Research Article

The influence of metabolism on the genotoxicity of catechol estrogens in three cultured cell lines

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The 2- and 4-hydroxy metabolites of 17β-estradiol (E2) and estrone (E1) are important for E2-mediated carcinogenesis due to the formation of genotoxic ortho-quinone metabolites. To assess the importance of metabolic conjugation for their genotoxicity, the DNA strand-breaking activity of the four catechol estrogens was determined in three cell lines with different activities of catechol-*O*-methyltransferase (COMT) and UDP-glucuronosyltransferase (UGT). Most DNA strand breaks were observed in V79 cells, which lack these metabolic activities. 2- and 4-hydroxy-E2 were 2.5 times more genotoxic than 2- and 4-hydroxy-E1. MCF-7 cells exhibit COMT activity, and the incidence of DNA strand breaks decreased with increasing methylation; only the 4-hydroxy metabolites of E1 and E2, which were poor substrates of COMT, exhibited low genotoxicity. HepG2 cells converted the catechol and methoxy metabolites of E2 to the respective E1 metabolites by 17β-hydroxysteroid dehydrogenase (HSD). Moreover, methylation and glucuronidation took place. Only 4-hydroxy-E1 elicited a weak genotoxic response in these cells. The extensive metabolism in HepG2 cells is proposed to account for the failure of catechol estrogens to induce DNA strand breaks. Thus, metabolism by COMT and UGT and, to a minor extent, by HSD is a major determinant for the genotoxicity of catechol estrogens in target cells.

Keywords: Alkaline unwinding / Catechol estrogens / Genotoxicity / Glucuronidation / Methylation

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1 Introduction

Over the years, the endogenous steroidal estrogen 17β-estradiol (E2) has been shown to induce various types of tumors in experimental animals [1, 2]. In humans, elevated level of E2 is a risk factor for cancer in hormone-dependent organs, particularly breast, endometrium, ovary, prostate, and testis [3 – 5]. Despite the clear association of E2 with cancer in humans and animals, the cellular and molecular mechanisms of estrogen-mediated carcinogenesis have not been fully elucidated to date. In addition to epigenetic events, *e.g.*, stimulation of cell proliferation mediated through estrogen receptors, there is growing evidence that estrogen-induced genetic damage may also play an important role [6 – 8]. In this context, the oxidative metabolism of E2 is of particular interest [6 – 13]. E2 is partly converted to estrone (E1) by the enzyme 17β-hydroxysteroid dehydrogenase (HSD), and both E2 and E1 are hydroxylated at various positions of the steroid moiety by cytochrome P450 (CYP)-dependent monooxygenases. Major phase I metabolites of E2 and E1 are the catechols 2-HO-E2/-E1 and 4-HO-E2/-E1 (Fig. 1). These catechol estrogens, which are carcinogenic in animals [14, 15] can be further oxidized to the respective 2,3- or 3,4-quinones, which have been shown to form DNA adducts [9, 12, 13]. In addition, the semiquinone/quinone intermediates generated from catechol estrogens can undergo redox-cycling to generate reactive oxygen species, which may also damage DNA [2, 6, 7]. In order to avoid genetic damage, quinones can be inactivated by reaction with glutathione, and catechol estrogens are conjugated through methylation by the enzyme catechol-*O*-methyltransferase (COMT) or through glucuronidation or sulfonation by the respective transferases [16 – 23]. Thus, the susceptibility of cells for genetic damage by steroid estrogens should depend on the rate of formation of catechol estrogens as well as their further metabolism (Fig. 1).

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Abbreviations: *t*-BuOOH, *tert*-butylhydroperoxide; **COMT,** catechol-*O*-methyltransferase; **E1,** estrone; **E2,** 17β-estradiol; **FCS,** fetal calf serum; **HSD,** 17β-hydroxysteroid dehydrogenase; **SAM,** *S*-adenosyl-L-methionine; **UDPGA,** uridine-5′-diphosphoglucuronic acid; **UGT,** uridine-5′-diphospho-glucuronosyltransferase

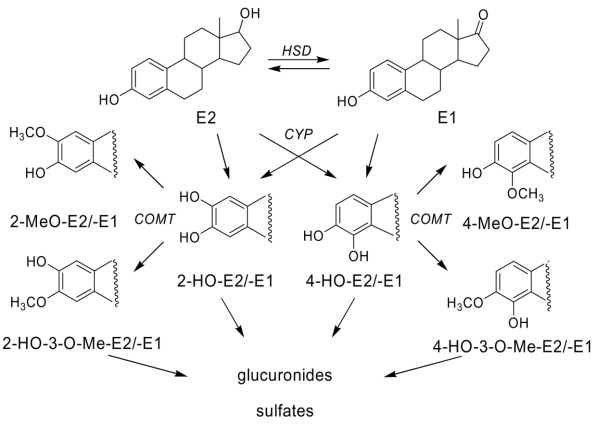

Figure 1. Formation of the catechol estrogens 2-HO-E2/-E1 and 4-HO-E2/-E1 and their subsequent metabolism through methylation, glucuronidation, and sulfonation.

