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Biological Activity of Genistein and Soy Extracts: Selective Induction of some but not all Estrogenic Responses in the Prepubertal Rat Uterus

[Actividad biológica de Genisteína y extractos de soya: inducción selectiva de algunas pero no todas las respuestas estrogénicas en el útero de la rata prepúber]

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Abstract: The existence of multiple kinds of estrogen receptors (ERs), involved in independent groups of responses, allows their dissociation and opens the possibility to selectively induce beneficial responses but not those considered at risk (cell proliferation). Based on the low hormone-dependent cancer mortality in Eastern Asia, attributed to high dietary intake of estrogenic isoflavones, we investigated whether genistein (G) or soybean extracts (S) selectively induce some, but not all estrogenic responses in the rat uterus, comparing its activity to that of estradiol-17β (E2). Prepubertal rats were treated with E2, G, concentrated S (Sc), diluted S (Sd), or vehicle, and uterine responses to estrogen were evaluated. Luminal epithelial and myometrial cell hypertrophy, and luminal epithelial RNA increase, were induced by E2, G or S. Uterine eosinophilia, endometrial edema and proliferation of 4 uterine cell-types were induced by E2 only. Results reveal that G and S induce some responses to estrogen but not others, suggesting their use as agents not displaying carcinogenic risk.

Keywords: estrogen, estradiol, genistein, soy extracts, uterus, selective stimulation.

Resumen: La existencia de múltiples tipos de receptores de estrógeno (ERs), involucrados en el desarrollo de grupos independientes de respuestas a estrógeno, permite su disociación y abre la posibilidad de inducir en forma selectiva respuestas benéficas pero no aquellas consideradas de riesgo (proliferación celular). Basado en la baja mortalidad por cánceres hormono-dependientes en el Este Asiático, atribuidos a una alta ingesta dietaria de isoflavonas estrogénicas, nosotros investigamos si la genisteína (G) o extractos de soja (S) inducen en forma selectiva algunas, pero no todas, las respuestas estrogénicas en el útero de rata, comparando su actividad con la del estradiol-17β (E2). Ratas prepuberales fueron tratadas con E2, G, S concentrado (Sc), S diluido (Sd) o vehículo, y las respuestas estrogénicas en el útero fueron evaluadas. Las hipertrofias celulares en epitelio luminal y miometrio, y el aumento de ARN en células del epitelio luminal fueron inducidas por E2, G o S. La eosinofilia uterina, el edema en estroma endometrial y la proliferación de 4 tipos celulares uterinos fueron inducidos sólo por E2. Los resultados revelan que G y S inducen algunas respuestas estrogénicas pero no otras, sugiriendo su uso terapéutico como agentes estrogénicos que no presentan riesgo de cáncer.

Palabras Clave: estrógeno, estradiol, genisteína, útero, estimulación selectiva

INTRODUCTION

It is generally accepted that hormonal replacement therapy in postmenopausal women, besides many beneficial therapeutic effects, increases the risk of breast or endometrial cancer, at least after a prolonged treatment (Lyytinen et al., 2006). This effect seems to be caused by target organ cell proliferation stimulation by estrogens used in hormone replacement therapy. Therefore, the finding of a compound mimicking beneficial responses to estrogen but not inducing cell proliferation is desirable.

If all responses to estrogen were mediated by the same mechanism and all ERs were identical, as it was first proposed for the cytosol-nuclear receptor-mediated genomic responses (Jensen & DeSombre, 1972), the possibilities to selectively induce therapeutically convenient responses to estrogen, but not those at risk (cell proliferation), would be scarce.

