Reduced isoflavone metabolites formed by the human gut microflora suppress growth but do not affect DNA integrity of human prostate cancer cells

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Dietary isoflavones, such as genistein and daidzein, are metabolised by the human gut microflora. Case–control studies have disclosed a link between the formation of the daidzein metabolite equol and prostate cancer risk. We evaluated the effects of genistein, daidzein and five metabolites on two prostate cancer cell lines by determining DNA integrity and cell growth. LNCaP cells contain the T877A androgen receptor mutation whereas Los Angeles prostate cancer (LAPC)-4 cells express the wild-type receptor, both of which may affect responses to isoflavones. DNA integrity was determined using the comet assay. Cell growth was assessed by staining DNA with 4,6-diamidino-2-phenylindole hydrochloride. Endogenous steroid hormones, but not isoflavones, induced DNA strand breaks. Dihydrotestosterone stimulated the growth of both cell lines. 17β-Oestradiol increased the growth of LNCaP but not LAPC-4 cells, pointing to an involvement of the T877A androgen receptor. Isoflavones did not stimulate growth in either prostate cancer cell line. However, the growth of LNCaP and LAPC-4 cells was suppressed by genistein (inhibitory concentration 50% (IC50) 45.2 μmol/l), but not of LNCaP cells. In conclusion, isoflavones do not damage DNA or promote growth of androgen-dependent prostate cancer cells. Several isoflavones, including the reduced daidzein metabolites equol and O-desmethylangolensin, suppress cancer cell growth. Taken together, these data suggest a contribution of gut-formed isoflavone metabolites to the beneficial effects of dietary isoflavones on prostate cancer risk.

Isoflavones: Prostate cancer: Cell growth: DNA damage

Incidence and mortalities of prostate cancer are low in many Asian countries but high in Western societies (Hsing et al. 2000; Jemal et al. 2002; Quinn & Babb, 2002). This has epidemiologically been attributed to the traditional Asian soyabased diet, since Asians who migrated to the USA became more likely to develop prostate cancer (Shimizu et al. 1991; Gomez et al. 2003). In three prospective cohort studies, the consumption of pulses or soya milk was associated with reduced prostate cancer risk (Dagnelie et al. 2004). Soya and soya products are extraordinarily rich in isoflavones (Liggins et al. 2000; Valsta et al. 2003). Asians consume up to 100 mg isoflavones/d, whereas Europeans usually consume less than 1 mg/d (van Erp-Baart et al. 2003). Similarly, plasma isoflavone concentrations are more than ten times higher in Asians than in Europeans or in Americans and reach up to 1 μmol total isoflavones/l in plasma (Morton et al. 2002; Akaza et al. 2004). Single-dose administration of purified isoflavones to postmenopausal American women resulted in increased plasma levels of up to 28 μmol genistein/l (Bloedon et al. 2002). Moreover, some isoflavones can accumulate in the prostate, reaching concentrations that are several times higher than the respective concentrations in plasma (Hedlund et al. 2005). Due to some structural similarities to steroid hormones, isoflavones are able to bind with human oestrogen receptors and to mimic or block oestrogenic transactivation activities (Kuiper et al. 1997, 1998). Based on these so-called phyto-oestrogenic effects, isoflavones were thought to contribute to the chemopreventive effects of the Asian diet (Ren et al. 2001)

Most of the published in vitro work has addressed effects of the major isoflavones genistein and daidzein. However, the exposure in vivo is more complex. Isoflavones can undergo metabolism by the human gut microflora (Fig. 1) and those products may help to prevent the development or progression of prostate cancer. For the first time in the present study we compared the biological activities of genistein, daidzein and five of their reduced gut metabolites, detected in human body fluids (Heinonen et al. 1999), using human prostate cancer cells. Beside their proposed beneficial properties, isoflavones are suspected to be genotoxic. In a number of in vitro studies, they induced chromosomal aberrations and DNA strand breaks in V79 Chinese hamster fibroblasts (Kulling & Metzler, 1997; Schmitt et al. 2003; Di Virgilio et al. 2004). A few studies, however, also revealed a genotoxic potential of isoflavones in human prostate (Mitchell et al. 2000) and colon cell lines (Pool-Zobel et al. 2000). In the present study we

Abbreviations: IC50, inhibitory concentration 50 %; LNCaP, Los Angeles prostate cancer; O-DMA, O-desmethylandolensin; Tris, tri(hydroxymethyl)-aminomethane.

