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Evaluation of Estrogenic/Antiestrogenic Activity of Ellagic Acid via the Estrogen Receptor Subtypes ERα and ERβ

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Ellagic acid is a plant-derived polyphenol, possessing antioxidant, antiproliferative, and antiatherogenic properties. Whether this compound has estrogenic/antiestrogenic activity, however, remains largely unknown. To answer this question, we first investigated the ability of ellagic acid to influence the activity of the estrogen receptor subtypes ERα and ERβ in HeLa cells. Cells co-transfected with an estrogen response element (ERE)-driven luciferase (Luc) reporter gene and an ERα- or ERβ-expression vector were exposed to graded concentrations of ellagic acid. At low concentrations (10^{-7} to 10^{-9} M), this compound displayed a small but significant estrogenic activity via ERα, whereas it was a complete estrogen antagonist via ERβ. Further evaluation revealed that ellagic acid was a potent antiestrogen in MCF-7 breast cancer-derived cells, increasing, like the pure estrogen antagonist ICI182780, IGFBP-3 levels. Moreover, ellagic acid induced nodule mineralization in an osteoblastic cell line (KS483), an effect that was abolished by the estrogen antagonist. Endometrium-derived epithelial cells (Ishikawa) showed no response to the natural compound by using a cell viability assay (MTT). These findings suggest that ellagic acid may be a natural selective estrogen receptor modulator (SERM).

KEYWORDS: Ellagic acid; estrogen receptor alpha; estrogen receptor beta; selective estrogen receptor modulator; osteoblasts

INTRODUCTION

Ellagic acid (**Figure 1**) is a dietary polyphenol present in abundance in strawberries (*1*−*3*). This compound exhibits antioxidant, antiproliferative, chemopreventive, and antiatherogenic properties, in a variety of tissues and cells, such as breast, colon, and prostate cancers, liver and lung tissues, and leukemia cells (*1*−*9*). Ellagic acid exerts its effects via activation of various signaling pathways, including apoptosis, protection from oxidative DNA damage, or LDL-oxidation and alteration of growth factor expression, as well as through the expression of p53, NF-kB, and PPAR family responsive genes (*4*, *5*, *7*, *8*, *10*, *11*).

Accumulating evidence suggests that some plant-derived polyphenols (flavonoids and phytoestrogens) interact with the estrogen receptor subtypes ERα and ERβ, exhibit estrogenic/antiestrogenic activities, and may play protective roles in cancer, inflammation, heart disease, and osteoporosis (*12*−*15*). These substances are considered "natural" selective estrogen receptor modulators (SERMs) and their use as an alternative for hormone therapy during menopause has recently expanded (*16*, *17*).

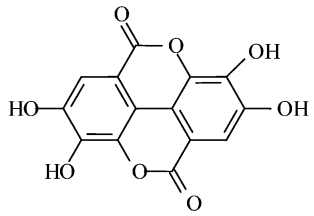

Figure 1. Chemical structure of ellagic acid.

Estrogen receptors (ERα and ERβ) belong to the nuclear receptor superfamily. They mediate the effects of estrogens by binding (as homodimers or heterodimers) either directly to DNA at their estrogen-response elements (EREs) or by protein−protein interactions with other transcription factors (i.e., AP-1, NF-kB) bound to their cognate DNA sequences, thus regulating the transcription of estrogen-responsive genes (*18*).

The ER isoforms exhibit a high homology in their ligand-binding domains, whereas they have only 18% homology in their aminoterminal domains. A plethora of data indicate that ERβ opposes the actions of ERα, a mechanism through which

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ERβ may play a protective role in breast cancer (*19*, *20*). Plant-derived flavonoids and phytoestrogens modulate ERα and ERβ activities differently and may act as estrogen agonists or antagonists or mixed agonists/antagonists, depending on tissue type and ligand concentration (*18*, *21*).

