The stimulation of cell proliferation by quercetin is mediated by the estrogen receptor

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Quercetin causes biphasic modulation of the proliferation of specific colon and mammary cancer cells. In this study, the possible involvement of the estrogen receptor (ER) in the stimulation of cell proliferation by quercetin was investigated. For this purpose, the effect of quercetin on cell proliferation was tested in ER-positive MCF-7 and T47D cells, and in ER-negative HCC-38 and MDA-MB231 cells. Quercetin stimulated proliferation of ER-positive cells only, suggesting this effect to be ER-dependent. In support of these results, quercetin induced ER-ERE-mediated gene expression in a reporter gene assay using U2-OS cells transfected with either ERα or ERβ, with $10^5 - 10^6$ times lower affinity than 17β-estradiol (E2) and $10^2 - 10^3$ times lower affinity than genistein. Quercetin activated the ERβ to a 4.5-fold higher level than E2, whereas the maximum induction level of ERα by quercetin was only 1.7 fold that of E2. These results point at the relatively high capacity of quercetin to stimulate supposed 'beneficial' ERβ responses as compared to the stimulation of ERα, the receptor possibly involved in adverse cell proliferative effects. Altogether, the results of this study reveal that physiologically relevant concentrations of quercetin can exert phytoestrogen-like activity similar to that observed for the isoflavonoid genistein.

Keywords: Biphasic effect / Estrogen receptor / Genistein / Proliferation / Quercetin

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1 Introduction

Quercetin (Fig. 1) is a well-known flavonoid, which can be found in a variety of fruits, vegetables, seeds, and nuts where it is mainly present as glycoside. The Western diet contains approximately 16 mg/day of quercetin [1]. The interest in quercetin as a functional food ingredient or food supplement is growing due to the presumed beneficial health effects of flavonoids and quercetin in particular, that include prevention of cancer and cardiovascular diseases [2, 3]. These conclusions are mainly based on *in vitro* studies reporting quercetin to be a strong antioxidant [4] and inhibitor of cancer cell proliferation [5, 6]. Recently, however, it was shown that quercetin causes a biphasic modulation of the proliferation of specific human colon cancer and breast cancer cell lines, known to express estrogen receptor (ER) mRNA [7, 8], including a stimulation of cell proliferation at the lower, physiologically relevant concentrations [9]. Analogous to quercetin, the isoflavone genistein (Fig. 1) also causes a biphasic effect on cell proliferation [10, 11]. This stimulation of cell proliferation by genistein, known to be a ligand of the ER [11, 12] and to stimulate ER-mediated gene expression, is only found in cells expressing the ER [13, 14]. The objective of the present study was to investigate whether, analogous to the mechanism underlying stimulation of cell proliferation by genistein, the

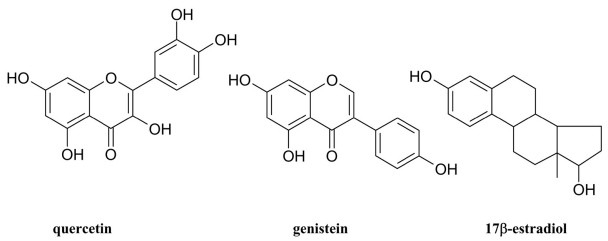

Figure 1. Molecular structures of quercetin, genistein, and 17β-estradiol.

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Abbreviations: CDTA, *trans*-1,2-diaminocyclohexane-*N,N,N′,N′*-tetraacetic acid; **DCC**, dextran-coated charcoal; **E2**, 17β-estradiol; **EBS**, estrogen binding site; **EEF**, estradiol equivalency factor; **ER**, estrogen receptor; **FCS**, fetal calf serum; **HBSS**, Hank's balanced salt solution; **LDH**, lactate dehydrogenase

ER is also involved in the stimulation of cell proliferation by quercetin.

The ER is a member of the superfamily of nuclear hormone receptors [15]. To date, three different subtypes of the ER are known; ERα and ERβ are the most studied ones and recently ERγ was discovered [16]. ERα and ERβ are expressed in many tissues, including liver, brain, bone, and especially in reproductive tissues [17]. Based on the finding that the ratio ERα/ERβ is higher in breast tumors than in normal tissue [18] and that by the formation of heterodimers ERβ modulates ERα transcriptional activity [19], the hypothesis arose that ERα might mediate the proliferative effects of estrogenic compounds, whereas ERβ might have inhibiting effects on this process [18].

