Induction of Uterine Adenocarcinoma in CD-1 Mice by Catechol Estrogens

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Abstract

Catechol estrogens may mediate estrogen-induced carcinogenesis because 4-hydroxyestradiol induces DNA damage and renal tumors in hamsters, and this metabolite is formed in the kidney and estrogen target tissues by a specific estrogen 4-hydroxylase. We examined the carcinogenic potential of catechol estrogen in an experimental model previously reported to result in a high incidence of uterine adenocarcinoma after neonatal exposure to diethylstilbestrol. Outbred female CD-1 mice were treated with 2- or 4-hydroxyestradiol, 17β-estradiol, or 17α-ethinyl estradiol on days 1–5 of neonatal life (2 μg/pup/day) and sacrificed at 12 or 18 months of age. Mice treated with 17β-estradiol or 17α-ethinyl estradiol had a total uterine tumor incidence of 7% or 43%, respectively. 2-Hydroxyestradiol induced tumors in 12% of the mice, but 4-hydroxyestradiol was the most carcinogenic estrogen, with a 66% incidence of uterine adenocarcinoma. Both 2- and 4-hydroxylated catechols were estrogenic and increased uterine wet weights in these neonates. These data demonstrate that both 2- and 4-hydroxyestradiol are carcinogenic metabolites. The high tumor incidence induced by 4-hydroxyestradiol supports the postulated role of this metabolite in hormone-associated cancers.

Introduction

Estradiol induces kidney tumors in Syrian hamsters (1). This animal model has been examined extensively to elucidate the mechanism of tumorigenesis by this natural hormone (2, 3). As part of such a mechanistic study, the induction of renal tumors by catechol estrogens has previously been examined to determine the role of these metabolites in this process. 4-Hydroxyestradiol was as carcinogenic as estradiol in the hamster kidney system, whereas 2-hydroxyestradiol did not induce any tumors (2, 4, 5). The applicability of this finding to human cancers has remained unclear because the hamster kidney is a mechanistic model but not an organ model for the genesis of human hormone-associated cancers, as, for instance, in the breast or uterus. Moreover, the concept of 4-hydroxyestradiol mediating cancer induction by hormones as determined in the hamster kidney system would be further strengthened if validated in another organ model more closely associated with human hormonal cancers.

In this investigation, we examined the carcinogenic activity of 2- and 4-hydroxyestradiol by studying the induction of uterine adenocarcinoma in CD-1 mice treated with either of these estrogen metabolites within the first 5 days of life. Uterine tumors are induced in this rodent strain 12–18 months after treatment with either estradiol or DES5 (6). This system is thus an excellent model for the study of the mechanism of induction of this neoplasm by hormones. For comparison with previous results, tumors were also induced by the natural hormone estradiol (6) and by the synthetic estrogen 17α-ethinyl estradiol. In addition, increases in uterine wet weight in the neonatal CD-1 mice were recorded for each of these estrogens to assess the hormonal activity of these compounds under our treatment conditions in the neonates. Our data offer insight into the role of catechol estrogen metabolites in the induction of uterine tumors.

Materials and Methods

Tumor Induction. Timed pregnant outbred female CD-1 [Crl:CD-1 (ICR) BR] mice near term were obtained from the breeding colony at National Institute of Environmental Health Sciences (Research Triangle Park, NC). Mice were individually housed in plastic cages with hard wood chip bedding under a 12-h light/12-h dark cycle in a temperature-controlled room (21°C–22°C) and fed NIH 31 mouse chow and fresh water ad libitum. All animal procedures complied with National Institute of Environmental Health Sciences animal care guidelines. At delivery, litters were adjusted to eight female pups/mom. Pups were given daily s.c. injections of 2 μg/pup/day of estrogen (2- or 4-hydroxyestradiol, 17β-estradiol, or 17α-ethinyl estradiol; Sigma Chemical Co., St. Louis, MO) dissolved in corn oil or corn oil alone (as a control) on days 1–5 of life. This estrogen dose was chosen because it has previously been shown to result in uterine carcinoma in 90% of aged mice treated with DES on days 1–5 of neonatal life (6). Mice were weaned at 21 days of age and housed four mice/cage. Mice were sacrificed by cervical dislocation at 12 or 18 months of age. Reproductive tract tissues were removed quickly, fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned at 6 μm. Tissue sections were stained with H&E and evaluated using light microscopy. If an area of pathological change was observed, additional sections were made for further evaluation. The number of mice with reproductive tract tumors was determined.

Estrogenicity of Catechol Estrogens in Neonatal Mouse. Pups were given daily s.c. injections of estrogen dissolved in corn oil or corn oil alone (as a control) on days 1–4 of neonatal life. A minimum of 10 pups/compound was used. On the morning of day 5, mice were sacrificed, and body weights and uterine weights were recorded. Data are expressed as the percentage of uterine wet weight increase over values in corn oil-treated control mice.

