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Effects of natural prenylated flavones in the phenotypical ER (+) MCF-7 and ER (−) MDA-MB-231 human breast cancer cells

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Abstract

The effect of seven natural prenylated flavones in DNA synthesis of two human breast cancer cell lines, the estrogen-dependent ER (+) MCF-7 and the estrogen-independent ER (−) MDA-MB-231 cells, was evaluated. Flavones with an isopentenyl group at C-8 and a ring linking C-3 and C-2′ presented a biphasic effect in DNA synthesis of ER (+) MCF-7 and displayed a stimulation at low concentrations (0.02–0.78 μM) whilst at higher concentrations (>3.12 μM) inhibition was observed. No stimulation was observed in ER (−) MDA-MB-231. In contrast, all the flavones exhibited an antiproliferative effect in both ER (−) and ER (+). Curiously, the inhibition of DNA synthesis was accompanied by a high capacity of these cells to reduce MTT, which was concurrent with the appearance of an intense intracytoplasmic vacuolization. The accumulation of the formazan product in these vacuoles could justify the enhancements of MTT reduction. The characterization of these vacuoles with the autophagic marker monodansylcadaverine (MDC) is consistent with autophagic vacuoles, which led to the suggestion that these flavones could induce autophagy in both ER (+) and ER (−) breast cancer cell lines.

Keywords: Prenylated flavones; *Artocarpus elasticus*; Estrogen receptors; Biphasic effect; Cytoplasmic vacuolization

1. Introduction

Flavonoids compose a large group of phenolic compounds, found in fruits, vegetables, nuts, seeds, herbs, spices, stems, flowers, as well as tea and red wine. These compounds display a remarkable spectrum of biological activities affecting various cellular systems (Middleton et al., 2000). The antiproliferative activity of flavonoids is well documented and has been extensively studied in several human tumor cell lines (Kuntz et al., 1999; Harborne and Williams, 2000; Middleton et al., 2000). However, studies on estrogen-dependent human breast cancer cell lines showed that some flavonoids, depending on the concentration used, exhibited a proliferative effect in addition to antiproliferative activity (Wang and Kurzer, 1997). This growth promoting effect has been attributed to an estrogen activity. The most well-known

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naturally occurring flavonoid with estrogen activity is the isoflavone genistein which has been shown to compete with estradiol for the estrogen receptor (Wang et al., 1996). This kind of similarity between flavonoids and estrogens explains the extensive research undertaken in recent years on this group of compounds for estrogen–antiestrogen activities.

Most studies involving phytoestrogens use the estrogen-dependent ER (+) MCF-7 human cancer cell line (Schmitt et al., 2001; Kinjo et al., 2004; Murata et al., 2004) in parallel with the estrogen-independent ER (−) MDA-MB-231 cells to prove the dependence of the stimulatory effect of these compounds on the estrogen receptors (Wang and Kurzer, 1997; Schmitt et al., 2001; Rowlands et al., 2002). In the ER (+) MCF-7 cells phytoestrogens normally exhibited a biphasic effect, which is expressed by a stimulatory effect on cell growth at low concentrations and an inhibitory effect at high concentrations (Wang and Kurzer, 1997). While the stimulatory effect of phytoestrogens seems to be mediated via estrogen receptors, the antiproliferative effect appears to involve an ER-independent cellular mechanisms (Collins-Burow et al., 2000). Most studies on the evaluation of the proliferative and antiproliferative properties of phytoestrogens use DNA synthesis or MTT assays (Wang and Kurzer, 1998; Wang and Lou, 2004). However, the MTT assay may represent a pitfall in measuring the antiproliferative effect because increases in cellular MTT-reducing activity have been reported in the presence of cell growth inhibition (Bernhard et al., 2003). The mechanism of MTT reduction is far from being clear. Although the reduction in MTT is generally attributed to mitochondrial respiratory chain activity, nonmitochondrial enzymes have also been implicated (Berridge and Tan, 1993). Moreover, MTT reduction in the intracellular vesicles, identified as endosomes and lisossomes formed during the autophagic process, was reported (Liu et al., 1997).

In previous work, while studying the cytotoxic effect of artelastin, a prenylated flavone isolated from *Artocarpus elasticus,* on MCF-7 cells (Kijjoa et al., 1996) it was noticed that this compound exhibited a biphasic effect in DNA synthesis of this ER (+) breast cancer cell line (Pedro et al., 2005) which was stimulatory at low concentrations and inhibitory at high concentrations, resembling the effect of phytoestrogens. Curiously, we found that concentrations of artelastin responsible for an antiproliferative effect were associated with an increased capacity of cells to reduce MTT, at the same time causing massive cytoplasmic vacuolization. These previous results prompted us to undertake a more thorough study of some of these findings. Consequently the aims of the present work were: (i) to evaluate if the biphasic effect exhibited by artelastin in DNA synthesis was equally present in a ER (−) breast cancer cell line; (ii) to extend the investigation of the effect in DNA synthesis to other prenylated flavones (Fig. 1), also isolated from *A. elasticus* (Kijjoa et al., 1996, 1998; Cidade et al., 2001) and structurally related to artelastin; (iii) to evaluate if the cytoplasmic vacuolization induced by flavone treatment was responsible for the increases detected in the cellular MTT reduction capacity; (iv) to evaluate if the cytoplasmic vacuoles could be related with the autophagic process.

2. Material and methods

2.1. Reagents

RPMI-1640, fetal bovine serum (FBS), L-glutamine, gentamicin and trypsin were supplied from Gibco Invitrogen Co. (Scotland, UK). Trypan blue, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO) and monodansylcadaverine (MDC) were from Sigma–Aldrich Co. (Saint Louis, U.S.A.). Sodium dodecyl sulfate (SDS), Entellan, methanol, paraformaldehyde and Hemacolor® were sourced from Merck (Darmstadt, Germany), [^{3}H]-thymidine from Amersham (Illinois, U.S.A.), dimethylformamide (DMF) from Romil Chemicals (England) and scintillation liquid from Perkin-Elmer (Boston, U.S.A.).

2.2. Plant material

A detailed description of the isolation, purification and identification of the seven prenylated flavones from the wood of *A. elasticus*, are published elsewhere (Kijjoa et al., 1996, 1998; Cidade et al., 2001). The purity of compounds was assessed to de ≥99% (HPLC). A stock solution of each flavone was prepared in DMSO and kept at −20 °C. Appropriate dilutions of flavones were freshly prepared just prior the different assays.

2.3. Cell cultures

Two human breast cancer cell lines were used, the ER (+) MCF-7 and the ER (−) MDA-MB-231. The MCF-7 cell line was provided by the National Cancer Institute (NCI, Bethesda, U.S.A.) and MDA-MB-231 was obtained from the American Type Culture Collection (ATCC, Manassas, U.S.A.). Cells were grown as monolayers and maintained in RPMI-1640 medium supplemented with 5% or 10% heat-inactivated FBS for MCF-7 and MDA-MB-231 respectively, 2 mM glutamine and 50 μg/ml of gentamicin, at 37 °C in a humidified atmosphere with 5% CO_2.

The exponential growing MCF-7 and MDA-MB-231 cells were obtained by plating 1.5×10^5cells/ml followed by 24 h incubation. The effect of the vehicle solvent (DMSO) was eval-