Isoflavone Genistein Modulates the Protein Expression of Toll-like Receptors in Cancerous Human Endometrial Cells

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Objective: *The present study aimed to investigate whether genistein, a potent phytoestrogen mainly found in soybean, modulated the expression of TLRs 2, 3, 4 and 9 proteins in human endometrial epithelial cell line RL95-2 under basal and polyinosinic-polycytidylic acid (poly I: C) stimulated conditions to mimic viral infection. The genistein effects were also compared with 17β-estradiol.*
Material and Method: *The RL95-2 cells were cultured in the estrogen-deprived media with or without poly I: C 30 min prior to incubation with genistein (10^{-7}, 10^{-6} or 10^{-5} M) or 17β-estradiol (10^{-9} M) for 48 h. The TLRs protein expression was analyzed by semi-quantitative Western blot.*
Results: *The cells expressed TLRs 3, 4 and 9 but a very low level of TLR2 proteins. Poly I: C significantly increased the TLRs 2 and 9 protein expressions whereas the TLRs 3 and 4 were reduced. Under basal condition, genistein at 10^{-7} M increased the TLR2 while 17β-estradiol decreased the TLR4. All concentrations of genistein and 17β-estradiol attenuated the poly I: C induced increase in the TLR2. By contrast, both genistein at 10^{-5} M and 17β-estradiol further potentiated the TLR4 suppressed by poly I: C. Only 17β-estradiol was found to antagonize the poly I: C-induced changes in TLRs 3 and 9.*
Conclusion: *Taken together, the present results that genistein increased the basal TLR2 and attenuated the viral component-induced TLR2 protein expression in human endometrial epithelial cells may indicate the potential role of this soy isoflavone in promoting the uterine immune function and probably alleviating the inflammation of endometrium following pathogen invasion.*

Keywords: *Phytoestrogen, Toll-like receptor, Endometrium, Estrogen, Poly I: C*

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Chronic infection of female reproductive tracts (FRT) has been suggested to be the cause of infertility, ectopic pregnancy, preterm labor and even cancer. This infection generally leads to immune responses of both innate and adaptive system. The innate immune system is the first line of defense against invading pathogens but important for the induction of the adaptive immune system to protect against re-exposure to the same pathogen[1]. The release of pro-inflammatory cytokines or chemokines i.e., IL-6, IL-8/CCL8 from the innate immune system such as epithelial cells which responds immediately against invading pathogens is the major chemical factor for stimulation of adaptive immunity[2]. However, chronic inflammation gradually induces inflammatory cytokines which are released to systemic circulation and mediated by specific receptors to produce the harmful effects[3].

Toll-like receptors (TLRs) are the pattern recognition receptors (PRRs) found on the innate immune cells and epithelia principally mediated the cytokines secretion after binding to pathogen-associated molecular patterns (PAMPs) molecules of microorganisms. To date, at least TLRs 1-10 are evidently identified in human. Each TLR specifically recognizes each type of bacterial, viral, and fungal components. TLR2, TLR4 and TLR5 recognize bacterial components, peptidoglycan, zymosan, lipopolysaccharide (LPS), Pam_3Cys-Ser-$(Lys)_4$ (Pam_3Cys) or flagellin, whereas TLR3, TLR7 and TLR8 recognize viral nucleotide sequences, both natural and synthetic products[4,5].

TLRs expression and function in endometrium

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both in vivo and in vitro are under the regulation of sex steroids, estradiol and progesterone[6,7]. Previous studies in human endometrial tissues have demonstrated the gene expression of TLR2, 3, 4 and 9 varying during the menstrual cycle. These gene expressions are suggested to correlate with cyclic changes of estrogen levels[8].