Up to now, the existence of at least two ERs, namely ERα and ERβ, is accepted (Kuiper et al., 1996; Wang et al., 1999; Damdimopoulos et al., 2008). However, several authors proposed that some of the responses to estrogen could be mediated by different kind of ERs. The earliest reports in this direction came from our Laboratories, describing estrogen binding by eosinophil leukocytes (Tchernitchin, 1967, 1973, 1979; Tchernitchin & Chandross, 1973). Further, it was proposed that the early migration of eosinophil leukocytes to the uterus under estrogen stimulation (Tchernitchin et al., 1974a), and several other non genomic responses to estrogen (Tchernitchin, 1983; Tchernitchin et al., 1985b, 1989) could be related to estrogen binding by the eosinophils (Tchernitchin, 1972; Tchernitchin et al., 1974b), through a novel mechanism (Tchernitchin, 1979; Galand et al., 1985; Tchernitchin et al., 1985b, 1989; Grunert et al., 1986; López et al., 1986). Other authors also proposed the existence of additional estrogen receptors (or binding proteins) and different mechanisms for hormonal action: cytoplasmic membrane ERs (Pietras & Szego, 1980; Nenci et al., 1981), type II cytoplasmic and nuclear ERs (Markaverich et al., 1981) and specific antiestrogen receptors (Sutherland et al., 1980).

The scene for hormone action is dramatically complex, however, it offers the possibility to induce separate responses to hormone administration by considering the kind of receptors involved or the mechanisms surrounding the estrogen responses. This dissociation of estrogenic responses was reported under the action of several agents or conditions: (a) the route of estrogen administration, allowing physiological hormone levels only locally or both locally and systemically (Tchernitchin & Galand, 1983); (b) the use of different estrogenic compounds, such as estriol (Tchernitchin et al., 1975b), estradiol-17α (Tchernitchin et al., 1989), diethylstilbestrol (Grunert et al., 1986), clomiphene (Grunert et al., 1987), nafoxidine (Galand et al., 1984, 1985), 2(OH)-estradiol-17β or 4(OH)estradiol-17β (Baumann et al., 1986); (c) the interaction with other hormones such as glucocorticoids (Tchernitchin et al., 1975a), progesterone (Grunert et al., 1984b), insulin (Steinsapir et al., 1982a) or thyroid hormones (Steinsapir et al., 1982b)); (d) the administration of various pharmaceuticals or biological reagents such as teophylline (Steinsapir et al., 1982c), bromocriptine (Unda et al., 1999); actinomycin D (Tchernitchin & Galand, 1982) or colloidal carbon (López et al., 1986); and (e) the exposure to environmental pollutants such as lead (Tchernitchin et al., 2003) or cadmium (Tchernitchin et al., 2008). This dissociation opens a therapeutic approach, to selectively induce required responses of estrogen stimulation, without the simultaneous induction of responses considered at risk (endometrial or mammary cell proliferation).

The basis for the present study were reports of a lower incidence of several hormonally dependent cancers in Asian women than in Western women (Adlercreutz, 2002a); this difference in incidence parallels the significantly higher amount of phytoestrogens – including soybean products – consumed by Eastern Asian women (Adlercreutz, 2002a; Wang & Murphy, 1994). Second- and third-generation descendants of women who migrated from Asia to Western countries have hormonally-dependent cancer risks similar to those of women in the host country, suggesting that lifestyle but not genetic factors could explain the lower cancer risk observed in women living in Asia (Probst-Hensch et al., 2000; Usui, 2006). In East and Southeast Asia the average intake of phytoestrogens is estimated to be more than ten times higher than in the United States or Europe (Adlercreutz, 2002b), and plasma isoflavone concentrations are higher in Japanese women than in Europeans, suggesting the possibility of their role in hormonally-dependent cancer prevention (Messina et al., 1994; Mense et al., 2008).

Based on the above evidence, the aim of the present study was to investigate the agonistic

estrogenic activity of G and S in the prepubertal rat uterus *in vivo*. In this model, we intended to find out whether these compounds are able to selectively induce some but not all responses to hormone stimulation in the uterus, for their potential use as therapeutic agents for hormonal replacement therapy devoid of hormonally-dependent cancer risk, at least, in the uterus.

