50 μM) or forskolin (10 μM). Sample solutions were collected at indicated times before and after application of genistein, forskolin or DMSO (control) for measurement of BD-2 concentration by ELISA. Values are mean ± SEM (n = 4-8). * $p<0.05$ was significant difference from control at each time point.

the endometrial tissues was much higher than those from the epithelial cells due to a greater number of epithelial cells and contribution of immune cells, which have been reported to express and secrete BD-1 and -2[20].

Generally, in most epithelial cells BD-1 is constitutively expressed while BD-2 is induced by pathogens or TLR ligands[8,21]. Schaefer et al in 2005 have reported that human endometrial epithelial cells secrete basal BD-2, which can be stimulated by TLR agonist[8]. In human amniochorionic membranes, BD 1-2 secretions have been induced by bacteria Gardnerella *vaginalis*[21]. Apart from pathogenic components, the BD 1-2 expressions are also regulated by sex steroid and hormonal change during menstruation. In human endometrial epithelial cells, the BD-1 is highly expressed during the secretory phase and the BD-2 during menstruation[11]. Additionally, the BD-2 expression in uterine epithelial cells are up-regulated by estradiol[10]. In other study, estradiol has been found to suppress the BD-2 expression[22], but increase LPS-induced BD-2 expression and secretion in vaginal epithelial cells[23]. These data indicates that the expression and secretion of BD 1-2 in female reproductive tract are regulated by estrogen.

As its chemical structure similar to endogenous estrogen, genistein or daidzein added to the apical or basolateral sides of the endometrial tissues was found to induce both apical BD-1 and -2 secretion. A similar pattern of BD-2 secretion in response to genistein was observed in the PEG cells. The maximal BD secretion in both cells and tissues was detected within 5-15 min after addition, suggesting the non-genomic effect of these isoflavones. Indeed, the mechanism responsible for genistein or daidzein induced BD secretion from the epithelial cells was poorly understood but such release could be due to exocytosis of pre-existing BD located near the site of secretion[7]. It is known that activation of cAMP or increasing of intracellular Ca^{2+} concentration plays a regulatory role in the exocytosis in secretory cells. In addition, genistein has been found to stimulate insulin secretion from pancreatic β-cells through cAMP activation[24], and induce Ca^{2+}-mediated apoptosis concentration in breast cancer cells[25]. This suggests the possibility that genistein and daidzein may acutely induce BD secretion by directly stimulating the exocytosis of pre-existing BD production through activations of cAMP or Ca^{2+} signaling.

Following peak response the uterine BD-1 and -2 levels induced by genistein gradually dropped and maintained above baseline up to one hour. This finding may also indicate the genomic effect of genistein on BD secretion. Although the gene expression of BD was not investigated in the present study, genistein has been shown to increase the expression of antimicrobial peptide cathelicidin in keratinocytes[16]. However, genistein suppresses IL-1β stimulated BD-2 expression in human corneal epithelial cells through its tyrosine kinase activity[18]. Further study regarding genomic and mechanistic action of soy isoflavones remains to be studied.

Our previous study in the PEG cells have shown that genistein-induced increase in I_{sc} is due to Cl^- secretion through CFTR activation[19]. The present results revealed that genistein induced the maximal Cl^- secretion at about the same time as the observed peak of BD-2 secretion, indicating that the BD-2 secretion from the endometrial epithelial cells may depend on the luminal Cl^- secretion. However, the findings that forskolin failed to induce the BD-2 secretion despite of a marked increase in Cl^- secretion indicate that the BD secretion may be irrelevant to the Cl^- secretion. On the other hand, there is coincidence

between genistein-stimulated BD secretion and Cl^- secretion. Indeed, the Cl^- secretion by endometrial epithelial cells provides the driving force for the fluid secretion. This mucosal epithelial secretions of fluid are also important components of non-specific innate immune defense as they help to flush out the pathogen off cell surface and protect epithelium from direct contact with infectious agent. Taken together, the coincidence secretions of BD and Cl^- will promote the potential function of endometrial epithelium to defense against the infection.

In conclusion, endometrial tissues and endometrial epithelial cells constitutively secrete BD-1 and -2 mostly at the apical side. These secretions were enhanced by potent constituent of soy isoflavones such as genistein or daidzein. Although the BD secretion appeared not to correlate with Cl^- secretion, these mucosal secretions induced by soy isoflavones may be useful for promoting mucosal innate immune defense which help protect the endometrium from invading pathogens.

What is already known on this topic?