Phytoestrogen is naturally occurring compound with its chemical structure similar to 17β-estradiol, an endogenous estrogen. Among soybean isoflavones, genistein is present in the highest quantities and relatively high bioavailability in the target tissues, the endometrium in particular[9]. Genistein exerts both estrogenic and antiestrogenic actions on the estrogen responsive organs and tissues depending upon the low ($\leq 10^{-6}$ M) or high ($>10^{-6}$ M) concentration ranges[10]. Therefore, the aim of the present study was to investigate whether genistein, a potent phytoestrogen mainly found in soybean, modulated the protein expression of TLR subtypes 2, 3, 4 and 9 in human endometrial epithelial cell line RL95-2. The TLR2, 3, 4 and 9 function to recognize peptidoglycan from Gram-positive bacteria, viral double-strand RNA, LPS from Gram-negative bacteria and unmethylated CpG DNA in bacteria, respectively[4,5]. The effects of low (10^{-7} or 10^{-6} M) and high concentration of genistein (10^{-5} M) were especially concerned as it may relate to the level of isoflavones found in many types of human diet. Since the endometrial tissues are usually contaminated with virus, the genistein effects on the TLR expression in the presence of a viral dsRNA analog polyinosinic-polycytidylic acid (poly I:C) to mimic viral infection were also investigated.

Material and Method

Materials

Genistein, 17β-estradiol, polyinosinic-polycytidylic acid (poly I:C), insulin, non-essential amino acid, *L*-glutamine 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 and fungizone were purchased from GIBCO BRL (Grand Island, NY, USA). Charcoal-stripped FBS was purchased from Biowest Co., (Miami, FL, USA).

Cell culture

Human endometrial cell carcinoma RL95-2 cells (ATCC® Number: CRL-1671™) were purchased from American Type cell culture collection (ATCC, VA, USA). These cells are epithelial origin with well-defined structures and able to form gland-like dome structure as well as present both cytoplasmic and nuclear estrogen receptors[11]. The cells (passage number 138-145) were maintained in DMEM containing 5% fetal bovine serum (FBS), 1% non-essential amino acid, 5 μg/ml insulin, 200 mM *L*-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin (standard media) and incubated at 37°C in a humidified 5% CO_2 in air. The culture media were changed every two days.

Cell treatment

The RL95-2 cells (10^6 cells) were seeded in a 50-mm plate. After reaching confluence, the cells were pre-conditioned in phenol red-free DMEM with 2% charcoal stripped FBS (2%csFBS) and other supplements similar to the standard media (estrogen-deprived media) for 48 h. Genistein (10^{-7}, 10^{-6}, 10^{-5} M), 17β-estradiol (10^{-9}) or DMSO as a vehicle control were then applied and incubated for 48 h. For the experiments with poly I:C stimulation, the cells were inoculated with poly I:C (10 μg/ml) to represent viral infection for 30 min prior to addition of genistein or 17β-estradiol for 48 h. After that, cells were collected for total protein isolation.

Western blot analysis

Total protein from the RL95-2 cells was extracted with lysis buffer (50 mM tris HCl, 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EGTA, 1 mM phenylmethylsulfonylfluoride, 20 mg aprotinin and 1 mM NaF, pH 7.4). Protein concentrations were determined using a bicinchoninic acid (BCA) protein assay kit (Pierce Chemical Co., USA) and the optical density was read at 562 nM by spectophotometer. Then 60 μg of total protein per sample was separated on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Bio-rad laboratories, Inc) and subsequently transferred to a polyvinylidene difluoride membrane (Pall Life Sciences, USA). The membrane was blocked with Tris buffer containing 5% non-fat dry milk at room temperature for 4 h, and incubated overnight at 4°C with a 1:200 dilution of rabbit anti-TLR2 antibody, goat anti-TLR3 antibody, rabbit anti-TLR4 antibody, goat anti-TLR9 antibody and housekeeping mouse anti β-actin (Santa Cruz Biotechnology Inc., USA). After washing, the membranes were incubated at room temperature for 1 h with anti-rabbit secondary antibody for TLR 2 and 4,

anti-goat secondary antibody for TLR3 and 9, and anti-mouse secondary antibody for β-actin, which is conjugated with horseradish peroxidase (Sigma Chemical Co). Finally, the immunoreactive blots were detected by clarity western ECL chemiluminescent substrate (Bio-rad laboratories, Inc) according to the manufacturer's instructions. The fluorescent band products were exposed for 8 minutes on Amersham™ hyperfilm ECL (Amersham-Pharmacia Biosciences) in dark room. The bands density was quantified by Scion Image software (Scion Corporation, Frederick, MD). All experiments were repeated at least three times.

Data analyses

All values are presented as mean ± standard error of mean (SEM), and n is the number of monolayers used in each experiment. The differences between control and experimental means were analyzed using a Student's t-test. The difference among means of more than two groups was analyzed by ANOVA and post hoc test by Student Newman Keuls (Prism 5.0 Graph Pad software, Inc San Diego, CA, USA). A value of $p<0.05$ was considered statistically significant.