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extended these findings to include several reduced isoflavone metabolites which have not yet been investigated in prostate cells. This new information was expected to reveal whether or not the reduction of soya isoflavones by the human gut microflora alters their genotoxic potential in prostate cells.

In addition to their ability to bind with oestrogen receptors, isoflavones were recently discovered to bind with the androgen receptor as well, but with weak affinity (Beck et al. 2003). Mutations in the gene encoding the androgen receptor are common events during prostate carcinogenesis, which may affect the binding capacity of the receptor (Suzuki et al. 2003). Therefore we investigated the activities of isoflavones in two prostate cancer models with different receptor phenotypes. The androgen receptor of LNCaP cells contains a well-characterised alteration of the amino acid sequence in its ligand-binding domain (T877A), resulting in an increased affinity to dihydrotestosterone while the affinity to dihydrotestosterone (Beck et al. 2003). Furthermore, even non-steroids, such as the anti-androgen flutamide, were found to act as receptor agonists (Taplin et al. 1999; Sack et al. 2001). The Los Angeles prostate cancer (LAPC)-4 cell line, in contrast, expresses the wild-type androgen receptor (Klein et al. 1997). On account of these different phenotypes, we proposed different responses to isoflavones between the cell lines. First, we evaluated the effects of isoflavones on cell growth. This endpoint is regulated with high complexity and therefore appears to be suitable to compare the general biological activities of the tested compounds. An inhibition of cell turnover may decrease the vulnerability to carcinogens and suppress the progression of malignant foci (Hedlund et al. 2003). In contrast, an isoflavone-mediated stimulation of cell growth would promote cancer progression.

Materials and methods

Materials

Genistein and daidzein were purchased from TCI Tokyo Kasai (Tokyo, Japan); dihydrogenistein, dihydrodaidzein and O-desmethylangolensin (O-ＤＭＡ) were obtained from Plantech Ltd (Reading, Berks, UK); equol and 4’,6’-diamidino-2-phenyildine hydrochloride were from Fluka (Buchs, Switzerland). Dihydrotestosterone, 17β-oestradiol and SybrGreen were purchased from Sigma (Seelze, Germany). All test compounds were dissolved in dimethyl sulfoxide and stored at −20°C. Cell culture media and supplements were purchased from Gibco (Karlsruhe, Germany), except the charcoal-stripped fetal calf serum (Biological Industries, Beit Haemek, Israel) and mibolerone (Perkin Elmer Life Sciences, Boston, MA, USA).

The genistein metabolite 6’-hydroxy-O-ＤＭＡ was synthesised at the Department of Organic Chemistry, University of Helsinki (Finland). In brief, 4’,7-dihydroxyisoflavone was reduced by lithium aluminium hydride (5·5 equivalents) in refluxing tetrahydrofuran. The reaction mixture was quenched into saturated ammonium chloride and neutralised with hydrochloric acid and extracted with ethyl acetate. After evaporation of the solvent the crude product was purified by flash chromatography, giving a good yield of 6’-O-ＤＭＡ. The product was characterised by proton and carbon NMR, low-resolution MS and high-resolution MS.

Cell lines and cell culture procedures

The LNCaP cell line was purchased from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). Cells were maintained in RPMI-1640 supplemented with 10% fetal calf serum, 1 g/l glutamine (2 mmol/l) and 1% penicillin-streptomycin. In order to avoid interference of phenol red or steroid hormones from the serum, medium was replaced by phenol red-free RPMI-1640 supplemented with 10% charcoal-stripped fetal calf serum, 1 g/l glutamine (2 mmol/l) and 1% penicillin-streptomycin, 24 h before treatment. The LAPC-4 cell line was a generous gift of Professor C. L. Sawyers, Department of Medicine, University of California (Los Angeles, CA, USA). These cells were maintained in Iscove’s modified Dulbecco’s medium supplemented with 10% charcoal-stripped fetal calf serum, 1 g/l glutamine (2 mmol/l) and 1% penicillin-streptomycin and mibolerone (2 mmol/l). Likewise, 24 h before treatment, medium was replaced by phenol red-free Iscove’s modified Dulbecco’s medium supplemented with 10% charcoal-stripped fetal calf serum, 1 g/l glutamine (2 mmol/l), 1% penicillin-streptomycin and mibolerone (2 mmol/l). Likewise, 24 h before treatment, medium was replaced by phenol red-free Iscove’s modified Dulbecco’s medium supplemented with 10% charcoal-stripped fetal calf serum, 1 g/l glutamine (2 mmol/l), 1% penicillin-streptomycin but no mibolerone. Steroid hormone depletion of the charcoal-stripped serum was ascertained by analyses of dihydrotestosterone (RIA-kit; DSL, Sinsheim, Germany) and 17β-oestradiol (AVIDA Centaur ACS; Bayer HealthCare, Leverkusen, Germany) performed at our department of clinical chemistry (<26 pmol 17β-oestradiol/l and 219 pmol dihydrotestosterone/l).