Ellagic acid carries in its chemical structure phenolic rings and ortho-dihydroxyl groups, which allow ER recognition and ER-mediated action. However, there is scant information regarding the estrogenic/antiestrogenic effects of ellagic acid in breast cancer cells, endometrial cancer cells, and osteoblasts or its interaction with the estrogen receptor subtypes ERα and ERβ. Therefore, we investigated the possibility that ellagic acid influences ERα- and ERβ-mediated signaling, and its estrogenicity/antiestrogenicity in breast and endometrial cancer cells, as well as osteoblasts. We used a panel of in vitro biological assays and examined the ability of ellagic acid (a) to induce, like antiestrogens, the Insulin Growth Factor Binding Protein-3 (IGFBP-3), a known estrogen-dependent marker in MCF-7 cells (*22*), (b) to stimulate the differentiation and mineralization of osteoblastic cells by histochemical staining for Alizarin Red-S (AR-S) (*23*), and (c) to inhibit cell viability of endometrial adenocarcinoma cells by use of MTT assay (*24*). The possibility of a genomic receptor-mediated (ERα or ERβ) effect was investigated by co-transfecting HeLa cells with an ERE-driven luciferase reporter gene and an ERα or ERβ expression vector.

MATERIALS AND METHODS

Plant Material. Pomegranate (*Punica granatum*) pericarps (peels) were collected from a mature tree, growing in Attica, Greece, in June 2002 and authenticated. A voucher specimen was deposited at the herbarium of Division of Pharmacognosy and Natural Products Chemistry, Department of Pharmacy, University of Athens. Ellagic acid can be easily isolated from pomegranate or strawberries. Due to the differences in concentration of ellagic acid in the varieties of strawberries and the difficulties in the identification of the cultivated species, we consider a safe choice to isolate it from pomegranate.

Extraction and Isolation. The dry powder of *P. granatum* pericarps (2 kg) was defatted with $CHCl_3$ and extracted with EtOH (80%), which yielded a brownish residue (120 g) on removal of the solvent. This extract was applied to a (R18 Si gel 60, 20−40 mm, Merck) reverse phase column (36 × 460 mm, Buchi) and eluted by H_2O followed by H_2O−EtOH mixtures (500 mL each mixture) of decreasing polarity to yield 12 major fractions. Further fractionation of Fr.10 on a MPLC RP column (15 × 230 mm, Buchi) using gradient H_2O−MeOH (150 mL each mixture) afforded ellagic acid (48 mg).

Plasmids and Transfections. HeLa cells (ATCC Cell Bank) were grown in Dulbecco's minimal essential medium (DMEM) (Gibco BRL, Thessalonica, Greece) supplemented with 10% fetal bovine serum (FBS) (Gibco BRL, Thessalonica Greece). Stock cultures were subcultured every 4−5 days using a 0.25% trypsin−0.02% EDTA solution (Gibco BRL, Thessalonica, Greece). Before each transfection experiment, cells were maintained for 2 days in DMEM Phenol Red (PR) free (-) containing 10% dextran-coated charcoal stripped serum (FBS DCC-treated). For each transfection experiment, 2×10^5 cells were plated per well in six-well dishes in DMEM PR (-) with 10% FBS DCC-treated. After 24 h, HeLa cells were transfected with 0.2 μg of ERα (hERα) or 0.2 μg of ERβ (pSG5-hERβ) and 0.2 μg ERE (3xERE-TATA-Luc) expression vectors by using Effectene Transfection Reagent (Qiagen, Athens, Greece) according to manufacturer's guidelines. After 24 h, cells were washed once with phosphate buffer saline (PBS) and 2 mL of DMEM PR (-) 10% FBS-DCC-treated was added, containing various final concentrations of estradiol (10^{-9} M) or ICI182780 (10^{-8} M) or 4-OH-tamoxifen (10^{-8} M) or substances (10^{-7} to 10^{-9} M). Co-incubation of ICI182780 (10^{-8} M) with estradiol (10^{-9} M) or ellagic acid (10^{-7} M) was also performed. Cells were harvested 24 h later and cell extracts were assayed for luciferase activity, using the Promega luciferase assay system (Promega, Athens, Greece) according to manufacturer's instructions.