Whether quercetin can be classified as a phytoestrogen is a matter of debate. Controversial findings have been reported on the binding affinity of quercetin for the ER [10–12, 14]. Furthermore, although quercetin appeared to affect processes following ER activation, including binding of the ER to the ERE in the DNA and translocation of the ER from the cytoplasm to the nucleus [20], quercetin appeared to have no [20–22] or very little effect [11] on ER-mediated gene expression in cell lines. However, luciferase reporter gene assays in which the activation of the ER was studied generally pointed at quercetin as an estrogen agonist, although the induction at a concentration of 1 μM was approximately a factor 30–50 times lower than by 17β-estradiol [12]. Finally, some studies using ER-positive cells showed no stimulation of cell proliferation mediated by quercetin [20, 23], whereas significant proliferation of ER-positive cell lines was reported in other studies [9, 21], the latter indicating the possible involvement of the ER in effects of quercetin on cell proliferation.

To obtain more insight into the possible role of the ER in the effects of quercetin on ER-mediated gene expression and cell proliferation, the ability of quercetin to induce ER/ERE-mediated gene expression was studied in reporter gene assays. For this purpose, two clones of the human osteosarcoma cell line U2-OS were used, transfected with ERα or ERβ, expressing physiological levels of either of these receptors [24]. Furthermore, the effect of quercetin on ER-mediated cell proliferation was investigated using the ER-positive cell lines MCF-7 and T47D [25], as well as the ER-negative cell lines MDA-MB231 [26] and HCC-38 [27]. To validate the conclusions, the effects of quercetin on cell proliferation obtained in all these cell systems were compared with the effects obtained for the model phytoestrogen genistein

2 Materials and methods

2.1 Materials

Dimethyl sulfoxide (DMSO) and quercetin dehydrate were purchased from Acros Organics (Pittsburgh, PA, USA). Magnesium carbonate ($(MgCO_3)_4Mg(OH)_2 \cdot 5H_2O$) was obtained from Aldrich (Saint Louis, MO, USA). Hygromycin and D-luciferin were obtained from Duchefa (Haarlem, The Netherlands). *Trans*-1,2-Diaminocyclohexane-*N,N,N′,N′*-tetraacetic acid monohydrate (CDTA) was obtained from Fluka (Buchs, Switzerland). Ascorbic acid, sodium bicarbonate ($NaHCO_3$), sodium hydroxide (NaOH), ethylenedinitrotetraacetic acid (EDTA · $2H_2O$; Titriplex), magnesium sulfate ($MgSO_4 \cdot 7H_2O$), and 1,4-dithiothreitol (DTT) were obtained from Merck (Darmstadt, Germany). Tris was obtained from Invitrogen Life Technologies (Paisley, Scotland). The cell culture media Dulbecco's modified Eagle medium (DMEM), Ham's F12, RPMI-1640, nonessential amino acids, gentamicin, geneticin, L-glutamine, sodium pyruvate, fetal calf serum (FCS), phosphate buffer solution (PBS), and Hank's balanced salt solution (HBSS) were obtained from Gibco (Paisley, Scotland). Adenosine triphosphate (ATP) was obtained from Roche (Mannheim, Germany). 17β-Estradiol, genistein, and tricine were obtained from Sigma. The human breast cancer cell lines MCF-7, T47D, MDA-MB-231, and HCC-38 were purchased from the American Type Culture Collection (Mannassas, VA, USA). The human osteosarcoma cell line U2-OS transfected with an expression vector encoding either human ERα or ERβ as well as with the estrogen-responsive reporter gene plasmid 3 × ERE-tata-Luc was made as previously described [24].

2.2 Cell culture

The human breast cancer cell lines T47D and HCC-38 were cultured in RPMI-1640 medium, supplemented with 10% FCS, 1 mM sodium pyruvate, 2 mM L-glutamine, and 50 μg/mL gentamicin. MCF-7 and MDA-MB-231 cells were cultured in DMEM, supplemented with 10% FCS and 50 μg/mL gentamicin. Cells were cultured at 37°C/5% CO_2 in a humidified atmosphere. The human osteosarcoma cell lines U2-OS-ERα and U2-OS-ERβ were cultured in a 1:1 mixture of DMEM and Ham's F12 medium, buffered with 1260 mg/L $NaHCO_3$, supplemented with 7.5% FCS and 0.5% nonessential amino acids. In addition, the medium for U2-OS-ERβ medium was supplemented with 200 μg/mL geneticin as a selection marker and medium for U2-OS-ERα was supplemented with both 200 μg/mL geneticin and 50 μg/mL hygromycin. Cells were cultured at 37°C and 7.5% CO_2 in a humidified atmosphere.

