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Preventive Effects of Isoflavones, Genistein and Daidzein, on Estradiol-17β-related Endometrial Carcinogenesis in Mice

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The effects of isoflavones (genistein and daidzein) on endometrial carcinogenesis in mice were investigated in two experiments. In the short-term experiment (2 weeks), single subcutaneous (s.c.) administration of genistein [1 mg/30 g body weight (b.w.)] significantly decreased the levels of estradiol-17β (E_2) (5 ppm in diet)-induced expression of c-jun, interleukin-1α (IL-1α) and tumor necrosis factor-α (TNF-α) mRNAs in the uteri of ovariectomized mice ($P<0.005$, $P<0.05$ and $P<0.01$, respectively). Daidzein significantly inhibited E_2-induced expression of c-fos and IL-1α ($P<0.01$, $P<0.01$ respectively). In the long-term experiment (30 weeks), 140 female ICR mice were given N-methyl-N-nitrosourea-containing solution (1 mg/100 g b.w.) and normal saline (as controls) into their left and right uterine corpora, respectively. They were divided into six groups; group 1 was given E_2 (in diet) alone. Group 2 was given E_2 and genistein (1 mg/30 g b.w., s.c., every four weeks). Group 3 was exposed to E_2 and daidzein (1 mg/30 g b.w., s.c., every four weeks). Groups 4 and 5 respectively received genistein and daidzein, and were kept on the basal diet. Group 6 was kept on the basal diet and served as a control. At the termination of the experiment, incidences of endometrial adenocarcinoma and atypical endometrial hyperplasia of the group given E_2 and genistein or daidzein were significantly lower than of the group with E_2 alone ($P<0.01$ and $P<0.05$, respectively). It is suggested that both genistein and daidzein have an inhibitory effect on estrogen-related endometrial carcinogenesis in mice, possibly by suppressing expression of estrogen-induced estrogen-related genes c-*fos* and c-*jun*, and internal cytokines IL-1α and TNF-α through a cytokine and estrogen receptor-mediated pathway.

Key words: Prevention — Isoflavones — Endometrial cancer — c-Fos/jun — Cytokines

Epidemiological studies have suggested that consumption of diets containing soybeans and soybean-based products reduce the risk of endometrial cancers[1)] and other cancers.[2,3)] Soybeans and soybean-related foods are a good source of phytochemicals, including phytoestrogens such as genistein and daidzein. It is well known that isoflavones have a variety of biological activities including anticancer effects.

Genistein (Fig. 1) is an inhibitor of protein tyrosine kinase which modulates several cellar activities and plays an important role in cell proliferation or transformation.[4,5)] This isoflavone is also known to inhibit the growth of not only breast cancer cell line MCF-7,[6,7)] but also a human colon tumor cell line.[8)] A related isoflavone, daidzein (Fig. 1) inhibits growth of human prostate cancer cells[9–12)] and induces apoptosis in human prostatic cancer cell lines.[13)]

Several studies have demonstrated the inhibitory effects of genistein and daidzein on carcinogenesis in mamma, skin, prostate and seminal vesicle.[14–17)] However, the effects of isoflavones on endometrial carcinogenesis have not been reported. In the present study, we examined the effects of genistein and daidzein on endometrial carcinogenesis in mice.

The transient expression of estrogen response genes, c-*fos*/*jun* is considered to be related to cellular proliferation and differentiation.[18–20)] Overexpression of c-fos/jun mRNA induced by estrogens in the uterine corpora of ovariectomized mice is known.[21–23)] Recently, we have shown that oriental herbal medicines such as *Glycyrrhizae radix* extract and *Juzen-taiho-to* suppress c-fos/jun expression in the uterine corpus and inhibit endometrial carcinogenesis in mice.[24,25)] Thus, inhibitory effects of *Glycyrrhizae radix* extract and *Juzen-taiho-to* on endometrial carcinogenesis induced by N-methyl-N-nitrosourea (MNU) and estradiol-17β (E_2) might be related to the suppression of overexpression of c-fos/jun induced by E_2 in the uterine corpus, because c-fos/jun is considered to be associated with cellular proliferation and differentiation.[18–20)] More recently, it was shown that genistein and daidzein inhibit expression and transcription of c-fos mRNA in some cell lines.[26–28)] Furthermore, there is evidence that internal