Results

Expression of TLRs 2, 3, 4 and 9 proteins under basal and poly I:C-stimulated conditions

The expression of TLRs 2, 3, 4 and 9 proteins was detected in human endometrial epithelial cell line (RL95-2 cells) as shown in Fig. 1. The TLR antibodies recognized the protein bands with approximate molecular mass of 100, 45, 75, 42 and 47 kDa for TLRs 2, 3, 4, 9 and internal protein β-actin, respectively. Using a semi-quantitative analysis as calculated by the ratio of the specific TLR to the β-actin band densities, it was found that the cells grown in the standard media (10% FBS) showed a relatively high basal level of TLRs 3 and 9 and a very low level of TLR2 protein. The expression of TLRs 2 and 3 in both standard and estrogen-deprived media (2%csFBS) was comparable, whereas the TLR4 was higher and the TLR9 was lower in the cells grown in the estrogen-deprived media than in the standard media. In both media conditions, the cells treated with poly I:C significantly increased the TLR2 and decreased the TLR3 protein expression as compared to the untreated cells. There were no significant changes in the TLRs4 and 9 expressions between the untreated and the poly I:C-treated cells grown in the standard media; however, the TLR4 was decreased and the TLR9 was increased in the poly I:C-treated cells grown in the estrogen-deprived media. The expression levels of four TLRs under poly I:C stimulation in both the standard media and the estrogen-deprived media were not significantly different ($p>0.05$; ANOVA followed by Student Newman Keuls' post-hoc test.

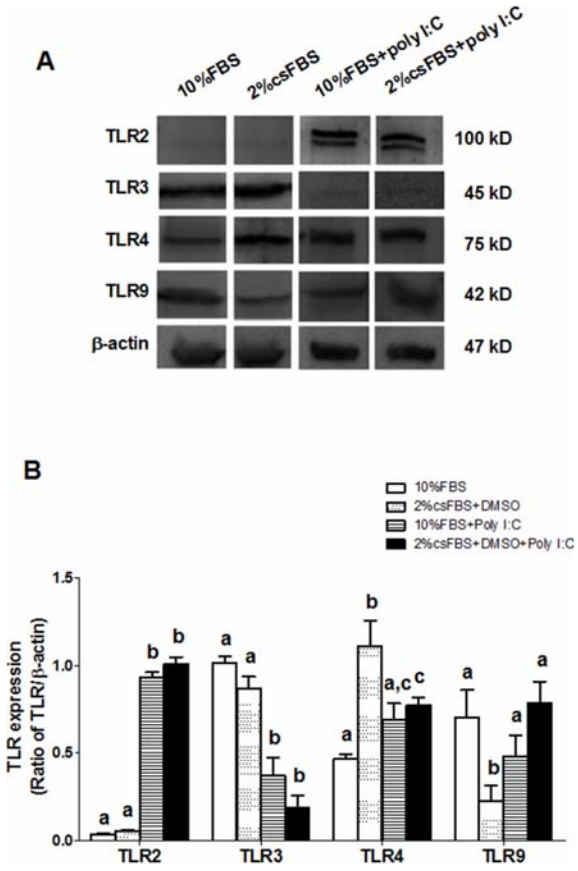

Fig. 1 (A) Western blot bands of TLRs2, 3, 4, 9 and internal control β-actin proteins in RL95-2 cells without or with poly I:C stimulation. Total proteins were isolated from the cells grown in the standard media (10%FBS) or estrogen-deprived media (2%csFBS) in the presence of poly I:C or vehicle control DMSO for 48 h. (B) Densitometric analysis of TLRs/β-actin band. Values are mean ± SEM (n = 3). Bars marked with same letter indicate no significant different, and different letters indicate statistically significant difference in each TLR group at $p<0.05$ using ANOVA followed by Student Newman Keuls' post-hoc test.

