Catechol estrogen metabolites and conjugates in different regions of the prostate of Noble rats treated with 4-hydroxyestradiol: implications for estrogen-induced initiation of prostate cancer

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Prostate carcinomas arise in 100% of Noble rats treated with estradiol and testosterone. We hypothesize that estrogens initiate prostate cancer mainly by formation of 4-catechol estrogens (CE), followed by their oxidation to catechol estrogen-3,4-quinones (CE-3,4-Q), which can react with DNA. To avoid cancer initiation, CE can be detoxified by catechol-O-methyltransferase (COMT), and CE-3,4-Q by conjugation with glutathione (GSH) or by reduction to CE, catalyzed by quinone reductase and/or cytochrome P450 reductase. To investigate the prostastic metabolism of estrogens, Noble rats were treated with the CE 4-hydroxyestradiol (4-OHE2) or estradiol-3,4-quine (E2-3,4-Q), and CE metabolites and conjugates were analyzed in the four regions of the prostate, which differ in susceptibility to carcinoma formation. Following treatment of rats with 4-OHE2 (6 μmol/100 g body weight in 200 μl of trioctanoin/dimethylsulfoxide (4:1) by intraperitoneal injection) for 90 min, the non-susceptible ventral (VP) and anterior (AP) prostate had higher levels of 4-methoxyCE and GSH conjugates than the susceptible dorsolateral prostate (DLP) and periurethral prostate (PUP). After treatment with the same molar amount of E2-3,4-Q, the VP and AP contained more GSH conjugates, 4-CE and 4-methoxyCE than the susceptible DLP and PUP. These results suggest that prostate areas susceptible to carcinoma induction have less protection by COMT, GSH, and quinone reductase and/or cytochrome P450 reductase, favoring reaction of CE-3,4-Q with DNA, presumably to initiate cancer.

Introduction

Exposure of women to high levels of estrogens has been related to increased incidence of breast cancer (1,2) and there is some evidence to suggest that estrogens are involved in prostate carcinogenesis as well (3). It is generally thought that estrogens, through receptor-mediated processes, affect the rate of cell proliferation, enabling genetic errors that lead to a malignant phenotype (2,4). Estrogens can also form 4-catechol estrogen (CE) metabolites, which can be oxidized to the reactive catechol estrogen-3,4-quinones (CE-3,4-Q) (5–7). Subsequent reaction of CE-3,4-Q with DNA generates depurinating adducts that lead to apurinic sites (5,7). Such apurinic sites can result in mutations that initiate cancer (8,9).

We hypothesize that estrogens initiate prostate cancer via formation of endogenous CE-3,4-Q (5,7). Some evidence in support of a role of estrogens in the initiation of prostate carcinogenesis comes from experiments using Noble (NBL) rats treated with testosterone plus estradiol (E2) (10). This combined treatment induces ductal adenocarcinoma of the prostate in 100% of the rats, whereas treatment with testosterone alone causes prostate cancer in only 40% of the rats. Furthermore, 5α-dehydrotestosterone, which unlike testosterone cannot be converted to E2 by aromatase (cytochrome P450 CYP19), results in only a 4% incidence, and treatment with E2 alone results in prostatic atrophy (ref. 11, unpublished results). The ductal carcinomas obtained in these experiments have been suggested to arise from estrogen-induced initiation and testosterone-produced promotion of prostate ductal tissue (3,10,12).