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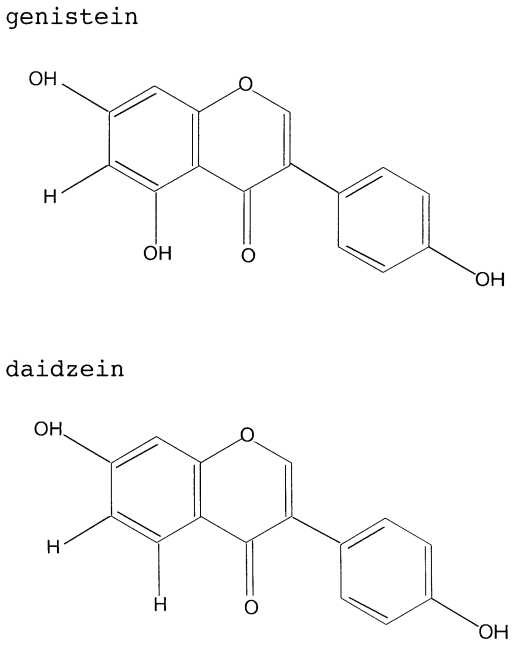

Fig. 1. The structural formulae of genistein and daidzein.

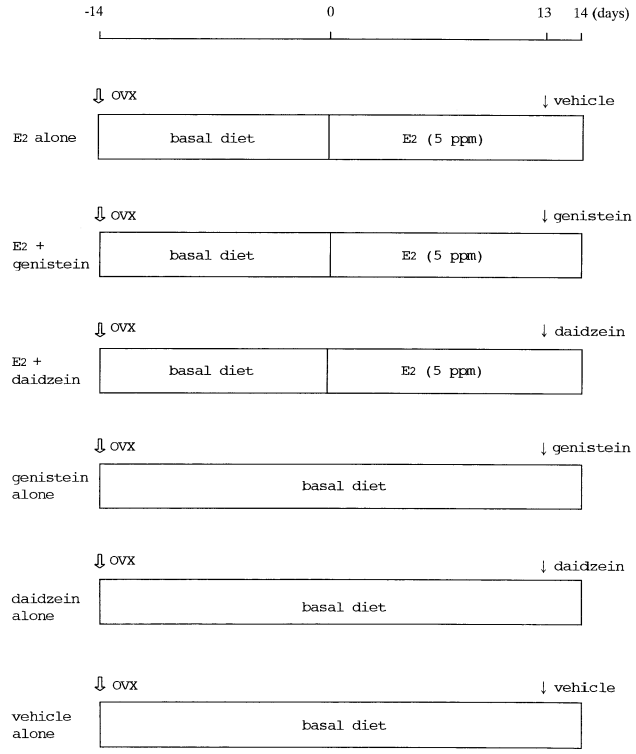

Fig. 2. Short-term experimental design. ⇩ : OVX, bi-lateral oophorectomy. ↓ : genistein or daidzein was subcutaneously injected at the dose of 1 mg/body. E_2: estradiol-17β.

cytokines, interleukin (IL)-1α and tumor necrosis factor (TNF)-α, are important factors for tumor promotion and progression in skin carcinogenesis.[29)]

In general, occurrence and growth of endometrial carcinoma are considered to be affected by estrogens. Isoflavones with estrogenic and anti-estrogenic properties are reported to be inhibitors of cell proliferation.[30)] The present study therefore attempted to address whether genistein and daidzein exert suppressive effects on mouse endometrial carcinogenesis by MNU and E_2. We also examined whether the effects are associated with expression of estrogen-related c-*fos*/*jun* genes or internal cytokines IL-1α and TNF-α mRNAs and their proteins, in the estrogenic milieu.

MATERIALS AND METHODS

Animals and chemicals Female ICR mice were purchased from Japan SLC Co. (Shizuoka). As a basal diet, Oriental MF (Oriental Yeast Co., Tokyo) was used. Diet and filtered tap water were available *ad libitum* throughout the experiment. E_2, and genistein (purity over 95%) and daidzein (purity over 97%) were purchased from Sigma Chem. Co. (St. Louis, MO) and Fujicco Co., Ltd. (Kobe), respectively. In the control MF diet, contents of E_2 and isoflavones (genistein and daidzein) were confirmed to be under 0.01 ppm.

