HHS Public Access

Author manuscript

Ann N Y Acad Sci. Author manuscript; available in PMC 2017 August 02.

Published in final edited form as:

Ann N Y Acad Sci. 2009 February ; 1155: 68–75. doi:10.1111/j.1749-6632.2008.03676.x.

Estrogen Metabolism and Breast Cancer A Risk Model

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Abstract

Oxidative metabolites of estrogens have been implicated in the development of breast cancer, yet relatively little is known about the metabolism of estrogens in the normal breast. We developed an experimental *in vitro* model of mammary estrogen metabolism in which we combined purified, recombinant phase I enzymes CYP1A1 and CYP1B1 with the phase II enzymes COMT and GSTP1 to determine how 17β-estradiol (E_2) is metabolized. We employed both gas and liquid chromatography with mass spectrometry to measure the parent hormone E_2 as well as eight metabolites, that is, the catechol estrogens, methoxyestrogens, and estrogen–GSH conjugates. We used these experimental data to develop an *in silico* model, which allowed the kinetic simulation of converting E_2 into eight metabolites. The simulations showed excellent agreement with experimental results and provided a quantitative assessment of the metabolic interactions. Using rate constants of genetic variants of CYP1A1, CYP1B1, and COMT, the model further allowed examination of the kinetic impact of enzyme polymorphisms on the entire metabolic pathway, including the identification of those haplotypes producing the largest amounts of catechols and quinones. Application of the model to a breast cancer case-control population defined the estrogen quinone E_2-3,4-Q as a potential risk factor and identified a subset of women with an increased risk of breast cancer based on their enzyme haplotypes and consequent E_2-3,4-Q production. Our *in silico* model integrates diverse types of data and offers the exciting opportunity for researchers to combine metabolic and genetic data in assessing estrogenic exposure in relation to breast cancer risk.

Keywords

estrogen; metabolism; breast cancer; risk; genotype; model

Introduction

Estrogens have long been recognized as the prime risk factor for the development of breast cancer,[1,2] but their assessment has not progressed beyond traditional exposure data, such as age, age at menarche, and age at first live birth. Although valuable in risk calculation,

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Conflicts of Interest

The authors declare no conflicts of interest.

current models of breast cancer risk prediction based on cumulative estrogen exposure do not reflect observations of and data on mammary estrogen metabolism.[3,4] Here we present a novel approach that is based on the molecular analysis of mammary estrogen metabolism.

Carcinogenesis is usually viewed as a stepwise process beginning with genotoxic effects (initiation) followed by enhanced cell proliferation (promotion). In the breast the main estrogen, 17β-estradiol (E_2), is both a substrate for the phase I enzymes cytochrome P50 (CYP) 1A1 and 1B1 and a ligand for the estrogen receptor (ER). In its dual role of substrate and ligand, E_2 has been implicated in the development of breast cancer by the way it simultaneously causes DNA damage via its oxidation products, the 2-OH and 4-OH catechol estrogens, and by how it stimulates cell proliferation and gene expression via the ER. Thus, E_2 and its oxidative metabolites are unique carcinogens that affect both tumor initiation and promotion.[5-8]

Experiments on estrogen metabolism, formation of DNA adducts, mutagenicity, cell transformation, and carcinogenicity have implicated that certain estrogen metabolites, especially the catechol estrogen 4-hydroxyestradiol (4-OHE_2), react with DNA via its quinone, causing mutations and initiating carcinogenesis.[8-18] It is important to note that the concentration of the 4-OHE_2 metabolite in human breast tissue is actually higher than that of the parent hormone, E_2, evidence that oxidative estrogen metabolism may be a critical factor in the development of human breast cancer.[14,19] Estrogen–DNA adducts have been detected in normal and malignant human breast tissues,[20,21] and we have recently provided direct experimental proof that oxidative metabolism of E_2 leads to the formation of 4-OHE_2 and deoxyribonucleoside adducts.[22] The collective evidence points to the importance of including mammary estrogen metabolism data into risk calculations, which is what we aim to accomplish with our new model.[23]

