Genotoxicity of the steroidal oestrogens oestrone and oestradiol: possible mechanism of uterine and mammary cancer development

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Oestrogens, including the natural hormones oestrone and oestradiol, induce various tumours in laboratory animals and have been recognized to be carcinogens in humans, raising the risk for breast and uterine cancer. As part of the search for the mechanism of hormone-induced carcinogenesis, various types of DNA damage have been detected which have been induced by oestrogens in cell-free systems, in cells in culture, or in vivo. Nevertheless, oestrogens have been postulated to act only as promoters of mammary carcinogenesis by receptor-mediated growth stimulation without consideration of their genotoxicity because these hormones failed to induce mutations in commonly used assays. More recently, oestradiol-induced numerical chromosomal changes (aneuploidy) and structural chromosomal aberrations have been detected in cells in culture and in hamster kidney, a target of oestrogen-induced cancer. In this animal model, oestradiol generates c-myc gene amplification and microsatellite instability. Mutations of the hprt gene have been induced by oestradiol in V79 cells and by catecholoestrogen metabolites in Syrian hamster embryo cells. Sequencing of this gene isolated from V79 mutant clones revealed point mutations and deletions. It is concluded that oestradiol plays a dual role as mutagen/carcinogen and as growth-stimulating hormone in the induction of tumours.

Key words: genotoxicity/mammary carcinogenesis/oestrogen-induced cancer/oestrogen-induced DNA damage/oestrogen-induced mutations

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Carcinogenicity of oestrogens

The natural hormones oestrone and oestradiol have clearly been recognized to be carcinogens in laboratory rodents and in humans (International Agency for Research on Cancer, 1987, 1999). In a variety of mouse and rat strains, oestradiol increases the incidence of tumours of the mammary or pituitary glands, uterus, cervix, vagina, testes, lymphoid system or bone (Noble et al., 1975; Highman et al., 1980, 1981; Huseby, 1980; Nagasawa et al., 1980; Inoh et al., 1985; Shull et al., 1997). In outbred hamsters, oestradiol and oestrone elicit a high incidence of kidney tumours (Kirkman, 1959).

In humans, prolonged oestrogen use unopposed by progestin has long been accepted by epidemiologists as a risk factor of endometrial adenocarcinoma (Greenwald et al., 1977; Key and Pike, 1988; Weiderpass et al., 1999). In contrast, early cohort studies failed to identify an association between serum oestrogen and breast cancer risk, presumably due to failings of the assay methods used (Wysowski et al., 1987; Garland et al., 1992). However, more recent epidemiological results reveal strong relationships between breast cancer risk and plasma or urinary oestrogen concentrations (Adlercreutz et al., 1994; Toniolo et al., 1995). Oestrogen medications also elevate breast cancer risk. Oral contraceptives have been estimated to increase breast cancer risk by approximately 3% per year of intake (relative risk estimate: 1.36) (Pike et al., 1993). A comparable increase of relative breast cancer risk has been obtained by a recent meta-analysis of more than 50 studies of hormone replacement therapy and breast cancer
risk (Collaborative group on hormonal factors in breast cancers, 1997). Oestrogen and progestin combinations are also carcinogenic to humans (International Agency for Research on Cancer, 1999; Ross et al., 2000).

Taken together, these data document that oestrogens, including the natural hormones oestradiol or oestrone, are carcinogens. They induce tumours in various strains of rats or mice, in hamsters, and in other species. Moreover, elevated plasma concentrations of oestrogen caused either by hormone medications or by an increased endogenous oestrogen production raise breast or uterine cancer risk in humans. This carcinogenic activity of oestrogenic steroids has now been recognized by the International Agency for Research on Cancer (IARC, 1987, 1999), which has classified oestrogens as carcinogens in humans.

It is important to recognize that oestrogens may be weak carcinogens compared with other more powerful carcinogens used in laboratory tests such as benzo[a]pyrene or 7,12-dimethylbenzanthracene. This weak carcinogenic activity is indicated by the relatively modest (30%) increase in relative breast cancer risk after taking oestrogen replacement therapy for 5–10 years. On the other hand, only slight increases in circulating oestrogen concentrations suffice to increase cancer risk. The number of women taking oestrogens either for birth control, for hormone replacement therapy or for other reasons represents a very large population. Therefore, a moderate cancer risk in a very large population translates into a sizeable number of women contracting this disease.