In the present study, the importance of phase II metabolism for the genetic toxicity of the four catechol estrogens has been investigated by measuring the induction of DNA strand breaks in three cell lines with different metabolic capabilities, *i.e.*, Chinese hamster lung fibroblasts (V79) and human cancer cells from breast (MCF-7) and liver (HepG2).

2 Materials and methods

2.1 Chemicals, cell lines, and culture conditions

The four catechol estrogens 2- and 4-HO-E2 and -E1 were purchased from Steraloids (Wilton, New Hampshire, USA). *tert*-Butylhydroperoxide (*t*-BuOOH) was from Merck (Darmstadt, Germany) and other biochemicals and reagents were from Sigma/Aldrich/Fluka (Taufkirchen, Germany). V79, MCF-7, and HepG2 cells were obtained from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). MCF-7 cells were cultured in DMEM with 5% fetal calf serum (FCS, Invitrogen, Karlsruhe, Germany), V79 in DMEM with 10% FCS and HepG2 cells in DMEM containing Ham's nutrient mixture F-12 (DMEM/F12) with 10% FCS at 37°C in a water-saturated atmosphere containing 5% carbon dioxide. All cell culture media contained 100 U/mL penicillin and 100 mg/mL streptomycin.

2.2 Chemical stability of catechol estrogens

Because catechol estrogens are prone to autoxidation, their stability was tested in DMEM/F12 medium at 20°C in the absence of FCS. The final concentrations were 2.5 μM for the catechol estrogens and 0.1% for DMSO. Aliquots of 2.5 mL were removed after time intervals of 60 min, 3′,5′, 3″, 5″-tetramethylbisphenol A was added as a standard to a final concentration of 0.4 μM, and the aliquots were extracted with 2.5 mL of ethyl acetate. The extracts were then evaporated to dryness under reduced pressure, the residues dissolved in 30 μL methanol and analyzed by HPLC (see Section 2.4).

2.3 Methylation and glucuronidation in cell lysate

For the preparation of cell lysate, MCF-7 and HepG2 cells were collected by trypsination and centrifugation, and washed with cold PBS. Aliquots containing 5×10^6 MCF-7 cells or 10×10^6 HepG2 cells in 50 μL PBS were lysed by cooling to −80°C. These cell lysates were used as source of the enzymes COMT and uridine-5′-diphospho-glucuronosyltransferase (UGT). For conducting methylation reactions with the lysate of MCF-7 cells, $MgCl_2$ (final concentration 4 mM), the catechol estrogen dissolved in DMSO (final substrate concentration 5 μM and final DMSO concentration 1%) and 875 μL 0.1 M potassium phosphate buffer pH 7.4 ("phosphate buffer") were added. After incubating the mixture at 37°C for 5 min, the reaction was started by adding *S*-adenosyl-L-methionine (SAM, final concentration 500 μM) in phosphate buffer to give a final volume of 1000 μL, and further incubated for 20 min at 37°C. The incubation mixture was extracted with 2×500 μL ethyl acetate, the combined extract evaporated to dryness, the residue dissolved in 30 μL methanol, and analyzed by HPLC (see Section 2.4). For conducting simultaneous methylation and glucuronidation reactions, 5 μg alamethicin in 40 μL phosphate buffer was added to the lysate of HepG2 cells and the mixture was placed on ice for 15 min. $MgCl_2$ (final concentration 10 mM), the β-glucuronidase inhibitor saccharolactone (final concentration 10 mM), the catechol estrogen dissolved in DMSO (final substrate concentration 50 μM and final DMSO concentration 1%), and 23 μL of phosphate buffer were added. After incubating the mixture at 37°C for 5 min, the reaction was started by adding SAM (final concentration 500 μM) and uridine-5′-diphosphoglucuronic acid (UDPGA, final concentration 4 mM) in phosphate buffer to give a final volume of 200 μL and further incubated for 60 min. The reaction was terminated by adding 200 μL 0.7 M glycin/HCl buffer pH 1.2. The incubation mixture was extracted with 2×500 μL ethyl acetate, the combined extract evaporated to dryness, the residue dissolved in 30 μL methanol, and analyzed by HPLC (see Section 2.4).