Experimental protocol for short-term assay Female ICR mice, 12 weeks of age, were ovariectomized at laparotomy under general anesthesia with diethylether. Two weeks later, the ovariectomized mice were divided into six experimental groups (5 mice in each) (Fig. 2). Groups 1, 2 and 3 were given the diet containing E_2 (5 ppm). Genistein and daidzein were given subcutaneously, to avoid interaction with oral administration of E_2. Mice of groups 2 and 3 received a single subcutaneous (s.c.) injection of genistein or daidzein at the dose of 1 mg/30 g body weight (b.w.), 24 h prior to resection of uteri on the 13th day, respectively. The dose of genistein or daidzein was calculated following a previous report in which a 10–20 mg/kg per day 6-day treatment with genistein and/or daidzein suppressed DNA adduct formation of the mammary glands in female ICR mice.[31)] The isoflavones were dissolved in ethanol, and mixed with sesame oil. The total volume of the emulsion/mouse was 0.2 ml/30 g b.w. Groups 4 and 5 were given the basal diet, and received a single injection of genistein or daidzein as described for groups 2 and 3, respectively. Group 6 was given the mixture of ethanol and sesame oil alone and served as a non-treatment control. Two weeks later, the uteri were resected and cut in half longitudinally. One half was quickly frozen in liquid nitrogen for the following experiments, and the other was submitted to pathological examination.

Reverse transcription-polymerase chain reaction (RT-PCR) Total RNA was isolated from frozen tissues by a guanidium thiocyanate-phenol-chloroform extraction method.[32] Total RNA (3 μg) was reverse-transcribed with Moloney murine leukemia virus reverse transcriptase (MMLV-RTase, 200 units, Gibco BRL, Gaitherburg, MO) in 20 μM Tris-HCl (pH 8.4), 50 μM KCl, 2.5 μM $MgCl_2$, 0.1 μg/ml bovine serum albumin, 10 μM dithiothreitol, and 0.5 μM deoxynucleotides to generate cDNAs, using random hexamers (50 ng, Gibco BRL), at 37°C for 60 min. The RT reaction mixture was heated at 94°C for 5 min to inactivate MMLV-RTase. For c-fos (320 bp) and TNF-α (369 bp) mRNA expression, 30 cycles of PCR were performed, consisting of 1 min at 94°C for denaturation, 1 min at 55°C for annealing, and 1.5 min at 72°C for extension. For c-jun (257 bp) and IL-1α (401 bp) mRNA expression, 25 cycles of PCR were performed, consisting of 1 min at 94°C for denaturation, 1 min at 55°C for annealing, and 1 min at 72°C for extension. The PCR reaction was carried out with reverse-transcribed cDNAs and 0.1 μM specific primers described below, using an Iwaki thermal sequencer TSR-300 (Iwaki Glass, Tokyo) with Vent DNA polymerase (New England Biolabs, Beverly, MA) in 10 μM KCl, 20 μM Tris-HCl (pH 8.8), 10 μM $(NH_4)_2SO_4$, 2 μM $MgSO_4$, 0.1% Triton X-100, and 0.15 μM deoxynucleotide phosphates. Twenty cycles of PCR for glyceraldehyde-3-phosphate dehydrogenase (GAPDH, a house-keeping gene) (252 bp) mRNA as an internal standard were performed similarly. The following oligodeoxynucleotides were synthesized as specific primers for PCR according to published information [cDNA for c-fos,[33] c-jun,[34] IL-1α,[35] TNF-α[36] and GAPDH[37]]: sense for c-fos, 5′-GCTTCTATAAAGGCGC-CAGCTGA-3′; anti-sense for c-fos, 5′-GACAGGAGAGC-CCATGCTGGAG-3′; sense for c-jun, 5′-GGAGTGGGA-AGGACGTGGCGC-3′; anti-sense for c-jun, 5′-TCCCAG-CCCTCCCTGCTTTGTG-3′; sense for IL-1α, 5′-GATG-GCCAAAGTTCCTGACTTG-3′; anti-sense for IL-1α, 5′-GCCTGACGAGCTTCATCAGTTT-3′; sense for TNF-α, 5′-AGGCAGGTTCTGTCCCTTTCA-3′; anti-sense for TNF-α, 5′-TCCACTTGGTGGTTTGCTACG-3′; and sense for GAPDH, 5′-CAAGGTCATCCCAGAGCTGAA-3′; anti-sense for GAPDH, 5′-GCAATGCCAGCCCCGGC-ATCG-3′.