### Genotoxicity of oestrogens

In early studies of the mechanism of tumour induction by oestrogens, binding of oestrogens to DNA and proteins has been observed (Jaggi et al., 1978). However, the nature of this binding had not been elucidated, and the mechanism of interaction of oestradiol or its metabolites with DNA had not been clarified. In subsequent studies during the 1980s and 1990s, various types of DNA damage induced by oestrogens have been detected. Several of these lesions have been caused by free radicals (Table I). For example, the catecholestrogen metabolites 2- and 4-hydroxyoestradiol or 2- and 4-hydroxyoestrone are redox-active and may be metabolically converted to corresponding semiquinone and further to quinone forms (structures are shown in Figure 1) (Liehr et al., 1986a; Roy and Liehr, 1988). Quinone intermediates in turn may be reduced by various reductases to semiquinones and hydroquinones (catechols). Alternatively, these semiquinones/quinones may enter into one-electron oxidations/reductions with molecular oxygen and form oxygen radicals (Roy and Liehr, 1988; Li et al., 1994). Consistent with these observations, various types of free radical-generated DNA lesions have been induced by oestradiol and/or catecholestrogens in vivo, in cells in culture, or in cell-free systems (Nutter et al., 1991, 1994; Han and Liehr, 1994a,b, 1995; Ho and Roy, 1994; Li et al., 1994; Wang and Liehr, 1995; Mobley et al., 1999), and by catecholestrogens, but not by parent hormones in cell-free systems (Han and Liehr, 1995) (Table I). These free radical-induced lesions include single-strand breaks of DNA, 8-hydroxylation of guanine bases, and DNA adducts formed by aldehyde decomposition products of lipid hydroperoxides. Two types of such DNA modifications have also been detected in mammary DNA of breast cancer patients: 8-hydroxylation of guanine bases and malondialdehyde–DNA adducts (Malins et al., 1993, 1995; Wang et al., 1996).

The covalent binding of oestrogen quinone reactive intermediates to guanine and adenine nucleic acid bases has also been investigated in cell-free test systems and in rats and hamsters in vivo (Table II) (Abul-Hajj et al., 1995; Stack et al., 1996; Cavalieri et al., 1997; Roy and Abul-Hajj, 1997; Shen et al., 1997). Quinone intermediates of 4-hydroxylated oestrogens have been shown to form unstable DNA adducts, which decompose and form potentially mutagenic apurinic sites, whereas 2-hydroxylated oestrogen metabolites generate more stable DNA adducts (Cavalieri et al., 1997). In studies of polycyclic hydrocarbon carcinogens, apurinic sites have been found to be much more mutagenic than the stable adducts (Chakravarti et al., 1995; Cavalieri and Rogan, 1998). Consistent with these observations, 4-hydroxyoestradiol is much more carcinogenic than 2-hydroxyoestradiol in