2.3 Luciferase assay

Confluent U2-OS cells were trypsinized and seeded in transparent 96-well plates (Nunclon, NUNC, Rochester, NY, USA) at a density of 10×10^4 cells/mL (U2-OS-ERα) or 7.5×10^4 cells/mL (U2-OS-ERβ) in a 1:1 mixture of

DMEM and Ham's F12 medium without phenol red, buffered with 1260 mg/L $NaHCO_3$ and supplemented with 5% dextran-charcoal-treated FCS (DCC-FCS) and 0.5% non-essential amino acids (100 μL/well). DCC-FCS was prepared by heat inactivation (30 min at 56°C) of FCS, followed by two 45-min DCC-treatments at 45°C as previously described [28]. Culture medium was refreshed after 24 h. Forty-eight hours after seeding, cells were exposed in triplicate to quercetin or genistein in the presence of 600 μM ascorbic acid for stabilization of the test compounds [9] at the indicated concentrations (final DMSO concentration, 0.1%) for 24 h at 37°C, 7.5% CO_2 in a humidified atmosphere. Ascorbic acid in a concentration of 600 μM proved to have no cytotoxic effects on U2-OS cells. To guarantee stability of the test compound for the whole incubation period under these conditions, the exposure medium was refreshed after 8 h. In addition, on each plate, cells were exposed in triplicate to a calibration curve of 17β-estradiol in the presence of 600 μM ascorbic acid, at the indicated concentrations (final DMSO concentration, 0.1%). After 24 h, the medium was removed and cells were washed with 100 μL/well 0.5 × PBS. Cells were lysed with 30 μL of a hypotonic low-salt buffer, pH 7.8, consisting of 10 mM Tris, 2 mM DTT, and 2 mM CDTA in nanopure water. Plates were put on ice for 10 min and subsequently frozen at –80°C. Before analysis, plates were thawed on ice and shaken to reach room temperature. Analyses were performed at room temperature in a Luminoskan (RS, Labsystems) as follows: first, background light emission of each plate was measured for 2 s. Then, 100 μL/well flashmix was added, after which light emission was measured for another 2 s and extinguished with 50 μL 0.2 M NaOH. The flashmix consisted of 20 mM tricine buffer, pH 7.8, supplemented with 1.07 mM $(MgCO_3)_4Mg(OH)_2 \cdot 5H_2O$, 2.67 mM $MgSO_4$, 0.1 mM EDTA · $2H_2O$, 2 mM DTT, 0.47 mM D-luciferin, and 5 mM ATP. Luciferase induction by quercetin and genistein was compared with the luciferase induction by the natural ligand for the ER, 17β-estradiol. To determine EC_{50} values of the compounds, curves were fitted using Slidewrite 6.10 for Windows. The EC_{50} was defined as the concentration of compound at which 50% of the maximum luciferase activity was reached. EC_{50} values were expressed as mean ± standard error. The estradiol equivalency factor (EEF) was calculated using Eq. (1):

$$\mathrm{EEF} = \frac{\mathrm{EC_{50}\ (estradiol)}}{\mathrm{EC_{50}\ (compound)}} \qquad (1)$$

2.4 Cell proliferation

Confluent cells were washed with HBSS, trypsinized, and seeded in transparent 96-wells plates (100 μL/well; Greiner, Frickenhausen, Germany) in plate medium. The composition of plate medium was similar to the culture medium described above, with the following modifications: phenol red or antibiotics were omitted, and instead of FCS, 5% DCC-FCS was added. The cells were incubated for 24 h at 37°C in a humidified atmosphere and 5% CO_2, as indicated above, to allow attachment. Plates having 60 – 80% confluent wells were used for experiments. After attachment, the culture medium was removed and 100 μL/well exposure medium was added. The composition of the exposure media was similar to the culture media described above, only without phenol red, FCS, and antibiotics. Exposure media were supplemented with quercetin or genistein from stock solutions in DMSO (final concentration, 0.5%) at the indicated concentration range, proven to give no cytotoxic effects in the lactate dehydrogenase (LDH)-leakage test (data not shown), the latter performed essentially as previously described [29]. Furthermore, 200 μM (MCF-7 cells), or 1 mM ascorbic acid (T47D, MDA-MB-231 and HCC-38 cells) was added for flavonoid stabilization [9]. Ascorbic acid in a concentration of 200 μM proved to have no cytotoxic effects on MCF-7 cells. To guarantee the presence of the test compound for the whole incubation period under these conditions, the exposure medium was refreshed after 8 h. After 24 h of incubation, the extent of proliferation was determined with the BrdU-labeling method using the Cell Proliferation ELISA, BrdU (colorimetric) kit from Roche Diagnostics (Mannheim, Germany). Results were expressed as percentage of the proliferation of control cells exposed to the solvent DMSO in culture medium containing ascorbic acid.

2.5 Statistical analysis

Statistical analysis of data was carried out using the Dunnett or Dunnett T3 test (analysis of variance, ANOVA). Differences were significant if $P < 0.05$.