Semi-quantitative analysis of c-fos, c-jun, IL-1α, and TNF-α mRNA expressions by Southern blotting of PCR products PCR products were applied to 1.5% agarose gel for electrophoresis at 50−100 V, then capillary-transferred to Immobilon transfer membrane (Millipore Corp., Bedford, MA) for 16 h. The membrane was dried at 80°C for 30 min, and UV-irradiated to tightly fix the products. The PCR products on the membrane were prehybridized in 1 *M* NaCl, 50 m*M* Tris-HCl (pH 7.6) and 1% sodium dodecyl sulfate (SDS) at 42°C for 1 h, and hybridized in the same solution with biotinylated oligodeoxynucleotide probes synthesized from the sequences between the specific individual primers of c-fos, c-jun, IL-1α and TNF-α at 65°C overnight. Specific bands hybridized with biotinylated probes were detected with Plex Luminescent Kits (Millipore Corp.) after exposure to X-ray films at room temperature for 10 min. The quantification of Southern blots was carried out with Bio Image (Millipore Corp.). The intensity of specific bands was standardized with that of GAPDH mRNA.

Immunohistochemical expression of c-fos, c-jun, IL-1α and TNF-α protein After having been fixed in 10% formalin, a half of the uterine corpus was processed for conventional staining. Briefly, the avidin-biotin-peroxidase complex was applied to the sections using a Vestain Kit (Vector, Burlingame, CA). The primary antibodies used were against the proteins of c-fos, c-jun, IL-1α and TNF-α (anti-rabbit polyclonal). Staining intensity was assigned as follows[23]: (+), positive; (+/−), minimally or randomly positive; (−), negative.

Experimental protocol for long-term effects of genistein and daidzein The protocol is shown in Fig. 3. A total of 140 female ICR mice, 12 weeks of age, under-

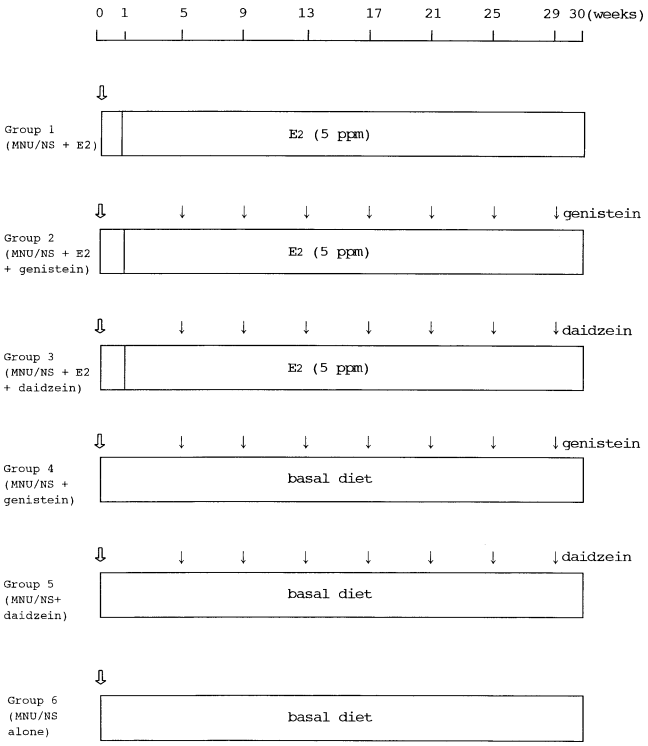

Fig. 3. Long-term experimental design. ⇩ : MNU solution (total volume: 0.1 ml) at a dose of 1 mg/100 g b. w. was injected into the left uterine tube and normal saline into the right. ↓ : genistein or daidzein was subcutaneously injected at the dose of 1 mg/30 g b.w.