Statistical Methods. Comparisons of uterine weights were made by Duncan’s multiple range test (7). The incidences of uterine adenocarcinoma were compared by Fisher’s exact tests (8).

Results

Carcinogenicity of Catechol Estrogens. Both the catechol estrogens examined, 2- and 4-hydroxyestradiol, induced tumors in the CD-1 mice (Table 1). 2-Hydroxyestradiol was more carcinogenic than estradiol, with 14% and 11% of the rodents developing uterine adenocarcinoma 12 and 18 months, respectively, after neonatal catechol estrogen administration. In contrast, 4-hydroxyestradiol was considerably more carcinogenic than 2-hydroxyestradiol and produced a 74% (12 months) and 56% (18 months) tumor incidence after treatment in the neonatal period. 17α-Ethynyl estradiol also induced more tumors (38% at 12 months and 50% at 18 months) than did estradiol. The type of tumor induced was, in all cases, similar to neoplasms produced by DES or estradiol, as described previously (6). Uterine adenocarcinoma in an 18-month-old mouse treated neonatally on days 1–5 of life with 4-hydroxyestradiol is shown in Fig. 1. The lesion is extensive, with irregularly shaped glands invading through the myometrium to the serosal surface of the uterus. There was a mixed population of squamous, cuboidal, and columnar cells present in this
lesion. In another animal, the tumor was composed of neoplastic cells that formed relatively well-defined glandular patterns in some areas, but endometrial glands were crowded together with little intervening stroma in some foci. Some glands contained secretive material, and some were lined by cells that were piling up (Fig. 2), but many glands appeared to be just nests of cells with no apparent lumen. Mitotic figures were frequently seen in this lesion. These uterine glands invaded through the myometrium to the serosal surface (arrow). Sample was stained with H&E (×4).

Hormonal Activity of Catechol Estrogens. Estradiol and 17α-ethinyl estradiol induced a 133% and 146% gain in uterine wet weights, respectively, over control values obtained with corn oil-treated animals (Table 2). 2-Hydroxyestradiol was less estrogenic than estradiol or ethinyl estradiol and produced a 107% increase of uterine wet weights over controls. In contrast, 4-hydroxyestradiol had a higher estrogenic potency in this system than the parent hormone and induced a 213% gain of uterine wet weights in these neonatal mice. These data demonstrate that both catechols are hormonally active estrogens in these neonatal mice.

Discussion

Our data demonstrate the powerful carcinogenic activity of 4-hydroxyestradiol as indicated previously in the hamster kidney tumor model (2, 4, 5). Tumor induction by this estrogen metabolite in the uterine endometrium is relevant to the human situation for the following reason: estrogen administration unopposed by progestin increases uterine cancer risk in humans (9, 10). By analogy, estradiol induces uterine adenocarcinoma in mice, which closely resembles the human endometrial neoplasm (6). Moreover, the predominant metabolic conversion of estradiol in human or rodent uterus is 4-hydroxylation (11, 12). The 9-fold higher tumor incidence in mice 12 and 18 months after 4-hydroxyestradiol administration compared to that in the estradiol-treated group supports the previous hypothesis that formation of this catechol estrogen represents a metabolic activation of estradiol to a (precursor of a) reactive intermediate (3, 13, 14). Consistent with this argument, several types of DNA damage are induced in vivo or in vitro by 4-hydroxyestradiol [as reviewed by Yager and Liehr (3) and Liehr (13, 14)]. The 60% higher estrogenic potency of 4-hydroxyestradiol compared to estradiol in the neonatal mouse uterus may contribute to tumorigenesis, but it is unlikely to be the sole characteristic of this metabolite necessary for the 9-fold increase in tumorigenesis. It is more likely that tumors are initiated by DNA alteration in combination with receptor-mediated proliferation of damaged cells, as proposed previously (3, 13, 14).

2-Hydroxyestradiol is also more carcinogenic than estradiol in this tumor model and is 80% as hormonally potent as the parent estrogen. Our data provide the first evidence for any carcinogenic activity of this catechol estrogen because it did not induce any tumors in the hamster kidney model (2, 4, 5). 2-Hydroxyestradiol has previously been shown to undergo metabolic redox cycling between quinone and hydroquinone (catechol) forms and to induce various forms of DNA alteration (11, 12). The 60% higher estrogenic potency of 4-hydroxyestradiol compared to estradiol in the neonatal mouse uterus supports the previous hypothesis that formation of this catechol estrogen represents a metabolic activation of estradiol to a (precursor of a) reactive intermediate (3, 13, 14).