### Table I. Oestrogen-induced indirect DNA damage in vitro or in rodents in vivo

<table>
<thead>
<tr>
<th>Type of DNA damage</th>
<th>Oestrone used</th>
<th>Cell-free systems, cells in vitro</th>
<th>In vivo</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single strand breaks</td>
<td>Oestrone-3,4-quinone</td>
<td>MCF-7 cells</td>
<td>Hamster</td>
<td>Nutter et al. (1991, 1994)</td>
</tr>
<tr>
<td></td>
<td>Oestradiol</td>
<td></td>
<td></td>
<td>Han and Liehr (1994a)</td>
</tr>
<tr>
<td></td>
<td>2- or 4-Hydroxyoestradiol</td>
<td>$\phi$X 174 RFI DNA</td>
<td>Hamster</td>
<td>Li et al. (1994)</td>
</tr>
<tr>
<td></td>
<td>2- or 4-Hydroxyoestradiol</td>
<td></td>
<td></td>
<td>Han and Liehr (1994a)</td>
</tr>
<tr>
<td></td>
<td>Oestradiol plus testosterone</td>
<td></td>
<td></td>
<td>Ho and Roy (1994)</td>
</tr>
<tr>
<td>8-Hydroxylation of guanine bases</td>
<td>2- or 4-Hydroxyoestradiol</td>
<td>DNA, Cu(II)SO$_4$</td>
<td>Hamster</td>
<td>Mobley et al. (1999)</td>
</tr>
<tr>
<td></td>
<td>4-Hydroxyoestradiol</td>
<td>DNA, microsomes</td>
<td>Hamster</td>
<td>Han and Liehr (1995)</td>
</tr>
<tr>
<td></td>
<td>4-Hydroxyoestrone</td>
<td>DNA, microsomes</td>
<td>Hamster</td>
<td>Han and Liehr (1995)</td>
</tr>
<tr>
<td></td>
<td>Equilenine-3,4-quinone</td>
<td>DNA, microsomes</td>
<td>Hamster</td>
<td>Han and Liehr (1995)</td>
</tr>
<tr>
<td></td>
<td>Oestradiol</td>
<td></td>
<td></td>
<td>Han and Liehr (1994b)</td>
</tr>
<tr>
<td></td>
<td>4-Hydroxyoestradiol</td>
<td></td>
<td></td>
<td>Han and Liehr (1994b)</td>
</tr>
<tr>
<td>Bulky DNA adducts (unknown structure)</td>
<td>Oestradiol</td>
<td></td>
<td>Hamster</td>
<td>Liewer et al. (1986d)</td>
</tr>
<tr>
<td>Oestradiol-induced malondialdehyde-DNA adducts</td>
<td>Oestradiol</td>
<td></td>
<td></td>
<td>Wang and Liehr (1994)</td>
</tr>
</tbody>
</table>
mouse and hamster models (Liehr et al., 1986b; Li and Li, 1987; Newbold and Liehr, 2000).

In summary, a large body of experimental evidence exists that oestrogens induce genotoxicity. This conclusion is supported by experiments in cell-free systems, in cells in culture, and in laboratory animals. Moreover, these data have been accumulated in more than half a dozen different laboratories in independent experiments. This evidence is only summarized here, but has been reviewed in more detail elsewhere (International Agency for Research on Cancer, 1987, 1999; Liehr, 2000). The DNA damage most likely arises from catecholoestrogen metabolites, because in cell-free systems only catecholoestrogens or their quinone intermediates (but not the parent hormones) are capable of covalently altering nucleic acid.

In vivo, this DNA damage is induced frequently only in organs of high rates of catecholoestrogen formation (Liehr, 2000).

Is genotoxicity of oestrogens relevant to the carcinogenic process?

Many carcinogenic chemicals such as polycyclic aromatic hydrocarbons are metabolically activated to reactive intermediates, which bind to DNA and induce various types of DNA damage (Cavalieri and Rogan, 1998). These genetic lesions may induce somatic mutations and ultimately result in tumour formation. This series of events has been clearly elucidated for chemical carcinogens (Cavalieri and Rogan, 1998), but could not be established as a mechanism of hormone-induced cancer (Li and Li, 1990). The primary contradicting evidence has been the failure of oestrogens or oestrogen metabolites to induce mutations in widely accepted bacterial or mammalian gene mutation assays (Table III). For instance, oestradiol, oestrone or any oestrogen metabolites including catecholoestrogens are not mutagenic in the Salmonella typhimurium reversion (Ames) assay (Lang and Redmann, 1979; Liehr et al., 1986b; Lang and Reiman, 1993). Moreover, oestradiol is not mutagenic in V79 Chinese hamster cells (Drevon et al., 1981). All of these tests have been carried out by several different investigators, and under different test conditions. All of these studies have confirmed a lack of mutagenic activity of oestrogens.

