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Effects of phytoestrogen on sexual development

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Phytoestrogen is an estrogenic compound that occurs naturally in plants. The most common sources of phytoestrogen are soybean products, which contain high levels of isoflavones. This compound, which has structural similarity with estrogen, can act as an estrogen receptor agonist or antagonist. Animal studies provide evidence of the significant effects of phytoestrogen on sexual development, including altered pubertal timing, impaired estrous cycling and ovarian function, and altered hypothalamus and pituitary functions. Although human studies examining the effects of phytoestrogen on sexual development are extremely limited, the results of some studies agree with those of the animal studies. In this paper, we review the possible mechanism of phytoestrogen action and the evidence showing the effects of phytoestrogen on sexual development in animal and human studies.

Key words: Phytoestrogens, Puberty, Sexual development

Introduction

Phytoestrogen is a plant-derived compound that mimics estrogen[1]. Phytoestrogens can be classified as isoflavones, coumestans, and lignans (Fig. 1). Isoflavones, which are the most extensively studied phytoestrogens, are found predominately in soybeans and soy product-containing foods[2]. The major isoflavones are genistein and daidzein, and their conjugates account for greater than 65% of the total isoflavones[3].

Phytoestrogen has significant binding affinity to estrogen receptor (ER), although much weaker than that of estradiol. Experimental animal studies have demonstrated several adverse effect of isoflavones on the reproductive system, such as premature pubertal onset[4,5], reduced fertility[6,7], altered estrous cyclicity[5,8], and disrupted pituitary responsiveness to gonadotropin releasing hormone (GnRH)[9]. Therefore, concerns over the possible adverse effects of phytoestrogens on reproductive health have been raised in humans as well.

Studies on the hormonal effects of isoflavones are especially important in Koreans because soy products are a major component of the Korean diet; in fact, the consumption of an isoflavone-containing diet such as soy-based infant formula and soy-fortified foods and beverages has recently increased. In this paper, we review the possible mode of actions of phytoestrogen and summarize the results of animal and human studies showing the effects of phytoestrogens on sexual development, especially in childhood.

Mode of phytoestrogen action and target tissues

Estrogens act through 2 subtypes of their receptors found in target tissues: ERα and ERβ. Both ERs are widely expressed in various mammalian tissues; however, there are some differences in their distribution. ERα is most abundant in the uterus, and smaller quantities are detected in the ovaries, Leydig cells of the testes, female mammary glands, bones, and hypothalamus[10,11]. ERβ expression is highest in ovarian granulose cells and the gastrointestinal tract, whereas it is moderate to low in spermatids, the prostate, epididymis,

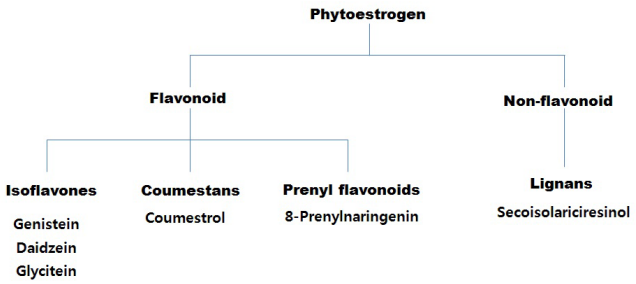

Fig. 1. Classification of phytoestrogens.

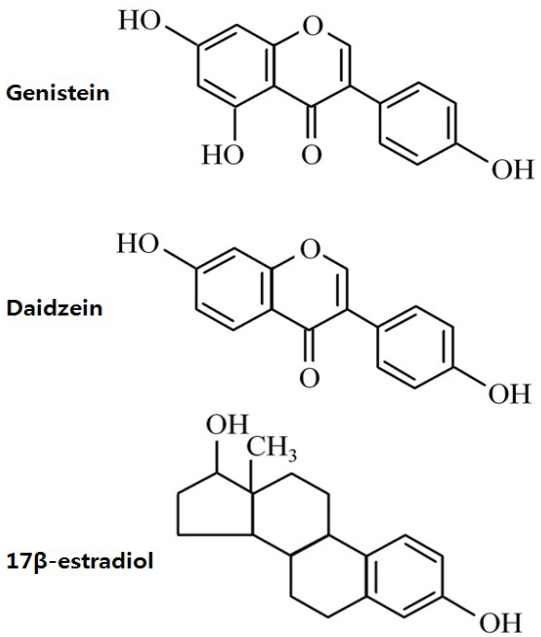

Fig. 2. Molecular structure of isoflavones.

female mammary glands, hypothalamus, and pituitary gland[11,12].