Breast Cancer Cell Studies. The Insulin Growth Factor Binding Protein-3 (IGFBP-3) has been shown to be down-regulated by estrogens and up-regulated by antiestrogens in MCF-7 breast cancer cells, an effect mediated via the estrogen receptor (*22*). MCF-7 cells (ATCC Cell Bank) were maintained in Dulbecco's minimal essential medium (DMEM) (Gibco BRL, Thessalonica, Greece) supplemented with 10% fetal bovine serum (Gibco BRL, Thessalonica, Greece). Cells were plated at an initial density of 25.000 cells/well in 24-plate multiwell plastic culture dishes and grown to confluence. After 3 days of cell culture, the media were changed to DMEM without Phenol Red supplemented with 2% FBS DCC (dextran-coated charcoal slurry)-treated.

After 24 h of cell culture, the media were changed and 300 μL of fresh DMEM PR free supplemented with 2% FBS DCC, containing in final concentrations estradiol 10^{-7} to 10^{-9} M (Sigma, Athens, Greece), ICI 182780 10^{-8} to 10^{-9} M (Tocris, Germany), or ellagic acid (10^{-6} to 10^{-8} M) were added and incubation followed. MCF-7 cells were also incubated with vehicle or ICI 182780 (10^{-8} M) or ellagic acid (10^{-7} M) in the presence of 10^{-8} M estradiol. The culture fluids were collected from the dish and centrifuged at 12000 rpm for 10 min. The supernatants were used for Insulin Growth Factor Binding Protein-3 (IGFBP-3) measurement. The cells were removed carefully from the dish using cell scrapers, collected in approximately 200 μL PBS buffer and counted using a hemocytometer plate. IGFBP3 was measured after 24 h of incubation. IGFBP3 was measured in all samples directly using an enzymatically amplified "two-step" sandwich-type immunoassay (DSL Diagnostic System Laboratories, Athens, Greece), according to manufacturer's instructions.

Bone Cell Study. The KS483 cell line is a nontransformed stable subclone of a parental cell line KS4 that has the ability to form mineralized nodules in vitro in the presence of estrogens (*23*). KS483 cells were grown in Phenol Red free α-minimum essential medium (α-MEM) (Gibco-BRL, Thessalonica, Greece) supplemented with 10% fetal bovine serum (Gibco-BRL, Thessalonica, Greece) and penicillin/streptomycin (Gibco-BRL, Thessalonica, Greece), in a CO_2 incubator (5% CO_2−95% air) at 37 °C and subcultured every 3−4 days at a dilution 1:5 to 1:6 using 0.125% trypsin/0.01% EDTA solution.

For our experiments, KS483 cells were seeded in 12 well plates (at a density of 45.000 cells/well) in α-MEM Phenol Red free, supplemented with 10% FBS DCC-treated. Three days after plating, cells reached confluence and were subsequently induced to differentiate by the addition to the culture medium of 50 μg/mL ascorbic acid (Sigma, Athens, Greece) and in the absence or presence of ellagic acid at four different concentrations (10^{-6} to 10^{-9} M). 17β-Estradiol was used as positive control at four different concentrations (10^{-6} to 10^{-9} M). Cells were also cultured with ICI182780 (10^{-7} M) in the presence of ellagic acid (10^{-8} M). β-Glycero-phosphate (Sigma, Athens, Greece) was added after day 10. The medium with the reagents was refreshed every 3−4 days for 24 days in total.

Alkaline Phosphatase Activity. At the end of the culture period, the cell layer was washed with PBS and then frozen (−20 °C). For the determination of alkaline phosphatase activity, cells were sonicated for 15 s in 0.1 M Tris buffer, pH 7.2, containing 0.1% Triton X-100. Alkaline phosphatase activity was measured by using *p*-nitrophenol phosphate as substrate (kinetic ALP/DGKC method). Total protein was also determined by Bradford method.

Assay for Mineralization. After 24 days, the cultures in 12-well plates were rinsed with PBS, followed by fixation with 5% formalin for 10 min, and stained for calcium deposition with Alizarin Red-S (solution 2% pH = 5.5) (Sigma, Athens, Greece) for 5 min. Mineralized nodules were counted by light microscopy at a 10-fold magnification.