went laparotomy under general anesthesia with diethyl-ether. MNU solution (total volume: 0.1 ml) at a dose of 1 mg/100 g b.w. was injected into the left uterine tube and normal saline into the right. One week after the MNU exposure, the animals were divided into six groups. Isoflavones were injected s.c. as for the short-term experiment. Group 1 (30 mice) was exposed to 5 ppm E_2-containing diet alone and received s.c. injections of vehicle alone. Group 2 (25 mice) was given E_2 (5 ppm in diet) and injected with genistein (1 mg/30 g b.w., s.c., every four weeks, seven times). Group 3 (25 mice) was treated with E_2 diet and injected with daidzein (1 mg/30 g b.w., s.c., every four weeks, seven times). The dose of genistein or daidzein in the long-term experiment was decided as the case of the short-term experiment. Group 4 (15 mice) received genistein injection and was kept on the basal diet. Group 5 (15 mice) was given daidzein and kept on the basal diet (as group 2). Group 6 (30 mice) was treated with vehicle alone as a control. In 30 weeks after the MNU exposure, all animals were killed and autopsied. All major organs, especially reproductive organs, were grossly inspected. The uterus, ovaries, vagina and other lesions suspected of being neoplastic and hyperplastic were cut in half. The tissues were submitted to histopathological examination. Tissues were sectioned in 3 μm thickness and stained with hematoxylin and eosin.

Histology of the uterine lesions According to the WHO

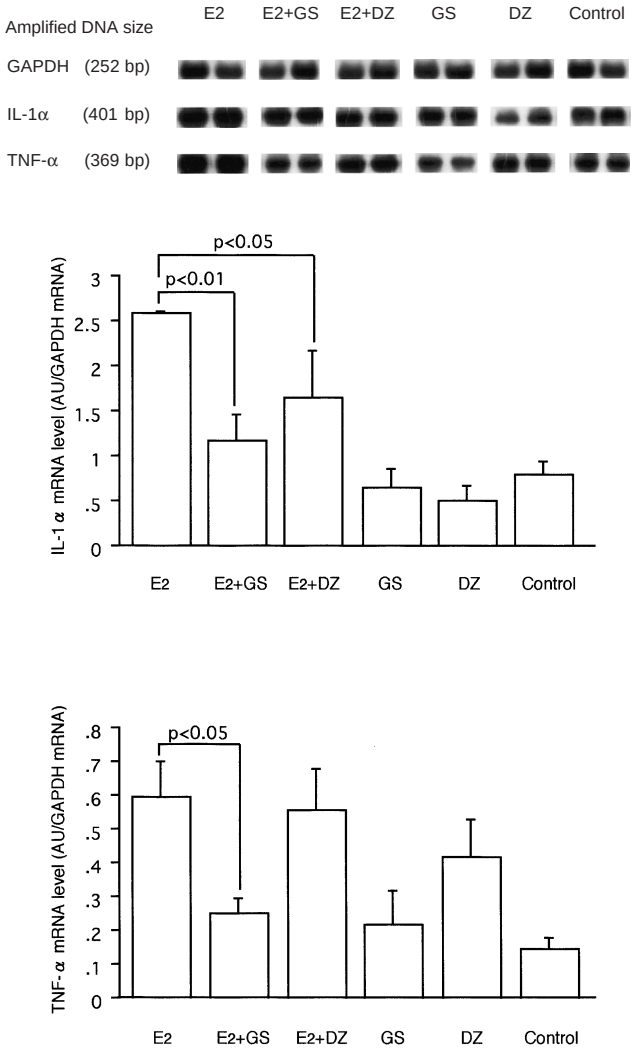

Fig. 4. Expression of IL-1α and TNF-α mRNA in the uterus of ovariectomized mice, treated continuously for two weeks with E_2 or E_2 plus genistein or daidzein, and genistein or daidzein alone. GS: genistein, DZ: daidzein.