The structure of isoflavones is closely similar to that of estrogens[13]. The presence of the 2 phenolic rings in isoflavones enables these compounds to bind to ERs (Fig. 2). Isoflavones exert estrogenic effects on a number of target organs that possess ERα and/or ERβ. Although the binding affinities to ERα and ERβ of isoflavones is much lower than those of 17β-estradiol, they can compete with 17β-estradiol for binding to the ERα and ERβ[1]. The binding affinity of genistein for ERβ is 87%, while that for ERα is 4%, while that of daidzein for ERβ and ERα is 0.5% and 0.1%, respectively[14]. The effect of phytoestrogen has been demonstrated to be either agonistic or antagonistic depending on its own concentrations[15] or the estrogen concentrations of the environment[16]. At low concentrations, genistein appears to act as an agonist, stimulating the proliferation of estrogen-dependent breast cancer cells, whereas high concentrations inhibit estradiol-stimulated cell growth[17]. Hwang et al.[16] showed that isoflavone metabolites act as ER agonists in a low-estrogen environment (postmenopausal level of estradiol) but act as ER antagonists in a high-estrogen environment (premenopausal level of estradiol).

Phytoestrogen exposure and sexual development in animals

A number of animal studies have shown the adverse effects of phytoestrogens on reproductive organs (Table 1). Exposure to genistein affects mammary gland development and differentiation in rodents. Chronic genistein exposure from gestation day (GD) 7 to postnatal day (PND) 50 has been shown to result in ductal/alveolar hyperplasia of the mammary gland in pups[18]. Neonatal female mice treated with genistein showed a differential effect on mammary gland growth according to genistein dosage: low dose-treated mice exhibited advanced maturation with increased ductal elongation, whereas high dose-treated mice showed reduced lobular alveolar development[19].

In the aspect of pubertal onset, the effects of phytoestrogens are quite different according to exposure time, dosage, and route. Signs of pubertal onset in rats/mice are vaginal opening in females and preputial separation in males. One study demonstrated that perinatal exposure to genistein did not affect the age of pubertal onset in male and female rats[20]. However, other studies found that dietary exposure to high doses of isoflavone from GD 1 to PND 21 induced the acceleration of vaginal opening in female offspring but did not affect preputial separation in male offspring[21,22]. Short-term exposure to high doses of isoflavone in the neonatal period has also been shown to expedite vaginal opening in female rats[5,8,23]. In fact, we recently found that exposure to high doses of genistein in the prepubertal period (from PND 21 until puberty) induces early vaginal opening in female rats[4].

The adverse effects of genistein exposure on the developing ovaries have been shown in rodent studies. At birth, mice have large oocyte nests, and during the first week of life, these oocyte nests dissociate into individual oocytes surrounded by granulose cells[24]. This ovarian differentiation process requires a postpartum decrease in estrogen and progesterone[24]. Neonatal treatment with estrogen compounds such as 17β-estradiol and genistein disrupts this process, leaving the oocytes together in nests and leading to multi-oocyte follicles (MOFs)[7,25,26]. Since MOF-derived oocytes have been observed to have reduced fertility rates during in vitro fertilization[27], an increased number of MOFs is one possible explanation for infertility in genistein-treated rodents[28].