Table 1: Induction of uterine adenocarcinoma in CD-1 mice by 2- and 4-hydroxyestradiol

Mice were given s.c. injections of the indicated compound (2 μg/pup/day) on days 1–5 of neonatal life and sacrificed at 12 or 18 months of age. Uterine tissues were examined for evidence of histological alterations using light microscopy. Values are presented as the number of mice with uterine adenocarcinoma/total number of animals and as the percentage of tumor incidence/group (shown in parentheses).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Incidence of uterine adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12 months</td>
</tr>
<tr>
<td>2-Hydroxyestradiol</td>
<td>3/21 (14%)</td>
</tr>
<tr>
<td>4-Hydroxyestradiol</td>
<td>14/19 (74%)</td>
</tr>
<tr>
<td>17β-Estradiol</td>
<td>0/5 (0)</td>
</tr>
<tr>
<td>17α-Ethynyl estradiol</td>
<td>9/24 (38%)</td>
</tr>
<tr>
<td>Control (corn oil)</td>
<td>0/12 (0)</td>
</tr>
</tbody>
</table>

* P < 0.05 versus corn oil controls (Fisher’s exact test).
* P < 0.01 versus corn oil controls (Fisher’s exact test).
These data have been published previously (6).

Table 2: Estrogenic potency of 2- and 4-hydroxyestradiol in the neonatal mouse uterus

Mice were given s.c. injections of 2 μg/pup/day of estrogen in corn oil or corn oil alone for controls on days 1–4 of neonatal life. They were sacrificed on day 5, and the uterine weight (wt)/body wt. ratio was determined. A minimum number of 10 pups/compound were included in each group.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Uterine wet wt./body wt. ratio × 100</th>
<th>Estrogenic potency in neonates (% uterine wet wt. gain)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.199 ± 0.03</td>
<td>100</td>
</tr>
<tr>
<td>2-Hydroxyestradiol</td>
<td>0.213 ± 0.03</td>
<td>107</td>
</tr>
<tr>
<td>4-Hydroxyestradiol</td>
<td>0.424 ± 0.06*</td>
<td>213</td>
</tr>
<tr>
<td>17β-Estradiol</td>
<td>0.265 ± 0.03</td>
<td>133</td>
</tr>
<tr>
<td>17α-Ethynyl estradiol</td>
<td>0.291 ± 0.04</td>
<td>146</td>
</tr>
</tbody>
</table>

* Data are expressed as the mean ± SE.
* Data are expressed as the percentage increase over control values.
* P < 0.05 versus corn oil controls and versus 2-hydroxyestradiol (Duncan’s multiple range test).
* This data has been published previously (6).

**Fig. 1.** Well-differentiated invasive uterine adenocarcinoma in an 18-month-old mouse treated neonatally with 4-hydroxyestradiol on days 1–5 of life. Note the extensive lesion with irregularly shaped glands, with little intervening stroma that extend through the myometrium to the serosal surface (arrow). Sample was stained with H&E (×4).

**Fig. 2.** Uterine carcinoma in an 18-month-old mouse treated neonatally with 4-hydroxyestradiol on days 1–5 of life. Note that the cells are piling up in some glands with an intact basal lamina (arrow), but other areas are invasive within the adjacent stroma. There is much nuclear pleomorphism, and mitotic figures (m) are frequent. Sample was stained with H&E (×20).
damage (15–18). Thus, the induction of uterine tumors in mice by this catechol is consistent with its DNA damaging potential (15–18) and is inconsistent with a designation as “a good estrogen” or antiestrogen (19, 20). The lack of tumor induction by 2-hydroxyestradiol in the hamster kidney system may have been due to rapid conjugation of this estrogen metabolite by catechol-O-methyltransferase, whereas in neonatal mice, this conjugating enzyme activity may not have reached activity levels prevalent in adults and thus may not have detoxified this reactive estrogen.

17α-Ethynyl estradiol was slightly more estrogenic than estradiol but induced a much higher tumor incidence than the natural hormone (50% and 10% incidence after 18 months, respectively). These data are inconsistent with the hamster kidney system, where this synthetic estrogen is only a poor carcinogen (1, 2, 5). This differential carcinogenic activity may be due to species differences in metabolism. In hamsters, the low carcinogenic activity of 17α-ethynyl estradiol is consistent with a decreased renal conversion of this synthetic estrogen to catechol metabolites (21). In contrast, our data in mice on the carcinogenic activity of 17α-ethynyl estradiol are in line with induction and promotion of liver tumors in rodents by 17α-ethynyl estradiol (22, 23). Moreover, 17α-ethynyl estradiol is the estrogen used in oral contraceptives and has been reported to raise the risk of breast cancer in humans (24–26). Consistent with this concept of breast cancer induction, 4-hydroxylation of estrogens predominates over 2-hydroxylation in human breast cancer by a specific estrogen 4-hydroxylase, that is detectable in tumors and normal mammary tissue (27).

In summary, these data are the first demonstration of the carcinogenic activity of 2- and 4-hydroxyestradiol in the mouse uterus. Furthermore, the potent carcinogenicity of 4-hydroxyestradiol in the mouse uterus adds support to the hypothesis that this estradiol metabolite plays a role in tumor induction in hormone-associated cancers.

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References


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