MATERIALS AND METHODS

Plant extracts, Phytoestrogens amd Hormones

Commercially available soybean *Glycine max* (L.) Merr. (*Leguminosae*) extract Solgen 40 was purchased from Solbar Plant Extracts, Ashdod, Israel. It contained 43.87% total isoflavones; 69% of total isoflavones were in genistin form. Pure genistein HPLC standard quality was purchased from Sigma. and estradiol-17β was purchased from Merck.

Experimental Animal Procedure

The experimental protocol was approved by the local Ethical Committee.

Sprague-Dawley rats were breaded and maintained in the vivarium of the University of Chile School of Medicine.

Female prepubertal (21-day-old) animals were treated s.c. with the vehicle (C) (0.3 mL 1:9 ethanol/ saline), estradiol-17β (E2) Merck (0.33 mg/kg b.wt.), genistein (G) Sigma (0.5 mg/kg b.wt.) or two different concentrations of Solgen 40 soybean ethanol extracts (S): diluted S (Sd) (0.06 mg genistin/kg) and concentrated S (Sc) (0.364 mg genistin/kg b.wt.). The dose of E2 was chosen from former studies in the same prepubertal rat model, assuring the maximal responses to hormone stimulation for all analyzed parameters of hormone stimulation in the uterus (Tchernitchin et al., 1975b, Grunert et al., 1986, 1987). The dose of G and the highest dose of S were chosen to assure an approximation to estradiol molar concentrations inducing the maximal response to hormone stimulation (0.01-0.3 mg/kg). Both doses of S were used to verify whether there were differences in the effects in the different uterine cell-types due to hypothetical presence of additional active agents in the extracts. Genistin quantification was based in the indirect valoration of G following 2M HCl hydrolysis and G quantification by HPLC (Franke et al., 1994; Irvine et al., 1998). The prepubertal rat model was chosen since the very low endogenous estrogen levels

in control animals assure absence of estrogenic responses; at that age all estrogen receptors and mechanisms were reported fully responding to hormone stimulation (Tchernitchin et al., 1980). 10 rats were used for each of the 10 experimental conditions.

Uteri were excised under ether anesthesia 6 or 24 h after treatment and fixed in neutral formalin for further histological process.

Histological, Histochemical and Morphometrical Procedure

Each uterine formalin fixed and dehydrated horn was cut in three pieces (superior, medium and caudal), that were paraffin-embedded together in a single paraffin block, so that 5 μm thick uterine cross sections from the above three pieces could be observed together in the same histological slide and evaluated all of them.

For each animal, one group of hydrated uterine cross sections was stained 1-3 min in hematoxylin, washed in several changes of tap water, and then quickly transferred to a saturated lithium carbonate solution, where they were kept for 1 min. Subsequently, they were stained in 1% eosin y aqueous solution, washed quickly in distilled water, and dehydrated in a graded series of ethyl alcohols, absolute ethanol and xylene (Grunert et al., 1984a). This procedure was used for eosinophil quantification (Tchernitchin et al., 1974a, 1985a), mitoses counting in the different uterine cell types (Grunert et al., 1986, 1987) morphometry (Grunert et al., 1984a), including computer assisted image analysis of luminal epithelial cell volume (Tchernitchin et al., 2003). Another group of hydrated uterine cross sections was stained with phosphate-buffered 0.01% acridine orange (pH 7.4) in distilled water for 5 min, differentiated in 0.1% (wt/v) calcium chloride and covered with phosphate buffer (pH 7.4). This stain allows RNA and DNA densitometry with a epifluorescence microscope under excitation light, λ 380-420 nm (Konarev, 1966).

Quantification of estrogenic responses

The following estrogenic responses were quantified in the uterus: uterine eosinophilia, percentage of eosinophils according to the distribution in different uterine histological layers and to their degree of degranulation; edema in deep endometrial stroma; luminal epithelial cell RNA content, luminal epithelial and myometrial cell

hypertrophy, and number of mitotic figures in luminal epithelium, glandular epithelium, endometrial stroma and myometrium.

