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Review

# Unbalanced metabolism of endogenous estrogens in the etiology and prevention of human cancer

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ABSTRACT

Among the numerous small molecules in the body, the very few aromatic ones include the estrogens and dopamine. In relation to cancer initiation, the estrogens should be considered as chemicals, not as hormones. Metabolism of estrogens is characterized by two major pathways. One is hydroxylation to form the 2- and 4-catechol estrogens, and the second is hydroxylation at the 16α position. In the catechol pathway, the metabolism involves further oxidation to semiquinones and quinones, including formation of the catechol estrogen-3,4-quinones, the major carcinogenic metabolites of estrogens. These electrophilic compounds react with DNA to form the depurinating adducts 4-$OHE_1(E_2)$-1-N3Ade and 4-$OHE_1(E_2)$-1-N7Gua. The apurinic sites obtained by this reaction generate the mutations that may lead to the initiation of cancer. Oxidation of catechol estrogens to their quinones is normally in homeostasis, which minimizes formation of the quinones and their reaction with DNA. When the homeostasis is disrupted, excessive amounts of catechol estrogen quinones are formed and the resulting increase in depurinating DNA adducts can lead to initiation of cancer. Substantial evidence demonstrates the mutagenicity of the estrogen metabolites and their ability to induce transformation of mouse and human breast epithelial cells, and tumors in laboratory animals. Furthermore, women at high risk for breast cancer or diagnosed with the disease, men with prostate cancer, and men with non-Hodgkin lymphoma all have relatively high levels of estrogen–DNA adducts, compared to matched control subjects. Specific antioxidants, such as *N*-acetylcysteine and resveratrol, can block the oxidation of catechol estrogens to their quinones and their reaction with DNA. As a result, the initiation of cancer can be prevented.


**Contents**



*Abbreviations:* BB, Big Blue; COMT, catechol-*O*-methyltransferase; CYP, cytochrome P450; CYP19, aromatase; DA-Q, dopamine quinone; DES, diethylstilbestrol; $E_1$, estrone; $E_2$, estradiol; $E_1(E_2)$-Q, estrone(estradiol) quinone; ERKO, estrogen receptor-α knocked out; GSH, glutathione; GST, glutathione-*S*-transferase; H, Harvey; NAcCys, *N*-acetylcysteine; NQO1 and NQO2, NAD(P)H quinone oxidoreductase 1 and 2; $OHE_1(E_2)$, hydroxyestrone(estradiol); PAH, polycyclic aromatic hydrocarbons; UPLC–MS/MS, ultraperformance liquid chromatography/tandem mass spectrometry.

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## 1. Introduction

Aliphatic and heteroaromatic molecules are widely represented in the human body, whereas aromatic molecules are rarely present. Organic chemistry is divided into three major classes of compounds: aliphatic, aromatic and heteroaromatic. Aromatic chemistry is the chemistry of benzene and polycyclic aromatic hydrocarbons. Heteroaromatic chemicals contain in their aromatic rings one or more heteroatoms, such as nitrogen. The few aromatic biomolecules have only one benzene ring, and include the estrogen hormones and the neurotransmitter dopamine.

Conversion of testosterone to estradiol ($E_2$) and androstenedione to estrone ($E_1$) constitutes the biosynthesis of estrogens, catalyzed by the enzyme aromatase, cytochrome P450 (CYP)19 (Fig. 1A). Dopamine is biosynthesized by hydroxylation of the amino acid L-tyrosine to L-Dopa, catalyzed by tyrosine hydroxylase, and subsequent decarboxylation to dopamine, catalyzed by L-Dopa decarboxylase (Fig. 1B).

The parent compound of aromatic chemistry is benzene, and it was found to induce leukemia a long time ago. The recognition that benzene is a human leukemogen required evaluation of large populations exposed to the chemical [1]. These data were obtained from Italian and Turkish workers in the shoemaking and printing industries, who had high incidences of acute myeloid leukemia [2]. More recently, the induction of non-Hodgkin lymphoma by benzene has been demonstrated [3,4]. Many polycyclic aromatic hydrocarbons (PAH) are carcinogenic, with potencies ranging from weak to very strong [5].

Metabolic activation of benzene and PAH to ultimate carcinogenic forms follows the principles of chemical carcinogenesis pioneered by James and Elizabeth Miller in the early 1960s [6,7], i.e., most chemical carcinogens (95%) are metabolically activated to electrophilic species that bind covalently to nucleophilic sites in DNA, forming predominantly DNA adducts of Ade and Gua. The other 5% are carcinogens that directly react with DNA without metabolic activation.

Most of the adducts of PAH are the depurinating adducts, which detach from DNA, leaving behind apurinic sites [8,9]. The apurinic sites can be erroneously repaired to give rise to mutations [10] that can initiate the cancer process. The sites of the depurinating adducts correlate with the sites of mutations in the Harvey (H)-*ras* oncogene [10]. The stable adducts, which remain in DNA unless removed by repair, are formed to a much smaller extent.