Antimicrobial peptides BD-1 and BD-2 mRNA and protein have been evidenced in human endometrial epithelial cells but only BD-2 secretion has been reported. The expression and secretion of BD 1-2 could be stimulated by several factors i.e. microbial components, cytokines, estrogen and hormonal changes during menstrual cycle.

What this study adds?

Endometrial tissues and endometrial epithelial cells are able to constitutively secrete BD-1 and -2. These secretions are stimulated by active dietary ingredients of soy isoflavones, i.e., genistein and daidzein, suggesting the potential roles in promoting endometrial innate immune function. Finally, the genistein-stimulated BD-2 secretion appears to coincidence with Cl^- secretion.

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Potential conflicts of interest

None.

References

1. Wira CR, Grant-Tschudy KS, Crane-Godreau MA. Epithelial cells in the female reproductive tract: a central role as sentinels of immune protection. Am J Reprod Immunol 2005; 53: 65-76.
2. Ganz T. Defensins: antimicrobial peptides of innate immunity. Nat Rev Immunol 2003; 3: 710-20.
3. Ochiel DO, Fahey JV, Ghosh M, Haddad SN, Wira CR. Innate Immunity in the female reproductive tract: role of sex hormones in regulating uterine epithelial cell protection against pathogens. Curr Womens Health Rev 2008; 4: 102-17.
4. Schroder JM, Harder J. Human beta-defensin-2. Int J Biochem Cell Biol 1999; 31: 645-51.
5. Yang D, Chertov O, Bykovskaia SN, Chen Q, Buffo MJ, Shogan J, et al. Beta-defensins: linking innate and adaptive immunity through dendritic and T cell CCR6. Science 1999; 286: 525-8.
6. King AE, Critchley HO, Kelly RW. Innate immune defences in the human endometrium. Reprod Biol Endocrinol 2003; 1: 116.
7. Valore EV, Park CH, Quayle AJ, Wiles KR, McCray PB Jr, Ganz T. Human beta-defensin-1: an antimicrobial peptide of urogenital tissues. J Clin Invest 1998; 101: 1633-42.
8. Schaefer TM, Fahey JV, Wright JA, Wira CR. Innate immunity in the human female reproductive tract: antiviral response of uterine epithelial cells to the TLR3 agonist poly(I:C). J Immunol 2005; 174: 992-1002.
9. Singh PK, Jia HP, Wiles K, Hesselberth J, Liu L, Conway BA, et al. Production of beta-defensins by human airway epithelia. Proc Natl Acad Sci U S A 1998; 95: 14961-6.
10. Fahey JV, Wright JA, Shen L, Smith JM, Ghosh M, Rossoll RM, et al. Estradiol selectively regulates innate immune function by polarized human uterine epithelial cells in culture. Mucosal Immunol 2008; 1: 317-25.
11. Fleming DC, King AE, Williams AR, Critchley HO, Kelly RW. Hormonal contraception can suppress natural antimicrobial gene transcription in human endometrium. Fertil Steril 2003; 79: 856-63.
12. Dixon RA. Phytoestrogens. Annu Rev Plant Biol 2004; 55: 225-61.
13. Guo TL, McCay JA, Zhang LX, Brown RD, You L, Karrow NA, et al. Genistein modulates immune responses and increases host resistance to B16F10 tumor in adult female B6C3F1 mice. J Nutr 2001; 131: 3251-8.
14. Regal JF, Fraser DG, Weeks CE, Greenberg NA.