Estrone (E1) and E2 are formed by aromatization of 4-androstene-3,17-dione and testosterone, respectively, catalyzed by CYP19 (Figure 1), and they are biochemically interconvertible by the enzyme 17β-estradiol dehydrogenase. E1 and E2 are metabolized via two major pathways: formation of CE and, to a lesser extent, 16α-hydroxylation (not shown in Figure 1). The CE formed are the 2- and 4-hydroxylated estrogens. In general, these two CE are inactivated, especially in the liver, by conjugating reactions such as glucuronidation and sulfation (not shown in Figure 1). The most common pathway of inactivation in extrahepatic tissues occurs by O-methylation catalyzed by the ubiquitous catechol-O-methyltransferase (COMT) (13). If formation of E1 and/or E2 is excessive, due to overexpression of CYP19 and/or the presence of excess sulfatase that converts the stored E1 sulfate to E1, increased formation of CE is expected. Formation of 2-CE is primarily catalyzed by CYP1A3 (14). Elevated expression of CYP1B1, the major enzyme responsible for formation of 4-CE (15,16), could dramatically increase this usually minor metabolic pathway. Additional estrogen imbalance can be generated by reduced methylation catalyzed by COMT, rendering the oxidative pathway of CE to CE-Q more competitive (Figure 1). If CE-Q are formed, they may be inactivated by conjugation with glutathione (GSH), catalyzed by glutathione-S-transferase. A second inactivating process for CE-Q is their reduction to CE by quinone reductase and/or cytochrome P450 reductase (17,18). If these two inactivating processes are insufficient, CE-Q may react with DNA to form stable (CE-2,3-Q) and depurinating (CE-3,4-Q) adducts (5,19). We have hypothesized that these depurinating adducts generate apurinic sites leading to mutations that may initiate many human cancers (9,20).
Using the NBL rat as a model for prostate studies, we report in this article the formation of CE metabolites and conjugates in four different regions of the prostate after treatment of the rats with 4-hydroxyestradiol (4-OHE2) or estradiol-3,4-quinone (E2-3,4-Q). This initial study had several purposes. One objective was to determine the feasibility of detecting and measuring these estrogen metabolites and conjugates in each region of the rat prostate. A second objective was to explore the disruption of the balance between estrogen activating and deactivating (protective) enzymes in this target organ. The prostatic areas in which tumors arise after treatment of NBL rats with E2 plus testosterone, namely the periurethral ducts of the dorsolateral prostate (DLP) and anterior prostate (AP) in the proximal region of the DLP and, particularly, the area of the periurethral prostate (PUP) (10), were expected to have less capacity to prevent the formation of reactive electrophilic CE-3,4-Q and/or their reaction with DNA. In contrast, the prostate areas not susceptible to cancer induction, namely the ventral prostate (VP) and distal region of the AP, were expected to provide a greater degree of protection by methyl-ation of 4-CE and inactivation of CE-3,4-Q by reduction to CE and/or reaction with GSH. The success of these initial studies, which used the proximate and ultimate carcinogenic metabolites of E2, warrants further studies with the parent E2.

Materials and methods

Materials

Male NBL (NBL/Cr) rats (11–12 weeks old) were obtained from Charles River (Raleigh, NC). 4-OHE1(E2) and 4-OCH3E1(E2) (Figure 1) were synthesized according to Dwivedy et al. (21), and 4-OHE1(E2)-2-glutathione (-SG), 4-OHE1(E2)-2-cysteine (Cys) and 4-OHE1(E2)-2-N-acetylcysteine (NACys) (Figure 1) were synthesized according to published procedures of...
Cao et al. (22). Enzymes and chemicals were purchased from Sigma (St Louis, MO). Certify II Sep-Pak cartridges were from Varian (Pal Alto, CA) and Luna(2) C18 reverse-phase HPLC columns from Phenomenex (Torrance, CA).

Methods

Treatment of rats. Groups of 10 NBL rats (~280–300 g in weight) were treated with 4-OHE2 or E2-3,4-Q (6 µmol/100 g body weight in 200 µl of trioctanoin/dimethylsulfoxide (4:1)) by intraperitoneal injection. Control animals were treated with the vehicle. The animals were killed after 90 min and four different regions of the prostate gland from each animal were immediately collected separately and frozen at ~80°C. The four regions of the prostate were pooled from the 10 rats for analysis as follows: VP (~200 mg/rat), AP (~110 mg/rat), DLP (~300 mg/rat) and PUP (also including the urethra, ~100 mg/rat).

Extraction of estrogen metabolites and conjugates. The VP, AP, DLP and PUP were pooled and processed separately. The pooled tissue was minced and ground to a fine powder in liquid nitrogen, suspended in 4 ml of 50 mM ammonium acetate, pH 5.0, and divided into two equal portions. Methanol was added to the samples to achieve a final concentration of 60% methanol in buffer, and the mixtures were extracted with 8 ml of hexane to remove any lipid (23). The aqueous phase was then diluted with 50 mM ammonium acetate, pH 4.4, containing 2 mg/ml ascorbic acid to an approximate final concentration of 30% methanol and applied to a Certify II Sep-Pak (200 mg) cartridge. The cartridge was first eluted with 3 ml of the buffer, followed by elution with 2 ml each of 20, 40 and 70% methanol in buffer, and fractions were collected. To minimize oxidation of the CE metabolites and conjugates, the eluting methanol/buffer mixture contained 2 mg/ml ascorbic acid. Collected fractions were analyzed by HPLC with electrochemical detection.