2.4 HPLC analysis

A Beckman system equipped with a binary pump, a UV detector, and Gold Nouveau Version 1.6 software for data collection and analysis was used. Separation was carried out on a 250×4.6 mm id, 5 μm, RP Luna C18 column (Phenomenex, Torrance, CA, USA). Solvent A was deionized water adjusted to pH 3.0 with formic acid, and solvent B was ACN. A linear solvent gradient was started directly

after injection, changing from 33% B to 50% B in 16 min, then to 100% B in 4 min, staying at 100% B for 4 min, and returning to initial conditions within 1 min. The flow rate was 1 mL/min and the UV detector was set to 280 nm.

2.5 Alkaline unwinding

DNA strand breaks induced by catechol estrogens and control substances were measured by alkaline unwinding according to Hartwig *et al*. [24]. 1×10^5 V79 cells were plated in 40 mm culture dishes and allowed to attach for 24 h in DMEM containing 10% FCS. This medium was then replaced by FCS-free DMEM to which catechol estrogen was added as DMSO solution (final concentrations of estrogen 5 – 50 μM and final DMSO concentration 0.5%), and the V79 cells were further incubated at 37°C for 90 min. In the case of MCF-7 and HepG2 cells, 1.4×10^5 cells were allowed to attach for 48 h in FCS-containing DMEM and DMEM/F12, respectively, prior to incubation with the catechol estrogens in FCS-free medium for 3 h. After removing the medium and washing the cells with cold PBS, alkaline unwinding was conducted by exposing the cells to an alkaline solution (pH 12.3) containing 0.03 M NaOH, 0.01 M Na_2HPO_4, and 0.9 M NaCl for 30 min in the dark at 20°C. The samples were then neutralized to pH 6.8 with 0.1 M HCl and sonicated for 15 s at 0°C prior to adding SDS to a final concentration of 0.05%. Separation of ssDNA and dsDNA was carried out on hydroxyapatite columns at 60°C. The DNA in both fractions was stained with Hoechst dye 33258 (final concentration 7.7×10^{-7} M) and the resulting fluorescence measured at 465 nm (excitation at 360 nm).

3 Results

3.1 Metabolism of catechol estrogens in cell lysates

The three cell lines used in the present study were selected because of their different metabolic capabilities for the catechol estrogens. Pilot studies carried out in our laboratory showed that all three cell systems lack monooxygenase activity, but differ in their ability to methylate or glucuronidate. Whereas V79 cells proved to be devoid of these conjugation reactions, MCF-7 cells were quite efficient for the methylation and HepG2 for the methylation and glucuronidation of catechol estrogens. These activities of MCF-7 and HepG2 cells were studied in more detail, using lysate from the respective cells rather than the intact cells themselves in order to obtain higher metabolic conversion rates.

When lysate from MCF-7 cells was fortified with SAM and incubated with the catechol estrogens, formation of methylated catechols was detected by HPLC analysis. This clearly showed that MCF-7 cells had active COMT. 2-HO-E2/-E1 gave rise to two methylation products each, *i.e.*,

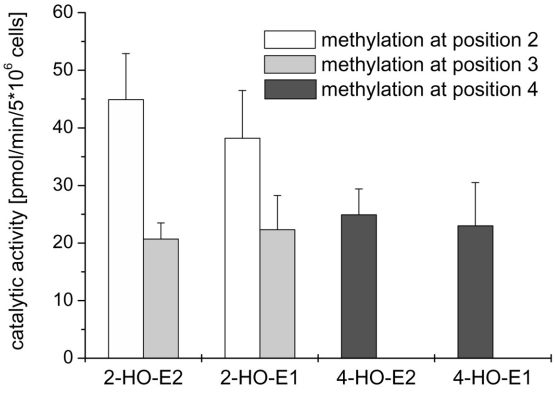

Figure 2. Activity of MCF-7 cell lysate for the methylation of catechol estrogens (5 μM). Data represent the mean ± SD of three independent experiments.

2-MeO-E2/-E1 and 2-HO-3-O-Me-E2/-E1 (Fig. 1). In contrast, only one methylated catechol, *i.e.*, 4-MeO-E2/-E1, was detected with 4-HO-E2 and 4-HO-E1. Comparison of the enzyme activities showed that 2-HO-E2/-E1 were much better substrates of COMT than 4-HO-E2/-E1 (Fig. 2). Addition of UDPGA to the lysate did not lead to the formation of glucuronidated catechol estrogens, indicating that MCF-7 cells are devoid of UGT activity.

