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Modulation of Aromatase Activity by Diet Polyphenolic Compounds

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Estrogens are involved in physiological actions related to reproduction, body fat distribution, and maintenance of bone mass and are also related to the pathogenesis of estrogen-dependent cancers. The aim of this work was to study the effect of polyphenols on estrogen synthesis. The effect of polyphenols and polyphenolic-rich beverages on aromatase activity was tested in JAR cells (a choriocarcinoma cell line) through the tritiated water release assay. Some of the tested polyphenols inhibited estrogen production, chrysin being the most potent. Additionally, we observed that red wine, alcohol-free red wine, green tea, and black tea (200 μL/mL) significantly decreased aromatase activity. No effect on aromatase expression, as assessed by western blotting and RT-PCR, has been detected after 24 h of treatment with any of the flavonoids under study. In conclusion, polyphenols are able to modulate aromatase activity and, consequently, estrogen synthesis. The knowledge of such interference may help to clarify some of the biological properties attributed to polyphenols and may be useful in prevention/treatment of estrogen-dependent disorders.

KEYWORDS: Aromatase; estrogens; polyphenolic compounds; tea; wine

INTRODUCTION

Estrogens influence several physiological processes both in males and females. The most obvious and best known of their actions is the regulation of reproductive functions. But estrogens have other not so obvious but also important activities. The more favorable conditions for cardiovascular health found in premenopausal women in comparison to men have been partially attributed to estrogens (*1*). There is also a clear involvement of these hormones in the formation and maintenance of bone in later life (*2*). Estrogen involvement in determining the amount and distribution of body fat (*3*) can also largely influence health, since obesity, especially the visceral type, influences the risk for cardiovascular diseases and diabetes (*4*). Additionally, estrogens have neuroprotective actions (*2*). On the other hand, estrogens have a profound impact in the development of estrogen-dependent cancers such as breast and prostate cancer (*5*). Concerning breast cancer, it is currently known that approximately 60% of premenopausal and 75% of postmenopausal breast cancer patients have estrogen-dependent tumors (*6*). Therapeutic approaches to these conditions may include one of two strategies to impair estrogen action: either blockade of the action of estrogens through estrogen receptor (ER) antagonists (or antiestrogens) or inhibition of their synthesis using modulators of aromatase activity (*7*).

Aromatase (EC.1.14.13) is a cytochrome P450 (CYP) enzyme that catalyses the aromatization of androgens (testosterone and androstenedione) to estrogens (estradiol and estrone, respectively) by three consecutive hydroxylation steps (*8*). It is most abundant in the ovary and placenta but exists also in other organs and tissues such as the brain and adipose tissue (*8*).

Polyphenols are a wide group of molecules present in almost all plant-based foods. Some classes of polyphenols have structural similarities to estrogens, being good candidates for a possible interference with estrogen-regulated cellular events (*9*).

Polyphenols of various food sources are being increasingly related to health, including inhibition of cancer initiation, promotion, and progression (*10*). Much interest has focused on wine and tea, polyphenolic-rich beverages, which are presently being recognized in epidemiological and experimental studies as possessing chemopreventive properties (*11*−*14*). Reduction of oxidative stress (*13*−*15*), angiogenesis inhibition (*16*), impairment of procarcinogen activation (*14*, *15*), and regulation of the cell cycle (*11*, *17*) are among the most common mechanisms attributed to these beverages that may explain their anticarcinogenic effects. However, little is known about their ability to interfere with estrogen synthesis (*17*−*22*).

The purpose of this work was to study the effect of polyphenolic compounds on estrogen synthesis. The effect of beverages rich in these polyphenolic compounds was also investigated. Choriocarcinoma-derived JAR cells were used to determine aromatase activity by tritiated water release assay. Because JAR cells express high levels of this enzyme, they are considered a good model for its investigation (*23*−*25*).