Dietary phytoestrogens have anti-inflammatory activity in a guinea pig model of asthma. Proc Soc Exp Biol Med 2000; 223: 372-8.
15. Zhang R, Li Y, Wang W. Enhancement of immune function in mice fed high doses of soy daidzein. Nutr Cancer 1997; 29: 24-8.
16. Park K, Kim YI, Shin KO, Seo HS, Kim JY, Mann T, et al. The dietary ingredient, genistein, stimulates cathelicidin antimicrobial peptide expression through a novel S1P-dependent mechanism. J Nutr Biochem 2014; 25: 734-40.
17. Pakalapati G, Li L, Gretz N, Koch E, Wink M. Influence of red clover (Trifolium pratense) isoflavones on gene and protein expression profiles in liver of ovariectomized rats. Phytomedicine 2009; 16: 845-55.
18. McDermott AM, Redfern RL, Zhang B, Pei Y, Huang L, Proske RJ. Defensin expression by the cornea: multiple signalling pathways mediate IL-1beta stimulation of hBD-2 expression by human corneal epithelial cells. Invest Ophthalmol Vis Sci 2003; 44: 1859-65.
19. Deachapunya C, Poonyachoti S. Activation of chloride secretion by isoflavone genistein in endometrial epithelial cells. Cell Physiol Biochem 2013; 32: 1473-86.
20. Duits LA, Ravensbergen B, Rademaker M, Hiemstra PS, Nibbering PH. Expression of beta-defensin 1 and 2 mRNA by human monocytes, macrophages and dendritic cells. Immunology 2002; 106: 517-25.
21. Zaga-Clavellina V, Martha RV, Flores-Espinosa P. In vitro secretion profile of pro-inflammatory cytokines IL-1beta, TNF-alpha, IL-6, and of human beta-defensins (HBD)-1, HBD-2, and HBD-3 from human chorioamniotic membranes after selective stimulation with Gardnerella vaginalis. Am J Reprod Immunol 2012; 67: 34-43.
22. Wira CR, Fahey JV, Ghosh M, Patel MV, Hickey DK, Ochiel DO. Sex hormone regulation of innate immunity in the female reproductive tract: the role of epithelial cells in balancing reproductive potential with protection against sexually transmitted pathogens. Am J Reprod Immunol 2010; 63: 544-65.
23. Han JH, Kim MS, Lee MY, Kim TH, Lee MK, Kim HR, et al. Modulation of human beta-defensin-2 expression by 17beta-estradiol and progesterone in vaginal epithelial cells. Cytokine 2010; 49: 209-14.
24. Liu D, Zhen W, Yang Z, Carter JD, Si H, Reynolds KA. Genistein acutely stimulates insulin secretion in pancreatic beta-cells through a cAMP-dependent protein kinase pathway. Diabetes 2006; 55: 1043-50.
25. Sergeev IN. Genistein induces Ca^{2+} -mediated, calpain/caspase-12-dependent apoptosis in breast cancer cells. Biochem Biophys Res Commun 2004; 321: 462-7.

การหลั่งสารเบต้าดีเฟนซินจากเนื้อเยื่อและเซลล์เยื่อบุผิวมดลูกที่เพิ่มขึ้นโดยสารไอโซฟลาโวนจากถั่วเหลือง

ยศวี ศรีสมบุญ, สุทธาสินี ปุญญโชติ, ฉัตรศรี เดชะปัญญา

วัตถุประสงค์: *เพื่อศึกษาว่าเนื้อเยื่อและเซลล์เยื่อบุผิวมดลูกสามารถหลั่งสารเบต้าดีเฟนซิน (BD)-1 และ -2 และสารไอโซฟลาโวนเจนิสทีอีน (genistein) และไดแอดซีน (daidzein) จากถั่วเหลืองสามารถส่งเสริมการหลั่งสาร BDs ได้หรือไม่ รวมทั้งตรวจสอบฤทธิ์ของ genistein ต่อการหลั่งคลอไรด์ที่สัมพันธ์กับการหลั่งสาร BDs*

วัสดุและวิธีการ: *เนื้อเยื่อบุผิวมดลูกหรือเซลล์เยื่อบุผิวมดลูกที่สร้างสารคัดหลั่งถูกนำมาติดตั้งเข้ากับ Ussing chamber เพื่อวัดค่าทางไฟฟ้ารวมทั้งเก็บตัวอย่างสารละลายจากทางด้าน apical และ basolateral ก่อนและหลังการให้สาร genistein หรือ daidzein เพื่อวัดระดับของสาร BD-1 และ -2 โดยใช้เทคนิค ELISA*

ผลการศึกษา: *เนื้อเยื่อและเซลล์เยื่อบุผิวมดลูกสามารถขับหลั่งสาร BD-1 และ -2 ส่วนใหญ่ทางด้าน apical การให้สาร genistein และ daidzein กระตุ้นการหลั่ง BD ทั้งสองชนิด ซึ่งวัดได้สูงสุดที่เวลา 5-15 นาที การหลั่ง BD ทางด้าน apical นั้นเกิดขึ้นควบคู่ไปกับการหลั่งของคลอไรด์ที่ถูกกระตุ้นโดยสาร genistein*

สรุป: *เนื้อเยื่อและเซลล์เยื่อบุผิวมดลูกมีส่วนรับผิดชอบในการป้องกันตัวเองจากการติดเชื้อโรคโดยการหลั่งสาร BD-1 และ -2 ซึ่งสามารถถูกกระตุ้นโดย genistein หรือ daidzein ผลที่ได้แสดงให้เห็นว่าสารไอโซฟลาโวนจากถั่วเหลือง อาจถูกนำมาใช้ประโยชน์ในการส่งเสริมการทำงานของระบบภูมิคุ้มกันแต่กำเนิดของเยื่อบุผิวมดลูกของมนุษย์ได้*