Figure 1. Effect of a treatment with estradiol-17ß, or phytoestrogens on myometrial (Fig 1A) and endometrial luminal epithelial (Fig. 1B) cell hypertrophy and on RNA content increase in endometrial luminal epithelial cells (Fig. 1C)
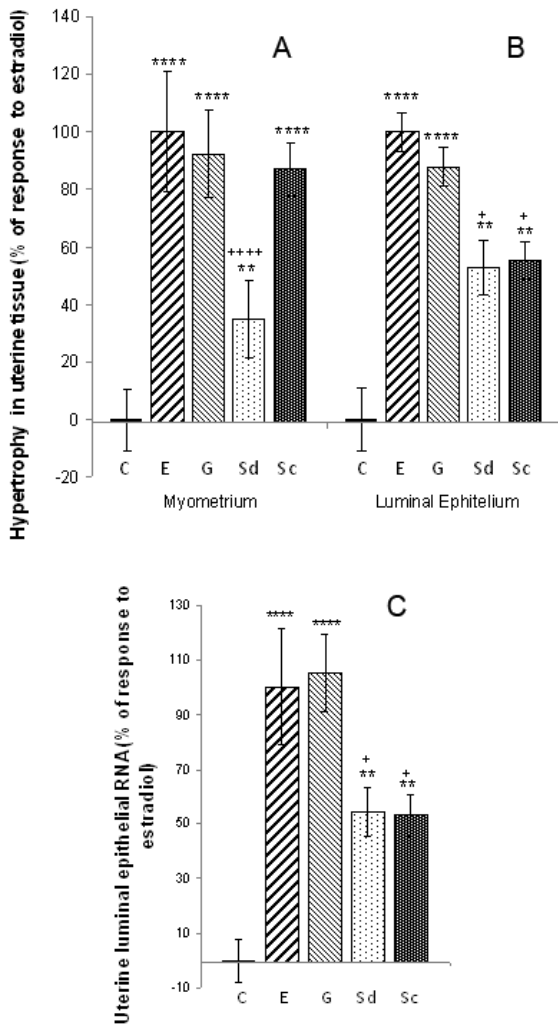

Figure 1

Prepubertal rats received s.c. 0,33 mg estradiol-17ß /kg b.wt (E), 0,5 mg genistein/kg b.wt. (G), soybean extract containing 0.06 mg genistin/kg b.wt.(Sd) or 0.364 mg genistin/kg b.wt. (Sc), or vehicle (C), and the uteri were excised 24 h thereafter under anesthesia. Bars indicate means (expressed as % of response to estradiol) ± standard error of the mean. Statistics: least significant difference *a posteriori* LSD test. +, p<0.05, **, p<0.01; **** or ++++, p<0.0001; *, comparisons to vehicle-treated controls; +, comparisons to estradiol-treated animals

The various parameters of estrogen stimulation were evaluated at times their maximal response occurs (Tchernitchin et al., 1974a, 2003; Grunert et al., 1984a). Uterine eosinophilia reaches maximal response at 6 or 24 h, endometrial edema reaches maximal response at 6 h and declines thereafter, uterine luminal epithelial RNA content, luminal epithelial and myometrial cell hypertrophy and uterine luminal epithelial, glandular epithelial, stromal and myometrial cell proliferation reach maximal level responses at 24 h after treatment.

Figure 2. Effect of a treatment with estradiol-17ß or phytoestrogens on edema in deep endometrial stroma (Fig. 2A) and uterine eosinophilia (Fig. 2B).
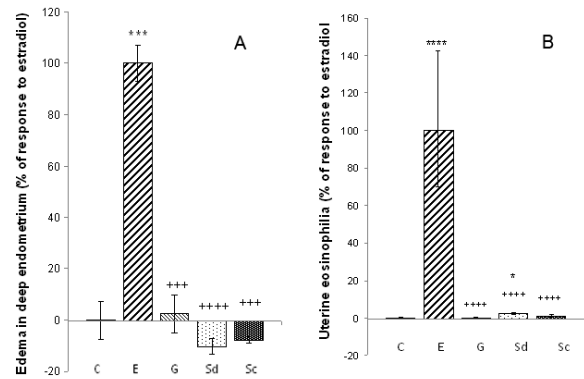