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MATERIALS AND METHODS

Materials. The following materials were used: [1β-^{3}H]androst-4-ene-3,17-dione (specific activity 25.3 Ci/mmol NEN Life Science Products, Boston, MA); 4-androstene-3,17-dione, 4-androstene-4-ol-3,17-dione, (+)-catechin, activated charcoal, chloroform, chrysin, daidzein, (−)-epicatechin, (−)-epigallocatechin-3-gallate, genistein, kaempferol, poly-L-ornithine hydrobromide, myricetin, β-NADH-Na_2, naringenin, sodium pyruvate, quercetin dihydrate, rutin hydrate, penicillin/streptomycin/amphotericin solution, trypsin−EDTA solution, trans-resveratrol, Tris−HCl (tris-[hydroxymethyl]-aminometane hydrochloride), HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) (Sigma, St. Louis, MO); DMSO (dimethyl sulfoxide), Triton X-100 (Merck, Darmstadt, Germany); Dextran 70 (Amersham Biosciences, Uppsala, Sweden). Red (RW) and white wines (WW) were from the Douro region (Portugal). Alcohol-free red (AF-RW) and white wines (AF-WW) were prepared by extracting ethanol from the intact wines (kindly prepared and supplied by Professor Paula Guedes de Pinho from ESBUC). Green tea (GT) and black tea (BT) were also purchased from the local market and prepared by the infusion of one tea bag in 250 mL of boiling water for 2 min (BT) and 5 min (GT).

Cell Culture. Choriocarcinoma-derived JAR cells were obtained from the American Type Culture Collection (ATCC HTB-144, Rockville, MD). Cells were maintained in humidified atmosphere of 5% CO_2−95% air and were grown in RPMI culture medium (Gibco BRL, Life Technologies, Gaithersburg, MD) supplemented with 2 mmol/L L-glutamine, 25 mM HEPES, 1 mmol/L sodium pyruvate, 4.5 g/L glucose, 10% heat-inactivated fetal bovine serum (56 °C, 30 min), 100 units/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin. Culture medium was changed every 2−3 days, and the culture was split when cells reached confluence. For subculturing, cells were incubated with 0.25% trypsin−EDTA solution (37 °C, 5 min), removed from the plate surface, and cultured in 22.1 cm^2 culture plates (Ø 60 mm, TPP, Trasadingen, Switzerland). For the experiments cells were split 1:6 and cultured in 12-well plates (3.66 cm^2, Ø 22.2 mm, TPP, Trasadingen, Switzerland) precoated with 0.1 g/L poly-L-ornithine (in 0.15 mol/L boric acid−NaOH, pH 8.5). Cells were used between passage number 24 and 50 after 3 days of culture (confluence), and each well contained 230−800 μg of cell protein.

Aromatase Assay. Aromatase activity was determined as described by Nakanishi et al. (*25*), through measuring the release of [^{3}H]H_2O during the aromatization of [^{3}H]androstenedione to estrone. Previous time-course analysis of aromatase reaction in JAR cells allowed us to determine the incubation time of 1 h as adequate. By kinetic analysis of the reaction we found the concentration of 54 nmol/L [^{3}H]-androstenedione to be adequate for modulation studies. The specificity of the reaction was tested through the incubation of JAR cells with the aromatase inhibitor 4-hydroxyandrostenedione (100 μmol/L). To study the acute effect of compounds on aromatase activity cells, after 72 h of culture, were preincubated in 750 μL of culture medium. Cells were then washed with 1 mL of serum-free medium, and the incubation began with the addition of 300 μL of serum-free medium with 54 nmol/L [^{3}H]androstenedione. Preincubation and incubation occurred at 37 °C, in a 5% CO_2−95% air atmosphere. After incubation for 1 h, culture plates were placed on ice (to stop the reaction) and 200 μL of incubation medium was removed and added to microtubes containing 500 μL of chloroform. Samples were vortexed for 60 s and centrifuged (9000*g*, 1 min). An aliquot of 100 μL of the aqueous upper phase was mixed with the same volume of a 5% charcoal/0.5% dextran 70 suspension, vortexed for 40 s, and incubated at room temperature for 10 min. After centrifugation for 15 min at 9000*g*, 150 μL of the supernatant was removed to determine the level of radioactivity, added to 8 mL of scintillation cocktail, and the [^{3}H]H_2O was measured by liquid scintillation counting.