Models of Mammary Estrogen Metabolism

Several investigators have proposed a qualitative model of mammary estrogen metabolism regulated by oxidizing phase I and conjugating phase II enzymes.[9,24] As shown in Figure 1, E_2 is oxidized to catechol estrogens by CYP1A1 and 1B1. These enzymes further oxidize the catechol estrogens to semiquinones and quinones. The highly reactive estrogen quinones form Michael addition products with deoxyribonucleosides.[25-27] Thus, estrogen quinones share a common feature of many chemical carcinogens, that is, the ability to covalently modify DNA.[12,28,29] Furthermore, estrogen semiquinones and quinones undergo redox-cycling, which results in the production of reactive oxygen species that can cause oxidative DNA damage.[30-32] It is postulated that the genotoxicity of the oxidative estrogen metabolism pathway is mitigated by alternate reactions of the metabolites with phase II enzymes. Specifically, COMT catalyzes the methylation of catechol estrogens to methoxy estrogens, which lowers the amount of catechol estrogens available for conversion to estrogen quinones.[33,34] In turn, the estrogen quinones undergo conjugation with glutathione (GSH) via the catalytic action of GSTP1.[35] The formation of GSH–estrogen conjugates would reduce the level of-estrogen quinones and thereby lower the potential for DNA damage.

The current model of mammary estrogen metabolism has several limitations. First, only single enzymes, for example, CYP1B1 and COMT, have been analyzed to date with simple substrate-product kinetics, which clearly generates an incomplete picture of the metabolic pathway. Second, while the model incorporates the functional roles of the phase I and II enzymes, it does so only qualitatively, and it remains uncertain how the enzymes interact quantitatively. Third, each of the phase I and II enzymes contains genetic polymorphisms.[33,36-38] Studies from several laboratories, including our own, have examined the functional implications of the polymorphisms on estrogen metabolism, again focusing on single enzymes.[33,34,38-40] Thus, the multitude of potential kinetic reactions resulting from the complex genetic variations of the phase I and II enzymes is completely outside the scope of the current model of estrogen metabolism. In contrast to the relatively small number of functional studies of estrogen metabolism multiple epidemiological studies have investigated breast cancer risk in relation to genetic variation in the critical enzymes involved in estrogen metabolism with inconsistent findings.[41,42] Such studies are limited by their ability to consider only one or two of the enzymes in the estrogen metabolic pathway. Furthermore, those studies that examined all of the component enzymes were not able to assess underlying metabolic interactions in the pathway.[43,44] The drawback of any purely genetic assessment is the lack of information about functional interactions inherent in complex metabolic pathways such as the estrogen metabolism pathway. Moreover, DNA analysis identifies the variant alleles but does not quantify the variation in the dynamics of the pathway. In contrast, the functional protein analysis will provide a quantitative assessment with each variation in protein structure likely to have a different effect. Thus, a pathway-based functional and quantitative approach is needed to overcome the current limitation in genotype assessment.

We recently developed an experimental *in vitro* model of mammary estrogen metabolism, in which we combined purified, recombinant phase I enzymes CYP1A1 and CYP1B1 with the phase II enzymes COMT and GSTP1 to determine how E_2 is metabolized.[45] We employed both gas and liquid chromatography with mass spectrometry (GC–MS and LC-MS) to measure the parent hormone E_2 as well as eight metabolites, i.e., the catechol estrogens, methoxyestrogens, and estrogen–GSH conjugates. We then used these experimental data to develop an *in silico* model of the metabolic pathway.[23] The *in silico* model allowed the kinetic simulation of converting E_2 into eight metabolites. The simulations showed excellent agreement with experimental results and provided a quantitative assessment of the metabolic interactions (Fig. 2). Using rate constants of genetic variants of CYP1A1, CYP1B1, and COMT, the model further allowed examination of the kinetic impact of enzyme polymorphisms on the entire estrogen metabolic pathway, including the identification of those haplotypes producing the largest amounts of catechols and quinones (Fig. 3).