Comet assay

As a general laboratory standard, we minimise carry-over of the solvent in order to avoid interference during the experiments. In the present study, all samples contained a final dimethyl sulfoxide concentration of 0·1%. To achieve this, stock solutions of the tested compounds (100 mmol/l) were first serially diluted in dimethyl sulfoxide and subsequently dissolved (1:1000) in cell culture medium to yield the required final compound concentrations. By applying this procedure, we noticed precipitation of some compounds at 100 μmol/l. Thus, compounds were only tested up to 80 μmol/l.

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Fig. 1. Metabolism of genistein and daidzein by human gut microflora. Genistein is reduced to dihydrogenistein which undergoes further reduction to 6’-hydroxy-O-ＤＭＡ. Daidzein is reduced to dihydrodaidzein which is further reduced to either O-desmethylangolensin (O-ＤＭＡ) or equol (modified according to and with permission of Heinonen et al. 1999).
a maximum concentration at which precipitation was not observed.

A total of 7·5 × 10⁶ cells were seeded in 75 cm² culture flasks and allowed to attach for 48 h. Medium was changed as described earlier. After 24 h, cells were harvested, washed with PBS, sampled to 1.0 × 10⁶ and incubated with test compounds (80 μmol/l) for 60 min at 37°C and 450 rpm. Thereafter, cells were washed and viability was determined by the trypan blue exclusion assay using a Neubauer haemocytometer. The following comet assay procedure has been described previously (Schaeferhenrich et al. 2003). In brief, incubated cells were placed on agarose-coated microscopic slides and covered with 0.7% low-melting agarose dissolved in PBS. Slides were exposed to a lysis solution (tri(hydroxy-methyl)-aminomethane (Tris)–HCl (10 mmol/l), Na₂EDTA in PBS. Slides were exposed to a lysis solution (tri(hydroxy-methyl)-aminomethane (Tris)–HCl (10 mmol/l), Na₂EDTA (1 mmol/l), NaCl (2.5 mol/l), 10% dimethyl sulfoxide, 1% Triton X-100, pH 10) for 60 min and subsequently incubated with alkaline electrophoresis buffer (Na₂EDTA (1 mmol/l), NaOH (300 mmol/l), pH 13) for 20 min. Thereafter, electrophoresis was carried out for 20 min (25 V, 300 mA). Slides were washed with neutralisation buffer (Tris-HCl (4.2 mol/l), Tris-Base (0.08 mol/l), pH 7.2) three times for 5 min. DNA was then stained with SybrGreen. The rate of DNA strand breaks was measured as ‘percentage DNA in comet tail’ (tail intensity). Samples were investigated in triplicate and, for each of the slides, fifty cells were evaluated using a Zeiss Axiolab HB50 microscope, COHU high performance CCD camera and the Komet 4.0 imaging and analysis system (Kinetic Imaging, Nottingham, Notts, UK). The means of one experiment were used to calculate the means of at least three independent reproductions.

**Cell growth assay**

Cells were seeded in ninety-six-well plates at a density of 8000 cells per well and allowed to attach for 48 h. Thereafter, medium was changed as described earlier; cells were incubated with the test compound (0.001–80 μmol/l). After 96 h, medium was removed and cells were fixed with methanol. Cell growth was determined from the content of residual DNA by 4′,6′-diamidino-2-phenylindole hydrochloride staining and fluorimetric detection at 360/465 nm using Tecan GENios. Results are expressed as percentage of cell growth compared with solvent control. To assure an appropriate growth of control cells, we first determined doubling times of both cell lines using these experimental conditions. LNCaP cells, 17β-oestradiol and dihydrotestosterone to increase DNA strand breaks in LNCaP cells (P<0.001), this effect did not reach statistical significance (Table 1). In contrast, the incubation with isoflavones had no effect on the rate of DNA strand breaks in either cell line (Table 2). Neither steroid hormones nor isoflavones affected cell viability during the comet assay experiments, as determined by trypan blue exclusion (data not shown).