Figure 2

Prepubertal rats received s.c. 0,33 mg estradiol-17ß /kg b.wt (E), 0,5 mg genistein/kg b.wt. (G), soybean extract containing 0.06 mg genistin/kg b.wt.(Sd) or 0.364 mg genistin/kg b.wt. (Sc), or vehicle (C), and the uteri were excised 6 h thereafter under anesthesia. In Fig. 2A (edema in deep endometrial stroma) bars indicate means from non-transformed data (expressed as % of response to estradiol) ± standard error of the mean. In Fig. 2B (uterine eosinophilia) bars indicate geometric means from log-transformed data (expressed as % of response to estradiol) ± standard error of the mean. Statistics: least significant difference *a posteriori* LSD test. *, p<0.05; *** or +++, p<0.001; **** or ++++, p<0.0001; *, comparisons to vehicle-treated controls; +, comparisons to estradiol-treated animals

To allow a comparison between the effects of genistein or soy extracts among the different parameters of estrogen stimulation, all responses were expressed as % of maximal response, i.e., the value of vehicle-treated animals was considered as 0% response, and the value of maximal response to E2 – 100% response.

For each animal, uterine eosinophilia (Tchernitchin et al., 1974a) was assessed in 30 uterine sections, distributed along the uterus

(proximal, medial and caudal); eosinophils were classified according to their location within the different uterine histological layers and to their degree of degranulation (Tchernitchin et al., 1985a; Grunert et al., 1986).

Edema in deep endometrial stroma (Grunert et al., 1984a) was evaluated by counting number of nuclei in 36 1054 μm^2 areas delimited by a standard circle located in the ocular piece of the microscope. As demonstrated in earlier reports (Grunert et al., 1984a), an increase in the reciprocal value of cell density (a decrease in cell counts within a standard area) in a location containing few cells and mainly extracellular space, reflects edema since an increase in its volume, but not in the cellular volume, traduces in an increase in the reciprocal value of cell density.

Myometrial cell hypertrophy (Grunert et al., 1984a) was also evaluated by counting the number of nuclei in 36 standard areas; previous reports (Grunert et al., 1984a) show that an increase in the reciprocal value of cell density in this location containing cell bodies and where extracellular space is negligible, reflects increase in cell size but not extracelullar edema.

Luminal epithelial cell hypertrophy was evaluated from digital micrographs from luminal epithelium, where cell limits were marked around groups of well defined epithelial cells boundaries; 80 cells were measured in each animal, using computer assisted image analysis to calculate luminal epithelial cell volume (Tchernitchin et al., 2003).

Luminal epithelial RNA content was evaluated from digital micrographs of acridine orange stained uterine sections, with an epifluorescence microscope Nikon EFD-3 under excitation light, λ 380-420 nm, using computer assisted morphometry and cell densitometry (Tchernitchin et al., 2003). 80 cells were measured for each animal.

Estrogen-induced mitotic response was evaluated as an increase in the number of mitotic figures in luminal epithelium, glandular epithelium, endometrial stroma and myometrium, and quantified for each animal in 18 uterine cross sections considering all three uterine pieces (Grunert et al., 1986, 1987).

Statistics

According to previous studies (Grunert et al., 1986) using the Tukey test of additivity (Snedecor & Cochran, 1967), data on some parameters of estrogen

stimulation needs to be submitted to logarithmic or square root transformation to normalyze data distribution. Accordingly, the log-transformation was performed on uterine eosinophil numbers, RNA content in luminal epithelial cells, and luminal epithelial cell volume (Grunert et al., 1986). Transformed (above responses to estrogen) and non-transformed (all other parameters of estrogen stimulation in the uterus) data were subjected to further statistical analysis.

Since multiple comparisons were performed between the different experimental conditions, transformed and non-transformed data were subjected to the least significant difference *a posteriori* (LSD) test. The common variance needed for this test was estimated from a one-way unbalanced analysis of variance (ANOVA).

