Phytochemicals Inhibit Catechol-O-Methyltransferase Activity in Cytosolic Fractions from Healthy Human Mammary Tissues: Implications for Catechol Estrogen-Induced DNA Damage

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Phytochemicals are natural dietary constituents of fruits and vegetables. Some of these phytochemicals are known to affect estrogen-metabolizing enzymes. In breast tissue, estradiol can be metabolized to the catechol estrogens 2- and 4-hydroxyestradiol (2-OHE2 and 4-OHE2). Catechol estrogens are suspected carcinogens potentially involved in the etiology of breast cancer. Catechol-O-methyltransferase (COMT) converts the catechol estrogens to their inactive methoxy derivatives (2-MeOE2 and 4-MeOE2). In this study we investigated the effects of several phytochemicals on COMT activity in cytosolic fractions of seven healthy human mammary tissues from reduction mammaplasty. Large interindividual variations were observed in the constitutive levels of COMT activity. However, in all cytosol samples the catalytic efficiency of COMT was greater for 2-MeOE2 formation than for 4-MeOE2 formation. The known COMT inhibitor Ro 41-0960 and several phytochemicals with a catechol structure (quercetin, catechin, and (-)-epicatechin) concentration-dependently inhibited COMT activity, while phytochemicals without a catechol structure (genistein, chrysin, and flavone) showed no effect up to 30 μM. Distinct interindividual variations were observed in sensitivity toward COMT inhibition among the various tissue samples, as was shown by the range in IC50 values for Ro 41-0960 (5–42 nM). The toxicological relevance of COMT inhibition and the effect of reduced inactivation of catechol estrogens was studied by determining the amount of catechol estrogen-induced DNA damage in MCF-7 cells using the comet assay. Catechol estrogens alone caused no increase of DNA damage compared with control treated cells. However, both Ro 41-0960 and quercetin caused a decrease of methoxy estradiol formation and an increase of catechol estrogen-induced DNA damage in MCF-7 cells. This suggests that phytochemicals with a catechol structure have the potential to reduce COMT activity in mammary tissues and may consequently reduce the inactivation of potentially mutagenic estradiol metabolites and increase the chance of DNA damage.

Key Words: phytochemicals; catechol estrogens; COMT; DNA damage.

During the last decades an increase has been observed in the occurrence of malignant neoplasms of the female breast in industrialized countries. Presently, it is one of the major causes of death among women in Western countries. In the etiology of breast cancer, estrogens play a key role in tumor development. The role of estrogen in carcinogenesis has been suggested to be dual; it may act by stimulating cell transformation and cell proliferation, and it may act as a tumor initiator through its metabolites by inducing damage to cellular macromolecules such as DNA (Cavaliere et al., 1997; Clemons and Goss, 2001; Lemon et al., 1992; Liehr, 1997; Yager, 2000). The tumor-initiating action of estrogens is believed to be a result of hydroxylation of the main estrogen in premenopausal women, 17β-estradiol (E2), to the catechol estrogens 2- and 4-hydroxyestradiol (Badawi et al., 2001; Martucci and Fishman, 1993; Ziegler et al., 1997). Unless inactivated, catechol estrogens can undergo oxidation to reactive quinones (Cavaliere et al., 1997). Quinones of 2-hydroxyestradiol (2-OHE2) can form stable DNA adducts that remain in the DNA unless repaired, but quinones of 4-hydroxyestradiol (4-OHE2) can form depurinating DNA adducts, a potential tumor-initiating event in human cancers (Cao et al., 1998; Cavaliere et al., 1997; Liehr, 1997; Yager, 2000). Catechol-O-methyltransferase (COMT) plays an important role in the inactivation of catechol estrogens (Ball and Knuppen, 1980; Weisz et al., 1998). COMT is a phase II enzyme involved in the inactivation of many endogenous catechol substrates by transferring a methyl group from S-adenosyl-L-methionine (SAM) to the substrate and thus converting them into their methoxy derivatives (reviewed by Männisto and Kaakkola, 1999).