This failure of oestrogens to induce gene mutations using commonly accepted point mutation assays has led to proposals of oestrogens as non-genotoxic epigenetic carcinogens (Nandi, 1978; Li and Li, 1990; Li, 1993). Specifically, the hormonal activity of oestrogens mediated by oestrogen receptors has been viewed as the basis of hormone-associated cancer. Oestrogen-induced tumour induction by uncontrolled stimulation of mammary epithelial cell proliferation has been proposed (Furth, 1982). More recently, this mechanistic proposal has been modified with an emphasis on oestrogen receptor-mediated proliferation of mammary epithelial cells carrying spontaneous replication errors (Feigelson and Henderson, 1996). In all these proposals, the role of oestrogen in breast carcinogenesis is confined to stimulation of hormone receptor-mediated proliferation.

The high incidence of oestrogen-induced aneuploidy without detectable and apparent gene mutations in Syrian hamster embryo cells has led to the proposal of a mechanism of oestrogen-induced cell transformation carrying the following features (Barrett et al., 1981; Barrett and Tsutsui, 1996): oestrogen is thought to disrupt microtubule assembly, resulting in anaphase abnormalities and non-disjunction. The resulting chromosomal aneuploidy has been proposed to induce cell transformation.

Figure 1. Formation and metabolic redox cycling of catecholoestrogens. 2-Hydroxyoestradiol is the major hydroxylated oestradiol metabolite in most mammalian species. The oestrogen 2-hydroxylase activity (1) forms mainly 2-hydroxyoestradiol and, by a lack of specificity of the enzyme, 15–20% 4-hydroxyoestradiol. This latter metabolite is also formed by oestrogen 4-hydroxylase, CYP1B1 (2). These catecholoestrogens may undergo metabolic redox cycling between quasiqone and quinone forms. The semiquinone intermediates are free radicals and may react with molecular oxygen to form superoxide radicals. These metabolic pathways are discussed in detail in a recent review (Liehr, 2000).
Is oestrogen-induced cancer an epigenetic process?

The epigenetic mechanisms of carcinogenesis by oestrogens are compelling in their simplicity, and have guided the research community well for many years. However, during the past one or two decades, experimental evidence has come to light which is inconsistent with tumour induction by oestrogens solely by hormone receptor-mediated processes or by numerical chromosomal changes.

(i) Proliferating cells of normal human mammary epithelium do not express oestrogen receptors, whereas oestrogen receptor-containing cells do not express proliferation markers (Clarke et al., 1997; Russo et al., 1999). These data demonstrate that oestrogen receptor-mediated processes may be linked indirectly to normal mammary cell growth, whereas oestrogen receptor-mediated stimulation of proliferation of breast cancer cells is well established. Furthermore, the Syrian hamster embryo cells used for mechanistic studies of hormonal carcinogenesis (Barrett et al., 1981; Barrett and Tsutsui, 1996) do not express measurable levels of oestrogen receptors (Korach and McLachlan, 1985). Therefore, the induction of aneuploidy and cell transformation by oestrogens in these cells did not require hormone receptors.

(ii) The induction of kidney tumours in Syrian hamsters by oestradiol has been decreased by inhibitors of oestrogen metabolism such as α-naphthoflavone or by free radical scavengers such as ascorbic acid (vitamin C) or butylated hydroxyanisole (BHA) (Liehr and Wheeler, 1983; Liehr et al., 1989, 1991). In addition, several synthetic oestrogens such as 2-fluoroestradiol or 17α-ethinylestradiol are poor carcinogens in this model system compared with the 100% tumour incidence induced by oestradiol, despite the comparable hormonal potency of these three oestrogens (Kirkman, 1959; Liehr, 1983; Liehr et al., 1986c). When the metabolism of the synthetic oestrogens was examined in the hope of detecting reasons for the differing carcinogenic activities, a decreased catechol oestrogen formation from 2-fluoroestradiol and 17α-ethinylestradiol was detected compared with rates of aromatic hydroxylation observed with the parent hormone (Ashburn et al., 1993; Zhu et al., 1993; Stalford et al., 1994). From these data, it has been concluded that hormonal activity of oestrogens via receptor-mediated pathways may be necessary, but not sufficient, for tumour induction. From the metabolism experiments indicating altered aromatic hydroxylation, it has been concluded that catechol oestrogen metabolites may play a crucial role in tumour initiation by oestrogens.

