Soybean isoflavones inhibit estrogen-stimulated gene expression in mouse uteri

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Summary

This study was performed to examine the inhibitory effects of soybean isoflavones on estrogen-stimulated gene expression of the uteri in ovarectomized mice. Especially when compared with the inhibitory effect of genistein and daidzein as aglycosides described in our previous report, subcutaneous administration of the glycoside genistin significantly decreased the levels of estradiol-17β (E_2) – induced expressions of c-jun, interleukin(IL)-1α and tumor necrosis factor (TNF)-α mRNAs ($p < 0.005$, $p < 0.05$ and $p < 0.05$, respectively) and seemingly proteins in the mice uteri, whereas the glycoside daidzin weakly inhibited E_2-stimulated expressions of c-fos and IL-1α. Both genistin and daidzin seemed to have a weaker inhibitory effect than that of genistein and daidzein on the expression of estrogen-stimulated genes. It is suggested that those glycosides are naturally derived and generally absorbed from plant foods and might prevent E_2-related endometrial carcinogenesis.

Key words: Soybean isoflavones; c-fos/jun; IL-1α, TNF-α.

Introduction

We have reported that soybean isoflavens, genistein and daidzein, have a preventive effect on estradiol-17β-related endometrial carcinogenesis in mice [1]. Isoflavones, including aglycones (genistein and daidzein) and their glycosides (genistin and daidzin) are naturally absorbed from soybeans and soybean-related foods, and exert a variety of biological activities including anti-cancer effects, such as inhibitory effects of genistein and daidzein on carcinogenesis in prostate, skin and bladder cancers [2-5].

In a E_2-related mouse endometrial carcinogenesis model, E_2 stimulates the expression of c-fos/jun, IL-1α and TNF-α mRNA and proteins, which are decreased by genistein and daidzein [1], resulting in a lower incidence of mouse endometrial (pre)neoplasia.

On the other hand, although genistin and daidzin as glycosides more naturally contained in soybeans and their related products [6], the biological effects of these glycosides remain to be clarified. To determine whether these glycosides as well as aglycones exert decreased effects in ovarectomized mice, the present experiment was performed.

Materials and Methods

Animals and chemicals

Female ICR mice were purchased from Japan SLC Co. (Shizuoka, Japan). Oriental MF (Oriental Yeast Co., Tokyo, Japan) was used as a basal diet. The diet and filtered tap water were available *ad libitum* throughout the experiment. Estradiol-17β (E_2) genistin, daidzin, genistein and daidzein (Figure 1) were purchased from Sigma Chem Co. (St. Louis, MO) and Fujicco Co., Ltd. (Kobe, Japan), respectively.

Experimental protocol

According to a previous study [1], the ovarectomized mice were divided into six experimental groups (6 mice each). Groups 1-5 were daily given a diet containing E_2 (5 ppm) for two weeks. Groups 2-4 received a single subcutaneous (s.c.) injection of those glycosides and aglycones at the dose of 1 mg/30 g body wgt, 24 hours prior to resection of the uteri on the 13th day. The dose of the agents was decided by the previous report that six daily 10-20 mg/kg treatments of genistein or daidzein suppress DNA adduct formations of mammary glands in female ICR mice [7]. Isoflavones were dissolved in ethanol, and mixed with sesame oil. Group 6 was given a mixture of ethanol and sesame oil alone as a non-treatment control. After two weeks treatments, the mice uteri were resected for the following experiments.

Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA isolation [8], RT reaction and PCR conditions of c-fos [9] c-jun [10], IL-1α [11], TNF-α [12] and GAPDH [13] were performed as described previously [1]. The primers of genes are shown in Table 1. Semi-quantitative analysis of the mRNA of the gene was carried out following our previously study [14].

Immunohistochemical expression of genes in the mouse uterus

After the tissues were fixed in 10% formalin, half of the resected uterine corpus was stained with conventional staining methods. Immunohistochemical expression of the proteins c-fos/jun, TNF-α and IL-1α was performed as described previously [1].

Statistical analysis

The analysis was done utilizing the Student's t-test.

