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Uterine phenotype of young adult rats exposed to dietary soy or genistein during development☆

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Abstract

Dietary soy intake is associated with protection from breast cancer, but questions persist on the potential risks of the major soy isoflavone genistein (GEN) on female reproductive health. Here, we evaluated intermediate markers of cancer risk in uteri of cycling, young adult Sprague–Dawley rats lifetime exposed to one of three AIN-93G semipurified diets: casein (CAS), soy protein isolate (SPI^+ with 276 mg GEN aglycone equivalents/kg) and CAS+GEN (GEN at 250 mg/kg). Postnatal day 50 (PND50) rats lifetime exposed to GEN or SPI^+ had similar uterine luminal epithelium height, myometrial thickness, endometrial gland numbers, endometrial immunoreactive proliferating cell nuclear antigen (PCNA), and serum estrogen and progesterone, as CAS-fed rats. GEN-fed rats showed modestly increased apoptosis in uterine glandular epithelium, compared to those of CAS- or SPI^+-fed groups. Diet had no effect on the uterine expression of genes for the tumor suppressors PTEN, p53 and p21, and the apoptotic-associated proteins Bcl2, Bax and progesterone receptor. Uterine tissue and serum concentrations of total GEN were higher in rats fed GEN than in those fed SPI^+. Human Ishikawa endocarcinoma cells treated with GEN-fed rat serum tended to exhibit increased apoptotic status than those treated with CAS-fed rat serum. Exogenously added GEN (0.2 and 2 μM) increased, while estradiol-17β (0.1 μM) decreased Ishikawa cell apoptosis, relative to untreated cells. Results suggest that lifetime dietary exposure to soy foods does not alter uterine cell phenotype in young adult rats, while GEN, by enhancing uterine endometrial glandular apoptosis in vivo and in vitro, may confer protection against uterine carcinoma. Given its limited influence on uterine phenotype of young adult females, GEN, when taken as part of soy foods or as supplement, should be favorably considered for other potential health benefits.

Keywords: Uterus; Soy; Genistein; Nutrition; Apoptosis; Endocarcinoma

1. Introduction

Numerous epidemiological studies have pointed to the breast cancer-protective effects of soy-based diets [1–4]. Although the molecular mechanisms underlying these effects remain ill-defined, this protection has been attributed, in part, to the soy isoflavone genistein (GEN) which exhibits estrogenic agonist/antagonist activity through estrogen receptor (ER)-α- and ER-β-mediated pathways [5,6]. The uterus, an estrogen-sensitive tissue, is also considered a target of GEN action [7,8]. However, a consensus on the positive or negative influences of GEN on uterine function remains lacking as studies to date using animal models have differed on the dose, age of first exposure, and duration and route of exposure for assessing possible benefits or risks [9–15]. Thus, while exposure to GEN has been shown to negatively impact the development of the female genital tract and subsequent fertility in a number of studies [9–12], other studies, by contrast, have demonstrated the lack of adverse reproductive effects of GEN, when taken in the diet or by injection [13–15]. Further, while subcutaneous administration of GEN to neonatal mice was associated with increased incidence of uterine adenocarcinoma [16], GEN administered by the same route was

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found in another study to be protective against estrogen-induced endometrial carcinogenesis in mice [17].

There have been no reports in humans on the adverse health effects from exposure to isoflavones in utero and postnatal. Asians, who are regular consumers of soy foods, have a lifetime of exposure to soy phytochemicals such as GEN; this level of exposure is relatively high in utero due to placental transfer of these phytochemicals from maternal circulation, decreases to very low levels during infancy as phytochemicals do not cross into the breast milk and significantly increases postweaning after initiation of traditional diets rich in soya products [18]. Female offspring of Asian women who consume GEN as part of a normal soya diet have been suggested to benefit from habitual soy intake [2–4,19–21], although limited direct evidence exists to link this to protection from risk of uterine dysfunctions such as endometrial carcinoma and endometriosis [22]. Indeed, only one epidemiological study has reported an analytical association between ingestion of phytoestrogenic compounds such as GEN and reduced risk of endometrial cancer [19]. Nevertheless, the potential health benefits of soy have encouraged women in the Western world to supplement their regular diets with GEN as a substitute for regular soy food consumption. However, the popular association of GEN and soyfoods is not entirely accurate; while native soy proteins have predominantly the isoflavone conjugate genistin, which undergoes intestinal metabolism to the aglycone and other molecular forms upon ingestion, GEN available in health food stores exists solely as the aglycone component. Moreover, soy proteins are also a rich source of the isoflavone daidzen, which has been demonstrated to exhibit biological effects in animal models in vivo and carcinoma cells in vitro [17,23,24].