(iii) Genetic experiments have been carried out more recently to define the role of oestrogen receptors in mammary carcinogenesis. For this purpose, oestrogen receptor knockout (ERKO) mice have been cross-bred with mice overexpressing the Wnt-1 gene, because these latter animals develop mammary tumours within a few months after birth (Bocchinfuso et al., 1999). In the cross-bred ERKO/Wnt-1 animals, the onset of mammary tumorigenesis was delayed but not eliminated. The authors concluded that Wnt-1 proto-oncogene expression in these animals induces mammary carcinogenesis, irrespective of oestrogen receptor status. Ovariecotomy of the ERKO/Wnt-1 animals reduced the circulating oestrogen concentrations in these animals, and also tumour induction. These data are consistent with a role of genotoxicity in oestrogen-induced mammary carcinogenesis.

(iv) Numerical chromosomal changes (aneuploidy) may well be a part of the genetic instability necessary for the development of cancer as proposed earlier (Lengauer et al., 1998), but by themselves are not sufficient for tumours to develop. This conclusion is based on earlier experiments (Endo et al., 1994) in which it was observed that Syrian hamster embryo cells carrying such chromosomal abnormalities were not capable of inducing tumours in nude mice. These authors concluded that other genetic changes (mutations) are necessary in addition to chromosomal abnormalities for cells to become tumorigenic.

Taken together, these data point to a complex mechanism of oestrogen-induced tumour induction, including mammary and uterine carcinogenesis. This complex mechanism may combine endocrine aspects such as receptor-mediated cell proliferation with tumour initiation by genotoxic events as outlined above.
Oestrogen genotoxicity in hormone-induced cancer

Table IV. More recent reports of oestradiol- or oestradiol metabolite-induced mutagenic activity

<table>
<thead>
<tr>
<th>Type of genetic mutation/ oestrogen</th>
<th>Test system</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numerical chromosomal aberrations (aneuploidy)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oestradiol</td>
<td>Syrian hamster embryo cells</td>
<td>Tsutsui et al. (1987, 1997)</td>
</tr>
<tr>
<td>Oestradiol</td>
<td>Human fibroblasts</td>
<td>Tsutsui et al. (1990)</td>
</tr>
<tr>
<td>Oestradiol</td>
<td>Syrian hamster kidney</td>
<td>Banerjee et al. (1992, 1994)</td>
</tr>
<tr>
<td>Structural chromosomal aberrations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oestradiol</td>
<td>Mouse genital tract</td>
<td>Hajek et al. (1989)</td>
</tr>
<tr>
<td>Oestradiol</td>
<td>Syrian hamster kidney</td>
<td>Banerjee et al. (1992, 1994)</td>
</tr>
<tr>
<td>2- and 4-Hydroxyoestriol</td>
<td>SHE cells</td>
<td>Tsutsui et al. (2000a)</td>
</tr>
<tr>
<td>2- and 4-Hydroxyoestriol</td>
<td>SHE cells</td>
<td>Tsutsui et al. (2000a)</td>
</tr>
<tr>
<td>Gene mutations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oestradiol</td>
<td>Gpt transgene, CH G12 cells</td>
<td>Su et al. (1998)</td>
</tr>
<tr>
<td>Oestradiol (10^{-10}M)</td>
<td>Hprt gene, CH V79 cells</td>
<td>Rajah and Pento (1994); Kong et al. (2000)</td>
</tr>
<tr>
<td>Oestradiol</td>
<td>Methotrexate resistance gene</td>
<td>Thibodeau et al. (1998)</td>
</tr>
<tr>
<td>2- and 4-Hydroxyoestriol</td>
<td>MCF-7 human breast cancer cells</td>
<td></td>
</tr>
<tr>
<td>4-Hydroxyoestriol</td>
<td>Hprt gene, SHE cells</td>
<td>Tsutsui et al. (2000a)</td>
</tr>
<tr>
<td>2- and 4-hydroxyoestriol</td>
<td>Hprt gene, SHE cells</td>
<td>Tsutsui et al. (2000b)</td>
</tr>
<tr>
<td>Gene amplification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oestradiol</td>
<td>c-myc gene, hamster kidney</td>
<td>Li et al. (1999)</td>
</tr>
<tr>
<td>Microsatellite instability</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oestradiol</td>
<td>Syrian hamster kidney</td>
<td>Hodgson et al. (1998)</td>
</tr>
<tr>
<td>Oestradiol</td>
<td>Human breast epithelial cells</td>
<td>Russo et al. (2001)</td>
</tr>
<tr>
<td>Loss of heterozygosity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oestradiol</td>
<td>Human breast epithelial cells</td>
<td>Russo et al. (2001)</td>
</tr>
</tbody>
</table>