3 Results

3.1 Effect of quercetin and genistein on ER-ERE-mediated luciferase activity in transfected U2-OS cells

To investigate whether quercetin and genistein can induce gene expression by interaction with ERα and/or ERβ, the effect of the two compounds was studied in reporter gene systems consisting of human osteosarcoma U2-OS cells, transfected with ERα or ERβ, and a luciferase reporter gene with an ERE-containing promoter region. Figures 2A and B show the effect of quercetin, genistein, and 17β-estradiol (E2) on luciferase activity mediated by ERα and ERβ, respectively. Comparing the affinities of quercetin, genistein and E2 for ERα and ERβ, it appeared that the EC_{50} of quercetin was approximately similar for ERα and ERβ, amounting to 11 ± 0.1 and 8 ± 0.7 μM, respectively. The

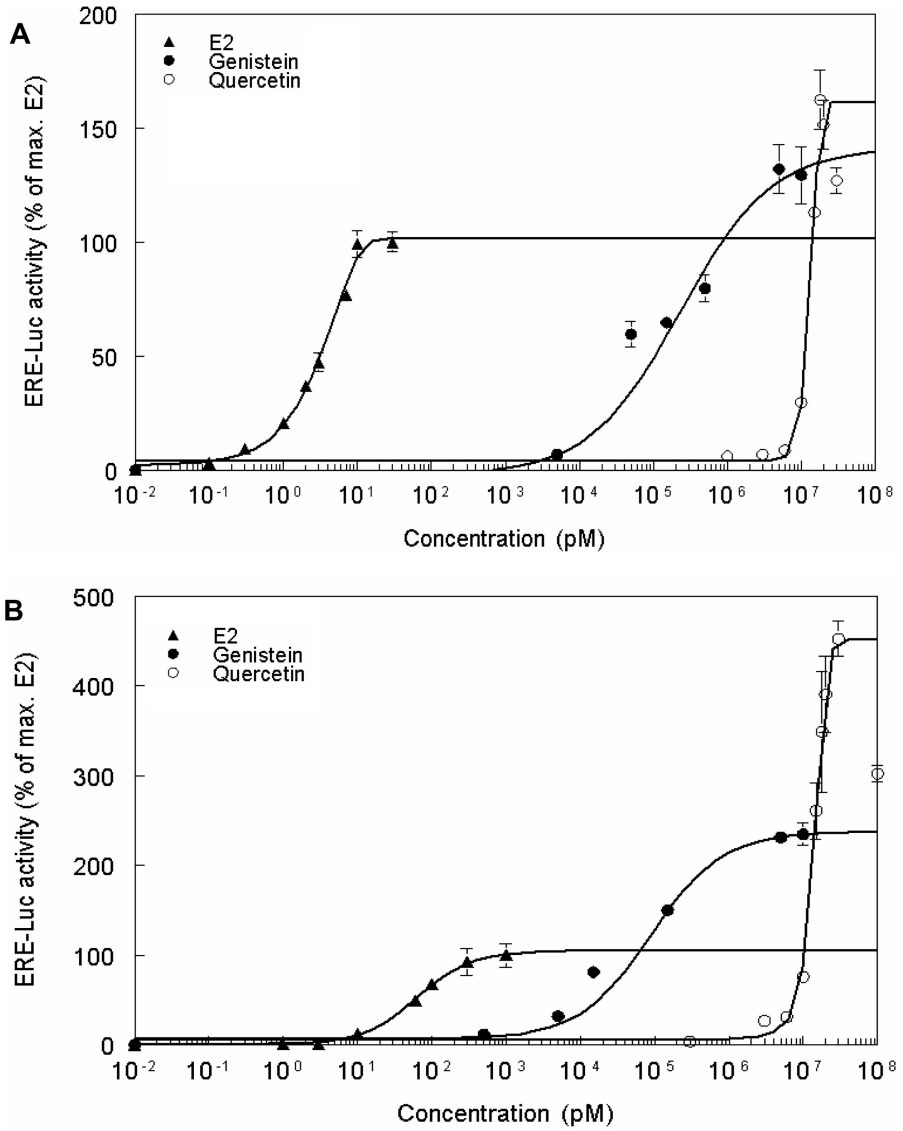

Figure 2. ER-ERE-mediated luciferase activity in (A) U2-OS-ERα and (B) U2-OS-ERβ cells exposed to quercetin or genistein for 24 h in the presence of ascorbic acid, compared to a reference curve of 17β-estradiol (E2).