Numerous studies have shown that exposure of rodents to genistein in the neonatal period caused abnormal estrous cyclicity[5,7,8] and altered ovulation[6,25]. These phenomena are mainly explained by the effect of genistein on the hypothalamic-pituitary-gonadal (HPG) axis,

Table 1. Animal Studies Investigating the Effects of Phytoestrogens on Sexual Development

Phytoestrogen	Species (gender)	Age at treatment	Dose, route	Effects	Reference
Geistein	Rat (F)	GD 7-PND 50	1,250 ppm, PO	Ductal/alveolar hyperplasia of MG	Delclos et al.[18]
Genistein	Mice (F)	PND 1-5	0.5 mg/kg/day, SQ 50 mg/kg/day	Advanced alveolar maturation of MG at 5 wk Reduced alveolar maturation of MG at 5 wk	Padilla-Banks et al.[19]
Genistein	Rat (F)	GD 15-PND 11	1,250 ppm, PO	No effect on VO Irregular estrous cyclicity (extended diestrus)	Takagi et al.[20]
Isoflavones	Mice (F)	GD 1-PND 21	0.05% isoflavone-diet	Early VO Increased incidence of MOFs	Takashima-Sasaki et al.[21]
Genistein	Rat (F)	GD 1-PND 21	0.02%, 0.1% genistein-diet	Early VO	Casanova et al.[22]
Genistein	Rat (F)	PND 1-5	1 mg/day, SQ	Early VO Irregular estrous cyclicity (extended estrus)	Kouki et al.[8]
Genistein	Rat (F)	PND 1-21	40 mg/kg/day, PND 1-7, SQ PND 8-21, PO	Early VO Irregular estrous cyclicity (extended estrus)	Lewis et al.[23]
Genistein	Rat (F)	PND 1-4	10 mg/kg/day, SQ	Early VO; Irregular estrous cyclicity; decreased GnRH activation	Bateman and Patisaul[5]
Genistein	Rat (F)	PND 21 -Puberal onset	10, 100 mg/kg/day, PO	Early VO	Lee et al.[4]
Genistein	Mice (F)	PND 1-5	1, 10, 100 micg, PO 1 micg 10, 100 micg	Increased MOFs at PND 19 Increased number of ovulated oocytes Decreased number of ovulated oocytes	Jefferson et al.[25]
Genistein	Mice (F)	PND 1-5	5, 20, 50, 100 mg/kg/day, PO	Increased incidence of MOFs in the neonate persisted into adulthood (4 mo old)	Cimafranca et al.[26]
Genistein	Rat (F)	PND 1-5	12.5, 25, 50, 100 mg/kg/day, PO	Increased incidence of MOFs; decreased fertility rate; no effect on VO	Nagao et al.[7]
Genistein	Mice (F)	PND 1-5	5, 50 mg/kg/day, PO 5 mg/kg/day 50 mg/kg/day	Irregular estrous cyclicity (extended estrus) Increased ovulation Decreased ovulation	Jefferson et al.[6]
Genistein	Rat (F)	PND 0-3	10 mg/kg/day, SQ	Decreased kisspeptin fiber density in AVPV early VO; increased MOFs at PND 24	Losa et al.[30]
Genistein	Rat (F)	PND 1-10	1-1,000 micg/day, SQ	10 micg increased LH release to GnRH 100-500 micg decreased LH release to GnRH	Faber and Hughes[9]
Genistein	Rat (M)	GD 7-PND 50	1,250 ppm 625-1,250 ppm 250-1,250 ppm	Decreased ventral prostate weight Delayed spermatogenesis Decreased sperm counts in the epididymis Ductal/alveolar hyperplasia	Delclos et al.[18]
Genistein	Rat (M)	GD 15-PND 11	1,250 ppm, PO	No effect on PPS No effect on weights of pituitary, adrenal, and ventral prostate	Takagi et al.[20]
Isoflavones	Mice (M)	GD 1-PND 21	0.05% isoflavone-diet	No effect on PPS and weight of testes	Takashima-Sasaki et al.[21]
Genistein	Rat (M)	GD 1-PND 21	0.02%, 0.1% genistein-diet	No effect on PPS and weights of testes and ventral prostate	Casanova et al.[22]
Genistein	Rat (M)	PND 1-5	12.5, 25, 50, 100 mg/kg/day, PO	No effect on PPS and fertility rate No histological change in the gonads	Nagao et al.[7]