In uterine eosinophil degranulation and distribution proportion studies, the χ^2 statistic was used to evaluate differences between the proportions. The percentage of degranulation and of eosinophils located in the various histological layers of the uterus were no considered in some experimental animals (i.e., control animals without estrogen treatment) due to the extremely low number of eosinophils in these experimental conditions, that does not allow a valid statistical analysis.

RESULTS

Cell hypertrophy and cell RNA content increase.

Myometrial hypertrophy was induced by E2, G and two concentrations of S. While hypertrophy reached with G or Sc was similar to that induced by E2, response reached with Sd was about half of that reached with E2 (Fig. 1A). Endometrial luminal epithelial cell hypertrophy (Fig. 1B) and RNA content (Fig. 1C) were induced by E2, G and both S. While responses similar to E2 were achieved by G only; the responses to Sc or Sd were about half of that obtained with E2.

Endometrial edema and uterine eosinophilia.

Edema in deep endometrial stroma was induced by E2 only (Fig. 2A). A normal intensity estrogen-induced uterine eosinophilia was observed following E2 treatment only, although a very slight but statistically significant response was also observed with the Sd (Fig. 2B). Eosinophil degranulation was significantly weaker with the Sd as compared to E2 (Fig. 3A). As compared to E2-treated animals where most uterine eosinophils were located

Figure 3. Effect of a treatment with estradiol-17ß or phytoestrogens on the proportions of degranulated and non-degranulated uterine eosinophils (Fig. 3A) and the proportions of uterine eosinophils located in the mesometrium and in the endometrium with myometrium (Fig. 3B).
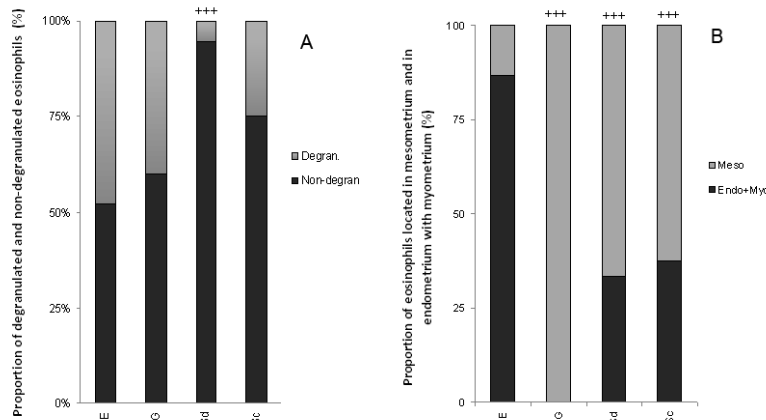

Figure 3

Prepubertal rats received s.c. 0,33 mg estradiol-17ß /kg b.wt (E), 0,5 mg genistein/kg b.wt. (G), soybean extract containing 0.06 mg genistin/kg b.wt.(Sd) or 0.364 mg genistin/kg b.wt. (Sc), or vehicle (C), and the uteri were excised 6 h thereafter under anesthesia. The proportions in controls are not shown because of the extremely low eosinophil numbers, which does not allow any valid statistical analysis. Statistics: χ^2 test; +++, p<0.001; comparisons to estradiol-treated animals.