CH = Chinese hamster; SHE = Syrian hamster embryo.

Oestrogen-induced gene mutations

As pointed out above, oestrogens induce tumours in laboratory animals and in humans (International Agency for Research on Cancer, 1987, 1999), transform cells in culture (Barrett et al., 1981; Barrett and Tsutsui, 1996; Russo et al., 2001) and are genotoxic, but have been reported not to induce mutations at specific genetic loci of commonly used point mutation assays. Moreover, the experimental evidence is not consistent with a mechanism of oestrogen-induced carcinogenesis solely based on oestrogen receptor-stimulated cell proliferation. Therefore, the induction of mutations by oestrogens or its metabolites has been re-examined, because oestrogens might have induced other genetic lesions than those commonly tested for by Ames and other bacterial or mammalian cell mutation assays. A wider definition of genetic lesions has been accepted in this review (Lengauer et al., 1998), because these authors have identified a mixture of various modifications at the chromosomal and gene levels to form the genetic basis of human cancer. These genetic lesions include chromosomal alterations such as numerical chromosomal changes (aneuploidy) and structural chromosomal aberrations, gene amplification, microsatellite alterations, and small changes of specific genes such as point mutations, insertions, and deletions. All these types of genetic lesions have now been documented for oestrogens or oestrogen metabolites (Table IV). For instance, chromosomal changes, i.e. aneuploidy and structural chromosomal aberrations have been documented for oestradiol both in cells in culture and in a target organ of oestrogen-induced cancer, the Syrian hamster kidney (Tsutsui et al., 1987, 1990, 1997, 2000a; Banerjee et al., 1992, 1994). In the same animal model, oestradiol induces c-myc gene amplification and microsatellite instability (Hodgson et al., 1998; Li et al., 1999). Low concentrations (10^{-10}M) of oestradiol induce hprt gene mutations of V79 cells. The mutation frequency is slightly elevated, yet statistically significant (Rajah and Pento, 1995; Kong et al., 2000) (Table IV). Deletions, point mutations (transitions and transversions) and an insertion have been identified by sequencing of the coding region of the hprt gene of V79 mutant clones (Kong et al., 2000). Mutations in the hprt gene of Syrian hamster embryo (SHE) cells have been induced by 4-hydroxyoestriol and 2- and 4-hydroxyoestriol, but not by the parent hormone oestrone or oestradiol (Tsutsui et al., 2000a). A 10- and 27-fold higher mutation frequency in the methotrexate resistance gene of MCF-7 breast cancer cells has been induced by 2- and 4-hydroxyoestriol, respectively, than by oestradiol (Thibodeau et al., 1998). Finally, others (Russo et al., 2001) transformed human breast epithelial cells by treatment with oestradiol. A genomic analysis of the transformed clones revealed microsatellite instability and loss of heterozygosity in chromosomes 3 and 11 (Russo et al., 2001). These reports demonstrate that steroido oestrogens induce all the different types of genetic lesions postulated previously (Lengauer et al., 1998) to be the basis of human cancer. In those studies where oestrogen metabolites have been examined, the 4-hydroxylated oestrogens (and to some degree 2-hydroxylated metabolites) elicit the highest mutagenic activity (Thibodeau et al., 1998; Tsutsui et al., 2000a).
Proposed mechanism of oestrogen-induced carcinogenesis

