The Different Effects of Phytoestrogens and Estrogen on Estrogen Receptor Expressions in the Uterus of Ovariectomized Rat

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Introduction
Phytoestrogens (i.e. genistein, daidzein) are bioactive compounds mainly found in soy bean. Their chemical structures are similar to estrogen; therefore, they can bind to estrogen receptor (ER) (1). There are two types of ER; ERα and ERβ. Phytoestrogen preferably binds to ERβ while estrogen prefers ERα than ERβ. In which ERα is mainly expressed in reproductive organs like uterus and breast. Previously, it had been reported that ER expression in the uterus could be changed according to hormone level such as during different phases of estrous cycle or when estrogen was given to an ovariectomized rat (2, 3, 4). Interestingly, the changes in ER expression had been implied as an indicator of cancer risk. Konduri and Schwarz (2007) found that the pancreatic cancer cell had higher expression of ERβ and resulting in the altered ratio of ERβ/ERα. Moreover, the increased in the level of ERα expression can inhibit the growth of ishikawa cell, the uterine cancer cell (6). It is then likely that the down or up regulation of ERα or ERβ, with correspondingly changes in ER ratio is responsible for the cancer malignancy. From the binding property of phytoestrogens, it is interesting to determine whether chronic treatment of genistein or daidzein can change the expression levels of estrogen receptor in the uterus of the ovariectomized rat.

Materials and Methods
Animals and Treatments: Female Wistar rats (180-200) were ovariectomized and randomly assigned into 4 groups receiving daily treatment of vehicle, estrogen (E2; 1 µg/kg), genistein (Gen; 0.25 mg/kg) or Daidzein (Dai; 0.25 mg/kg). Four weeks following ovariectomy, all rats were sacrificed and the uterus were weighed, quick frozen in liquid nitrogen and kept at -20°C for western blot analysis. Measurement of ER α and β protein: The frozen uterus was homogenized in lysis buffer containing protease inhibitor cocktail (Sigma, USA) and centrifuged, then the supernatant was collected. The amount of protein was measured with modified Lowry’s assay using commercial test kit (Biorad Laboratories). Samples (50-75 µg protein/lane) were resolved by 9% SDS-PAGE and electrophoretically transferred to polyvinylidene difluoride membranes in Tris-glycine transfer buffer. Blotted membranes were then blocked with 5% nonfat powdered milk in Tris-buffered saline for 4 hours at room temp. For identification of proteins, membranes were washed and incubated overnight at 4°C with the primary antibodies diluted in 1% milk. The primary antibodies were polyclonal anti-ERα (Santa Cruz, USA), polyclonal anti-ERβ (Santa Cruz) at the dilution of 1:2,500 and monoclonal anti-β-actin (Sigma) at the dilution of 1:100,000. Following the primary antibody incubation, the membranes were washed and then incubated in 1:5,000 horseradish peroxidase-conjugated secondary antibodies at room temperature for 1 h. This incubation step was terminated with several washes and the immunoreactive protein bands were visualized using chemiluminescence technique (ECL Plus; Amersham Biosciences). Membranes were exposed to film (Hyperfilm-ECL) for times adequate to visualize chemiluminescent bands. Differences in protein immunoreactivity between treatments were determined by scanning densitometry in proportion to β-actin immunoreactive bands (Scion Image; Scion Corporation, USA). All samples were repeated in duplicate. Statistical analysis: The data are expressed as mean ±SEM and evaluated with one-way ANOVA followed by Duncan’s multiple comparison tests.

Results and Discussion
Four weeks after ovariectomy, the uterine weight in the vehicle group was lowered than the E2 group indicating that E2 contained a uterotrophic effect as shown in Figure 1. Gen and Dai had no uterotrophic effect as the uterine weights were not differed from veh (Fig. 1). This data was consistent with previous report (7).

References
Immunoreactive bands corresponding to ERα and ERβ were presented in the ovary and the uterus at the molecular weight of 66 and 53 kDa, respectively. Chronic treatment of estrogen resulted in increased level of ERα when compared to vehicle treated group (Fig. 2). On the other hand, both genistein and daidzein had no effect on ERα levels (Fig. 2). The ERβ protein levels were not affected by any treatment. This data has revealed that estrogen not only contained the uterotrophic effect but also up-regulated the ERα level in the uterus; whereas, phytoestrogens had no effects on both the uterus and the amount of ERα. This data implied that the phytoestrogen may be used as an alternative to estrogen as it is unlikely to affect ER ratio which can lead to abnormal cell growth.

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References