MTT Assay [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] Method. Cultures of Ishikawa cells (ECACC, No. 99040201) were grown in Dulbecco's minimal essential medium (DMEM) (Gibco-BRL, Thessalonica, Greece) supplemented with 10% fetal bovine serum (FBS) (Gibco-BRL, Thessalonica, Greece), 50 units/mL penicillin, and 50 μg/mL streptomycin (Gibco-BRL, Thessalonica, Greece) in T-75 cm^2 flasks at 37 °C, 85% humidity, and 5% CO_2 atmosphere. Subcultures were carried out every 3−4 days using a 0.25% trypsin and 0.02% EDTA solution (Gibco-BRL, Thessalonica, Greece). Cell viability was estimated by a modification of the MTT assay (*24*).

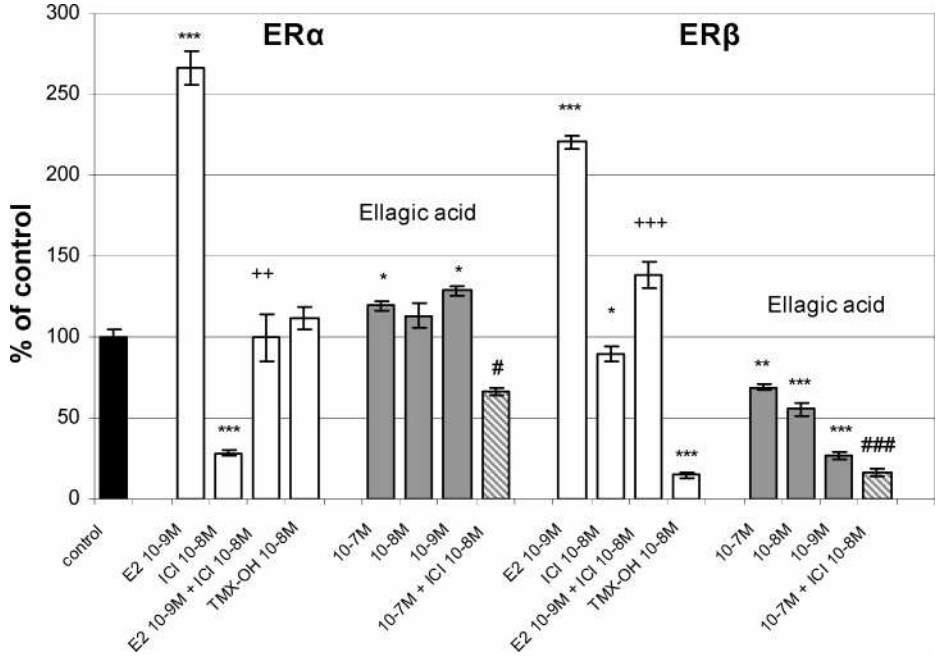

Figure 2. Effect of estradiol, ICI182780, and ellagic acid on luciferase activity in HeLa cells, transfected with ERα or ERβ and an EREs-containing reporter vector. HeLa cells were exposed to estradiol (10^{-9} M), ICI182780 (10^{-8} M), 4-OH-tamoxifen (10^{-8} M), or ellagic acid (10^{-7} to 10^{-9} M). Co-incubation of ICI182780 (10^{-8} M) with estradiol (10^{-9} M) or ellagic acid (10^{-7} M) also occurred. Results are expressed as percentage of control (vehicle). Columns and bars represent mean ±SD of the results of three experiments. *Significantly different from vehicle control (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). $^{+}$Significantly different from E_2 10^{-9} M ($^{++}p < 0.01$, $^{+++}p < 0.001$). $^{\#}$Significantly different from ICI182780 10^{-8} M ($^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$).