Low COMT activity has been associated with increased breast cancer risk (reviewed by Yue et al., 2003). There are several ways in which COMT activity might be altered. Lachman et al. have described a low-activity form of COMT resulting from a genetic polymorphism (Lachman et al., 1996). A single nucleotide substitution in codon 108 causes an amino acid transition (Val → Met) which results in a high- (Val/Val) or low-activity (Met/Met) form of the COMT enzyme with a three-to-four-fold difference in activity. COMT activity can also be inhibited by substrate competition for the enzyme. There are many naturally
occurring substrates for COMT in the body, but some exogenous compounds have also been identified as substrates for the enzyme. For example, certain catechol metabolites of PCBs have been shown to inhibit COMT activity (Garner et al., 2000). In addition, many dietary catechols, such as phytochemicals, can be a substrate for COMT. Phytochemicals are a diverse group of chemicals which can be found in fruits and vegetables. This group of biologically active compounds occurs in high concentrations in our diet, and the daily intake can comprise a few hundreds of milligrams per day (Hollman and Katan, 1999). As a result, submicromolar plasma levels can be reached (Hollman and Katan, 1999; Rein et al., 2000; Warden et al., 2001). Phytochemicals have been shown to possess antioxidant, anticancer, and antiviral properties. Because of these properties, they are generally regarded as safe, and many phytochemicals are sold in high concentrations as dietary supplements with recommended intake levels that exceed normal daily intake up to a 100-fold. However, in addition to the beneficial properties, phytochemicals may also affect various enzyme activities. For example, Zhu and Liehr described the effect of quercetin, a phytochemical found in many food items, on COMT activity in male Syrian hamsters (Zhu and Liehr, 1996). In hamsters fed with quercetin, a decreased COMT activity was found that resulted in increased catechol estrogen concentrations in the kidneys and subsequent enhancement of estradiol-induced tumorigenesis.

In the present study, we investigated COMT activity in healthy mammary tissues, where COMT plays an important role in the inactivation potentially genotoxic catechol estrogens. We studied the constitutive rates of O-methylation of catechol estrogens and the effects of phytochemicals on this activity in healthy human mammary tissue cytosol. We hypothesized that phytochemicals with a catechol structure, like quercetin, catechin, and (-)-epicatechin (chemical structures in Fig. 1), make a suitable substrate for the COMT enzyme and thus potentially inhibit the formation of methoxyestrogens. We also investigated the effects of several phytochemicals without a catechol structure (genistein, chrysin, and flavone) on COMT activity. Ro 41-0960, a known selective COMT inhibitor, was used as a standard positive control. COMT inhibition results in decreased activity of catechol estrogens, which in turn may lead to increased DNA damage. Therefore, we studied the implications of decreased COMT activity caused by phytochemicals on catechol estrogen-induced DNA damage by performing the alkaline comet assay using the malignant human mammary tumor cell line MCF-7.

MATERIALS AND METHODS

Chemicals and reagents. S-adenosyl-L-methionine (SAM), dithiothreitol (DTT), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Ro 41-0960 (2’-fluoro-3,4-dihydroxy-5-nitrobenzophenone), quercetin, catechin, (-)-epicatechin, chrysin, genistein, and flavone were obtained from Sigma (St. Louis, MO). Estrogen standards were obtained from Steraloid Inc. (Newport, RI), and N.O-bis(trimethylsilyl) trifluoroacetamide/trimethylchlorosilane (BSTFA/TMCS) was purchased from Supelco (Bellefonte, PA).