affinity of genistein for the ERs appeared to be higher than that of quercetin. Genistein showed a higher affinity for ERα than for ERβ, with EC_{50} values of 9 ± 0.1 and 58 ± 6 nM, respectively. Previously, EC_{50}-values of 6 and 20 nM were determined [12]. For E2, EC_{50} values of 3 ± 0.3 pM and 58 ± 2 pM were found for ERα and ERβ, respectively, which is comparable to values from earlier studies, amounting to 5 and 50 pM, respectively [12]. Consequently, the EEFs of quercetin for ERα and ERβ were 2×10^{-7} and 7×10^{-6}, respectively, whereas the EEFs of genistein for ERα and ERβ were 5×10^{-5} and 7×10^{-3}, respectively. Thus, the affinities of both genistein and quercetin for the ERs were lower than that of E2, the affinity of quercetin for the ERα being a factor 10^7 lower, that for ERα a factor 10^6 lower. The affinity of genistein for ERα and ERβ was a factor 10^5 and 10^3 lower, respectively, than that of E2.

Although the affinities of quercetin and genistein for both ERs were lower than that of E2, the maximum induction reached was higher. For ERα, maximum induction by quercetin and genistein were, respectively, 1.7 and 1.4 times higher than that of E2. For ERβ, the difference with E2 was even higher, with maximum induction by quercetin and genistein being, respectively, 4.5 and 2.4 times higher than that by E2. At quercetin and genistein concentrations above respectively $1.8–5 \times 10^7$ pM and 1×10^7 pM, ERE-Luc activity decreased, which was probably due to cytotoxicity of the test compounds. Altogether, the results show that both quercetin and genistein can induce ER-mediated gene expression in the presence of ERα or ERβ, although with lower affinity than E2. The maximum induction that can be reached with these compounds is, however, significantly higher than with E2.