Our *in silico* model is pertinent to the numerous epidemiological studies that have examined the association of genetic variants of enzymes involved in estrogen metabolism with breast cancer risk.[41,42] These studies were handicapped because they investigated only one or two enzymes, but even those examining all enzymes have been fundamentally limited by not having the means to assess the underlying metabolic interactions.[43,44] Our model attempts to fill this gap, and we applied it to a hospital-based case-control population that was analyzed previously with respect to CYP1A1, CYP1B1, and COMT genotypes.[43,46,47] Here we went

beyond genotypes and used the model to determine for each woman the effect of her composite CYP1A1, CYP1B1, and COMT haplotypes on estrogen metabolite production. Inherited variations in enzyme genotype persist throughout life and can therefore be regarded as constants for each individual.

Application of the model to a breast cancer case-control population (221 invasive breast cancer cases, 217 controls) defined the estrogen quinone E_2-3,4-Q as a potential risk factor. In this exploratory study, the model identified a subset of women with an increased risk of breast cancer based on their enzyme haplotype and consequent E_2-3,4-Q production (Fig. 4).[23] Whether the E_2-3,4-Q is an independent risk factor, as suggested by our exploratory study, will need to be confirmed by a larger separate study.

Summary

Carcinogenesis is usually viewed as a stepwise process that begins with genotoxic effects (initiation) followed by enhanced cell proliferation (promotion). The main estrogen, E_2, is a substrate for oxidizing enzymes such as CYP1B1 and a ligand for the ER. In its dual role of substrate and ligand, E_2 has been implicated in the development of breast cancer by its simultaneously causing DNA damage via its oxidation products, the catechol estrogens, and estrogen quinones and by stimulating cell proliferation and gene expression via the ER. Thus, E_2 and its oxidative metabolites are unique carcinogens that affect both tumor initiation and promotion (Fig. 5).[5-8]

Basing further research on our kinetic-genomic model, it should be possible for investigators to develop more refined risk prediction models that integrate known reproductive and lifestyle factors with predicted exposure to E_2-3,4-Q as determined by inherited variation in critical genes involved in the estrogen metabolic pathway. Being based upon a validated laboratory model and high quality epidemiologic data, this research has the potential to significantly enhance our ability to predict breast cancer risk in individual women—a potential with direct implications for breast cancer screening and earlier detection of disease.

Acknowledgments

Supported by NIH (1R01CA/ES83752, U54CA113007) and the Vanderbilt Integrative Cancer Biology Center.