Effects of genistein and 17β-estradiol on the expression of TLR proteins under basal and poly I:C-stimulated conditions

To determine whether genistein and 17β-

estradiol modulates the basal expression of TLR proteins, the RL95-2 cells culturing in the estrogen-deprived media were treated with genistein or 17β-estradiol for 48 h. As shown in Fig. 2, the TLR2 expression was only significantly increased in response to genistein at 10^{-7} M, but not to other concentrations of genistein used or 17β-estradiol 10^{-9} M as compared to the control. However, the expression of TLRs 3, 4 and 9 was not affected by genistein treatment. Likewise, 17β-estradiol treatment had no effect on the protein expression of TLRs 3, 4 and 9 except a significant reduction of the TRL4 expression when compared to control cells or cells treated with genistein at 10^{-7} or 10^{-6} M (Fig. 2C).

The results from Fig. 1 revealed that the estrogen-deprived cells exposure to poly I:C alone was found to up-regulate the TLRs 2 and 9 and down-regulate the TLRs 3 and 4 proteins as compared to the untreated cells. All concentrations of genistein and 17β-estradiol significantly decreased the TLR2 protein stimulated by poly I:C (Fig. 3A). While genistein having no effect on the TLR3, 17β-estradiol was found to slightly increase the TLR3 suppressed by poly I:C (Fig. 3B). By contrast, high concentration of genistein (10^{-5} M) or 17β-estradiol further decreased the poly I:C-suppressed TLR4 expression (Fig. 3C). The poly I:C-induced increase in the TLR9 protein expression was not affected in the presence of genistein, but was significantly reduced in 17β-estradiol treatment (Fig. 3D).

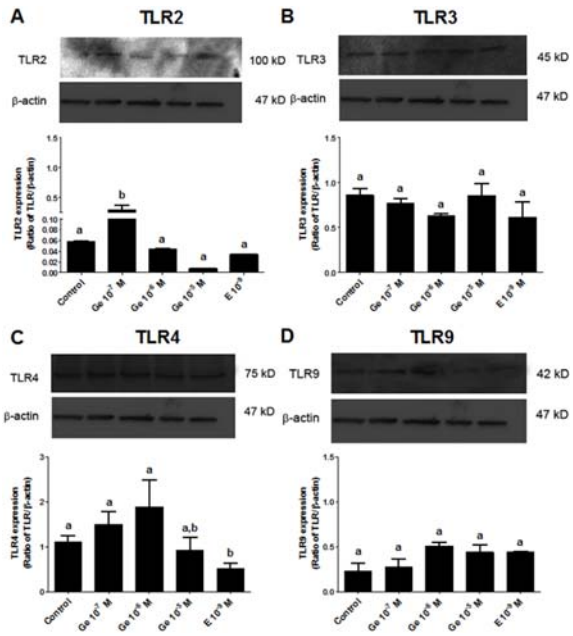

Fig. 2 Western blot and densitometric analysis of TLR2 (A), TLR3 (B), TLR4 (C) and TLR9 (D) to β-actin proteins in response to genistein or 17β-estradiol in RL95-2 cells. Total proteins were isolated from the cells grown in the estrogen-deprived media with DMSO (Control), genistein (Ge) or 17β-estradiol (E) at indicated concentrations for 48 h. Values are mean ± SEM (n = 3). Bars marked with same letter indicate no significant different, and different letters indicate statistically significant different at $p<0.05$ using ANOVA followed by Student Newman Keuls' post-hoc test.

Discussion

Recognition of pathogen by TLRs is an important function of the innate immunity in