GD, gestational day; PND, postnatal day; PO, per oral; MG, mammary gland; SQ, subcutaneous injection; VO, vaginal opening; MOFs, multi-oocyte follicles; GnRH, gonadotropin releasing hormone; AVPV, anteroventral periventricular nucleus; PPS, preputial separation.

upon which normal estrous cyclicity is dependent[6] and from which a mid-cycle surge of luteinizing hormone (LH) for ovulation is derived[29]. The response of the HPG axis in the neonatal period to genistein exposure seems to be dose-dependent. One study demonstrated that exposure to low doses of genistein in the neonatal period increased GnRH-induced LH release, whereas a high dose of genistein decreased GnRH-induced LH release[9]. Similarly, mice treated neonatally with low doses of genistein showed increased LH production and enhanced ovulation[6,25], whereas rats exposed to high doses of genistein in the neonatal period demonstrated decreased GnRH activation and kisspeptin fiber density in the hypothalamus[5,30]. These data suggest that exposure to genistein in

the neonatal period disrupts HPG function in a dose-dependent manner.

Relatively few studies have demonstrated the effects of phytoestrogen on male sexual development in rodents. Delclos et al.[18] showed decreased ventral prostate weight and delayed spermatogenesis in male rats after genistein exposure from conception to puberty. However, exposure for short duration (GD 1 to the prepubertal period) did not affect pubertal timing, gonadal weight/histology, or fertility rate in male rodents[7,20-22].

Phytoestrogen exposure and sexual development in children

Human phytoestrogen exposure is highly dependent on the consumption of soy-based foods. The traditional Asian diet includes greater amounts of soy products such as tofu, soy sauce, and tempeh than the typical Western diet; therefore, the Asian population has higher concentrations of isoflavone in the urine and plasma than the Western population[31-33]. Because phytoestrogens cross the placenta, fetuses are exposed to them through maternal dietary consumption[34]. After birth, infants are exposed to phytoestrogens though breastfeeding; however, the most extensive exposure to phytoestrogens occurs via soy-based formula feeding[35].

A few studies have investigated the relationship between phytoestrogen exposure and sexual development in children, and an association was mainly reported in girls (Table 2). Neonatal consumption of soy-based formula was independently associated with premature thelarche in Puerto Rican girls[36]. Zung et al.[37] also reported that

Table 2. Human Studies Investigating the Effects of Phytoestrogens on Sexual Development

Study population (sample size)	Method	Findings	Reference
Puerto Rican, girls aged 6 mo-8 yr (130 PT cases, 130 controls)	Cross-sectional study Assessment of breast bud	Positive association between PT and consumption of soy-based formula	Freni-Titulaer et al.[36]
Israel, female infants (total 694, soy formula feeding 92)	Cross-sectional study Assessment of breast bud	Positive association between PT and consumption of soy-based formula	Zung et al.[37]
US, infants (35 girls)	Follow-up study Assessment of breast bud, vaginal wall cells	Girls fed soy-formula showed re-estrogenization at 6 months in vaginal wall cell maturation index	Bernbaum et al.[38]
US, females aged 20-34 yr (128 fed soy, 268 fed cow milk formula in infancy)	Retrospective cohort study Assessed pubertal timing by telephone interview	No difference in pubertal timing between groups	Strom et al.[39]
US, New York girls aged 9 yr (192)	Cross-sectional study Assessment of Tanner stages	A negative trend for urine daidzein and genistein levels with breast development	Wolff et al.[40]
German, girls (119)	Cohort study Assessment of dietary intake, breast development	A negative trend for dietary isoflavones with breast development in girls No association for urinary isoflavone levels with pubertal markers	Cheng et al.[41]
UK, girls (2,920)	Prospective study Assessed age at menarche by annual questionnaire	Girls fed soy-formula in early infancy have an increased risk of menarche	Adgent et al.[42]
Korea, girls (108 CPP cases, 91 controls)	Case-control study Assessed Tanner stage, bone age, GnRH stimulation test	A positive association between CPP risk and high serum isoflavone level	Kim et al.[43]
US, infants (37 boys)	Follow-up study Assessment scrotal anatomy or testicular volume	No difference in genital development	Bernbaum et al.[38]
US, males aged 20-34 yr (120 fed soy, 295 fed cow milk formula in infancy)	Retrospective cohort study Assessed pubertal timing by telephone interview	No difference in pubertal timing between groups	Strom et al.[39]
German, boys (108)	Cohort study Assessment of dietary intake, gonadal development	No association with gonadal development in boys No association for urinary isoflavone levels with pubertal markers	Cheng et al.[41]
UK, boys (total 7,928, 51 hypospadias cases)	Prospective study; Assessed life style and dietary practices by questionnaire during pregnancy	Increased risk of giving birth to a boy with hypospadias in vegetarian mothers compared with omnivors	North and Golding[45]