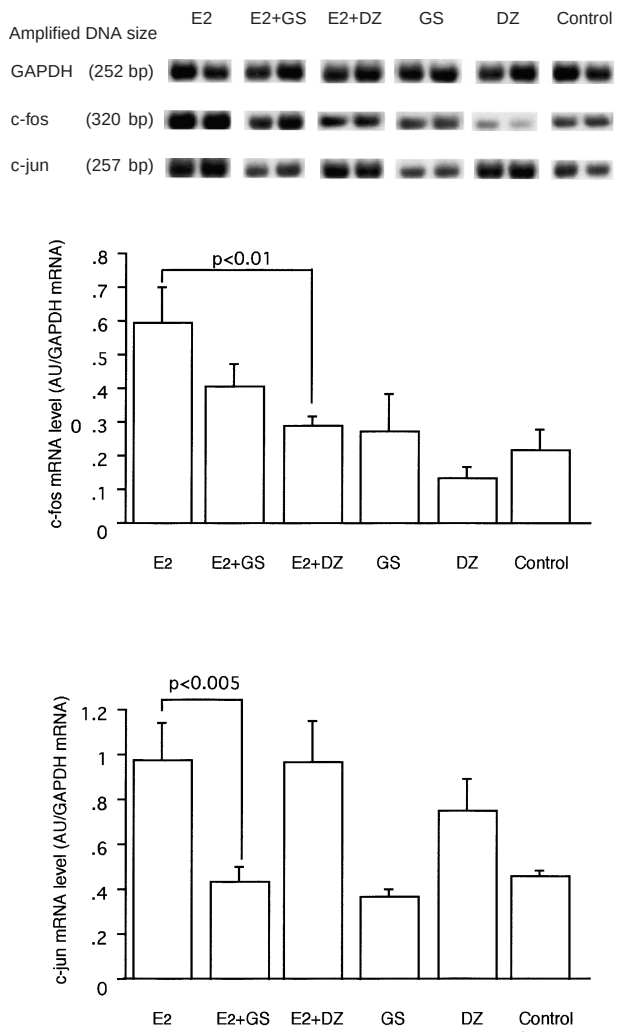

Fig. 5. Expression of c-fos and c-jun mRNA in the uterus of ovariectomized mice, treated continuously for two weeks with E_2 or E_2 plus genistein or daidzein, and genistein or daidzein alone. GS: genistein, DZ: daidzein.

criteria,[38] uterine endometrial lesions were divided into four groups: a) endometrial hyperplasia, simple; b) endometrial hyperplasia, complex; c) atypical endometrial hyperplasia; d) adenocarcinoma.

Statistical analysis Statistical analysis was done using the k^2 test or Student's t test.

RESULTS

The levels of IL-1α and TNF-α, and c-fos and c-jun mRNA expressions in the short-term assay are shown in Figs. 4 and 5, respectively. The data were determined for five animals for each group. Genistein significantly decreased the E_2-induced expression of c-jun ($P<0.005$) or cytokine IL-1α ($P<0.05$) and TNF-α ($P<0.05$), while daidzein inhibited c-fos ($P<0.01$) and IL-1α ($P<0.01$). Genistein or daidzein also tended to decrease the expression of c-fos or c-jun and TNF-α mRNA levels.

The immunohistochemical expressions of IL-1α, TNF-α, c-fos and c-jun proteins are summarized in Tables I and II. The c-fos, c-jun, IL-1α and TNF-α proteins were prominently expressed in the glandular cells of uteri in mice treated with E_2, but were decreased by genistein or daidzein.

In the long-term experiment, six mice in group 1, two in group 2, six in group 3, one in group 5, four in group 6 died within 15 weeks, yet no pathological abnormality other than pneumonia was found. The remaining animals survived until the termination of the experiment, and were enrolled as effective animals (Table III). No significant differences of mean body weight were found among groups 1, 2 and 3, and 4, 5 and 6. The mean wet weights of left and right uterine corpora of groups 1 and 2 were smaller than that of group 3 ($P<0.001$, $P<0.05$, respectively), and those of groups 4 and 5 were also significantly smaller than that of group 6 ($P<0.001$, $P<0.05$, respectively).

Histological properties of endometrial adenocarcinoma and hyperplasia were similar to those reported previously.[23] All endometrial adenocarcinomas were well or moderately differentiated types. The incidence of the preneoplastic and neoplastic endometrial lesions is summarized in Table IV. The incidences of adenocarcinoma and atypical hyperplasia of the treated side uterine corpus of groups 1 and 2 (treated with E_2 plus genistein or daidzein) were significantly lower than that of group 3 ($P<0.01$, $P<0.05$), respectively. Those of groups 4 and 5, and 1 and 2 (control side) also tended to be decreased compared with

Table I. Immunohistochemical Expression of IL-1α and TNF-α of Ovariectomized Mouse Uterus in Each Group

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+genistein	+/−	+/−	+/−	+/−	+/−	+/−
3	E_2+daidzein	+/−	+/−	+/−	+	+/−	+/−
4	Genistein alone	+/−	−	−	+/−	−	−
5	Daidzein alone	+/−	−	−	+/−	+/−	−
6	Vehicle alone	+/−	−	−	+/−	−	−

(+), positive; (+/−), minimally or randomly positive; (−), negative.