Briefly, cells were plated in their growth medium at a density of 10000 cells/well in 96 flat bottomed well-plate. Twenty-four hours after plating, ellagic acid was added at final concentrations ranging from 400 to 0.16 μM in DMEM Phenol Red free. After 48 h of incubation, the medium was replaced with MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Sigma, Athens, Greece) dissolved at a final concentration of 1 mg/mL in serum-free, Phenol Red free medium, for an additional 4 h of incubation. Then, the MTT-formazan was solubilized in 2-propanol and the optical density was measured at a wavelength of 550 nm and a reference wavelength of 690 nm.

RESULTS

Ellagic acid was obtained from the pericarps of pomegranate (*Punica granatum*). The isolated compound was identified as ellagic acid by comparison of its spectroscopic data (^{1}H and ^{13}C NMR) with those of the literature (*25*).

ERα- and ERβ-Mediated Reporter Activity. Figure 2 demonstrates the effect of vehicle control (in the absence of compounds), estradiol, pure antiestrogen ICI182780, and ellagic acid on luciferase activity on HeLa cells transfected with ERα or ERβ and EREs. Estradiol was used as control for agonist activity whereas ICI 182780 was used as a control for antagonist activity. ERα or ERβ bound to EREs in the absence of ligand, an activity which was set as 100% (vehicle control), respectively. In HeLa cells transfected with ERα, incubation with estradiol significantly increased ($p < 0.001$) luciferase activity at a concentration of 10^{-9} M compared to vehicle control. On the other hand, the pure antiestrogen ICI 182780 significantly reduced luciferase activity at the concentration of 10^{-8} M ($p < 0.001$), whereas 4-OH-tamoxifen did not show any statistically significant effect. In ERα-transfected cells, co-incubation of ICI 182780 (10^{-8} M) with estradiol (10^{-9} M) abolished the stimulatory effect of estradiol in a statistical significant way ($p < 0.01$). In ERα-transfected cells, ellagic acid significantly increased ($p < 0.05$) the basal luciferase activity at the 10^{-7} and 10^{-9} M concentrations. Moreover, co-incubation of ellagic acid (10^{-7} M) with ICI 182780 (10^{-8} M) abolished the inhibitory effect of the latter via ERα in a statistically significant manner ($p < 0.05$).

In ERβ-transfected cells, incubation with estradiol at a concentration of 10^{-9} M significantly increased luciferase activity ($p < 0.001$). ICI 182780 reduced luciferase activity at the concentration of 10^{-8} M ($p < 0.05$). Co-incubation of ICI 182780 (10^{-8} M) with estradiol (10^{-9} M) abolished the stimulatory effect of estradiol in a statistically significant manner ($p < 0.001$). 4-OH-tamoxifen significantly reduced the basal luciferase activity ($p < 0.001$). Ellagic acid alone significantly reduced the basal luciferase activity ($p < 0.01-p < 0.001$) at a concentration range of 10^{-7} to 10^{-9} M in ERβ-transfected cells. Moreover, co-incubation of ellagic acid (10^{-7} M) with ICI182780 (10^{-8} M) enhanced the inhibitory effect of the latter ($p < 0.001$). These results indicate that ellagic acid may act as an estrogen antagonist mainly via ERβ.

Breast Cancer Cell Studies. Figure 3 demonstrates the effect of vehicle control (in the absence of compounds), estradiol, ICI182780, and ellagic acid on the IGFBP-3 levels secreted by MCF-7 cells. The dose response curve showed that estradiol reduced IGFBP-3 levels significantly at a concentration of 10^{-7} M ($p < 0.01$) and 10^{-8} M ($p < 0.001$). On the other hand, the pure antiestrogen ICI 182780 significantly increased the IGFBP-3 levels at 10^{-8} M ($p < 0.01$) and 10^{-9} M ($p < 0.001$) concentrations, an effect abolished by incubation with estradiol 10^{-8} M ($p < 0.01$). Ellagic acid significantly increased the IGFBP3 levels in MCF-7 cells at a concentration of 10^{-6} M ($p < 0.01$), 10^{-7} M ($p < 0.001$), and 10^{-8} M ($p < 0.01$). Co-incubation of the ellagic acid (10^{-7} M) with estradiol (10^{-8} M) abolished the stimulatory effect of ellagic acid on IGFBP3 levels, like ICI182780, in a statistically significant way ($p < 0.01$), implicating an ER-mediated antiestrogenic effect in MCF-7 cells.