Effect of Compounds. To test the effect of compounds on the activity of aromatase, the compounds (or vehicle) were present during the preincubation (2 h) and incubation (1 h) periods. Chrysin, daidzein, epigallocatechin-3-gallate, genistein, kaempferol, myricetin, naringenin, quercetin, rutin, and resveratrol were dissolved in DMSO, and catechin and epicatechin were dissolved in ethanol. The maximal concentration of the solvents in incubation media was 1% (v/v).

Effect of Beverages. To study the effect of beverages on aromatase activity, JAR cells were preincubated and incubated in the presence of 200 μL/mL of each beverage. Controls were run for all tested beverages: 12% ethanol (v/v) in 5 g/L tartaric acid (pH 7.4) for wines and 0.9% NaCl (w/v) for tea and AF beverages. All incubation media pH were verified and adjusted to 7.4. The final concentration of ethanol was 2.40% (v/v) for wines.

Cell Viability Determination. Cell viability was assessed by measuring lactate dehydrogenase (LDH) activity, as described by Bergmeyer and Bernt (*26*). The release of the intracellular enzyme LDH into the media was used to calculate cell viability. In brief, LDH activity was derived by measuring the oxidation of NADH at 340 nm during the reduction of pyruvate to lactate. Optical density values were determined for 2 min, and the rate of reduction was calculated. Released LDH is a stable enzymatic marker that correlates linearly with cell death. To determine total LDH activity, cells from control cultures were solubilized with 300 μL of 0.1% (v/v) Triton X-100 (in 5 mmol/L Tris−HCl, pH 7.4) and placed for 30 min at 37 °C. This cell-associated LDH activity was added to the LDH activity released from control cultures, and the total activity was considered to represent 100% cell death. The amount of LDH present in the medium was then calculated as a percentage of the total, which determines the percent cell death in that sample.

Protein and RNA Extraction. JAR cells were grown for 24 h and then treated for another 24 h with the different polyphenols dissolved in the culture medium. Total protein and RNA were extracted using Tripure isolation reagent (Roche, IN), according to the producer's instructions. Proteins were dissolved in 1% SDS solution, and RNA was dissolved in water (DEPC-treated) and stored at −80 °C.

Western Blotting. The protein solution was diluted (1:1) in loading buffer (Bio-Rad Laboratories) containing 2% mercapthoethanol (v/v) and run (8 μg) in a 10% SDS−page gel. The proteins in the gel were transferred to a nitrocellulose membrane (Hybond C-Extra, Amersham Biosciences). The membrane was blocked in PBS with 0.05% (v/v) of Tween 20 (PBS-T) containing 5% (w/v) fatfree powder milk and incubated with goat antiaromatase polyclonal antibody (1:1000), then incubated with bovine antigoat polyclonal antibody HRP-conjugated (1:2000). Detection was performed with ECL reagent (Amersham Biosciences). β-Actin primary antibody hybridization was made by the same procedure. All antibodies were purchased to Santa Cruz Biotechnologies (Santa Cruz, CA).