Table II. Immunohistochemical Expression of c-fos and c-jun of Ovariectomized Mouse Uterus in Each Group

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+genistein	+/−	+/−	+/−	+/−	+/−	+/−
3	E_2+daidzein	+/−	+/−	+/−	+/−	+/−	+/−
4	Genistein alone	+/−	+/−	−	+/−	−	−
5	Daidzein alone	+/−	−	−	+/−	+/−	−
6	Vehicle alone	+/−	−	−	+/−	−	−

(+), positive; (+/−), minimally or randomly positive; (−), negative.

Table III. Mean Body Weight and Mean Weight of Left (Treated) and Right (Control) Uterine Corpora of Mice in Each Group

Group	Treatment	Initial number of animals	Effective number of animals[a]	Body weight (g)	Wet weight of uterine corpora (g)	
					Left	Right
1	MNU/NS[b]+E_2 alone	30	24	42.8±5.0	0.71±0.25	0.35±0.16
2	MNU/NS+E_2+genistein	25	19	42.3±5.9[c]	0.13±0.07*	0.11±0.05**
3	MNU/NS+E_2+daidzein	25	23	45.0±5.2	0.12±0.09*	0.12±0.07**
4	MNU/NS+genistein	15	15	38.0±5.0	0.09±0.03*	0.10±0.05**
5	MNU/NS+daidzein	15	14	40.0±5.0	0.08±0.04*	0.10±0.05**
6	MNU/NS alone	30	26	48.0±5.9	0.47±0.33	0.33±0.20

a) Animals that survived more than 15 weeks.
b) NS: normal saline.
c) Mean±SD.
* $P<0.001$, ** $P<0.05$, compared with each control group.

Table IV. Incidence of Neoplastic and Preneoplastic Endometrial Lesions of the Left and Right Uterus of Mice in Each Group

Group	Treatment	Number of mice	Left (MNU-treated side)				Right (control side)			
			ADC[a]	AtH[b]	EH, complex[c]	EH, simple[d]	ADC	AtH	EH, complex	EH, simple
1	MNU/NS+E_2	24	8 (33.3%)	16 (66.7%)	23 (95.8%)	22 (91.7%)	2 (8.3%)	7 (29.2%)	23 (95.8%)	22 (91.7%)
2	MNU/NS+E_2 +genistein	19	0 (0%)*	5 (26.3%)**	18 (94.7%)	16 (84.2%)	0 (0%)	4 (21.1%)	18 (94.7%)	13 (68.4%)
3	MNU/NS+E_2 +daidzein	23	1 (4.3%)*	4 (17.4%)**	22 (95.7%)	16 (69.6%)	0 (0%)	5 (21.7%)	22 (95.7%)	13 (56.5%)
4	MNU/NS+ genistein	15	0 (0%)	4 (26.7%)	14 (93.3%)	5 (33.3%)	0 (0%)	2 (13.3%)	14 (93.3%)	3 (20.0%)
5	MNU/NS+ daidzein	14	0 (0%)	3 (21.4%)	12 (85.7%)	4 (28.6%)	0 (0%)	2 (14.3%)	13 (92.9%)	3 (21.4%)
6	MNU/NS alone	26	3 (11.5%)	8 (30.8%)	19 (73.1%)	6 (23.1%)	1 (3.8%)	3 (11.5%)	12 (46.2%)	2 (7.7%)

a) ADC: adenocarcinoma; *b*) AtH: atypical endometrial hyperplasia; *c*) EH, complex: endometrial hyperplasia, complex; *d*) EH, simple: endometrial hyperplasia, simple.
* $P<0.01$, ** $P<0.05$, compared with group 1.

the respective control group. Meanwhile, the incidences of endometrial hyperplasia, complex or simple in groups 1 and 2, and 3 and 4 showed almost no differences compared with the corresponding control group.