In contrast to MCF-7 cells, lysates of HepG2 fortified with SAM and UDPGA gave rise to the formation of both methylated catechol estrogens and glucuronidated metabolites (Fig. 3) upon incubation with the catechol estrogens. Moreover, HepG2 cells are able to convert E2, its catechol, and methoxy metabolites to the respective E1 metabolites by the enzyme HSD. Therefore, incubation with 2-HO-E2 and 4-HO-E2 led to the additional detection of 2-HO-E1 and 4-HO-E1 as well as their methylation and glucuronidation products (Fig. 3). Notable differences in the conjugation of the positional isomers of catechol estrogens were observed: whereas 4-HO-E2 and 4-HO-E1 were predominantly glucuronidated, 2-HO-E2 and 2-HO-E1 were preferentially conjugated through methylation (Fig. 3).

The metabolites of the catechol estrogens were also determined in the cell culture medium of MCF-7 and HepG2 cells after 60 and 120 min incubation, respectively (data not shown). Whereas methylation products could be extracted from the medium with ethyl acetate, we did not succeed in extracting the small amount of glucuronides. However, the pattern of methylated catechol estrogens observed in the medium closely resembled that obtained with cell lysate (Fig. 3), showing that cell lysate is a suitable metabolic surrogate for intact cells.

3.2 Induction of DNA strand breaks

3.2.1 Control experiments

Because catechol estrogens are prone to autoxidation, their stability under the experimental conditions of the genotox-

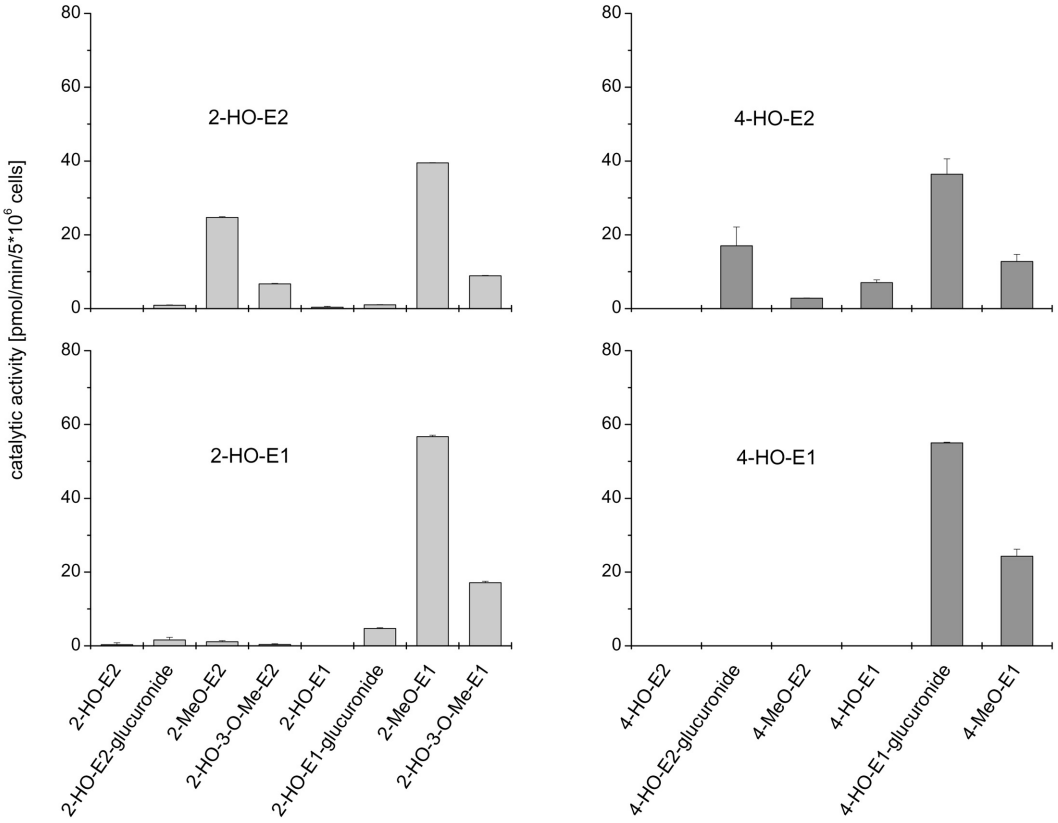

Figure 3. Activity of HepG2 cell lysate for the glucuronidation and methylation of the four catechol estrogens (50 μM). Data represent the mean ± SD of three independent experiments.