Bone Cell Study. Figure 4 demonstrates the effect of vehicle control (in the absence of compounds), estradiol, and ellagic

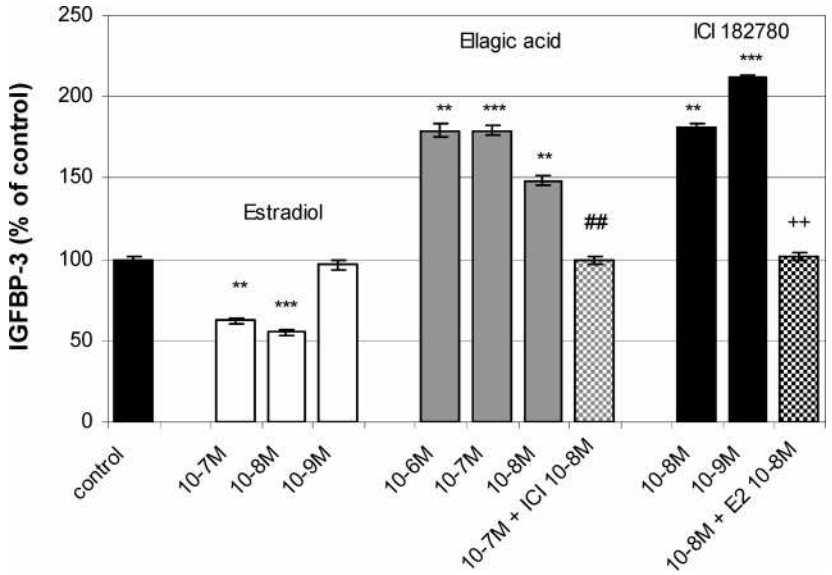

Figure 3. Effect of estradiol, ellagic acid, and ICI182780 on $IGFBP_3$ producing by MCF-7 breast cancer-derived cells. MCF-7 were cultured with vehicle control, estradiol (10^{-7} to 10^{-9} M), ellagic acid (10^{-6} to 10^{-8} M), or ICI182780 (10^{-8} to 10^{-9} M) for 24 h. Cells were also treated with ellagic acid (10^{-7} M) or ICI182780 (10^{-8} M) in the presence of estradiol E_2 (10^{-8} M). Results are expressed as percentage of control (vehicle). Columns and bars represent mean ±SD of the results of four cultures. *Significantly different from vehicle control (**$p < 0.01$, ***$p < 0.001$). $^{+}$Significantly different from ICI182780 10^{-8} M ($^{++}p < 0.01$). $^{\#}$Significantly different from ellagic acid 10^{-7} M ($^{\#\#}p < 0.01$).