PAH have two major mechanisms of metabolic activation to form ultimate carcinogens: one is formation of radical cations, and the other is formation of bay-region diol-epoxides [8,9]. A third mechanism of metabolic activation, which produces extremely weak ultimate carcinogens, generally involves compounds containing one or two benzene rings. In these compounds, activation occurs through formation of electrophilic catechol quinones, which react with DNA by Michael addition to form adducts. This mechanism of activation occurs with benzene [11,12], naphthalene [13,14], $E_1(E_2)$ [15–17], diethylstilbestrol (DES) [18], hexestrol [19], and dopamine [11,12] (Fig. 2). In this mechanism, the benzene ring is enzymatically oxidized to form a phenol. A second hydroxylation leads to formation of a catechol, followed by a third oxidation to form the ultimate carcinogenic metabolite, an *ortho*-quinone (Fig. 2). The electrophilic *ortho*-quinone reacts with the purine bases of DNA to form N3Ade and N7Gua adducts (Fig. 2).

## 2. Genotoxicity of estrogens

Exposure to estrogens has been epidemiologically associated with increased risk of breast cancer, and evidence of a dose–response relationship has been found [20,21]. Induction of prostate adenocarcinomas in 100% of Noble rats implanted with $E_2$ plus testosterone, vs. 40% of rats treated only with testosterone, led to the hypothesis that $E_2$ initiates and testosterone promotes the development of prostate tumors [22].

In relation to cancer initiation, estrogens should be considered as other chemicals, namely, their physico-chemical and biochemical properties lead them to follow the principles of chemical carcinogenesis elucidated by the Millers [6,7], rather than considering them as hormones. Substantial evidence supports a genotoxicity paradigm for the initiation of cancer by endogenous estrogens. Specific oxidative metabolites of estrogens can react with DNA and generate the critical mutations that lead to the initiation of cancer (Fig. 3) [16,17,23–29]. Two major pathways of metabolism of estrogens are the formation of catechol estrogens, 2-hydroxy(OH)$E_1(E_2)$ and 4-$OHE_1(E_2)$, and the formation of 16α-$OHE_1(E_2)$ [30]. If the catechol estrogens are not conjugated, they can lead through oxidation to semiquinones and quinones (Q, Figs. 3 and 4). Both the $E_1(E_2)$-2,3-Q and $E_1(E_2)$-3,4-Q react with DNA to form DNA adducts, but the 3,4-Q are more reactive with various nucleophilic groups of DNA than the 2,3-Q (Fig. 4) [16,17,23,26]. Depurination of the 4-$OHE_1(E_2)$-1-N3Ade and 4-$OHE_1(E_2)$-1-N7Gua adducts generates apurinic sites in the DNA. Error-prone repair of these apurinic sites may lead to specific mutations [27–29] that can initiate breast, prostate and other types of human cancer (Fig. 3) [31].

Carcinogenicity testing of the endogenous estrogens $E_1$ and $E_2$ and their catechols demonstrated that they induce cancer in hormone dependent and independent organs [32–36]. This paradigm suggests that specific critical mutations generate abnormal cell proliferation leading to cancer, rather than estrogen receptor-mediated cell proliferation giving rise to random cellular mutations. The specificity of these critical mutations arises from the intercalated complex between estrogens and DNA before conversion to a covalent bond between them, as demonstrated with DES [18].

## 3. Metabolism of estrogens

Metabolism of estrogens is characterized by a balanced homeostatic set of activating and deactivating pathways (Fig. 5). Aromatization of androstenedione and testosterone, catalyzed by aromatase (CYP19), yields $E_1$ and $E_2$, respectively. Excess estrogen is stored as $E_1$-sulfate. $E_1$ and $E_2$ are interconverted by 17β-hydroxysteroid dehydrogenase, and they are metabolized by hydroxylation at the 2- or 4-position to form 2-$OHE_1(E_2)$ or 4-$OHE_1(E_2)$. CYP1A1 preferentially hydroxylates $E_1$ and $E_2$ at the 2-position, whereas CYP1B1 almost exclusively catalyzes the formation of 4-$OHE_1(E_2)$ [37–39]. The most common pathway of conjugation of catechol estrogens in extrahepatic tissues is *O*-methylation, catalyzed by catechol-*O*-methyltransferase (COMT) [40]. If the activity of COMT is low, competitive oxidation of the catechol estrogens to $E_1(E_2)$-2,3-Q and $E_1(E_2)$-3,4-Q by CYP or peroxidases can increase (Fig. 5).

Oxidation of semiquinones to quinones can also be mediated by molecular oxygen. Reduction of estrogen quinones to semiquinones, catalyzed by CYP reductase, completes the redox cycle (Fig. 5). In this process, the molecular oxygen is reduced to superoxide anion radical, which is converted to $H_2O_2$, yielding hydroxyl radicals in the presence of $Fe^{++}$. The hydroxyl radicals first generate lipid hydroperoxides, which can act as unregulated cofactors of CYP, leading to an abnormal increase in the oxidation of the catechol estrogens to their quinones. Thus, redox cycling can be a major contributor to the formation of $E_1(E_2)$-Q, which are the ultimate carcinogenic metabolites of estrogens.

The 4-$OHE_1(E_2)$ have greater carcinogenic potency than the 2-$OHE_1(E_2)$ [33–35], an effect that cannot be attributed to formation