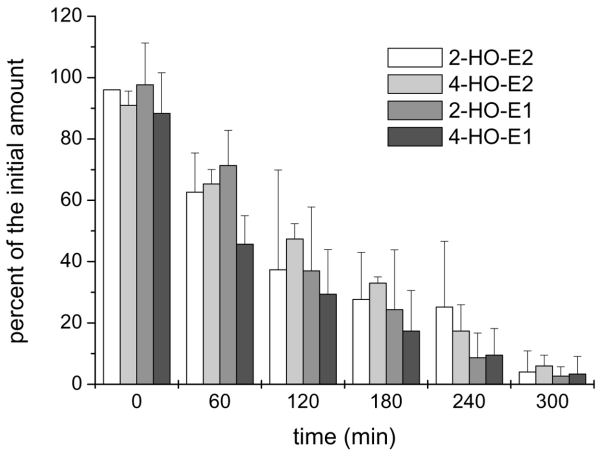

Figure 4. Stability of the catechol estrogens (2.5 μM) in DMEM/F12 without FCS at 20°C. Data represent the mean ± SD of three independent experiments.

icity study was first clarified. When the compounds were incubated with the medium without FCS in the absence of cells for various periods of time, a time-dependent decline was observed (Fig. 4). About 60% of the initial amounts had disappeared after 120 min and 75% after 180 min. Therefore, the incubation time with the cells (see Section 3.2.2) was limited to 180 min for MCF-7 and HepG2 cells, and to 90 min for V79 cells, because the doubling time of V79 is about half of that of the other two cell lines.

In order to ensure the susceptibility of the three cell lines for the chosen genotoxic endpoint, V79, MCF-7, and HepG2 cells were incubated with a known inducer of DNA strand breaks, *i.e.*, *t*-BuOOH [25]. *t*-BuOOH gave rise to DNA strand breaks in each of the cell lines, although to a different extent: MCF-7 ($19.8 \pm 4.0 \times 10^3$ strand breaks *per* cell) and HepG2 ($21.8 \pm 1.2 \times 10^3$) appeared to be about five times more susceptible than V79 cells ($4.1 \pm 0.8 \times 10^3$).

3.2.2 Catechol estrogens

V79, MCF-7, and HepG2 cells were incubated with each of the four catechol estrogens at concentrations ranging from 5 to 50 μM, and the induction of DNA strand breaks was measured using the method of alkaline unwinding. The results are depicted in Fig. 5. All four catechol estrogens caused a concentration-dependent induction of DNA strand breaks in V79 cells. The catechols of E2 were clearly more potent than the catechols of E1, but no difference was observed between the 2-hydroxylated and the 4-hydroxylated isomers. In MCF-7 cells, the genotoxic activity of the four catechol estrogens was much lower than in V79 cells, and significant effects were only observed for 4-HO-E1 and

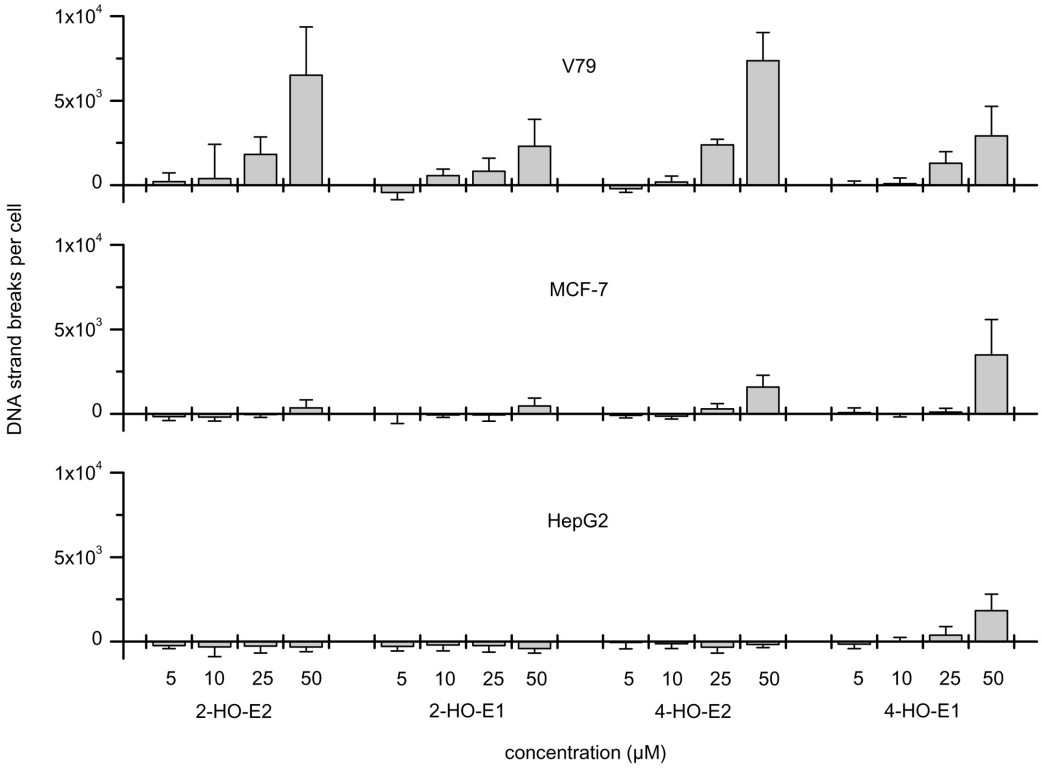

Figure 5. DNA strand breaks induced by the catechol estrogens (5 – 50 μM) in V79, MCF-7, and HepG2 cells. Data represent the mean ± SD of three independent experiments.