Sample preparation. Tissues from reduction mammoplasty (n = 7) were obtained from the Antonius Hospital (Nieuwegein, the Netherlands). The study was approved (number TME/Z-02.09) by the medical ethical committee of the hospital. All women gave permission for the removed tissue to be used by an informed consent. Tissues were diagnosed as histologically normal breast tissue by a pathologist. Upon arrival in our laboratory, fresh tissues were snap frozen in liquid nitrogen and stored at −70°C until use. Before preparation of cytosolic fractions, the tissues were thawed at 4°C and kept on ice. Adipose tissue was removed with a surgical knife, and the remaining parenchyma was cut into small pieces. The tissue pieces were weighed, and 3 ml cold phosphate buffer (50 mM, pH 7.6 containing 0.1 mM EDTA) was added per mg tissue. This mixture was homogenized with a Potter–Elvehjem Teflon-glass homogenizer. Cytosolic fractions were prepared through ultracentrifugation (Beckman L7-55). Homogenates were first centrifuged at 10,000 × g for 15 min at 4°C to remove the cell debris and remaining adipose tissues. Subsequently, the supernatant was centrifuged at 100,000 × g for 75 min at 4°C to separate the cytosolic (supernatant) from the microsomal (pellet) fractions. Aliquots of the cytosolic fractions were stored at −70°C until analysis. Protein contents of the fractions were determined by the method of Lowry et al. (1951) using bovine serum albumin (BSA) as protein standard.

COMT activity. In order to study the O-methylation activity, cytosolic protein (300 μg) was incubated with 50 mM phosphate buffer (pH 7.6), 5 mM MgCl2, 150 μM SAM, 1 mM DTT, and various concentrations of a phytochemical or the solvent vehicle (0.1% v/v MeOH) to a final volume of 492.5 μl. Reaction mixtures were incubated at 37°C for 5 min before the reaction was started by adding 2-0HE2 and 4-OHE2 (3.75 μM each). After 30 min, the reaction was stopped by generating the reaction tubes on ice. The metabolite extraction procedure was adapted from Spink et al. and performed as described previously (Spink et al., 1990; van Duursen et al., 2003). Briefly, the internal standard (20 μl equinil, 10 μM) was added, and 2- and 4-MeOE2 were extracted with dichloromethane. Trimethylsilyl derivatives of the estrogens were prepared and analyzed by GC/MS. Peak areas were determined at m/z 446 and 340 for 2- and 4-MeOE2, respectively. Peak identification and quantification was performed with the corresponding standards.

Cell lines and cell culture. MCF-7 cells were obtained from the American Type Culture Collection (Rockville, MD, USA) and cultured in Dulbecco’s Modified Eagle’s Medium supplemented with 0.01 mg/ml insulin, 5% fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were cultured in a humidified atmosphere with 5% CO2 at 37°C.

Cell viability. The cell viability was determined by measuring the capacity of the cells to reduce MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to the blue-colored formazan (Denizot and Lang, 1986). Cells were cultured in a humidified atmosphere with 5% CO2 at 37°C.

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Alkaline single-cell gel electrophoresis (comet) assay. The effect of COMT inhibition on DNA damage caused by catechol estrogens was determined using the single-cell gel electrophoresis (comet) assay as described by Singh et al., with some modifications (Singh et al., 1988). For this assay, 5 × 105 MCF-7 cells were plated onto 12-well plates and placed in a humidified atmosphere with 5% CO2 at 37°C. The next day, cells were exposed for 5 h to serum-free medium containing the solvent vehicles (ethanol, methanol, and DMSO, total of 0.17% v/v) and catechol estrogens (7.5 μM 2-OHE2 and 4-OHE2), Ro 41-0960 (10 μM),
and quercetin (10 µM or 30 µM), alone or in combination. Then, media were removed and analyzed for methoxy estradiol concentrations as described above. The cells were washed with PBS, and 100 µl trypsin was added. As soon as the cells detached, 1 ml of warm medium containing 5% FBS was added, and the cells were suspended and transferred to a 1.5-ml Eppendorf cup. The cell suspension was briefly centrifuged, and 1000 µl of the supernatant was removed. The remaining cells were gently resuspended, and a 10 µl aliquot was added to 90 µl warm 0.5% low-melting agarose. This mixture was spread onto a frosted slide covered with 1.5% normal melting agarose and placed on an ice-cold glass plate to solidify. Then, the slides were placed in freshly prepared cold lysis solution (2.5 M NaCl, 0.1 M Na₂EDTA, 10 mM Tris containing 1% Triton-X, and 10% DMSO, pH 10) for 1 h at 4°C. After this, the slides were kept in the dark to prevent DNA damage by exposure to direct light. Subsequently, the slides were placed in a horizontal slide holder in an electrophoresis unit containing cold electrophoresis