RT-PCR. Five micrograms of RNA was used as the template for cDNA production through the incubation with reverse transcriptase (Reverase, Bioron GmbH) for 1 h at 45 °C, in 10 μmol/L random hexamers, 0.375 mmol/L per dNTP, 3 mmol/L $MgCl_2$, 75 mmol/L KCl, 50 mmol/L Tris−HCl, pH 8.3, 10 mmol/L dithiothreitol, and 40 units of RNase inhibitor (RNaseOUT; Gibco BRL), followed by 10 min at 95 °C to inactivate the enzyme. Samples were incubated for 30 min at 37 °C with 0.1 mg/mL RNAse (Sigma). PCR amplification was performed in the presence of 2 mmol/L $MgCl_2$, 0.5 mmol/L each primer, 0.2 mmol/L dNTPs, 2 U of Taq DNA polymerase (DFS-Taq DNA polymerase, Bioron GmbH), and 4 μL of RT product, in a final volume of 50 μL. Simultaneous amplification of CYP19 and the invariant housekeeping gene GAPDH was performed. Amplification started with denaturation at 94 °C for 5 min and 30 cycles of denaturation at 94 °C for 90 s, annealing at 60 °C for 90 s, elongation at 72 °C for 60 s, and a final elongation at 72 °C for 15 min. Primers were purchased from Metabion International (Martinsried, Deutschland). CYP19 primer set sequences were 5′-GCA TAT TGG AAA TGC TGA TCG CAG-3′ and 5′-TAA CGA GGA TGG CTT TCA TCA TCA CC-3′. GAPDH primer set sequences were 5′-ACT GGC GTC TTC ACC ACC AT-3′ and 5′-TCC ACC ACC CTG TTG CTG TA-3′. The predicted sizes of the PCR products were (in bp): 462 (CYP19) and 682 (GAPDH). PCR products were visualized on a 1.6% agarose gel with ethidium bromide staining. The expression of aromatase was normalized to the expression of GAPDH of each sample and compared.

Protein Determination. The protein content of cell monolayers was determined as described by Bradford (*27*), with human serum albumin as the standard.

Calculation and Statistics. Results are expressed as arithmetic means ± SEM or geometric means with 95% confidence intervals. For