Table 1. Correlation of the metabolic capabilities of cells with DNA strand breaks induced by catechol estrogens

	Metabolic activity for		DNA strand breaks induced by 50 μM				
Cell line	Methylation	Glucuronidation	*t*-BuOOH	2-HO-E2	2-HO-E1	4-HO-E2	4-HO-E1
V79	–	–	++	++++	++	++++	++
MCF-7	+	-	+++++++	(+)	(+)	+	++
HepG2	+	+	+++++++	–	–	–	+

4-HO-E2 at the highest concentrations (Fig. 5). In HepG2 cells, only 4-HO-E1 at 50 μM induced a low level of DNA strand breaks.

4 Discussion

The catechol estrogens are major metabolites of E2 and believed to act as genotoxic agents in the mechanism of E2-mediated carcinogenesis [2, 7, 9]. In the present study, we have shown that the catechol estrogens of E2 and E1 cause DNA strand breaks in three different mammalian cell lines, but the extent of this genotoxic effect appears to depend on the structure of the catechol estrogen and on the metabolic capabilities of the cells (Table 1). The most pronounced genotoxic effects were observed in V79 cells (Fig. 5), although this cell line was least susceptible to the DNA strand-breaking activity of the directly acting clastogen *t*-BuOOH. The catechol estrogens of E2 were clearly more genotoxic than the catechol estrogens of E1 by a factor of almost 3 in V79 cells. As V79 are devoid of conjugating enzymes, it is assumed that the observed differences reflect the intrinsic genotoxic potencies of the catechol estrogens.

MCF-7 and HepG2 cells, which were five times more susceptible to *t*-BuOOH, exhibited a much lower genotoxic response to the catechol estrogens than V79 (Fig. 5). Moreover, the 2-hydroxylated compounds were virtually inactive in these two cell lines, and 4-HO-E1 had the highest genotoxicity. This can best be explained when the metabolic capabilities of the two cell lines are taken into account: in cell lysate of MCF-7 cells, 2-HO-E2/-E1 gave rise to two methylation products each, *i.e.*, 2-MeO-E2/-E1 and 2-HO-3-O-Me-E2/-E1. In contrast, only one methylation product, *i.e.*, 4-MeO-E2/-E1, was detected with 4-HO-E2 and 4-HO-E1 (Fig. 2). Moreover, the catalytic activity for the methylation of the 2-HO-estrogens was higher than for the

methylation of the 4-HO-estrogens. The failure of COMT to methylate the 3-hydroxyl group of 4-HO-estrogens has been observed before [26]. Also, it has been repeatedly reported that 2-HO-E2/-E1 are much better substrates of COMT than 4-HO-E2/-E1 [10, 27, 28]. Thus, in our study, good substrates of COMT, *i. e.*, 2-HO-E2 and 2-HO-E1, had a lower DNA strand-breaking activity than 4-HO-E2/-E1. The importance of COMT for preventing the genotoxicity of catechol estrogens has recently also been demonstrated with Syrian hamster embryo cells by Hirose *et al*. [27]. Likewise, it has been shown in E2-treated MCF-7 cells that inhibition of COMT leads to an increase in oxidative DNA damage mediated by the catechol estrogens [28].

The metabolism of catechol estrogens in HepG2 cells proved to be more complex than in MCF-7 cells (Fig. 3): in addition to methylation, HepG2 cells are able to convert E2, its catechol and methoxy metabolites to the respective E1 metabolites by the enzyme HSD, and to form glucuronides. Whereas 4-HO-E2 and 4-HO-E1 were predominantly glucuronidated, 2-HO-E2 and 2-HO-E1 were inactivated through methylation. The preferential glucuronidation of the 4-hydroxy estrogens can be explained by their poor methylation, as discussed above; moreover, HepG2 cells express UGT2B7 which specifically glucuronidates 4-HO-E2/-E1 [22, 29, 30]. The extensive metabolism in HepG2 cells is proposed to account for the failure of three of the catechol estrogens to induce DNA strand breaks in these cells; only 4-HO-E1 was weakly genotoxic at the highest concentration, indicating incomplete inactivation under these conditions (Fig. 5).