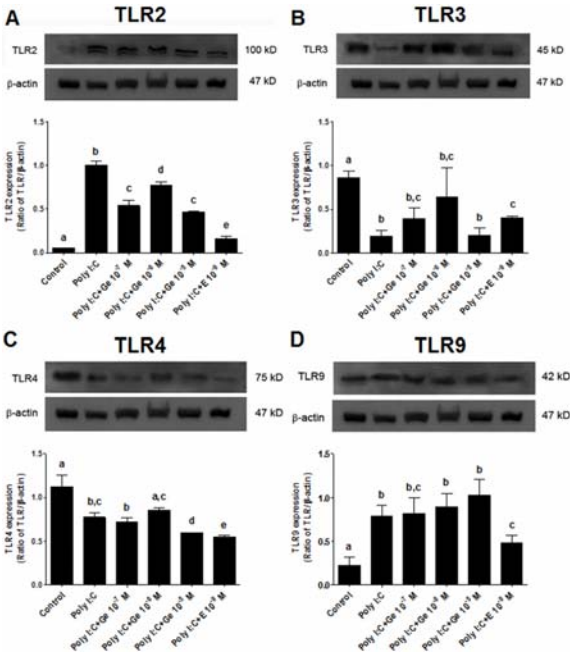

Fig. 3 Western blot and densitometric analysis of TLR2 (A), TLR3 (B), TLR4 (C) and TLR9 (D) to β-actin proteins in response to genistein or 17β-estradiol in RL95-2 cells under poly I:C stimulation. Total proteins were isolated from the cells grown in the estrogen-deprived media and pretreated with DMSO (Control) or poly I:C for 30 min followed by genistein (Ge), 17β-estradiol (E) at indicated concentrations for 48 h. Values are mean ± SEM (n = 3). Bars marked with same letter indicate no significant different, and different letters indicate statistically significant different at $p<0.05$ using ANOVA followed by Student Newman Keuls' post-hoc test.

endometrium which helps protect against uterine infection. The present study demonstrated that the expression of TLRs 2, 3, 4 and 9 was detected in the RL95-2 cells which is in agreement with the mRNA expression in human endometrial tissue[8,12]. The high basal level of TLR3 and very low level of TLR2 protein expression were equivalent in cells cultured either in standard or estrogen-deprived media, indicating the TLRs2 and 3 expressions are independent of estrogen. By contrast, the expression of TLRs4 and 9 appeared to depend on cell media conditions. Depletion of estrogen induced up-regulation of the TLR4 protein, indicating the suppressive effect of estrogen on the TLR4 expression. This is correlated with our finding that treatment with physiological concentration of 17β-estradiol significantly decreased the TLR4 expression (Fig. 2C). On the other hand, the TLR9 expression which was significantly decreased in the estrogen-deprived condition was not affected by 17β-estradiol treatment. This suggests that the TLR9 expression is not regulated by estrogen, but may be due to yet unidentified factors in the serum. Hirata and coworkers also report no alterations of *TLRs 2, 3 and 9* mRNA expressions in response to 17β-estradiol treatment for 72 h in human endometrial epithelial cells[8].

The present finding with TLR4 protein expression contrasts with lack of *TLR4* mRNA expression and response to lipopolysaccharide (LPS) previously found in the RL95-2 cells[13]. However, the expression of *TLR4* mRNA and proteins has been detected in human endometrium and endometrial epithelial cells[8,13], and yet was decreased with estradiol treatment[8]. Although the *TLR4* mRNA expression is not investigated in the present study, the low expression of TLR4 in response to 17β-estradiol suggests the functional expression of TLR4. The difference in the TLR4 expression in RL95-2 cells is possibly due to conditioned media, cell population, treatment period, and other unknown factors.

Poly I:C is a synthetic double-stranded RNA (dsRNA) commonly used to mimic viral infection. It is recognized by TLR3 inducing the activation of NF-κB and the production of pro-inflammatory cytokines and chemokines[5,14]. This TLR3 agonist has been shown to produce a strong inflammatory response and expression of other TLR subtypes[15]. In the current study, the RL95-2 cells inoculated with poly I:C produced a marked increase in the TLR2 and decrease in the TLR3 in both standard and estrogen-deprived media. The poly I:C-induced TLR2 expression is consistent with the studies in human lung epithelial cells which demonstrate the up-regulation of the TLR2 mRNA and proteins following poly I:C exposure[15,16]. However, poly I:C has been demonstrated to increase the TLR3 mRNA and proteins[15] which is also in contrast with the present finding. The different response in the TLR3 expression by poly I:C is unknown. It is possible that a constant contact with viral dsRNA like poly I:C in endometrium may result in a low level of TLR3 expression and reduced responsiveness of endometrial epithelial cells to its own ligand, which could occur in the same manner as TLR4 hyporesponsiveness to LPS in intestinal epithelial cells[16]. Alternatively, the poly I:C may be recognized by non-TLR3 like the cytosolic RNA helicase retinoic acid-inducible gene (RIG)-like receptors (RER) and melanoma-differentiation-associated gene 5 (MDA5). These RER and MDA5 have been shown to mediate the potent inhibitory effect of poly I:C on TGFβ-induced profibrotic activity in fibroblast which is independent of TLR3[17].