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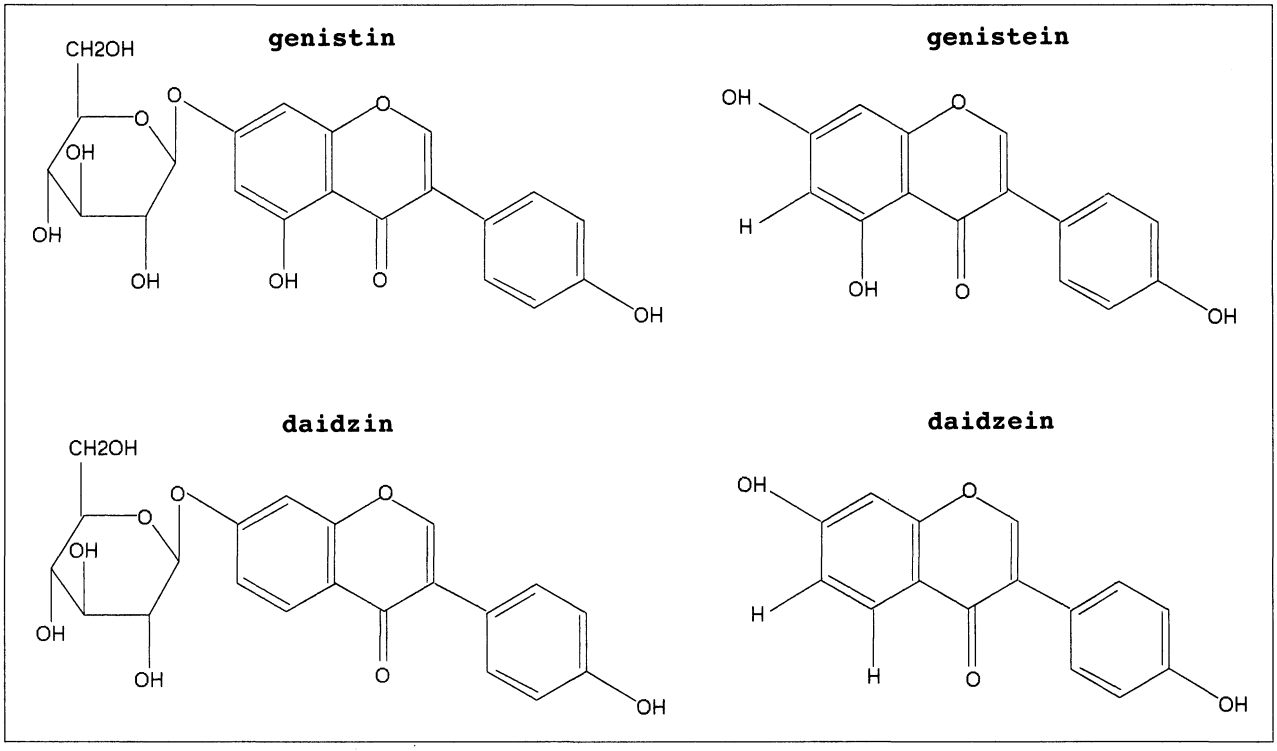

Figure 1. — The structural formula of soybean isoform isoflavones, genistin, daidzin, genistein and daidzein.

Table 1. — *Sequences of primers.*

Primes	Nucleotide sequences
c-fos sense	5'-CTTACGCCAGAGCGGGAATG-3'
c-fos antisense	5'-AAGCCTCAGGCAGACCTCCA-3'
c-jun sense	5'-GGAGTGGGAAGGACGTGGCGC-3'
c-jun antisense	5'-TCCCAGCCCTCCCTGCTTTGTG-3'
IL-1α sense	5'-GATGGCCAAAGTTCCTGACTT-3'
IL-1α antisense	5'-GCCTGACGAGCTTCATCAGTTT-3'
TNF-a sense	5'-AGGCAGGTTCTGTCCCTTTCA-3'
TNF-a antisense	5'-ACCACTTGGTGGTTTGCTACG-3'
GAPDH sense	5'-CAAGGTCATCCCAGAGCTGAA-3'
GAPDH antisense	5'-GCAATGCCAGCCCCGGCATCG-3'

Results

Uterine mRNA expressions of c-fos/jun, IL-1α and TNF-a were significantly increased in E_2 treatment compared with the control (Figures 2-5). Corresponding to the change of mRNA levels, the uterine protein expressions of c-fos/jun (Table 2), IL-1α and TNF-α (Table 3) seemed to be increased in the E_2 treatment. Genistin significantly decreased the E_2-induced expression of c-jun, IL-1α and TNF-α mRNAs (Figures 3, 4 and 5), whereas daidzin did not significantly suppress E_2-induced gene expression. The immunohistochemical stainings of the proteins showed similar tendencies to those of the mRNA levels (Tables 2 and 3). Generally, aglycones seemed to inhibit the E_2-induced factors (genes) more than glycosides did.