In the United States, where newborns consuming commercially available soy formula are exposed to high (micromolar) levels of GEN for a limited period in the first year of life [25,26], the adverse consequence of this early GEN exposure on long-term uterine health remains a major concern. Thus, information gleaned from an analysis of molecular and cellular parameters modified in the young adult uterus by prolonged and early exposure to dietary soy proteins and associated isoflavones, a regimen simulating the Asian diet may aid in resolving the issues surrounding their effects on women's reproductive health. In the current study, we evaluated intermediate markers of cancer risk, namely, proliferation status, apoptotic index and expression of specific growth regulatory genes, in uteri of cycling, young adult Sprague–Dawley rats lifetime exposed to a diet of soy protein isolate (SPI^+) or a control diet (CAS) supplemented with GEN, relative to those fed the control CAS diet. Further, we compared the apoptosis-inducing effects of exogenously added GEN, estradiol-17β and of sera from rats fed GEN-enriched diets using human Ishikawa epithelial carcinoma cells to discern the potential of GEN for cancer chemoprevention in the endometrium, as has been suggested for the mammary gland [1,18,27].

2. Materials and methods

2.1. Rats, diets and tissue collection

All animal procedures were approved by the University of Arkansas for Medical Sciences Animal Care and Use Committee. Time-mated Sprague–Dawley rats were purchased from Charles River Laboratories (Wilmington, MA) and kept individually in polycarbonate cages in rooms under controlled temperature (24°C), humidity (40%) and light (12-h light/dark cycle). Rats at gestation day (GD) 4 were randomly assigned to semipurified isocaloric diets which were made according to the AIN-93G diet formula [28], except that corn oil replaced soy bean oil and contained as sole protein source, either casein [20% (w/w) CAS; New Zealand Milk Products, Santa Rosa, CA] (Group 1) or soy protein isolate [20% (w/w) SPI+] (a gift from Solae Company, St. Louis, MO) (Group 2). Diet containing SPI^+ had 276 mg GEN (aglycone) equivalents per kilogram. A third diet group (Group 3) contained CAS [20% (w/w)] as sole protein source to which was added aglycone GEN (Sigma, St. Louis, MO) at 250 mg/kg; the latter resulted in serum GEN levels (~1.5 μM; Table 1) that were within the range of those found in humans consuming a normal soya diet [29]. Animals were provided food and water ad libitum. At delivery, pups were randomly selected from litters of five to seven dams of the same diet group, and 10 pups (five males and five females) were assigned to each dam for suckling. The offspring were weaned to the same diets as their mothers and were continued on these diets throughout the study. We have previously reported that females fed these diets exhibited normal 4- to 5-day estrous cycles, as evaluated by daily vaginal smears beginning at postnatal day (PND) 32 [18,30,31]. Moreover, females in all three groups consumed the same amounts of food, and their body weights did not significantly differ with diet from birth until PND50 (data not shown). At PND50, females ($n=10$ per diet group) were sacrificed, and uteri were collected and weighed. For each female, the left uterine horn was immediately homogenized in TriZol reagent (Invitrogen, Carlsbad, CA), and the homogenate frozen at −80°C for later RNA isolation, while the right horn was fixed overnight in 10% neutral buffered formalin for subsequent morphometry and immunohistochemistry. The male rats were used in an unrelated study.

Table 1
Total genistein content in PND50 rat uterus and serum as a function of diet[a]

Diet	Tissue[b] (nmol/g)	Serum[c] (μmol/L)
CAS	0.02±0.01	0.01±0.01
SPI^+	0.33±0.11*	0.44±0.10*
CAS+GEN	0.63±0.24*	1.47±0.04*

[a] Values are mean±S.E.M.
[b] Tissues were from $n=4$ individual rats per diet group.
[c] Sera were pooled from $n=6$ rats per diet group and were analyzed in triplicate.
* Different from CAS, $P<.05$ (t test).