HPLC analysis. Analyses were carried out by using a Luna(2) C18 reverse phase column (250 × 4.6 mm, 5 µm) on an HPLC system equipped with dual ESA Model 580 solvent delivery modules, an ESA Model 540 autosampler and a 12-channel ESA CoulArray electrochemical detector (ESA, Chelmsford, MA) (23). The oxidation potentials of the electrochemical cells were set at 0, 30, 80, 140, 230, 320, 410, 500, 580, 650, 710 and 750 mV, with respect to the internal standard electrode, for channels 1 to 12. A linear gradient starting from 100% acetonitrile/methanol/water/1 M ammonium acetate, pH 4.4 (15:5:70:10) to 90% acetonitrile/methanol/water/1 M ammonium acetate, pH 4.4 (50:20:20:10) over 50 min was employed to separate the compounds, at a flow rate of 1 ml/min. Estrogen metabolites and conjugates from biological samples were identified by comparison with authentic standards (Figure 1), based on their retention time, as well as peak height ratios between the dominant peak and the peaks in the two adjacent channels (23). Data analysis was carried out by using ESA CoulArray software.

Results and discussion

This is the first study on the metabolism of estrogens in the prostate of the NBL rat model to investigate the possible role of estrogen metabolism in the initiation of prostate cancer. We used treatment with 4-OHE2 to determine the ability of the prostate to metabolize this CE and compared the four regions of the prostate in which carcinomas develop, the DLP and PUP, or do not develop, the VP and AP, upon treatment of the rats with E2 and testosterone. The results of this study are interpreted on the assumptions that the metabolism occurs in the prostate and that the injected compound is distributed more or less evenly throughout the prostate. No E1, E2 or their metabolites or conjugates was detected in prostate tissue from the control group (treated with vehicle) for either experiment described below.

Upon treatment of the rats with 6 µmol of 4-OHE2/100 g body weight for 90 min, the two regions of the prostate in which tumors do not develop (VP and AP) had higher levels of 4-methoxy CE than the two regions in which carcinomas are induced by E2 and testosterone (DLP and PUP, Table I and Figure 2). This finding suggests that the protective enzyme COMT is more effective in the VP and AP than in the DLP and PUP. In addition, the level of GSH conjugates was higher in the non-susceptible VP and AP compared with the susceptible DLP and PUP. The interpretation of these findings could be that the two non-susceptible regions of the prostate metabolize the 4-CE to CE-3,4-Q more efficiently than the susceptible regions or that the protection by GSH and/or glutathione-S-transferase is more abundant in the VP and AP than in the DLP and PUP.

To investigate further these possibilities and the level of the protective enzymes quinone reductase and/or cytochrome P450 reductase, which reduce CE-Q to CE (Figure 1), rats were treated with 6 µmol of E2-3,4-Q/100 g body weight for 90 min.

<table>
<thead>
<tr>
<th>Metabolite/conjugate</th>
<th>Ventral prostate</th>
<th>Anterior prostate</th>
<th>Dorosilateral prostate</th>
<th>Periurethral prostate and urethra</th>
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<tbody>
<tr>
<td>4-OHE2</td>
<td>352</td>
<td>277</td>
<td>554</td>
<td>172</td>
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<tr>
<td>4-OHE1</td>
<td>9</td>
<td>30</td>
<td>8</td>
<td>ND</td>
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<td>4-CH3E2</td>
<td>389</td>
<td>627</td>
<td>485</td>
<td>242</td>
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<tr>
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<td>786</td>
<td>230</td>
<td>194</td>
<td>129</td>
</tr>
<tr>
<td>4-OHE2-2-SG</td>
<td>32</td>
<td>70</td>
<td>8</td>
<td>12</td>
</tr>
</tbody>
</table>

*aResults are the average of two experiments in which the data varied by 10–30%.