PT, premature thelarche; US, United States; UK, United Kingdom; CPP, central precocious puberty.

premature thelarche was more prevalent among soy formula-fed infants. In addition, the estrogenic activity of soy-based formula in infants involves vaginal cell maturation[38]. Vaginal wall cells show maximal maturation index at birth due to maternal estrogen and then rapidly lose the estrogen effect by the age of 1 month. This low maturation index is maintained in breast milk- or cow-based formula-fed infants; in contrast, the vaginal maturation index rises again in infants fed soy-based formula by the age of 6 months, indicating the estrogen activity of soy-based formula.

There are conflicting results in regard with pubertal timing. A telephone survey of young adults who had been fed soy formula during the infancy reported no significant changes in pubertal maturation[39]. Two studies reported that phytoestrogen exposure was associated with delayed puberty in girls. A cross-sectional study of 192 healthy 9-year-old girls found that delayed breast development was observed in girls with high concentrations of urinary daidzein and genistein[40]. A prospective cohort study reported that high isoflavone intake was associated with delayed breast development in German girls[41]. On the other hand, recent studies support that phytoestrogen exposure may induce earlier pubertal maturation. A prospective longitudinal study that enrolled pregnant women in the United Kingdom reported that early soy-fed infants had earlier menarche compared with girls fed breast milk or other types of formula[42]. We recently demonstrated that high serum isoflavone levels are associated with central precocious puberty in Korean girls[43].

These conflicting results reflect the complex influence of phytoestrogens on the HPG axis. The effects of isoflavones on puberty seems be dependent on several factors, such as the amount of isoflavones consumed, timing of isoflavone exposure, and endogenous estrogen status of individuals. As discussed earlier, genistein exposure alters GnRH activation and pituitary responsiveness; specifically, a low dose of genistein increased GnRH-induced LH release, whereas a high dose of genistein decreased GnRH-induced LH release[9]. In the peripubertal period when endogenous estradiol levels are low, phytoestrogen exposure could induce increased sensitivity of the pituitary gland to GnRH challenge[9], whereas exposure after pubertal onset could interfere with gonadotropin release as demonstrated in premenopausal women[44].

For boys, one epidemiologic study found that maternal vegetarian diets containing large amounts of isoflavones are associated with hypospadias in male offspring[45]. However, most studies failed to show a significant association between phytoestrogen exposure and pubertal onset[39,41].

Summary

Several animal studies have demonstrated significant effects of phytoestrogen on sexual development, including altered pubertal timing, impaired estrous cyclicity and ovarian function, and altered function of the HPG axis. Human studies are also examining the possible effects of isoflavone exposure on sexual development in childhood. Especially during infancy, which is a critical period for growth and development, soy formula feeding may exert estrogenic effects on multiple organs including the mammary glands, gonads, and brain. In human studies, it is difficult to absolutely control other factors influencing clinical outcomes, such as diet, genetic factors, or other environmental disruptors. Further prospective human studies are needed that control these factors as much as possible to reveal the potential health effects of soy-based formulas or other sources of phytoestrogens on sexual development in children.

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