FIG. 1. Chemical structures of the compounds used in this study; phytochemicals with a catechol structure (quercetin, catechin, and (-)-epicatechin), without a catechol structure (flavone, chrysin, and genistein) and the known COMT inhibitor Ro 41-0960.
solution (0.3 M NaOH, 0.001 M EDTA) for 25 min and then electrophoresed for 25 min at 25 V, 290–310 mA. Then, the slides were washed three times with a sterile neutralization buffer (0.4 M Tris/HCl, pH 7.5) and dehydrated for 10 min in 100% ethanol. The slides were kept in a dark box at 4°C until analysis. Prior to analysis, the slides were stained with ethidium bromide (20 μg/ml). Analysis was performed under a fluorescence microscope using a 20× objective and a filter of 450–490 nm equipped with a digital camera. Of each treatment 175–200 cells (four slides per treatment, 40–50 cells per slide) were analyzed and the tail moment (comet tail length × % tail DNA) was determined using the PC image-analysis program Casp described by Konca et al. (2003).

**Data analysis.** Enzyme kinetic parameters (V max and K m, values in pmol/min/mg protein and μM, respectively) were calculated with Prism 3.0 (GraphPad Software, San Diego, CA). Statistical significance of difference of the mean was determined by the Student’s t-test. Variance and differences among the means were determined by a one-way ANOVA with a Tukey-Kramer Multiple Comparisons test using GraphPad InStat 3.06 (GraphPad Software Inc., San Diego, CA).

## RESULTS

**Constitutive COMT activity**

Enzyme kinetics were studied in samples 1 and 4 by determining the MeOE 2 formation at various equimolar concentrations of 2- and 4-OHE 2. The concentration of SAM, the methyl donor for O-methylation, in the incubation mixture was 150 μM, which was a saturating concentration (data not shown). After 30 min, MeOE 2 concentrations were determined. In both samples the catalytic efficiency of COMT was higher for 2-MeOE 2 formation than for 4-MeOE 2 formation. In sample 1, catalytic efficiencies (V max/K m) were 3.0/5.0 = 0.6 and 1.3/9.5 = 0.1 for 2- and 4-MeOE 2 formation, respectively. In sample 4, catalytic efficiencies were 16.2/9.4 = 1.7 and 4.2/19.7 = 0.2 for 2- and 4-MeOE 2 formation, respectively. In both samples, the rate of methoxy formation was linear up to a concentration of 25 μM for at least 45 min (data not shown). An incubation of 30 min with 7.5 μM catechol estrogens was chosen to study the constitutive COMT activity and the effects of phytochemicals.

The constitutive rates of methylation of catechol estrogens in the various tissue samples are shown in Table 1. The mean 2-MeOE 2 formation of seven tissues was 8.12 ± 1.32 pmol/min/mg protein with a range of activity of 2.14–19.03 pmol/min/mg protein. For 4-MeOE 2, the mean metabolite formation and range of activity were 1.83 ± 0.29 and 0.37–3.81 pmol/min/mg protein, respectively. ANOVA analysis showed significant differences in 2- and 4-MeOE 2 formation between the tissue samples (p = 0.0004 and p = 0.0044, respectively). Further analysis showed that cytosol from tissue sample 3 had substantially higher rates of methoxy estradiol formation compared with other tissue samples. However, despite the variation in rates of methylation, the ratio of 4-MeOE 2/2-MeOE 2 formation at 7.5 μM 2- and 4-OHE 2 was not significantly different statistically between the tissues (ANOVA analysis, p = 0.125). The average 4-/2-MeOE 2 ratio in seven tissue samples was 0.26 ± 0.02.