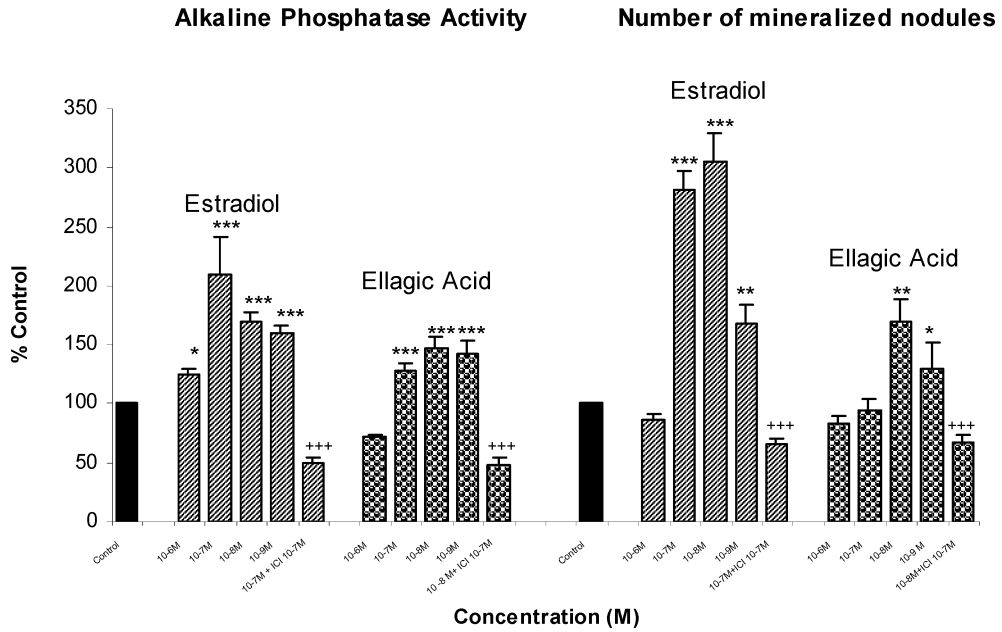

Figure 4. Effect of estradiol, ellagic acid, or ICI182780 on alkaline phosphatase activity and on mineralization of extracellular matrix in KS 483 cells. Cells were exposed to vehicle control, estradiol (10^{-6} to 10^{-9} M), ellagic acid (10^{-6} to 10^{-9} M), and ICI182780 (10^{-7} M) in the presence of ellagic acid (10^{-8} M) and estradiol (10^{-7} M). Results are expressed as percentage of control (vehicle). Columns and bars represent mean ±SD of the results of four cultures. *Significantly different from vehicle control (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). $^{+}$Significantly different from ellagic acid 10^{-8} M ($^{+++}p < 0.001$).

acid on the alkaline phosphatase activity and on the mineralized nodules formation. Ellagic acid significantly increased ($p < 0.001$) alkaline phosphatase activity at concentrations of 10^{-7} to 10^{-9} M with the higher effect at a concentration of 10^{-8} M. Treatment with ICI 182780 (10^{-7} M) in the presence of ellagic acid (10^{-8} M) or estradiol (10^{-7} M) abolished the positive effect on the alkaline phosphatase activity ($p < 0.001$). Treatment with ellagic acid stimulated significantly the mineralization at concentrations of 10^{-8} M ($p < 0.01$) and 10^{-9} M ($p < 0.05$), whereas concentrations of 10^{-6} and 10^{-7} M exhibited no effect. Treatment with ICI 182780 (10^{-7} M) in the presence of ellagic acid (10^{-8} M) or estradiol (10^{-7} M) abolished the positive effect on mineralization ($p < 0.001$).

Endometrial Cancer Cell Study. The dose−response curve concerning the effect of ellagic acid on Ishikawa cell viability revealed that at all tested concentrations there was no statistical significant stimulation above control and no inhibitory effect on cell proliferation either (data not shown).

DISCUSSION

We assessed ellagic acid as a natural SERM molecule by using a series of reliable, previously established, widely accepted cell culture systems and sensitive estrogen responsive markers (*26*, *27*). In HeLa cells, ERβ had a lower activity in response to E_2 than ERα, which is in agreement with previous reports

(*28*). The pure estrogen antagonist ICI182780 inhibited ERα- and ERβ-induced transactivation, as expected. ERα and ERβ responded differently to ellagic acid, which displayed a low but significant estrogenic activity on ERα and complete antagonist activity on ERβ at low concentrations (10^{-7} to 10^{-9} M). This is similar to the estrogen agonist/antagonists activities of tamoxifen, 4-OH-tamoxifen, and raloxifen, which display low but significant estrogenic activity in hERα- but only antagonism in hERβ-reporter cell systems (*28*). Thus, ellagic acid, a plant polyphenol, functions like the known partial agonists/antagonists tamoxifen, 4-OH-tamoxifen, and raloxifen. The differences in the responsiveness between ERα and ERβ to ellagic acid may be attributed to hERβ having an AF1 domain that allows only partial agonism in the presence of ellagic acid, similar to tamoxifen (*29*).