Figure 4. Effect of a treatment with estradiol-17ß or phytoestrogens on the number of mitotic figures in endometrial luminal epithelium (Fig. 4A), endometrial glandular epithelium (Fig. 4B), endometrial stroma (Fig. 4C) and myometrium (Fig. 4D).
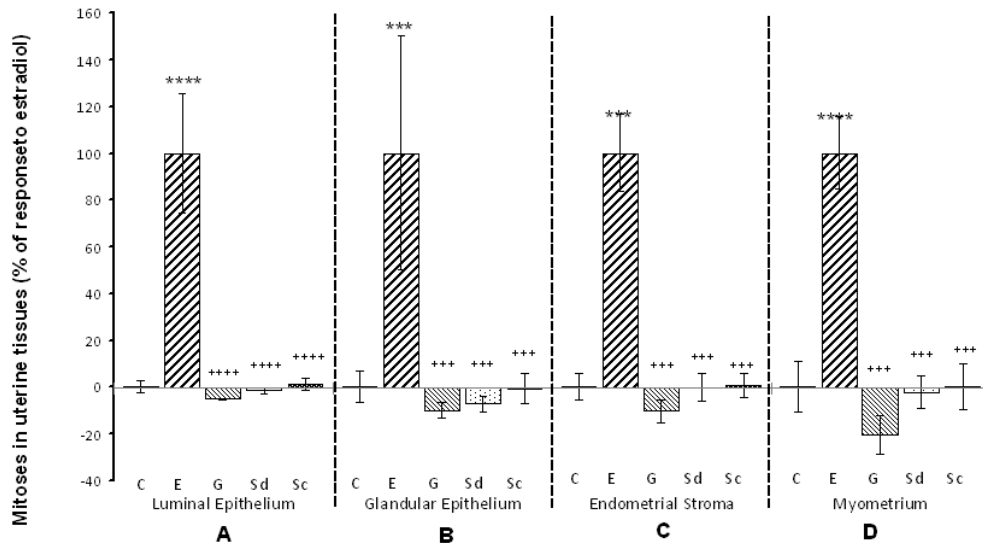

Figure 4

Prepubertal rats received s.c. 0,33 mg estradiol-17ß /kg b.wt (E), 0,5 mg genistein/kg b.wt. (G), soybean extract containing 0.06 mg genistin/kg b.wt.(Sd) or 0.364 mg genistin/kg b.wt. (Sc), or vehicle (C), and the uteri were excised 24 h thereafter under anesthesia. Bars indicate means (expressed as % of response to estradiol) ± standard error of the mean. Statistics: least significant difference *a posteriori* LSD test. *** or +++, p<0.001; **** or ++++, p<0.0001; *, comparisons to vehicle-treated controls; +, comparisons to estradiol-treated animals.

in endometrium with myometrium, in S-treated animals there were more eosinophils in the mesometrium, and following G treatment all the eosinophils were in the mesometrium (Fig 3B).. Eosinophil degranulation and distribution within the uterine histological layers data is not shown in control animals because of the extremely low eosinophil counts that do not allow any valid statistical analysis.

Uterine cell proliferation.

Uterine cell proliferation (Fig 4), was induced in uterine luminal epithelium (Fig. 4A), uterine glandular epithelium (Fig. 4B), endometrial stroma (Fig. 4C) and myometrium (Fig. 4D) by E2 only, but not by G or the soy extracts.

DISCUSSION

Present results reveal a dissociation of estrogenic responses by either G and S. While the increase in RNA content in luminal epithelial cells and cell hypertrophy in luminal epithelial and myometrial cells were strongly induced by E2, G, Sd or Sc, uterine eosinophilia, endometrial edema and cell proliferation in luminal epithelium, glandular epithelium, endometrial stroma and myometrium were induced by E2 only, but not by G or S.

A myriad of explanations may be proposed to explain the selective induction of just some responses to estrogen but not others. Subtle differences in ER conformations caused by interaction with different ligands, which interact with different co-activators or co-repressors within the cell (Charlier et al., 2009). Presence of different molecular chaperones eg FKBP51, FKBP52, Cyp40, which may be incorporated in the inactivated ER complexes from the different cell types (Reynolds et al., 1999; McKeen et al., 2010). Or the existence of different kind of ERs mediating different responses to hormone stimulation (Tchernitchin 1972, 1983; Galand et al., 1985; Tchernitchin et al., 1985b, 1989; Grunert et al., 1986; López et al., 1986). Present results, do not disagree with any of the above hypotheses. According to our hypothesis, some receptors may display high affinity for any specific phytoestrogens while other receptors may display low affinity for the same compounds, explaining the induction of some responses only. Alternatively to the above explanations, it is possible to speculate that

the effects of G and/or S are not responses mediated by ERs. To evaluate this possibility, a possible antagonistic effect of these agents on receptor mediated responses to estrogen is currently investigated. Preliminary results from experiments with coadministration of G and E2 suggest competitive interaction with responses induced by E2 (manuscript in preparation).