**COMT Inhibition by Ro 41-0960**

Because substantial interindividual variation in methylation rates was observed, a concentration-response curve was made with Ro 41-0960, a known COMT inhibitor, for every tissue sample. IC 50 values for every tissue sample are listed in Table 1. Large variations between the tissue samples in responsiveness to Ro 41-0960 were observed. Sample 4 was the least responsive to Ro 41-0960, with an IC 50 value for COMT inhibition of 42.1 nM, and this IC 50 value was significantly different from samples 3 and 7. Although COMT has a higher constitutive activity with 2-OHE 2 than with 4-OHE 2 as substrate, inhibition of methylation occurred in the same order of magnitude for both 2- and 4-MeOE 2 formation (Fig. 2).

## Table 1

<table>
<thead>
<tr>
<th></th>
<th>2-MeOE 2 formation (pmol/min/mg protein)</th>
<th>4-MeOE 2 formation (pmol/min/mg protein)</th>
<th>4-MeOE 2/2-MeOE 2</th>
<th>IC 50 (nM) for Ro 41-0960</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>5.38 ± 1.45a</td>
<td>1.13 ± 0.14</td>
<td>0.21 ± 0.06a</td>
<td>33.6 ± 5.8a</td>
</tr>
<tr>
<td>Sample 2</td>
<td>2.17 ± 0.31</td>
<td>0.37 ± 0.05</td>
<td>0.17 ± 0.03</td>
<td>11.4 ± 5.0</td>
</tr>
<tr>
<td>Sample 3</td>
<td>19.03 ± 4.81a</td>
<td>3.81 ± 1.21c</td>
<td>0.20 ± 0.08</td>
<td>5.1 ± 2.7</td>
</tr>
<tr>
<td>Sample 4</td>
<td>5.38 ± 2.35</td>
<td>1.16 ± 0.24</td>
<td>0.22 ± 0.10</td>
<td>42.1 ± 14.3f</td>
</tr>
<tr>
<td>Sample 5</td>
<td>2.14 ± 0.47</td>
<td>0.67 ± 0.11</td>
<td>0.30 ± 0.02</td>
<td>27.5 ± 9.8</td>
</tr>
<tr>
<td>Sample 6</td>
<td>9.10 ± 1.34</td>
<td>2.31 ± 0.30</td>
<td>0.27 ± 0.03</td>
<td>8.6 ± 1.5</td>
</tr>
<tr>
<td>Sample 7</td>
<td>9.36 ± 1.30</td>
<td>2.53 ± 0.41</td>
<td>0.27 ± 0.04</td>
<td>5.8 ± 2.0</td>
</tr>
</tbody>
</table>

Note: After incubation of cytosol with an equimolar concentration of 7.5 μM 2- and 4-OHE 2.

- Mean ± standard error of the mean of 4 to 7 determinations.
- Mean IC 50 value (nM) of two duplicate determinations ± SEM.
- Significantly different from sample 1 and 4 (p < 0.05) and sample 2 and 5 (p < 0.01).
- Significantly different from sample 2 and 5 (p < 0.05).
- Significantly different from sample 3 and 7 (p < 0.05).
Effects of Phytochemicals on COMT Activity

The \(O\)-methylation of 7.5 \(\mu\)M 2- and 4-OHE\(_2\) in cytosolic fractions of human mammary tissues was concentration-dependently inhibited by quercetin, catechin, and epicatechin, as was expected based on their catechol structure (Fig. 3). While Ro 41-0960 fully inhibited COMT activity at the highest concentration tested (0.3 \(\mu\)M) in all tissue samples, the phytochemicals decreased COMT activity to about 30–40\% of the control activity at the highest concentrations tested (10–100 \(\mu\)M). IC\(_{50}\) values for COMT inhibition were 0.48 \(\mu\)M (tissue #1), 1.64 (tissue #1) and 1.96 \(\mu\)M (tissue #3) for quercetin, catechin, and epicatechin, respectively (Table 2). Phytochemicals without catechol structure were less potent COMT inhibitors. Genistein and chrysin appeared to reduce COMT activity slightly at the highest concentration tested (30 \(\mu\)M). Flavone, on the other hand, decreased COMT activity in tissue sample 3 to 40\% of the control levels at the highest concentration tested (100 \(\mu\)M) with an IC\(_{50}\) value of 5.5 \(\mu\)M. ANOVA analysis showed that only the differences in IC\(_{50}\) values for quercetin and flavone were statistically significant \((p < 0.05)\). However, in tissue sample 5, which was less responsive to COMT inhibition by Ro 41-0960, no inhibitory effect on COMT activity could be detected (data not shown).