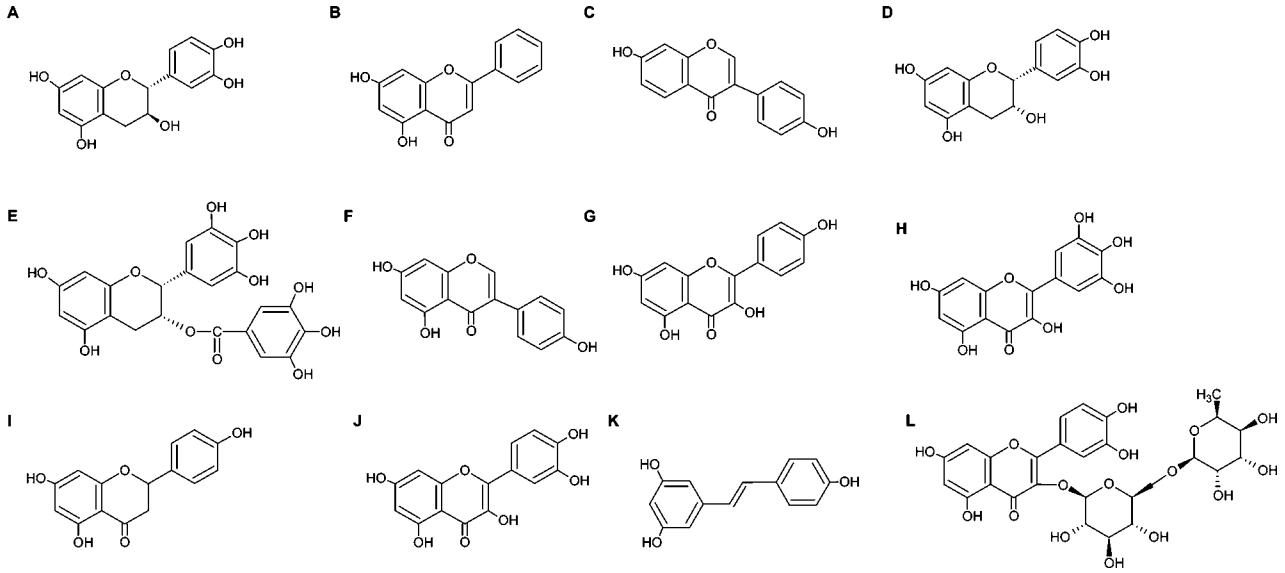

Figure 1. Chemical structure of the polyphenols tested: (**A**) catechin, (**B**) chrysin, (**C**) daidzein, (**D**) epicatechin, (**E**) epigallocatechin-3-gallate, (**F**) genistein, (**G**) kaempferol, (**H**) myricetin, (**I**) naringenin, (**J**) quercetin, (**K**) resveratrol, and (**L**) rutin.

Table 1. Inhibition of Aromatase by Phenolic Compounds (IC_{50})[a]

phenolic compound	IC_{50} (95% CI) μmol/L
chrysin	6.1 (3.0–12.4)
naringenin	7.5 (3.2–17.5)
quercetin	84.6 (58.4–122.4)
kaempferol	161.6 (86.0–304.0)
myricetin	164.1 (87.2–309.1)
resveratrol	296.3 (196.5–446.6)
epigallocatechin-3-gallate	1770 (1501–2088)

[a] JAR cells were preincubated (2 h) and incubated (1 h) in the presence of the test compound (or vehicle). The incubation was carried out with 54 nM [^{3}H]androstenedione, at 37 °C. Results represent geometric means with 95% confidence intervals ($n = 6$).

the comparison of several groups the one-way analysis of variance (ANOVA) test was used, followed by Bonferroni's test when the distribution was normal or the nonparametric Kruswal−Wallis and the posthoc Dunns test when the distribution was not normal. To compare two treatments, Student's *t* test was applied. Differences were considered to be statistically significant when $P < 0.05$.

RESULTS

Phenolic Compounds. Tested compounds included a flavone (chrysin), a flavanone (naringenin), isoflavones (daidzein and genistein), flavonols (kaempferol, myricetin, quercetin, and rutin), flavanols (catechin, epicatechin, and epigallocatechin-3-gallate), and a stilbene (resveratrol) (**Figure 1**). After 3 h in contact with JAR cells, all these compounds inhibited aromatase activity. To compare inhibitory strength, cells were incubated with different concentrations of these compounds, and the concentration required for 50% inhibition of aromatase activity (IC_{50}) was determined. Chrysin, naringenin, and quercetin were the most potent polyphenols in inhibiting aromatase activity (**Table 1**). Kaempferol, myricetin, and resveratrol did also markedly inhibit aromatase, while epigallocatechin-3-gallate had a less-pronounced effect, with an IC_{50} of 1770 (1501−2088) μmol/L (**Table 1**). For catechin, epicatechin, genistein, and rutin it was not possible to assess the IC_{50}; therefore, the maximal aromatase activity reduction (% of control) was determined. Catechin (1 mmol/L), epicatechin (2 mmol/L), genistein (1 mmol/L), and rutin (1 mmol/L) reduced aromatase activity by 31, 40, 26, and 23%, respectively (**Table 2**). None of the tested compounds interfered with cell viability (results not shown). Concerning aromatase expression as determined by western blotting and RT-PCR, no effect of any of the polyphenols was found after treatment of JAR cells with the compounds for 24 h (results not shown).

Table 2. Inhibition of Aromatase by Catechin, Epicatechin, Genistein, and Rutin[a]

phenolic compound	aromatase activity (% of control)	conc mmol/L
catechin	64.2± 5.5%[b]	1
epicatechin	60.5 ± 2.1%[b]	2
genistein	74.0 ± 6.0%[b]	1
rutin	77.9 ± 3.7%[b]	1

[a] JAR cells were preincubated (2 h) and incubated (1 h) in the presence of the test compound (or vehicle). The incubation was carried out with 54 nM [^{3}H]androstenedione, at 37 °C. Results represent means ± SE ($n = 6$). [b] $P <$ 0.05 vs control.