The absence of estrogen induced uterine eosinophilia, and one of the responses proposed to be mediated by the eosinophils in the uterus (endometrial edema), may be explained by the lack of recognition of the mesometrium vascular endothelium by the eosinophils - the main site of migration of eosinophils towards the uterus (Soto et al., 1979) - or by the decrease in eosinophils mobility through uterine extravascular space towards myometrium and endometrium. It was suggested that eosinophil recognition of uterine (mainly mesometrial) endothelium is a process mediated by high affinity ERs located in the surface of the eosinophils (Tchernitchin et al., 1985b). The lack of recognition of the uterine mesometrium may be explained either by a low affinity displayed by the phytoestrogen for this receptor or by its antagonist action. An inhibition of eosinophil redistribution through the uterus from mesometrium, observed in the present study (increased proportion of eosinophils remaining in the mesometrium in animals treated with Sc, Sd or G) may be explained by the decrease in eosinophil degranulation that was observed in Sc treated rats, taking into consideration that hydrolytic enzyme released from degranulating eosinophils are required for eosinophil migration through uterine ground substance (Grunert et al., 1984b; Tchernitchin et al., 1985b, 1989).

A comparison of luminal epithelium and myometrium also reveals differences under the effect of different concentrations of S. In the myometrium, while Sc induced a full cell hypertrophy similarly to E2, in luminal epithelium Sc induced a much weaker cell hypertrophy or RNA content increase than E2. This may reflect a difference between luminal epithelium and myometrial ERs, displaying different affinities for some of the S active components, or a possible competitive inhibition by glycones or other phytoestrogens present in the extracts. The soybean ethanol extracts mainly contain the glucoside genistin, which is supposed to be devoid of estrogenic activity since it most probably does not

enter the intracellular space. Soybean genistin taken orally hydrolyzes in the intestine to its aglycone form genistein and enters circulation as such (Kelly et al., 1995), displaying estrogenic activity (Zhang et al., 1999). Further studies are necessary to evaluate this possibility, and investigate whether part of the genistin is hydrolized in the tissue to release the aglycone or other metabolites.

Irrespectively to the mechanisms involved in the interaction of G or S with the uterus, the dissociation of estrogenic responses by phytoestrogens suggests their possible therapeutic application to induce clinically needed responses without inducing risk responses. Estrogen-induced cell proliferation is the main risk side effect for hormone replacement therapy in postmenopausal women, which may initiate or stimulate tumour growth. Therefore, absence of cell-proliferation in uterine luminal epithelial, glandular epithelial cells, endometrial stroma and myometrium by S and by G itself, suggests they do not share the estrogens' increased risk for cancer development in postmenopausal women, at least in the uterus. Further studies are needed to investigate the effects of G and S in other estrogen target organs.

CONCLUSIONS

Present results reveal, for the first time, a dissociation of responses to estrogen by phytoestrogens, While estradiol induces an increase in RNA content in luminal epithelial cells, cell hypertrophy in luminal epithelial and myometrial cells, uterine eosinophilia, endometrial edema and cell proliferation in luminal epithelium, glandular epithelium, endometrial stroma and myometrium, G and S only induce an increases in RNA content in luminal epithelial cells and cell hypertrophy in luminal epithelial and myometrial cells, but not the remaining estrogenic responses.

The selective induction of some estrogenic responses but not others under the effect of G and S suggests their possible therapeutic application as agents not inducing cell proliferation in the uterus, therefore, not displaying the increased risk of cancer development in this organ. Work is in progress to evaluate the expression of apoptotic proteins such as Ki67 as well as the expression of estrogen receptors α and β.

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