Table 2. — *Immunohistochemical expression of c-fos and c-jun of uteri of ovarectomized mice after two weeks' feeding a diet with E_2, E_2 plus genistin, genistein, daidzin or daidzein.*

Group treatment	c-fos			c-jun		
	Glandular cells	Luminal cells	Stromal cells	Glandular cells	Luminal cells	Stromal cells
1 E_2 alone	+	+	±	+	±	±
2 E_2 + Genistin	+	+	±	+	±	±
3 E_2 + Daidzin	+	±	±	+	±	±
4 E_2 + Genistin	±	±	±	±	±	±
5 E_2 + Daidzin	±	±	±	±	±	±
6 No treatment	±	–	–	±	–	–

(+), positive; (±), minimally or radomly positive; (–), negative.

Table 3. — *Immunohistochemical expression of IL-1α and TNF-α of uteri of ovarectomized mice after two weeks' feeding a diet with E_2, E_2 plus genistin, genistein, daidzin or daidzein.*

Group treatment	IL-1α			TNF-α		
	Glandular cells	Luminal cells	Stromal cells	Glandular cells	Luminal cells	Stromal cells
1 E_2 alone	+	+	±	+	±	+
2 E_2 + Genistin	±	±	±	+	±	±
3 E_2 + Daidzin	+	+	±	+	+	+
4 E_2 + Genistin	±	±	±	±	±	±
5 E_2 + Daidzin	±	±	±	+	±	±
6 No treatment	±	–	–	±	–	–

(+), positive; (±), minimally or radomly positive; (–), negative.