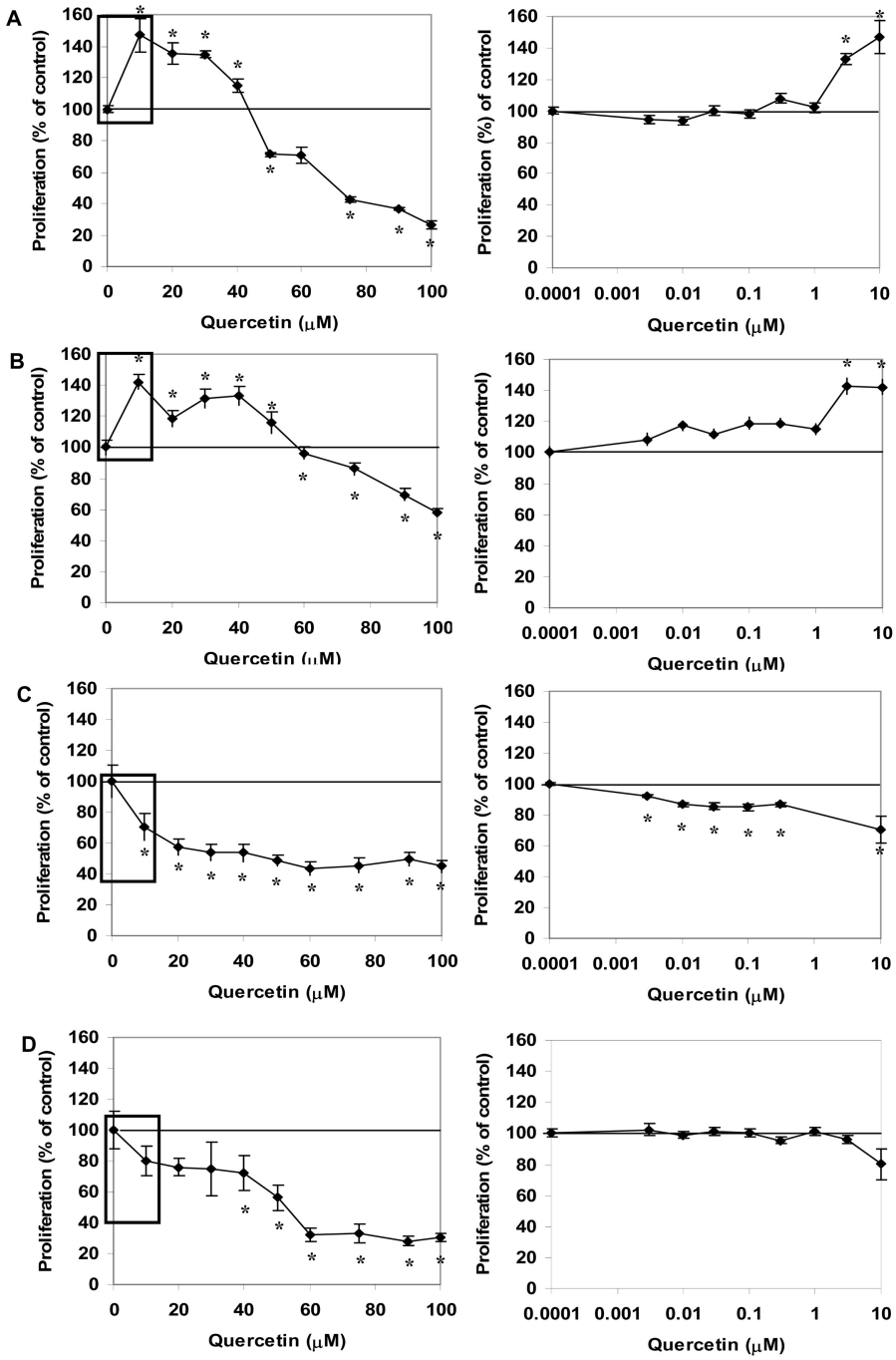

Figure 3. Effect of quercetin in the presence of ascorbic acid on the proliferation of (A) T47D, (B) MCF-7, (C) MDA-MB-231, and (D) HCC-38. Graphs on the right are results obtained at the concentration range in the boxes in the graphs on the left. * Significantly different from control at $P < 0.05$.

3.2 Effect of quercetin and genistein on the proliferation of ER-positive and ER-negative cell lines

In order to validate the results obtained in the reporter gene systems, an *in vitro* system with a physiologically more relevant endpoint was used to investigate whether the ER is involved in the stimulating effect of quercetin on cell proliferation. Because no functional ER was detected in original U2-OS cells [24] and because E2 proved to have no effect on proliferation of original U2-OS cells, or U2-OS cells transfected with ERα or ERβ [30], these types of U2-OS cells were not used for proliferation experiments because these observations point at the possible absence of an ER-dependent proliferation machinery. Figures 3 and 4 show the effects of quercetin and genistein, respectively, on the proliferation of the ER-positive cell lines T47D (A) and MCF-7 (B), and of the ER-negative cell lines MDA-MB-231 (C) and HCC-38 (D). The concentration range tested proved to have no effect in the LDH-leakage assay for all