**Modulation of prostate cell growth**

The experimental setup of our growth assay and the sensitivity of cells lines were verified by the responses to steroid hormones. Fig. 2 (A) shows biphasic effects of dihydrotestosterone in both cell lines after 96 h incubation. However, their magnitudes of effects differed markedly. The growth of LNCaP cells increased by 86%, whereas the growth of LNCaP cells increased by 36%. Higher concentrations decreased the growth of both cell lines, which was significant for LNCaP cells at 40 and 80 μmol/l. In contrast, 17β-oestradiol increased the growth of LNCaP cells by about 90% but had no stimulating effects in LAPC-4 cells. 17β-Oestradiol between 20 and 80 μmol/l decreased the growth of LAPC-4 cells (Fig. 2 (B)).

**Discussion**

In the present study we investigated the isoflavones genistein, daidzein and their reduced metabolites formed by the human gut microflora for effects on DNA integrity and growth of prostate cancer cells. We did not find any genotoxic potential of the tested isoflavones but confirmed reports showing growth-inhibiting effects of equol in prostate cells (Mitchell et al. 2000; Hedlund et al. 2003). Furthermore, we revealed that O-DMA is another gut-formed daidzein metabolite with potential to suppress prostate cancer cell growth.
Table 1. Effects of human steroid hormones (80 μmol/l) on the rate of DNA strand breaks in LNCaP and Los Angeles prostate cancer (LAPC)-4 cells (Mean values with their standard errors)

<table>
<thead>
<tr>
<th>Hormone</th>
<th>LNCaP</th>
<th>LAPC-4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tail intensity (%)‡</td>
<td>Tail intensity (%)‡</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td>Control‡</td>
<td>5.0</td>
<td>0.4</td>
</tr>
<tr>
<td>17β-Estradiol</td>
<td>8.9</td>
<td>0.1</td>
</tr>
<tr>
<td>Dihydrotestosterone</td>
<td>7.9</td>
<td>1.1</td>
</tr>
</tbody>
</table>

* P values from unpaired t tests (two-tailed) by comparison of the individual hormones with control (n = 4).
† Percentage migrated DNA as determined by comet assay after 60 min treatment.
‡ Solvent control (0.1 % dimethyl sulfoxide).

Table 2. Effects of isoflavones (80 μmol/l) on the rate of DNA strand breaks in LNCaP and Los Angeles prostate cancer (LAPC)-4 cells (Mean values with their standard errors)

<table>
<thead>
<tr>
<th>Isoflavone</th>
<th>LNCaP</th>
<th>LAPC-4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tail intensity (%)‡</td>
<td>Tail intensity (%)‡</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td>Control‡</td>
<td>4.8</td>
<td>0.4</td>
</tr>
<tr>
<td>Genistein</td>
<td>7.3</td>
<td>2.3</td>
</tr>
<tr>
<td>Dihydrotestosterone</td>
<td>6.2</td>
<td>1.4</td>
</tr>
<tr>
<td>6'-OH-O-DAZA</td>
<td>6.1</td>
<td>1.7</td>
</tr>
<tr>
<td>Daidzein</td>
<td>4.6</td>
<td>0.5</td>
</tr>
<tr>
<td>Dihydrodaidzein</td>
<td>4.5</td>
<td>0.5</td>
</tr>
<tr>
<td>O-DAZA</td>
<td>4.9</td>
<td>0.7</td>
</tr>
<tr>
<td>Equol</td>
<td>5.3</td>
<td>0.3</td>
</tr>
</tbody>
</table>

6'-OH-O-DAZA, 6'-hydroxy-O-desmethyleangolensin; O-DAZA, O-desmethyleangolensin.
* P values from unpaired t tests (two-tailed) by comparison of the individual isoflavones with control (n = 3).
† Percentage migrated DNA as determined by comet assay after 60 min treatment.
‡ Solvent control (0.1 % dimethyl sulfoxide).