The data discussed above demonstrate that hormone-induced cancer including mammary and uterine cancer in humans may be a very complex disease characterized by an interplay of mutagenic and hormonal effects of oestrogens. The simple mechanistic proposals of the past, which emphasized only one or the other effects of hormones, may have served us well then, but they are not consistent with the experimental evidence available today. The genotoxic and mutagenic activities of oestrogens described above are consistent with and delineate a dual role of oestrogens as carcinogens (tumour initiators) and as hormones (stimulators of cell proliferation). The following proposal of oestrogen-induced carcinogenesis is in line with all the hormonal and genotoxic effects of oestrogens described above and is illustrated in Figure 2. In cells expressing oestrogen 4-hydroxylase activity, oestradiol or oestrone is converted to 4-hydroxylated oestrogen metabolites. If these catechol-oestrogens are not detoxified by phase II enzyme activities such as catechol-O-methyltransferase, UDP glucuronosyl transferase or sulphotransferase, they may undergo metabolic redox cycling between quinone and hydroquinone (i.e. catechol) forms (Liehr et al., 1986a; Roy and Liehr, 1988; Liehr, 2000). The semiquinone intermediates in this redox cycle are free radicals, which generate more oxygen radicals and may induce multiple forms of DNA damage. The quinone intermediates may bind covalently to nucleic acids and form potentially mutagenic DNA adducts or apurinic sites. Multiple genetic lesions may be induced at the chromosome or gene level by these various types of genotoxicity. The induction of DNA damage and mutations by reactive metabolic intermediates of oestrogens highlights the metabolic balance of cells between hormone biosynthesis, hormone action and catabolic conversion to biologically harmless products as a way to control deleterious events. Tumours may arise from cells which have suffered a loss of cellular control over hormone biosynthesis and metabolism, and subsequently have been transformed by oestrogen-induced mutational changes and at the same time have responded to hormone receptor-mediated stimulation of cell proliferation. It is possible that oestrogens may indirectly stimulate cell proliferation by more complex endocrine regulatory mechanisms, since proliferating normal mammary epithelial cells do not express oestrogen receptors.

Conclusions

In summary, the data discussed above indicate that the natural hormones oestradiol and oestrone are weak mutagens and weak carcinogens compared with more powerful polycyclic hydrocarbon carcinogens used in the laboratory. This conclusion is supported by the relatively modest increase in breast cancer risk induced by long-term intake of oestrogen medications and by elevated endogenous oestrogen production. It is not inconsistent with the tumour incidence observed in animal models. The genotoxicity and mutagenicity studies of oestrogens point to the catecholoestrogen metabolites as the precursors of DNA damaging semiquinone/quinone reactive intermediates. Hormonal, receptor-mediated effects may complete the carcinogenic process in cells altered by mutagenic lesions. Therefore, oestrogens must be considered complete carcinogens, which may induce tumours by a dual action as inducers of genotoxicity and of cell proliferation. There are synthetic oestrogens, which are poorly carcinogenic in the hamster kidney tumour system compared with the 100% tumour incidence induced by oestradiol and which have...
well-maintained hormonal potency (Kirkman, 1959; Liehr, 1983; Liehr et al., 1986c). The existence of such oestrogens and the possibility of separating hormonal from carcinogenic activities indicate that cell proliferation is a necessary (but not sufficient) event in the course of cancer development. This conclusion is consistent with previous evaluations of the role of cell proliferation in oncogenesis (Farber, 1995; Huff, 1995).

Only a weakly carcinogenic/mutagenic activity may be expected of naturally occurring hormones, and this may be the maximal deleterious effect tolerated by living organisms. A higher tumorigenic activity of endogenous oestrogens would have provided an evolutionary disadvantage and would not have permitted the existence of higher life forms, including that of the human species. Humans living in the modern world may be exposed to oestrogenic drugs, natural or synthetic oestrogens in foods and endocrine disruptors in the environment, which may add to the biological effects of endogenously produced oestrogenic hormones, and thus may raise breast and uterine cancer risks by the mechanisms described.

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Oestrogen genotoxicity in hormone-induced cancer