Beverages. RW, WW, AF-RW, AF-WW, GT, and BT were tested on JAR cells to determine their putative effect on aromatase activity. In comparison with basal activity (16.6 ± 3.0 pmol/mg protein/h), all beverages, with the exception of WW and AF-WW, were able to significantly decrease aromatase activity (**Figure 2**). This modulation was, in all cases, independent of changes in cell viability, which was not affected by these treatments. RW and AF-RW were able to markedly inhibit aromatase activity (to 56.9 ± 4.0% and 49.4 ± 2.8% of control, respectively). GT and BT did also reduce aromatase activity in JAR cells (to 55.8 ± 10.4% and 57.7 ± 9.9% of control, respectively).

DISCUSSION

It is no longer a matter of debate that food habits largely influence biological events with strong repercussion in individual health. For example, the Mediterranean diet, which has been the subject of scientific interest for the past few years, is believed to result in lower incidence of cancer and cardiovascular diseases (*28*, *29*).

A feature that makes this such a special kind of diet is its high levels of polyphenols. Wine and tea are polyphenol-rich

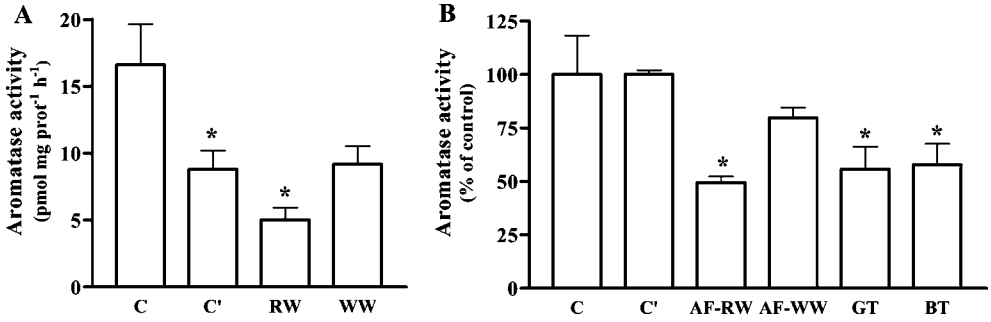

Figure 2. Effect of beverages on aromatase activity. (**A**) Wine ($n = 6$; C, no treatment; C′, 12% (v/v) ethanol control; RW, red wine; WW, white wine). $^{*}P < 0.05$ vs C, C′, and WW. (**B**) Alcohol-free beverages ($n = 6$; C, no treatment; C′, 0.9% NaCl (w/v) control; AF-RW, alcohol-free red wine; AF-WW, alcohol-free white wine; GT, green tea; BT, black tea). $^{*}P < 0.05$ vs C and C′. Confluent JAR monolayers were preincubated at 37 °C for 2 h and then incubated for 1 h in the presence of 200 μL/mL of beverages (or vehicle). The incubation was carried out with 54 nM [^{3}H]androstenedione, at 37 °C. Results represent means ± SEM.

beverages which have been reported to possess cancer-preventive activity. Some of the mechanisms by which this effect occurs have already been elucidated and include antioxidant, antiinflamatory, antimutagenic, antiangiogenic, antiestrogenic, and cell cycle regulating actions (*10*, *13*, *16*).

Thus, we considered it to be of interest to know which kind of effect diet polyphenols would have on estrogen synthesis. To do so, JAR cells were incubated with increasing concentrations of selected polyphenols and the IC_{50} was determined for each compound.

Most of the polyphenols tested could inhibit aromatase, chrysin, naringenin, quercetin, kaempferol, myricetin, and resveratrol showing the strongest inhibitory ability. Although chrysin, the most potent inhibitor of aromatase activity, with an IC_{50} of around 6 μM, has not yet been identified in any of the beverages tested here, it is present in foodstuffs that are part of human diet (*30*). Naringenin, which is especially abundant in citrus fruits, but can be found in small amounts in red wine (RW), was also found to be a good inhibitor of aromatase. These results are in agreement with what has been described by others (*31*−*33*).