Because of the large variations among the tissue samples in responsiveness to COMT inhibition, the potencies of the phytochemicals to inhibit COMT activity were calculated relative to the inhibitory potency of Ro 41-0960 in the same tissue sample (Table 2). For quercetin, catechin, epicatechin, and flavone the relative potencies were 0.07 (tissue #1), 0.021 (tissue #1), 0.0026 (tissue #3), and 0.0009 (tissue #3), respectively.

Catechol Estrogen-Induced DNA Damage in MCF-7 Cells

To investigate the implications of possible COMT inhibition by quercetin in whole cells, the effects of quercetin on catechol
estradiol formation in MCF-7 cells was 148.1 pmol/h/10^6 cells for 2-MeOE2 and 4-MeOE2, respectively. Constitutive methoxy estrogen levels were determined in the culture media of MCF-7 cells used for the comet assay. DNA damage could be attributed to decreased COMT activity, as shown by decreased COMT inhibition resulting in a decreased methoxy estradiol formation compared with control cells. A concentration-dependent decrease in methoxy estradiol formation was seen when cells were co-incubated with 10 and 30 μM quercetin, respectively, compared with vehicle-treated control cells.

To study whether the increase in catechol estrogen-induced DNA damage could be attributed to decreased COMT activity, methoxy estrogen levels were determined in the culture media by GC/MS analysis. An equimolar concentration of catechol estrogens (7.5 μM), catechol estrogens together with quercetin (30 μM) or Ro 41-0960 (10 μM), or a combination of the two COMT inhibitors, did not cause cytotoxicity in MCF-7 cells, as determined by the MTT test (data not shown). Incubation of MCF-7 cells with catechol estrogens, Ro 41-0960, or quercetin alone did not cause a significant increase of DNA damage compared with the solvent vehicle-treated cells (Fig. 4). The extent of background DNA damage was significantly increased (by about 200%) in catechol estrogen-exposed cells when COMT was inhibited by 10 μM Ro 41-0960 (p < 0.05). When quercetin was added to the cells together with Ro 41-0960, catechol estrogen-induced DNA damage increased even further, in an apparent concentration-dependent manner, compared with Ro 41-0960-treated cells. A concentration-dependent increase of catechol estrogen-induced DNA damage was also seen after incubation with catechol estrogens and quercetin alone. Catechol estrogen-induced DNA damage levels increased 76 and 160% when cells were co-incubated with 10 and 30 μM quercetin, respectively, compared with vehicle-treated control cells.

To study whether the increase of catechol estrogen-induced DNA damage could be attributed to decreased COMT activity, methoxy estrogen levels were determined in the culture media of the MCF-7 cells used for the comet assay. Constitutive methoxy estradiol formation in MCF-7 cells was 148.1 ± 5.4 and 85.5 ± 4.9 pmol/h/10^6 cells for 2-MeOE2 and 4-MeOE2, respectively (Fig. 5). Incubation with 10 μM Ro 41-0960 inhibited methoxy estradiol formation by about 98% compared with control cells. Incubation with both Ro 41-0960 and quercetin did not significantly change methoxy estradiol formation compared with Ro 41-0960 alone (data not shown). In culture media of MCF-7 cells incubated with catechol estrogens and quercetin, a concentration-dependent decrease in methoxy estradiol formation compared with control cells was seen. Incubation with 10 μM quercetin resulted in a 46% and 18% decrease in 2- and 4-MeOE2 formation, respectively. For 30 μM the decrease was 82% and 73% for 2- and 4-MeOE2 formation, respectively.

These data indicate that the increase in catechol estrogen-induced DNA damage in MCF-7 cells shown in the comet assay was due to COMT inhibition resulting in a decreased inactivation of the catechol estrogens, as shown by decreased methoxy estradiol formation.