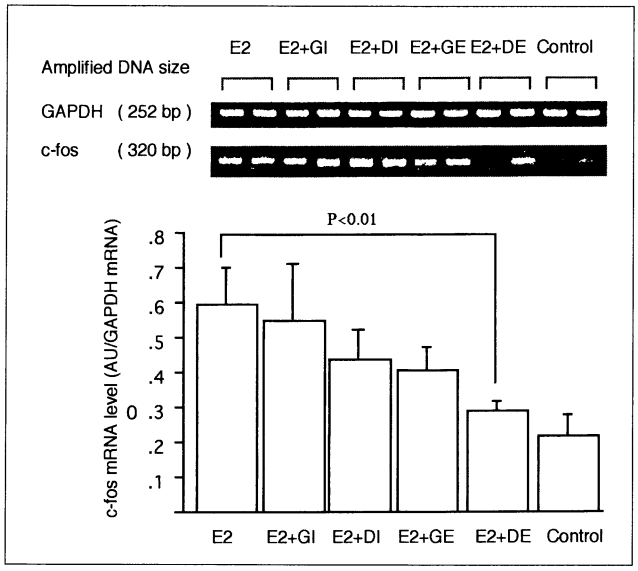

Fig. 2

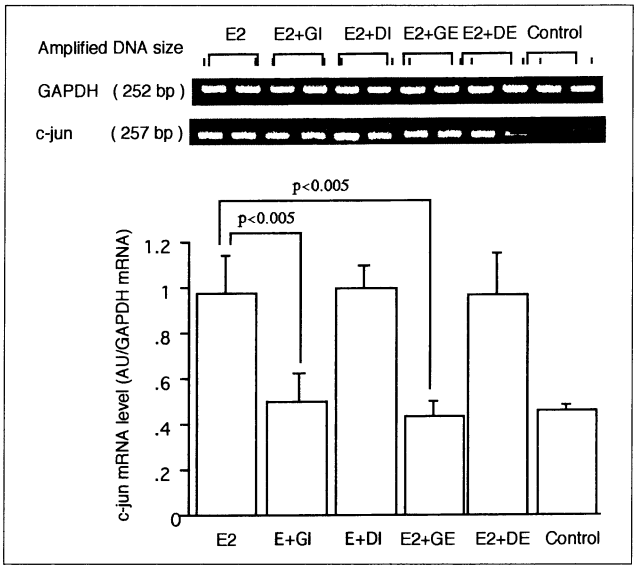

Fig. 3

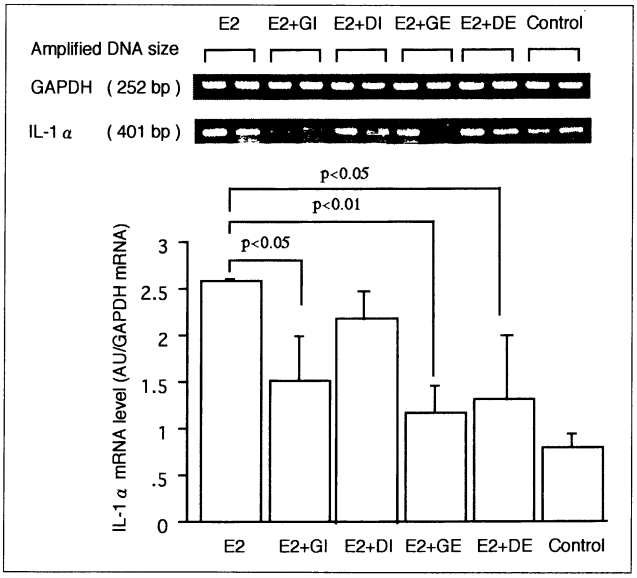

Fig. 4

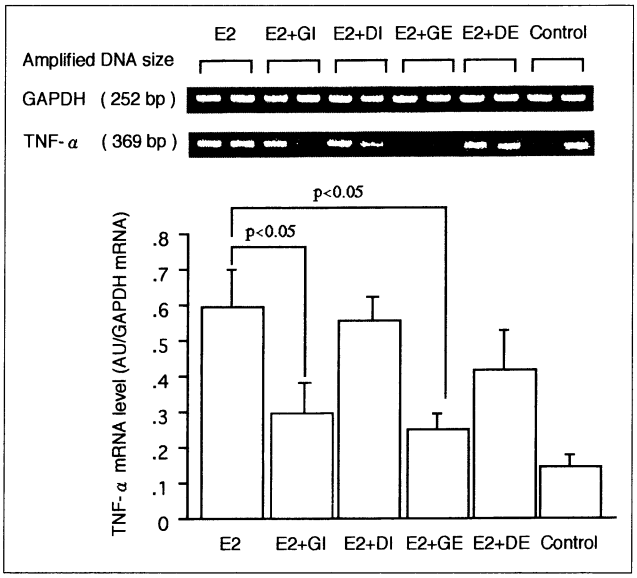

Fig. 5

Figure 2. — Expression of c-fos mRNA in the uterus of ovarectomized mice, treated continuously for two weeks with E_2 or E_2 plus genistin, daidzin, genistein and daidzein or no treatment. GI, genistin; DI, daizin; GE, genistein and DE, daidzein.
Figure 3. — Expression of c-jun mRNA in the uterus of ovarectomized mice, treated continuously for two weeks with E_2 or E_2 plus genistin, daidzin, genistein and daidzein or no treatment. GI, genistin; DI, daizin; GE, genistein and DE, daidzein.
Figure 4. — Expression of IL-1α mRNA in the uterus of ovarectomized mice, treated continuously for two weeks with E_2 or E_2 plus genistin, daidzin, genistein and daidzein or no treatment. GI, genistin; DI, daizin; GE, genistein and DE, daidzein.
Figure 5. — Expression of TNF-α mRNA in the uterus of ovarectomized mice, treated continuously for two weeks with E_2 or E_2 plus genistin, daidzin, genistein and daidzein or no treatment. GI, genistin; DI, daizin; GE, genistein and DE, daidzein.

Discussion

It is known that the expression of c-fos and c-jun is related to carcinogenesis in several animal models [15-17]. The internal cytokines such as IL-1α and TNF-α are suggested to be involved in both the promotion and progression of carcinogenesis [18-21]. In this study, the expression of those genes stimulated by E_2 was confirmed in endometrial carcinogenesis in mice as demonstrated in our previous study [1], while inhibition of those gene expressions was related to the prevention of mouse endometrial cancer. The present study demonstrated an inhibitory effect of some soybean glycosides on estrogen-stimulated genes. Although the inhibitory effects of glycosides seemed to be weaker than those of aglycones, genistein and daidzein, glycosides might be metabolized by or to aglycones *in vivo* and work on E_2-related pathways. Glycosides (daidzin and genistin) derived from the natural isoflavone, are metabolized to aglycones in vivo by two intestinal bacteria, respectively [22]. In subcutaneous administration glycosides could exert the effects directly or after metabolized to genistin and daidzin, which might be more effective. Isofalvonoid glycosides

are contained in foods, fruits, vegetables and soybeans, and can exert an inhibitory effect on the expression of E_2-stimulated genes, resulting in prevention of endometrial carcinogenesis.

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