In earlier reports, plant-derived polyphenols have shown variable dose-response curves in ERα- and ERβ-mediated effects in ERE-driven reporter gene assays. For example, some tea catechins, particularly epigallocatechin gallate (EGCG), were antiestrogenic for ERα at high doses (5×10^{-6} M) and estrogenic for ERα at low doses (5×10^{-7} to 10^{-9} M) in the presence of E_2. However, the ERβ response to the same phytoestrogens was estrogenic for a wide concentration range (5×10^{-6} to 5×10^{-9} M). Catechins alone, on the other hand, induced luciferase activity through neither ERα nor ERβ (*30*). Resveratrol, a polyphenol found naturally in grapes, binds ERα and ERβ with comparable affinity, but it transactivates ERα and ERβ differently in an ERE sequence-dependent manner (*31*−*33*). The phytochemical polyphenol lindleyin, on the other hand, transactivates ERβ with a higher potency than ERα, whereas glabrene, a polyphenol isolated from the licorice root, is a natural estrogen agonist that activates ERα-mediated transcription in vitro (*34*, *35*). Recently, it was reported that phytochemical glyceollins exerted antihormonal effects through ERα and ERβ (*36*). Moreover, a new phytoestrogen, namely, 4-ethoxymethylphenol, transactivated ERα with a higher potency than it did ERβ (*37*). Our findings and the aforementioned results indicate that plant polyphenols influence ERα and ERβ activity in different ways, quite likely causing different allosteric conformations of ERα and ERβ and that nature is rich in selective hERα and hERβ ligands.

Due to the similarities between ellagic acid and the synthetic SERMs tamoxifen, 4-OH-tamoxifen, and raloxifen in the HeLa cell system, we thought that ellagic acid might be a natural SERM. Thus, we further examined its effects on the bone mineralization process and its antitumorigenicity in breast and endometrial cancer cells, by using sensitive, postreceptor, tissue-specific response in vitro assays reflective of a SERM profile. The use of KS483 cells and the measurement of alizarin red-S staining reflect possible osteoblastic activity of a test compound, whereas the production of IGFBP-3 by MCF-7 cells is a well-established marker for screening compounds acting as estrogen agonists/antagonists and cell growth promoters. The MTT test, on the other hand, tests the effect of a substance on cell viability and proliferation.

Ellagic acid-induced mineralization of osteoblasts, inhibited estrogen-mediated activity by increasing the IGFBP-3 production by breast cancer cells and showed no effect on endometrial cell proliferation. These data indicate that ellagic acid exhibits the characteristic effects of a SERM compound. The divergent biologic activities of ellagic acid, being an agonist in bone cells, but an antagonist in breast cancer cells, may be attributed to the recruitment of tissue-specific ERα or ERβ coactivators or corepressors, as well as to varying proportions of tissue ERα and ERβ levels (*18*). Another plant compound, genistein, like estradiol and 4-ethoxymethylphenol, is influenced by certain ER modifiers such as cofactors, coactivators, and corepressors (*37*, *38*).

In an earlier report, ellagic acid suppressed glucocorticoid-induced luciferase activity. The mechanism for this is not clear but it is plausible that ellagic acid might change several steroid receptor-mediated signaling systems, by means of which it may exert beneficial health effects (*39*−*41*). In conclusion, ellagic acid is a natural selective ERα and ERβ ligand, exhibiting SERM properties similar to those of synthetic SERMS. As a natural hormone, ellagic acid warrants further investigation.

ABBREVIATIONS USED

ERα, Estrogen Receptor α; ERβ, Estrogen Receptor β; EREs, Estrogen Response Elements; IGFBP-3, Insulin Growth Factor Binding Protein-3; SERM, Selective Estrogen Receptor Modulator; FBS, Fetal Bovine Serum; DCC, Dextran-Coated Charcoal; DMEM, Dulbecco's Minimal Essential Medium; PR, Phenol Red; a-MEM, a-Minimum Essential Medium; PBS, Phosphate Buffer Saline; ALP/DGKC, Alkaline Phospatase/German Society of Clinical Chemists; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

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