**ND: not detected.
Table II. Estrogen metabolites and conjugates in the prostate of rats treated with E_2-3,4-Q

<table>
<thead>
<tr>
<th>Metabolite/conjugate</th>
<th>pmol/g tissue</th>
<th>Ventral prostate</th>
<th>Anterior prostate</th>
<th>Dorsolateral prostate</th>
<th>Periurethral prostate and urethra</th>
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<tr>
<td>4-OHE_2</td>
<td>98</td>
<td>211</td>
<td>281</td>
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<td>4-OHE_1</td>
<td>29</td>
<td>22</td>
<td>27</td>
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<tr>
<td>4-OCH_3E_2</td>
<td>240</td>
<td>465</td>
<td>190</td>
<td>161</td>
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<tr>
<td>4-OCH_3E_1</td>
<td>515</td>
<td>100</td>
<td>63</td>
<td>70</td>
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<tr>
<td>4-OHE_2-2-SG</td>
<td>48</td>
<td>124</td>
<td>35</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>4-OHE_2-2-Cys</td>
<td>35</td>
<td>64</td>
<td>30</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>4-OHE_2-2-NAcCys</td>
<td>40</td>
<td>55</td>
<td>20</td>
<td>15</td>
<td></td>
</tr>
</tbody>
</table>

*Results are the average of two experiments in which the data varied by 10–30%.

Because the ultimate reactive E_2-3,4-Q was injected, not only the 4-OHE_2-2-SG conjugate was detected, but also its hydrolytic products, 4-OHE_2-2-Cys and 4-OHE_2-2-NAcCys (Table II), which are derived from the mercapturic acid biosynthesis pathway (24). The total CE-3,4-Q conjugates, namely, 4-OHE_2-2- SG, 4-OHE_2-2-Cys and 4-OHE_2-2-NAcCys, were once again more abundant in the two non-susceptible regions (VP, 123 pmol/g tissue, and AP, 243 pmol/g tissue, Table II) than in the susceptible ones (DLP, 85 pmol/g tissue, and PUP, 55 pmol/g tissue) (Figure 3), suggesting that the levels of protection by GSH and/or glutathione-S-transferase in the susceptible areas are lower. This result implies that the DLP and PUP conjugate relatively less CE-3,4-Q, increasing the likelihood of the CE-3,4-Q to react with DNA.

The sum of the 4-CE and 4-methoxyCE in each region of the prostate in E_2-3,4-Q-treated rats can be considered a measure of quinone reductase and/or cytochrome P450 reductase activity. The levels in the non-susceptible VP (882 pmol/g tissue) and AP (798 pmol/g tissue) were higher than those in the susceptible areas (DLP, 561 pmol/g tissue, and PUP, 330 pmol/g tissue) (Table II), suggesting better protection by quinone reductase and/or cytochrome P450 reductase in the VP and AP than in the DLP and PUP. As already observed when rats were treated with 4-OHE_2, the level of 4-methoxyCE in the non-susceptible areas was higher than in the susceptible regions (Figure 3), indicating again that the protective action of COMT is more abundant in the non-susceptible regions of the prostate. Incidentally, the level of 4-methoxyE_1 was higher than that of 4-methoxyE_2 in the VP, but not in the AP, DLP or PUP, after treatment with either 4-OHE_2 or E_2-3,4-Q (Tables I and II). At present, we cannot explain this observation, except to suggest differences in expression of 17β-hydroxysteroid dehydrogenases in the various regions of the prostate may be involved (25). In summary, these two experiments reveal that the two regions of the NBL rat prostate not susceptible to cancer induction, the VP and AP, are better protected than the susceptible regions at the CE level by methylation and at the CE-Q level by better conjugation with GSH and more effective reduction to CE.

Conclusions

The combined metabolism results from this initial study of treatment of NBL rats with 4-OHE_2 or E_2-3,4-Q suggest that the two regions of the prostate susceptible to cancer induction have a lower level of protection by COMT-catalyzed methylation of CE and less protection by quinone reductase and/or cytochrome P450 reductase, which reduce the endogenous carcinogenic CE-3,4-Q. We think that a relative imbalance in estrogen homeostasis, already observed in hamster kidney (26), leads to decreased protection at both the CE and CE-Q levels in the two susceptible prostate regions. This would favor reaction of CE-3,4-Q with DNA, presumably to generate oncogenic mutations.

Further studies are planned to examine the different metabolic profiles in the four regions of the prostate upon treatment of NBL rats with E_2, the compound known to induce prostate cancer when administered in conjunction with testosterone. Such studies are far more complex because of the variety of estrogen metabolites and conjugates that must be analyzed. At the same time, we will measure the activity and expression of the enzymes involved in biotransformation of E_2, with the objective of determining how estrogen activation and deactivation become unbalanced in the regions of the prostate susceptible to induction of cancer.

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References


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