Genotoxic activities of the test compounds were studied at high concentrations of 80 μmol/l, which have never been reached in vivo. In one study with postmenopausal women, supplementation of purified soya isoflavones (up to 16 mg genistein/kg body weight) resulted in plasma peak concentrations of up to 28.1 μmol total genistein/l and up to 90.0 μmol total daidzein/l (Bloedon et al. 2002). Thus, total soya-isoflavone plasma concentrations of 40 μmol/l might be possible. Another study in men revealed that isoflavone concentrations in prostate fluid are several times higher than in plasma (daidzein, 5.1-fold; dihydrodaidzein, 5.6-fold; equol, 21.7-fold), pointing to an accumulation of isoflavones in prostate tissue. However, we demonstrated that dihydrotestosterone stimulated the growth of both cell lines. Further in vitro and in vivo work is needed to fully evaluate the genotoxic potential of isoflavones.

In vivo, genistein administration did not induce DNA strand breaks in mice splenocytes, even though plasma concentrations increased up to 9.2 μmol/l (Record et al. 1995). Within a human intervention study, supplementation of isoflavones increased concentrations of genistein in plasma individually up to 27 μmol/l, which did not affect DNA integrity of primary lymphocytes (Miltyk et al. 2003). Thus, these studies do not support the hypothesis that isoflavones are genotoxic in vivo. Further in vitro and in vivo work is needed to fully evaluate the genotoxic potential of isoflavones.

We also determined effects on cell growth and demonstrated that there were different responses to steroid hormones. Dihydrotestosterone stimulated the growth of both cell lines.
However, equol possibly accumulates in the prostate, reaching peak concentrations of 13.5 \text{nmol/l} in the prostate fluid (Morton et al. 1997). In the present study, equol at 5 \text{nmol/l} significantly decreased LAPC-4 cell growth by 32 %, which is half of the equol concentration found to reduce LAPC-4 cell growth in a study of Hedlund et al. (2003). Moreover, that study revealed that equol treatment at 1 \text{nmol/l} decreases growth of benign prostatic epithelial primary cells, making those cells less vulnerable to cancer initiation (Hedlund et al. 2003). In the present study, equol at 10 \text{nmol/l} also reduced growth of LNCaP cells, which confirms previous reports (Mitchell et al. 2000; Hedlund et al. 2003). However, the concentration of 5 \text{nmol/l} has also been shown to decrease LNCaP growth, as reviewed by Adlersreutz (2002). Thus, equol seems to exert the most potent growth-inhibiting activity in comparison with the other tested compounds. Recently reported in vivo data also indicated chemopreventive activities of equol. Three case–control studies disclosed that prostate cancer patients are less likely to produce this reduced daidzein metabolite than healthy controls (Akaza et al. 2002, 2004; Ozasa et al. 2004).

Fig. 2. Modulation of prostate cell growth by human steroid hormones. LNCaP (○) and Los Angeles prostate cancer (LAPC)-4 (□) cells were treated for 96 h and growth was determined from the content of residual DNA by 4′,6-diamidino-2-phenylindole hydrochloride staining. (A) Dihydrotestosterone increased growth of LNCaP cells by 36 % at 0.001 \text{nmol/l} but decreased growth between 40 and 80 \text{nmol/l} (n 5). In LAPC-4 cells, dihydrotestosterone at 0.01 \text{nmol/l} increased growth by 86 % (n 3). (B) 17β-oestradiol increased growth of LNCaP cells at 0.001–1 \text{nmol/l} by about 90 % (n 4) whereas growth of LAPC-4 cells decreased between 20 and 80 \text{nmol/l} (n 4). Data are mean values of relative cell growth compared with control (0·1 % dimethyl sulfoxide), with their standard errors represented by vertical bars. Mean values were significantly different from control: * P < 0·05, ** P < 0·01 (one-way ANOVA and Dunniff’s post hoc test).
We further observed that O-DMA had growth-suppressing activities in LAPC-4 cells, but not in LNCaP cells. We believe that distinct capabilities to metabolise O-DMA, for example, conjugation and elimination, are responsible for the observed differences. Our current work is directed to prove this assumption.

Wiseman et al. (2004) determined isoflavone concentrations in plasma following a 10-week consumption of a soya-rich diet (104 mg isoflavones/d) and found about 200 nmol O-DMA/l and 170 nmol equol/l (values approximated from figures). Only 35% of the subjects were capable of producing equol (‘equol-producers’). This subset had mean plasma concentrations of 364 nmol/l (Wiseman et al. 2004). There are also considerable interindividual variations in the production of O-DMA; accordingly the term ‘O-DMA-producers’ seems to be reasonable as well (Watanabe et al. 1998). In a recent study by Hedlund et al. (2005) about 95% of the subjects were found to be capable of producing O-DMA. After consuming a soya beverage (50 mg isoflavones/d) for 1 week, concentrations of O-DMA individually increased up to 367 nmol/l in plasma and 1229 nmol/l in prostate fluid (Hedlund et al. 2005).