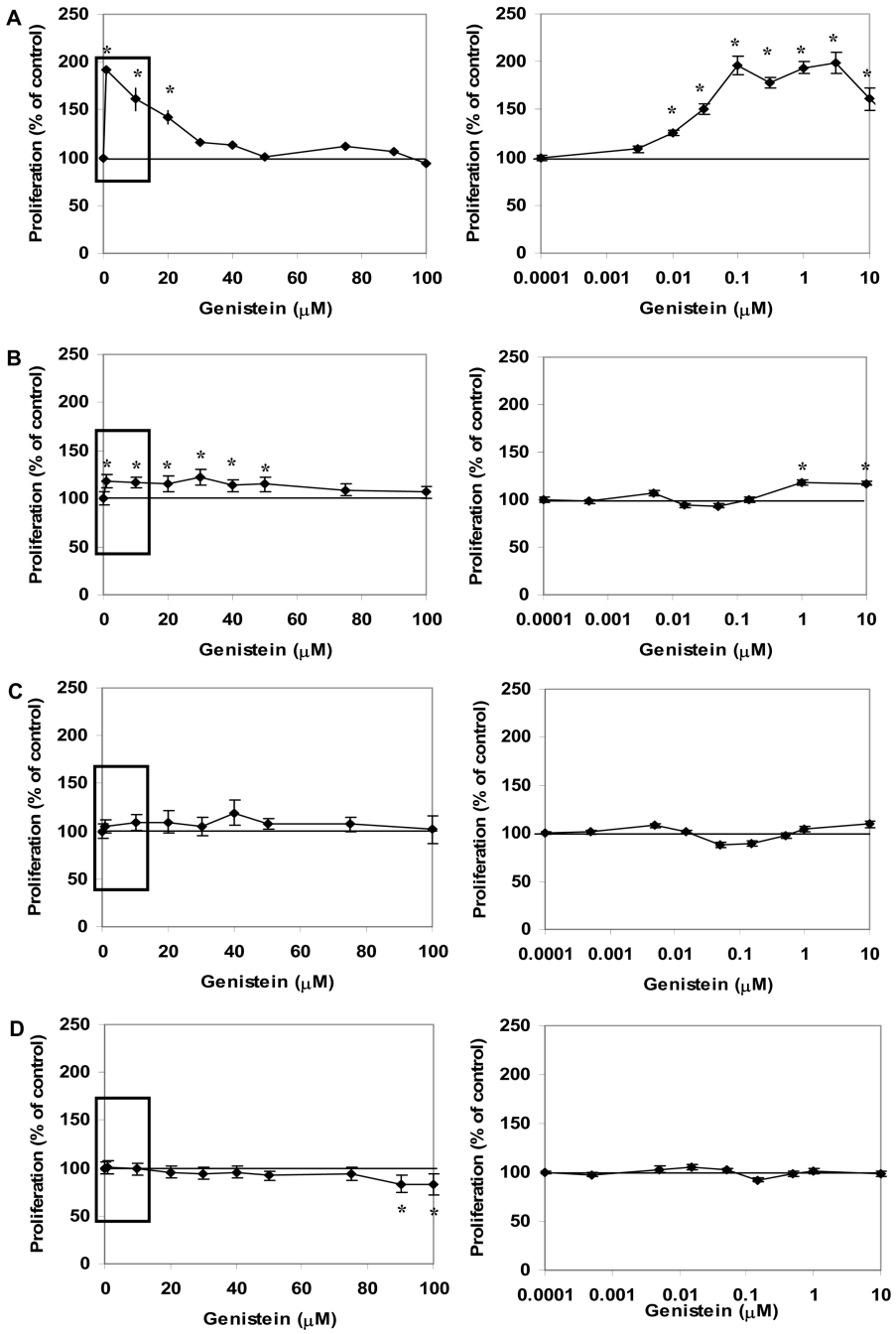

Figure 4. Effect of genistein in the presence of ascorbic acid on the proliferation of (A) T47D, (B) MCF-7, (C) MDA-MB-231, and (D) HCC-38. Graphs on the right are results obtained at the concentration range in the boxes in the graphs on the left. * Significantly different from control when $P < 0.05$.

cell lines tested (data not shown). In the ER-negative cell lines, quercetin inhibited proliferation over the entire concentration range tested (Fig. 3). From these results, it can be concluded that the inhibition of cell proliferation caused by quercetin is not dependent on the presence of an ER. However, a biphasic effect on cell proliferation was found for the cell lines expressing an ER, with a stimulating effect of quercetin on cell proliferation up to approximately 150% at relatively low concentrations. At concentrations higher than 45 or 55 μM for T47D and MCF-7 cells respectively, quercetin inhibited cell proliferation (Fig. 3).

In contrast with quercetin, genistein (Fig. 4) showed no significant effect on the proliferation of ER-negative cell lines in the concentration range tested. However, genistein stimulated cell proliferation in the ER-positive cell lines with a maximum of approximately 200% in T47D and 120% in MCF-7. In the higher concentration range, genistein had no significant effect on cell proliferation. In view of the fact that the stimulating effect of both quercetin and genistein on cell proliferation was only seen in cell lines expressing the ER, these results suggest that the stimulating effect of quercetin and genistein on cell proliferation is mediated by the ER.

4 Discussion

Recent studies showed that quercetin has a biphasic effect on the proliferation of the mammary carcinoma cell line MCF-7 and on the colon carcinoma cell lines HCT116 and HT29 [9]. Because these cell lines express ER-mRNA [7, 8] and because the ER is involved in the stimulation of cell proliferation by the phytoestrogen genistein [11, 12], the hypothesis that the ER mediates the stimulating effect of quercetin on cell proliferation was investigated in this study.

First the capacity of quercetin to activate the ER and to induce subsequent gene expression was investigated using a reporter gene assay in U2-OS cells transfected with either ERα or ERβ. Quercetin, and for comparison also genistein, both induced ER/ERE-mediated luciferase activity in both ERα and ERβ receptor systems, although with higher EC_{50} values than E2, which is in line with previous studies [12, 22]. It is important to note that both compounds have a higher induction factor in both cell systems than E2. This result was reported before for genistein but claimed to be absent for quercetin [12]. The fact that in our study quercetin was stabilized by the presence of ascorbic acid [9] might account for the enhanced quercetin-induced gene expression seen in the present study. The control incubation, relative to which proliferation was expressed, excludes possible effects on cell proliferation of ascorbic acid itself.

To validate the results with a physiologically more relevant endpoint than luciferase activity, the effects of quercetin and genistein on cell proliferation were tested in human breast cancer cell lines, including the ER-positive cell lines MCF-7 and T47D and the ER-negative cell lines HCC-38 and MDA-MB-231. In the ER-positive cell lines E2 has been reported to modulate cell proliferation in a biphasic manner [25]. Similar to E2, low concentrations of quercetin enhanced the proliferation of the ER-positive cell lines only, thereby showing that the stimulating effect of quercetin on cell proliferation is ER-dependent. The isoflavone genistein, used in this study as model phytoestrogen, also displayed stimulating effects on cell proliferation in ER-positive but not in ER-negative cell lines. The fact that quercetin showed similar effects in both the reporter gene assay and the proliferation assay as the well-established phytoestrogen genistein [11 – 14] indicates that quercetin may also be considered a phytoestrogen.