In addition to the enzymatic activities focussed on in this study, other enzymes are known to be involved in the metabolism of catechol estrogens, *e. g.*, glutathione *S*-transferase, which catalyzes the formation of glutathione adducts of quinones, and NAD(P)H:quinone oxidoreductase, which reduces quinones to the respective catechols [6, 7, 16]. The activities of these enzymes may also be different in the three cell types, as may be their antioxidant status, and estrogen receptor levels. Future studies should address the importance of these factors for the genotoxicity of catechol estrogens.

A caveat for all cell culture studies involving incubation with catechol estrogens or other catechols is the instability of these compounds due to autoxidation. In fact, the amount of the catechol estrogens decreased under the conditions of incubation with a half-life of about 100 min (Fig. 4). However, this should not compromize the results of this study because of the short incubation times and the fact that the instability was the same for all four catechol estrogens.

In conclusion, because comparative studies on the genotoxicity of all four catechol estrogens are rare, it was the aim of this study to compare the intrinsic genotoxicity of 2-HO-E2/-E1 and 4-HO-E2/-E1 and to assess the effect of metabolic methylation and glucuronidation on genotoxicity. In the absence of metabolism, the catechol estrogens of E2 are clearly more genotoxic than the catechol estrogens of E1. Therefore, conversion of 2-HO-E2 or 4-HO-E2 to the respective catechols of E1 by the enzyme HSD must be classified as a metabolic pathway lowering genotoxicity. Because the genotoxicity of all four catechol estrogens was markedly decreased in cells with active COMT or UGT, methylation and glucuronidation must be assumed to represent the most effective inactivating metabolic pathways of catechol estrogens. The results of this study lend further support to the notion that not only metabolic activation but also inactivation in target cells is a major determinant for the genotoxicity and carcinogenicity of estrogens.

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The authors have declared no conflict of interest.

5 References

[1] International Agency for Research on Cancer, Sex hormones (II), *IARC Monogr.* 1979, *21*, 139 – 343.

[2] Roy, D., Cai, Q., Felty, Q., Narayan, S., Estrogen-induced generation of reactive oxygen and nitrogen species, gene damage, and estrogen-dependent cancers. *J. Toxicol. Environ. Health B* 2007, *10*, 235 – 257.

[3] Bernstein, L., Ross, R. K., Endogenous hormones and breast cancer risk. *Epidemiol. Rev.* 1993, *15*, 48 – 65.

[4] Thomas, H. V., Reeves, G. K., Key, T. J., Endogenous estrogen and postmenopausal breast cancer: A quantitative review. *Cancer Causes Control* 1997, *8*, 922 – 928.

[5] Henderson, B. E., Ross, R. K., Pike, M. C., Casagrande, J. T., Endogenous hormones as a major factor in human cancer. *Cancer Res.* 1982, *42*, 3232 – 3239.

[6] Yager, J. D., Liehr, J. G., Molecular mechanisms of estrogen carcinogenesis. *Annu. Rev. Pharmacol. Toxicol.* 1996, *36*, 203 – 232.

[7] Yager, J. D., Endogenous estrogens as carcinogens through metabolic activation. *J. Natl. Cancer Inst.* 2000, *27*, 67 – 73.

[8] Cavalieri, E., Frenkel, K., Liehr, J. G., Rogan, E., Roy, D., Estrogens as endogenous genotoxic agents – DNA adducts and mutations. *J. Natl. Cancer Inst. Monographs* 2000, *27*, 75 – 93.

[9] Cavalieri, E., Rogan, E., Catechol quinones of estrogens in the initiation of breast, prostate and other human cancers: Keynote lecture. *Ann. N. Y. Acad. Sci.* 2006, *1089*, 286 – 301.

[10] Bolton, J. L., Thatcher, G. R. L., Potential mechanisms of estrogen quinone carcinogenesis. *Chem. Res. Toxicol.* 2008, *21*, 93 – 101.

[11] Devanesan, P., Todorovic, R., Zhao, J., Gross, M. L., *et al*., Catechol estrogen induced conjugates and DNA adducts in the kidney of male Syrian golden hamster treated with 4-hydroxyestradiol: Potential biomarkers for estrogen-initiated cancer. *Carcinogenesis* 2001, *22*, 489 – 497.

[12] Li, K. M., Todorovic, R., Devanesan, P., Higginbotham, S., *et al*., Metabolism and DNA binding studies of 4-hydroxyestradiol and estradiol-3,4-quinone in vitro and in female ACI rat mammary gland in vivo. *Carcinogenesis* 2004, *25*, 289 – 297.

[13] Zahid, M., Kohli, E., Saeed, M., Rogan, E., Cavalieri, E. L., The greater reactivity of estradiol-3,4-quinone vs estradiol-2,3-quinone with DNA in the formation of depurinating adducts: Implication for tumor-initiating activity. *Chem. Res. Toxicol.* 2006, *19*, 164 – 172.