Hence, the present study disclosed growth-suppressing activities of O-DMA at concentrations, which have not to date been determined in vivo. Further, we did not find any epidemiological data, for example, case–control studies, supporting a possible relationship between the human O-DMA exposure and the risk of prostate cancer. Therefore, our work might be seen as a first hint of chemopreventive properties of O-DMA in prostate cells. Additional studies are needed to further elucidate the biological activities of this compound.

We should note that the present study was designed to compare the biological activities of soya isoflavones and their reduced gut metabolites. The observed suppressive effects on cell growth, however, could result from various mechanisms, such as inhibition of cell proliferation or induction of apoptosis, which we have not analysed in detail. The present study has disclosed that this group of soya-related compounds possesses a number of interesting properties in prostate cells. For one, the tested isoflavones do not directly induce DNA damage in prostate cells. Isoflavones also do not appear to promote the growth of prostate cancer cell foci, regardless of whether those cells express the mutated T877A or the wild-type androgen receptor. This is important, since prostate cancer is often unrecognised for years due to a lack of clinical symptoms. The reductive gut metabolism of daidzein enhances its growth-suppressive activities in prostate cancer cells. Despite the potent growth-suppressive activities of genistein, its reduced metabolites have only little effect.

**Fig. 3. Modulation of prostate cell growth by genistein and its reduced gut metabolites.** LNCaP (●) and Los Angeles prostate cancer (LAPC)-4 (○) cells were treated for 96 h and growth was determined by quantifying residual DNA with 4′,6-diamidino-2-phenylindole hydrochloride staining. (A) Genistein decreased growth of LNCaP by ≥21% from 10 μmol/l (n = 4) and of LAPC-4 cells by ≥34% from 20 μmol/l (n = 4). (B) Dihydrogenistein did not affect growth of LNCaP cells (n ≥ 3). (C) 6′-Hydroxy-O-desmethylangolensin decreased growth of LNCaP at 80 μmol/l by 32% (n = 4) whereas it had no effect in LAPC-4 cells (n ≥ 3). Data are mean values of relative cell growth compared with control (0-1% dimethyl sulfoxide), with their standard errors represented by vertical bars. Mean values were significantly different from control: * P < 0.05, ** P < 0.01 (one-way ANOVA and Dunnnett’s post hoc test).
Taken together, the present in vitro data support a possible preventive role of soya-based isoflavones in the progression of prostate cancer. Our findings also point to a possible contribution of the reduced daidzein gut metabolites, equol and possibly O-DMA, by inhibiting growth of malignant foci. The growth-suppressing activities of genistein and equol occur at physiologically relevant concentrations and therefore are probably of significance in vivo.

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The authors wish to thank Professor Charles L. Sawyers (University of California, Los Angeles, CA, USA) for providing LAPC-4 cells. The present study was carried out with financial support from the Commission of the European Communities specific RTD programme ‘Quality of Life and Management of Living Resources’ (project no. QLK-2000-00 266). It does not necessarily reflect its views and in no way anticipates the Commission’s future policy in this area. We are grateful to Dr William M. Pool for editorial assistance.

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Fig. 4. Modulation of prostate cell growth by daidzein and its reduced gut metabolites. LNCaP (●) and Los Angeles prostate cancer (LAPC)-4 (○) cells were treated for 96 h and growth was determined from the content of residual DNA by 4',6-diamidino-2-phenylindole hydrochloride staining. (A) Daidzein decreased growth of LNCaP by 36 % at 80 μmol/l (n 4) and of LAPC-4 cells by ≥39 % from 40 μmol/l (n 3). (B) Dihydroadaidzein did not affect growth of prostate cells (n ≥3). (C) O-Desmethylangolensin had no effect on LNCaP cell growth (n 3) but decreased growth of LAPC-4 cells by ≥37 % from 20 μmol/l (n 3). (D) Equol decreased growth of LNCaP by 42 % from 10 μmol/l, and of LAPC-4 cells by ≥32 % from 5 μmol/l. Data are mean values of relative cell growth compared with control (0·1 % dimethyl sulfoxide), with their standard errors represented by vertical bars. Mean values were significantly different from control: *P < 0·05, **P < 0·01 (one-way ANOVA and Dunnett’s post hoc test).
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