Flavonols quercetin, kaempferol, myricetin, and rutin may also be found in wine and tea (*13*, *34*). The first three produced a strong reduction, while the latter had almost no effect on aromatase activity. Because rutin is a rhamnoside of quercetin, its crossing of the plasma membrane depends on the hydrolysis of the sugar moiety by β-glucosidases (*35*) which do not exist in JAR cells. Other authors have also found no effect of 100 μmol/L of rutin on aromatase activity (*19*).

Catechins are the most abundant polyphenols found in all three types of beverages tested. Catechin and epicatechin are mainly present in wine (*34*), and epigallocatechin-3-gallate is more abundant in tea, especially in GT (*13*). Satoh et al. (*22*) found that epigallogatechin-3-gallate reduced aromatase activity in placental microsomes with an IC_{50} of 60 μmol/L (vs 1770 μmol/L found in this study). The fact that, in our experimental model, this compound has to cross the plasma membrane to penetrate the cell may explain the differences of potency between our and other results.

Genistein and daidzein are isoflavones and had previously been described as having a poor aromatase inhibitory ability (*33*), which is corroborated by our results. In fact, daidzein produced no inhibition of aromatase when tested up to 1 mmol/L. Genistein (1 mmol/L) reduced aromatase activity only slightly, to 74% of that of the control.

Some of these compounds possess aromatase inhibitory activity in concentrations above the levels found after the ingestion of polyphenol-rich usual sources. However, the results show the possibility of compounds from plant-based foods to interfere with estrogen synthesis. Even if not of interest for nutritional reasons, the pharmacological interest is indisputable.

As expected from the results obtained in the presence of polyphenol compounds, the present work showed that RW and tea inhibited aromatase activity in JAR cells.

Wine is produced from the fermentation of grapes of the species *Vitis vinifera*. During this process grape skins and seeds are kept in contact with growing ethanol concentrations for longer or shorter periods of time, in the production of RW or WW, respectively. This will largely determine the polyphenolic content of the wine (more than 1000 mg/L in RW and less than 50 mg/L in WW) (*34*). The incubation of JAR cells with RW resulted in strong inhibition of aromatase, as opposite to WW, which had no effect on this enzyme. This difference suggests that polyphenols may be involved in aromatase modulation.

In using the beverages in direct contact with the cells, we have tried to maintain the original properties of the beverage. With the manipulation of the wine matrix, fractionating or evaporating and reconstituting the beverages, we could possibly lose compounds or the interaction between them.

Aromatase catalyses a critical step for estrogen production from circulating androgens (*8*). Estrogens have a role in estrogen-dependent cancers such as some endometrium and breast cancers (*6*, *35*), and estrogen synthesis blockade has unquestionable value as a therapeutic approach in breast cancer management (*36*).

In most epidemiological studies, alcohol consumption is dose-dependently related to the increase in the risk of developing certain forms of cancer (e.g., digestive tract, liver, and breast) (*37*). However, in the specific case of breast cancer there are some studies (*12*) suggesting that the risk varies according to the type of alcoholic beverage consumed, spirits being related to an increased risk, whereas RW decreased breast cancer risk. Interestingly, in a breast cancer family cohort, WW consumption showed a positive association and RW an inverse association with breast density, a known risk factor for breast cancer (*12*). Our results showed that the effect of AF beverages (AF-RW and AF-WW) was not significantly different from that of the same alcohol-containing beverages. This means that their ethanol content is not responsible and is not important for the observed aromatase inhibitory effect.

When tested alone, ethanol, the main ethanol present in wines, was able to significantly decrease aromatase activity. However, as just shown, the effect of the beverages with and without ethanol on aromatase activity was the same.