[14] Liehr, J. G., Fang, W. F., Sirbasku, D. A., Ari-Ulubelen, A., Carcinogenicity of catechol estrogens in Syrian hamster. *J. Steroid Biochem.* 1986, *24*, 353 – 356.

[15] Newbold, R. R., Liehr, J. G., Induction of uterine adenocarcinomas in CD-1 mice by catechol estrogens. *Cancer Res.* 2000, *60*, 235 – 237.

[16] Zhu, B. T., Conney, A. H., Functional role of estrogen metabolism in target cells: Review and perspectives. *Carcinogenesis* 1998, *19*, 1 – 27.

[17] Raftogianis, R., Creveling, C., Weinshilboum, R., Weisz, J., Estrogen metabolism by conjugation. *J. Natl. Cancer Inst. Monographs* 2000, *27*, 113 – 124.

[18] Hachey, D. L., Dawling, S., Roodi, N., Parl, F. F., Sequential action of phase I and phase II enzymes cytochrome P450 1B1 and glutathione S-transferase P1 in mammary estrogen metabolism. *Cancer Res.* 2003, *63*, 8492 – 8499.

[19] Dawling, S., Roodi, N., Mernaugh, R. L., Wang, X. Y., Parl, F. F., Catechol-O-methyltransferase (COMT)-mediated metabolism of catechol estrogens: Comparison of wild-type and variant COMT isoforms. *Cancer Res.* 2001, *61*, 6716 – 6722.

[20] Bergman-Jungeström, M., Wingren, S., Catechol-O-methyltransferase (COMT) gene polymorphism and breast cancer risk in young women. *Br. J. Cancer* 2001, *85*, 859 – 862.

[21] Guillemette, C., Bélanger, A., Lépine, J., Metabolic inactivation of estrogens in breast tissue by UDP-glucuronosyltransferase enzymes: An overview. *Breast Cancer Res.* 2004, *6*, 246 – 254.

[22] Lépine, J., Bernard, O., Plante, M., Têtu, B., *et al*., Specificity and regioselectivity of the conjugation of estradiol, estrone, and their catecholestrogen and methoxyestrogen metabolites by human uridine diphospho-glucuronosyltransferases expressed in endometrium. *J. Clin. Endocrin. Metab.* 2004, *89*, 5222 – 5232.

[23] Adjei, A. A., Weinshilboum, R. M., Catecholestrogen sulfation: Possible role in carcinogenesis. *Biochem. Biophys. Res. Commun.* 2002, *292*, 402 – 408.

[24] Hartwig, A., Klyszcz-Nasko, H., Schlepegrell, R., Beyersmann, D., Cellular damage by ferric nitrilotriacetate and ferric citrate in V79 cells: Interrelationship between lipid peroxidation, DNA strand breaks and sister chromatid exchanges. *Carcinogenesis* 1993, *14*, 107 – 112.

[25] Sohn, J. H., Han, K. L., Choo, J. H., Hwang, J. K., Macelignan protects HepG2 cells against tert. – butylhydroperoxide-induced oxidative damage. *Biofactors* 2007, *29*, 1 – 10.

[26] Dawling, S., Hachey, D. L., Roodi, N., Parl, F. F., In vivo model of mammary estrogen metabolism: Structural and kinetic differences between catechol estrogens 2- and 4-hydroxyestradiol. *Chem. Res. Toxicol.* 2004, *17*, 1258 – 1264.

[27] Hirose, Y., Tsutsui, T. W., Ohno, M., Barrett, J. C., Tsutsui, T., Effects of a catechol-*O*-methyltransferase inhibitor on catechol estrogen-induced cellular transformation, chromosome aberrations and apoptosis in Syrian hamster embryo cells. *Int. J. Cancer* 2007, *120*, 1627 – 1633.

[28] Lavigne, J. A., Goodman, J. E., Fonong, T., Odwin, S., *et al*., The effect of catechol-*O*-methyltransferase inhibition on estrogen metabolite and oxidative DNA damage levels in estradiol-treated MCF-7 cells. *Cancer Res.* 2001, *61*, 7488 – 7494.

[29] Cheng, Z., Rios, G. R., King, C. D., Coffman, B. L., *et al*., Glucuronidation of catechol estrogens by expressed human UDP-glucuronosyltransferases (UGTs) 1A1, 1A3 and 2B7. *Toxicol. Sci.* 1998, *45*, 52 – 57.

[30] Pfeiffer, E., Graf, E., Gerstner, S., Metzler, M., Stimulation of estradiol glucuronidation: A protective mechanism against estradiol-mediated carcinogenesis? *Mol. Nutr. Food Res.* 2006, *50*, 385 – 389.