Additionally, poly I:C was found to decrease the TLR4 and increase the TLR9 protein expression as observed in the estrogen-deprived media while having no effects in the standard media. The decreased TLR4 expression and function by poly I:C has been reported in macrophages[18] and human airway epithelial cells[15]. Although a study of the TLR9 expression is limited in the epithelial cells, poly I:C is shown to up-regulate the cell surface expression of TLR9 in murine dendritic cells[19]. The present findings suggest that the TLR4 and TLR9 expressions mediated by poly I:C in this RL95-2 cells are differentially regulated depending on estrogen and conditioned media.

Without poly I:C exposure, low concentration of genistein (10^{-7} M) was found to up-regulate the TLR2 expression whereas 17β-estradiol had no effect. As structurally similar to endogenous estrogen, genistein elicits an estrogen or anti-estrogenic effects through estrogen receptor dependent pathway[20]. In addition, the estrogen receptor-independent pathways via inhibitions of tyrosine kinase, DNA topoisomerase II and phophatidylinositol turnover may be responsible for its action[21]. Thus, lack of the TLR2 expression in response to 17β-estradiol treatment or estrogen-depleted media suggests the up-regulation of TLR2 protein expression by genistein could be mediated by the estrogen receptor-independent pathway, possibly due to the direct effect of genistein on TLR2 gene expression or the indirect effect through the release of specific cytokine (IL6) that acts as an autocrine signaling molecule to induce TLR2 up-regulation as

has been evidenced in bronchial epithelial cells[22].

Genistein at all concentrations was found to attenuate the TLR2 protein expression induced by poly I:C. Although genistein had no effect on the basal TLR4 expression, high concentration of genistein (10^{-5} M) further decreased the poly I:C-suppressed TLR4 expression. Poly I:C acts as a potent stimulus for release of several cytokines and chemokines involved in TLR signaling and up-regulation of TLR2 in the lung epithelial cells[22]. Recently, the anti-inflammatory effect of genistein has been shown to be mediated by inhibition of TLR4 expression and cytokine release through NF-κB signaling pathway in microglia[23]. Thus, our results raise the possibility that genistein may inhibit or interfere the signaling molecules involved in TLR3 signaling pathway resulting in attenuation of specific cytokine release and consequently TLR2 and TLR4 protein expression. Our findings regarding 17β-estradiol down-regulation of TLRs 2, 4 and 9 in the presence of poly I:C suggest its anti-inflammatory effects in response to viral infection. Lesmeister et al[24] have shown that treatment of endometrial epithelial cell lines with 17β-estradiol has no effect on TLR3 expression but it suppresses the production of proinflammatory cytokines and chemokines resulting from TLR3 stimulation with poly I:C. Thus, the modulatory effect of 17β-estradiol on the TLR protein expression following poly I:C exposure may be mediated directly through gene expression and/or indirectly via alteration of the cytokine release induced by TLR3 signaling.

The expression and activation of TLRs has been reported to relate with cell death and injuries, pathogenic conditions, and cancers[25,26]. Zhou et al[27] reported that TLR2 mRNA expression in tumor ovarian was higher than normal ovary. The low level TLR2 protein expression in endometrium cells has been proposed for maintaining the healthy uterus[28]. The present findings that genistein at specific concentration moderately increased the low basal level of TLR2 protein may provide an ability of endometrium to defend against bacteria. Additionally, the alleviation of poly I:C-induced TLR2 by high concentration of genistein (10^{-5} M) or 17β-estradiol may help attenuate excessive activation of TLRs which may trigger uncontrolled immune responses contributing to the patho-physiology of diseases. Thus these results suggest the beneficial effects of phytoestrogen to provide not only a proper innate immune function but also alleviation of the inflammation of endometrium following pathogen invasion.

In conclusion, although the functional relevance of TLRs expression and health-related diseases have not well understood, the current results demonstrate the variable expression of TLRs2, 3, 4 and 9 proteins which are differentially expressed upon stimulation with viral component poly I:C. Treatment with soy isoflavone genistein increased the TLR2 and attenuated the poly I:C-induced TLR2 protein expression in human endometrium could be an important piece of evidence for application of this substance to promote proper innate immune response which is capable of detecting microbial components of invading pathogens.

What is already known on this topic ?