GT is the beverage prepared by the infusion of dry leaves of *Camellia sinensis*, which is also rich in polyphenols. For the

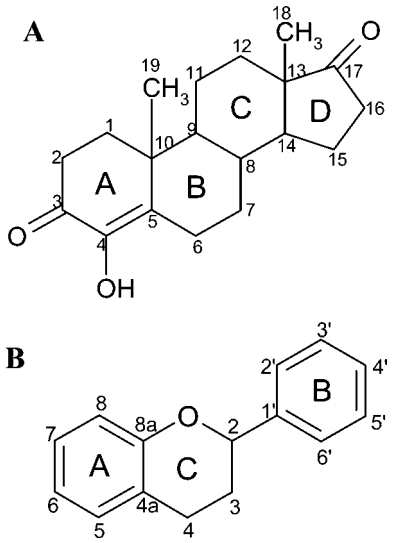

Figure 3. Structural homology between the aromatase substrate (**A**) and the basic flavonoid structure (**B**).

production of BT, leaves are allowed to oxidize prior to drying, which results in the production of new compounds (*13*). There have been several studies concerning GT and BT's ability to reduce the genesis of estrogen-dependent or -independent cancers such as ovarian and skin cancer (*38*, *39*). In the present study, GT and BT did significantly inhibit aromatase, and this may have impact on the development of breast cancer.

Thus, the results obtained in the presence of polyphenolic compounds may partially account for the inhibition of aromatase activity observed in the presence of wine and tea. However, it is important to bear in mind that these beverages are complex mixtures of polyphenols and other compounds may contribute to their effect. The occurrence of additive/synergistic effects is also possible.

Despite their effects on aromatase activity, when the same polyphenols were tested for 24 h in JAR cells, no effect on aromatase expression was observed. Thus, the modulation of aromatase activity by polyphenols might be due to their interaction with the enzyme or with the metabolic pathways regulating its activity, without effects on the expression of the *CYP19* gene. In fact, it has been suggested that polyphenolic rings A and C mimic aromatase substrate rings C and D, being thus able to inhibit aromatase (**Figure 3**) (*40*). Curiously, in our study, the compounds with the strongest inhibitory effects possessed a carbonyl group on the C4 of ring C. This feature may be relevant for the interaction of polyphenols with aromatase. The disturbance of the CYP catalytic cycle is also possible, since polyphenols are antioxidant molecules that may either reduce NADPH or O_2 availability, decreasing the possibility of the aromatase reaction to proceed. This is supported by the fact that some of the tested beverages, or their compounds, are able to inhibit other CYP isoenzymes, as demonstrated for RW (*41*, *42*), tea (*15*), and resveratrol (*42*, *43*).

Although detailed knowledge on polyphenol bioavailability is still lacking, there is evidence that after consumption of polyphenol-rich foods or beverages these compounds appear in plasma in concentrations varying with the polyphenol in question and the amount ingested (*43*). Although in this study some of the polyphenols showed aromatase-inhibiting activities in concentrations far above those present in plasma after consumption of a single serving of drink, one should not discard the possibility of those plasma levels being higher after the simultaneous ingestion of polyphenols from several sources. Furthermore, once several compounds have been shown to be able to inhibit aromatase, it is possible that these molecules exert additive effects, producing a measurable and significant reduction of estrogen synthesis in vivo.

In conclusion, RW and tea are able to modulate aromatase activity, which may, at least in part, be explained by the polyphenols contained in these beverages. The interference of these products with estrogen synthesis may make them interesting for the prevention/treatment of estrogen-dependent disorders such as breast cancer.

ABBREVIATIONS USED

AF, alcohol-free; BT, black tea; CYP, cytochrome P450; DEPC, diethyl pyrocarbonate; ER, estrogen receptor; GAPDH, glyceraldehyde phosphate dehydrogenase; GT, green tea; LDH, lactate dehydrogenase; NADPH, nicotinamide−adenine dinucleotide (reduced form); RW, red wine; WW, white wine.

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