**DISCUSSION**

**Constitutive COMT Activity and Inhibition by Ro 41-0960**

In this study we compared constitutive COMT activities in seven healthy human mammary tissue samples. In line with other studies, we found a higher catalytic activity of COMT with 10 μM quercetin. Catechol estrogen-induced DNA damage in MCF-7 cells and the effect of COMT inhibition. MCF-7 cells were incubated with 7.5 μM catechol estrogens (CE), 10 μM Ro 41-0960, and/or quercetin (10 or 30 μM). Exposure of the cells to UV light (1 min) was used as a positive control. The experiment was performed twice in duplicate, and 40–50 comets per slide were analyzed. Data are represented as mean Tail Moment (comet tail length × % of total DNA in the comet tail) of four slides ± SEM. * Significantly different from vehicle control-treated and CE-treated cells (p < 0.05). # Significantly different from vehicle control-treated and CE-treated cells (p < 0.05).

**TABLE 2**

<table>
<thead>
<tr>
<th>Tissue sample number</th>
<th>QUE IC50 (μM)</th>
<th>CAT IC50 (μM)</th>
<th>GEN IC50 (μM)</th>
<th>EPI IC50 (μM)</th>
<th>CHR IC50 (μM)</th>
<th>FLA IC50 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.48 ± 0.19</td>
<td>1.64 ± 0.84</td>
<td>NA</td>
<td>1.96 ± 0.44</td>
<td>NA</td>
<td>5.49 ± 1.92</td>
</tr>
<tr>
<td>2</td>
<td>33.6 ± 5.8</td>
<td>33.6 ± 5.8</td>
<td>11.4 ± 5.0</td>
<td>5.1 ± 2.7</td>
<td>5.1 ± 2.7</td>
<td>5.1 ± 2.7</td>
</tr>
<tr>
<td>3</td>
<td>0.070</td>
<td>0.021</td>
<td>NA</td>
<td>0.0026</td>
<td>NA</td>
<td>0.0009</td>
</tr>
</tbody>
</table>

Note: Quercetin (QUE), catechin (CAT), epicatechin (EPI), chrysin (CHR), genistein (GEN) and flavone (FLA).

*RPs are calculated by dividing the IC50 value for COMT inhibition by Ro 41-0960 by the IC50 value of the phytochemical in cytosol from the corresponding tissue sample.

*Significantly different from IC50 value for flavone.
reported that quercetin acted as inhibitor of COMT activity inhamster kidney cytosol, with IC50 values of 8 μM and 2 μM atsubstrate concentrations of 10 μM 2-OHE2 and 4-OHE2, respectively (Zhu and Liehr, 1996). We found in this study lower IC50values for COMT inhibition with quercetin (0.5 μM for both2-OHE2 and 4-OHE2). This is probably due to differences in studydesign; we added both 2- and 4-OHE2 together to thecytosol, and we used human COMT. Zhu and Liehr concludedthat quercetin acted as a noncompetitive inhibitor of COMTactivity by competing for SAM. Quercetin and other phytochemicalscontaining a catechol structure have shown to be a substratefor COMT and, thus, compete for cofactors necessary for O-methylation of the substrate, such as SAM (Zhu et al., 2000).Upon O-methylation, a methyl group from SAM is transferred tothe catechol substrate resulting in S-adenosyl-L-homocysteine(SAH). An increasing concentration of SAH was shown to (noncompetitively) inhibit the association of the methyl donor SAMwith COMT. This might also explain why phytochemicalsreduced COMT activity to 60% of the control activity, whileRo 41-0960 fully inhibited methylation. Ro 41-0960 is a poorsubstrate for COMT, but it binds tightly to the catalytic site ofthe enzyme, thus inhibiting methylation of other substrates withoutdepletion of cofactors (Backstop et al., 1989; Ding et al., 1996).