The expression and function of TLRs 2, 3, 4 and 9 have been evidenced in human endometrial cells and tissues, and these TLRs expression have been regulated by sex steroid hormones and the menstrual cycle.

What this study adds ?

The present study reveals the TLRs 2, 3, 4 and 9 proteins are differentially expressed upon stimulation with viral component poly IC. It also provides new findings regarding the significant role of soy isoflavone genistein on up-regulation of the basal TLR2 and attenuation of the viral component-induced TLR2 protein expression in human endometrial epithelial cells.

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Potential conflicts of interest

The authors have declared that no conflict of interest exists.

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ผลของสารไอโซฟลาโวนเจนิสทีอีนต่อการแสดงออกของโปรตีนโทลไลค์รีเซฟเตอร์ในเซลล์มะเร็งเยื่อบุผิวมดลูกของมนุษย์

นรธีร์ บัวทอง, สุทธาสินี ปุญญโชติ, ฉัตรศรี เดชะปัญญา

วัตถุประสงค์: เพื่อศึกษาว่าเจนิสทีอีน (genistein) ซึ่งเป็นสารไฟโตเอสโตรเจนที่พบมากในถั่วเหลืองสามารถปรับเปลี่ยนการแสดงออกของโปรตีน TLRs 2, 3, 4 and 9 ในเซลล์เยื่อบุผิวมดลูกของมนุษย์ (RL95-2) ได้หรือไม่ภายใต้สภาวะปกติและสภาวะที่กระตุ้นด้วย polyinosinic-polytidylic acid (poly I: C) ที่เลียนแบบการติดเชื้อไวรัสโดยเปรียบเทียบผลของ genistein กับ 17β-estradiol

วัสดุและวิธีการ: นำเซลล์ RL95-2 ไปเพาะเลี้ยงในน้ำเลี้ยงเซลล์ที่พร่องเอสโตรเจนที่มีหรือปราศจาก poly I: C เป็นเวลา 30 นาที ก่อนใส่ genisten (10^{-7}, 10^{-6} or 10^{-5} M) หรือ 17β-estradiol (10^{-9} M) เป็นเวลา 48 ชั่วโมง วิเคราะห์การแสดงออกของโปรตีน TLRs ด้วยเทคนิค semi-quantitative Western blot

ผลการศึกษา: เซลล์มีการแสดงออกของโปรตีน TLRs 3, 4 และ 9 แต่พบโปรตีน TLR2 น้อยมาก การให้ poly I: C มีผลเพิ่มการแสดงออกของ TLR2 และ 9 ได้อย่างมีนัยสำคัญทางสถิติแต่กลับลดการแสดงออกของ TLR3 และ 4 ภายใต้ภาวะปกติพบว่าการให้ genistein ที่ความเข้มข้น 10^{-7} M เพิ่มการแสดงออกของโปรตีน TLR2 ขณะที่ 17β-estradiol ลดการแสดงออกของ TLR4 ในภาวะที่เซลล์ถูกกระตุ้นด้วย poly I: C พบว่า genistein ทุกความเข้มข้นและ 17β-estradiol สามารถยับยั้งการเพิ่มขึ้นของโปรตีน TLR2 ที่ถูกเหนี่ยวนำโดย poly I: C อย่างไรก็ตามทั้ง genistein (10^{-5} M) และ 17β-estradiol กลับส่งเสริมผลของ poly I: C ที่ทำให้เกิดการลดลงของ TLR4 โดยมีเพียง 17β-estradiol เท่านั้นที่ต่อต้านการแสดงออกของโปรตีน TLR3 และ 9 ที่ถูกเหนี่ยวนำด้วย poly I: C

สรุป: จากผลของ genistein ที่สามารถเพิ่มการแสดงออกของโปรตีน TLR2 และลดการแสดงออกโปรตีน TLR2 ที่ถูกกระตุ้นด้วยองค์ประกอบของไวรัสในเซลล์เยื่อบุผิวมดลูกของมนุษย์ แสดงให้เห็นถึงบทบาทที่สำคัญของสารไอโซฟลาโวนจากถั่วเหลืองชนิดนี้ ในการส่งเสริมการทำงานของระบบภูมิคุ้มกันของมดลูกและยังอาจช่วยลดการอักเสบของเยื่อบุมดลูกจากการรุกรานของเชื้อโรคได้