The present study suggests that the ER is involved in the stimulation of cell proliferation by quercetin. The mechanism behind ER-mediated stimulation of cell proliferation by quercetin is still unclear. However, the results of the reporter gene assay showed that quercetin is capable of activating ER-ERE-mediated gene expression in a concentration range comparable to the range in which stimulating effects of quercetin on cell proliferation were seen. Nevertheless, controversial findings have been reported on the binding affinity of quercetin for the ER [11, 12, 14], which raises the question whether quercetin can activate the ER by acting as a ligand of the receptor [22], or whether a different mechanism is involved. Another possibility is that quercetin affects the phosphorylation state of various components in the signal transduction cascade involved in ER activation, by interference with the activity of kinases [31 – 34].

The results of the present study also show that quercetin inhibits cell proliferation at the higher concentrations in all cell lines studied, which suggests that the inhibition of cell proliferation by quercetin is not dependent on the ER. Different mechanisms underlying the inhibition of cell proliferation by quercetin have been proposed, including DNA strand breakage [35], cell cycle arrest [36], and/or the induction of apoptosis [6, 36], possibly by influencing the activity of various kinases, including phosphatidylinositol-3-kinase, tyrosine protein kinase and protein kinase C [33, 34]. Another plausible mechanism of inhibition of cell proliferation, which is also in line with the estrogenic character of quercetin, is the interaction of quercetin with so-called type II estrogen binding sites (EBS) [37, 38], probably involved in the inhibition of estrogen-stimulated growth *in vivo* [38]. In contrast with previous data [11], genistein, contrary to quercetin, did not inhibit cell proliferation at concentrations up to 100 μM in the present study. However, similar to quercetin, genistein is also known as a protein kinase inhibitor [39] and a ligand for type II EBS with subsequent inhibitory effect on cell proliferation [11]. Up to now, however, no differences in efficiency of kinase activity inhibition or type II EBS-mediated inhibition of cell proliferation between quercetin and genistein have been reported that might account for the differences in effects seen [36, 40].

Phytoestrogens are generally associated with health-promoting effects, including the inhibition of breast and endometrial cancer [41], cardiovascular diseases, and osteoporosis [42]. The present data, however, suggest that quercetin and also genistein have a stimulating effect on cell proliferation of ER-containing cells at the lower physiologically relevant concentrations [43, 44]. Of importance to note is the fact that quercetin and genistein appeared able to activate the ERβ to a 4.5- and 2.4-fold higher level, respectively, than E2, whereas the maximum induction levels of ERα were only 1.7- and 1.4-fold higher than that of E2. This is especially interesting given the hypothesis that ERα might mediate the proliferative effects of estrogenic compounds, whereas ERβ might have protective effects on this process [18] by the formation of heterodimers of ERα and ERβ [19]. Thus, the results of the present study point at the relatively high capacity of the flavonoids to stimulate 'beneficial' ERβ responses as compared to their stimulation of ERα, the receptor possibly involved in adverse cell proliferative effects. This might be an explanation for the

inverse correlation between cancer incidence and consumption of fruits and vegetables, rich in this type of bioactive compounds [45, 46]. Whether the extent of cell proliferation in the ER-containing cells also matches their relative level of ERα as compared to ERβ, remains an interesting topic for further research and awaits identification and quantification of the ER types present in MCF7 and T47D cells used in the present study.

Nevertheless, the fact that quercetin has estrogenic potency suggests that it might interact with the endocrine function. Although phytoestrogens generally have a lower affinity for ER activation than E2 as reflected by their EC_{50} values, their respective physiological concentrations are such that values in the order of the EC_{50} values can be expected for both types of compounds [43, 47]. The present results show that the maximum induction by quercetin and genistein is even 2- to 5-fold higher than that by E2 at these physiologically relevant concentrations. Because in general, phytoestrogens other than quercetin or genistein may also reach concentrations that are 1000-fold higher than E2 concentrations *in vivo* [48] and because additive effects of estrogenic compounds are known [49], the cumulative effect of several compounds with weak estrogen agonism may result in significant effects *in vivo* [50], especially because phytoestrogens may interfere with the development of the fetus [51]. Several animal studies revealed possible adverse health effects of phytoestrogens, including stimulation of tumor growth in mice [52] and neurobehavioral effects in primates resulting in altered patterns of agonistic and social behavior [53]. In view of these effects and the fact that consumption of large amounts of food supplements may dramatically increase the dietary load of quercetin, careful attention should be paid to the balance between beneficial and potentially adverse effects of this flavonoid.

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