DISCUSSION

Genistein has been reported to inhibit both the initiation and promotion stages of skin carcinogenesis.[16] Genistein and daidzein are also known to have chemopreventive potentials against carcinogenesis of mammary gland and prostate.[14, 15, 17] However, the effects of isoflavones on endometrial carcinogenesis have not been studied before.

It was reported that genistein and daidzein in the diet are metabolized to glucuronidated products in the small intestine.[39] Free genistein or daidzein as well as the corresponding glucuronidated products are absorbed, and the compounds are excreted in free form or as metabolites.[39, 40] In this study, genistein and daidzein were given subcutaneously, as has been done in a number of previous studies.[14, 15, 41] It is thought that such isoflavones administered s.c. can be directly absorbed into the blood. This may be an advantage of s.c. exposure compared to dietary exposure, for experimental purposes.

In this study, genistein and daidzein suppressed the expression of internal cytokines IL-1α and TNF-α mRNAs as well as that of the proteins. Both isoflavones inhibited estrogen-mediated expression of c-fos and c-jun mRNAs and oncoproteins in the uterine corpora of ovariectomized mice, like other anti-estrogenic compounds.[23–25] It is suggested that TNF-α and IL-1α act as growth factors in the skin or in colon carcinogenesis.[29, 42] There is evidence that

TNF-α stimulates tumor promotion and progression of initiated cells or preneoplastic cells.[43–46] Hence, analysis of inhibition of TNF-α mRNA expression is considered to be valuable for studies of cancer prevention, in particular for establishing the mode of action of chemopreventive agents.[47]

The mean uterine weight in the groups treated with both genistein and daidzein at the doses used in this study was significantly lower than that of the corresponding control group. The dose of isoflavones used in this study seems to be higher than those in previous reports.[16, 17] In this study, estrogenic effects of isofavones to the endometrium were not found. The mean wet weights of uterine corpora varied widely among the groups. In general, estrogen exposure is suggested to generate an increase in the weight of the uterine corpus. The major reason for the increase of mean uterine corpus weight in some groups is presumably the frequent appearance of endometrial lesions including carcinoma in such groups. The incidence of endometrial hyperplasia, complex, or simple was not increased in the groups treated with isoflavones. Since isoflavones are known to possess both estrogenic and anti-estrogenic properties,[40] the result that only anti-estrogenic effects on endometrial carcinogenesis were found is considered to be related to the dose used in this study.

In this study, the incidences of adenocarcinoma and atypical hyperplasia in animals treated with E_2 and genistein or daidzein were significantly lower than in those treated with E_2 alone in the long-term experiment. However, the incidences of endometrial hyperplasias, complex or simple showed no significant differences due to the treatment with genistein or daidzein. Atypical hyperplasia is considered to be a direct precursor of adenocarcinoma of the endometrium.[21] This inhibitory effect of isoflavones might be related to suppression of the internal cytokines IL-1α and TNF-α, as well as estrogen-related genes c-*fos* and c-*jun*.

It was reported that a low dose of genistein stimulates cell growth but a high dose has a reverse action in certain cell lines.[7, 26] Similar biological activity of genistein was confirmed in the case of E_2-induced DNA synthesis.[6] Genistein is also suggested to compete more strongly with E_2 for binding to estrogen receptor (ER)-β than to ER-α,[48] and to decrease ER mRNA levels.[6] This may be one of reasons why genistein or daidzein inhibits endometrial carcinogenesis. A recent study has shown that soybean-related food may decrease the level of ovarian hormones without decreasing luteinizing hormone (LH)/follicle-stimulating hormone (FSH) levels.[49] This decrease may be involved in the suppressive effect on endometrial carcinogenesis.

This study demonstrated an inhibitory effect of genistein and daidzein on endometrial carcinogenesis. The present data suggest that the inhibitory effect is related to the suppression of IL-1α and TNF-α, as well as c-fos and c-jun expression. It is also implied that genistein or daidzein exerts the anti-carcinogenic effect through mechanisms relating to not only inhibition of ER-mediated estrogenic actions, but also other mechanism(s) relating to IL-1α or TNF-α. Isoflavones, genistein and daidzein could be promising agents for prevention of human endometrial cancers.

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