The tested phytochemicals with a catechol structure, quercetin,catechin, and (-)-epicatechin, all reduced COMT activity, butlarge differences in inhibitory potency were found. This might bea result of structural differences of these phytochemicals, butinterindividual variations among the tissue samples inCOMT activity and responsiveness toward inhibition might alsoplay a role. Interindividual variation between the tissue sampleswas especially apparent with flavone, which showedCOMT inhibition in sample 3, but not in sample 5. In an attemptto correct for interindividual variations in sensitivity towardCOMT inhibition, we calculated the potency of a phytochemicalrelative to the potency of Ro 41-0960 to inhibit COMT activity inthesame tissue sample. The relative potencies (RPs) varied lessthan a 100-fold with the RPs of the three phytochemicals with a catecholstructure (e.g. quercetin, catechin, and epicatechin) beinghigher than the RP of flavone, the phytochemical without a catecholstructure. However, we did not study the potencies of allphytochemicals in all the tissue samples. As a result, it is not clearwhether the RPs represent the differences between individuals or differences between the potencies of the phytochemicals. Therefore, the calculated inhibition potencies of the phytochemicals, both absolute and relative to Ro 41-0960, should beconsidered with care.

**Catechol Estrogen-Induced DNA Damage in MCF-7 Cells**

Although COMT activity was inhibited by the phytochemicals in cytosol from healthy mammary tissues, the question wasraised if this inhibition is relevant in a more complex system suchas whole cells. We showed that incubation with Ro 41-0960or quercetin caused a significant increase in DNA damage by

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**FIG. 5.** Methoxy estradiol formation (pmol/h/10⁶ cells) in MCF-7 cells after incubation with 7.5 μM catechol estrogens (CE) or co-incubation with CE and Ro 41-0960 (10 μM) or quercetin (10 or 30 μM). Data are represented as means ± SD (n = 3). * Significantly lower than CE-treated cells.

**Effect of Phytochemicals on COMT Activity**

In this study we showed that phytochemicals with a catecholstructure are capable of inhibiting COMT activity in cytosolicfractions of healthy human mammary tissues. Zhu and Liehr
catechol estrogens compared with catechol estrogens alone. Chen et al. also showed by the comet assay a low potency of another catechol estrogen, 4-hydroxyestrone (4-OHE₁), to induce DNA damage in MCF-7 cells (Chen et al., 2000). They mainly attributed this low potency to the fact that 4-OHE₁ does not auto-oxidize and requires oxidative enzymes to generate the highly reactive quinone. However, our study suggests that the inactivation of catechol estrogens plays an important role in the potential of these compounds to cause DNA damage. We showed that a decrease in inactivation of the potentially genotoxic catechol estrogens by COMT inhibition caused a significant increase in catechol estrogen-induced DNA damage. Our data concur with the results described by Lavigne et al. (2001). They found a clear association between catechol estrogen levels and 8-oxo-dG levels in MCF-7 cells after treatment with estradiol and the COMT inhibitor Ro 41-0960. These data show that catechol estrogens have the potential to induce DNA damage, but that this is strongly dependent on the cellular capacity for inactivation by COMT.

Implications for Breast Cancer Development

Phytochemicals are often studied in relation with hormone-dependent cancers such as breast cancer. The low breast cancer incidence in Asian countries is often attributed to the soy-rich diet, which contains high concentrations of isoflavones like genistein. On the other hand, some studies describe a deleterious effect of certain phytochemicals in women with breast cancer. The low breast cancer incidence caused a significant increase in catechol estrogen-induced DNA damage. Our data concur with the results described by Katan, 1999a). It is not unlikely that higher levels can be reached, since quercetin plasma levels return to basal levels after about 20 h, so repeated consumption of high levels of quercetin can result in accumulation in the blood. Furthermore, the COMT-inhibiting properties of quercetin also resulted in decreased inactivation of potentially genotoxic catechol estrogens and an increase in catechol estrogen-induced DNA damage. Yet, it is difficult to predict the effects of excessive phytochemical intake in individuals, as large variations in COMT activity and responses to COMT inhibition between the various breast tissue samples were found. Nevertheless, this study shows that adverse effects of high levels of certain phytochemicals are not unlikely. Therefore, high intake of phytochemicals, for example through dietary supplements, should be considered with care.

REFERENCES