Financial Support

This work was partially supported under grant U54CA113007

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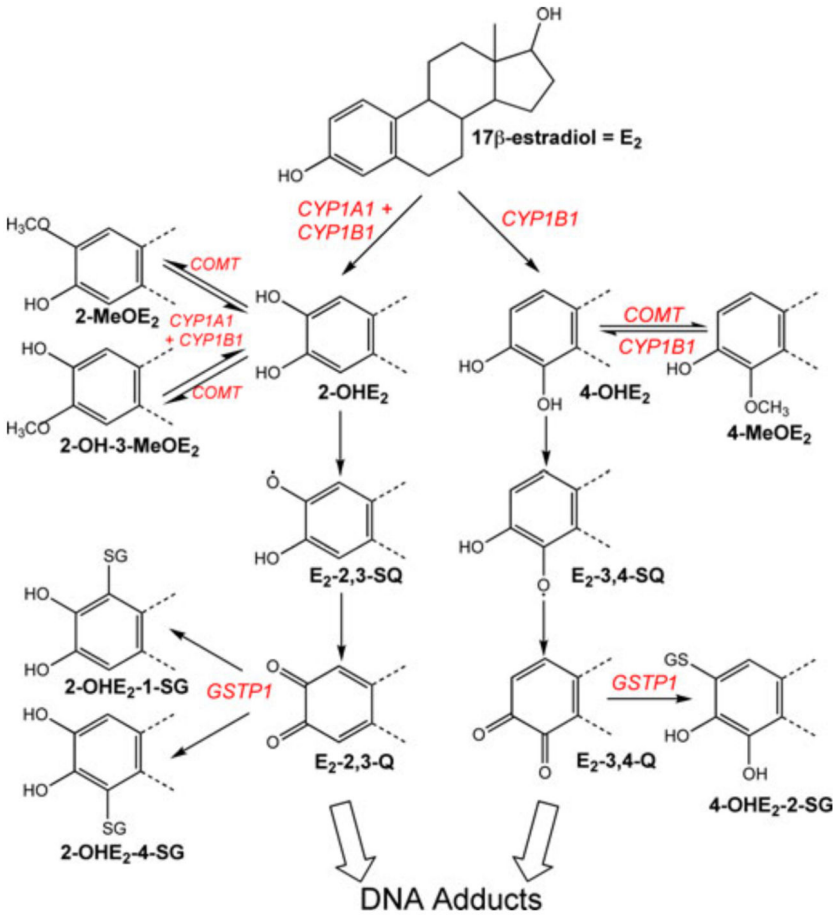

Figure 1.

Oxidative estrogen metabolism causes DNA adduct formation. The estrogen metabolism pathway is regulated by oxidizing phase I and conjugating phase II enzymes. CYP1A1 and CYP1B1 catalyze the oxidation of E_2 to catechol estrogens 2-OHE_2 and 4-OHE_2. The catechol estrogens are either methylated by COMT to methoxyestrogens (2-$MeOE_2$, 2-OH-3-$MeOE_2$, 4-$MeOE_2$) or further oxidized by CYPs to semiquinones (E_2-2,3-SQ, E_2-3,4-SQ) and quinones (E_2-2,3-Q, E_2-3,4-Q). The estrogen quinones are conjugated by GSTP1 to GSH-conjugates (2-OHE_2-1-SG, 2-OHE_2-4-SG, 4-OHE_2-2-SG). Alternatively, the quinones can form quinone-DNA adducts (e.g., 4-OHE_2-N7-guanine, 2-OHE_2-N2-deoxyguanosine) or cause oxidative adducts (e.g., 8-OH-deoxyguanosine) via reactive oxygen species resulting from redox-cycling between semiquinones and quinones. The three adducts and their estrone (E_1) and adenine counterparts have been identified in human breast tissues.[20,21] Recently, we demonstrated experimentally that CYP1B1-mediated oxidation of E_2 in the presence of deoxyguanosine caused the formation of the 4-OHE_2-N7-guanine adduct.[22] Our results provide direct evidence that metabolism of the parent hormone can initiate DNA damage.

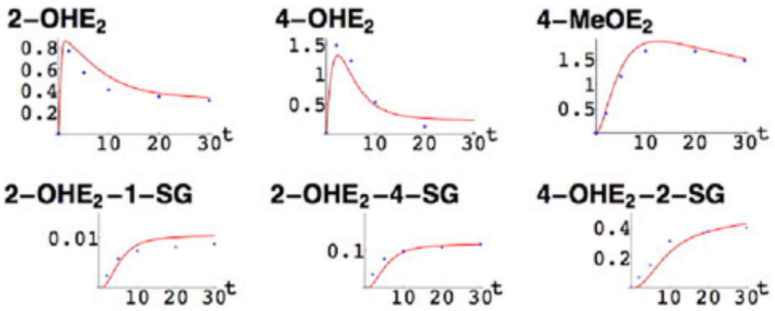

Figure 2.

Comparison of mathematical model with experimental data. The red curves are plots of the solutions to the nonlinear system of differential equations and the blue dots are experimental data.[45] As shown, the model allowed simulations of all reactions in the pathway, which agreed well with the experimentally determined results.[23]

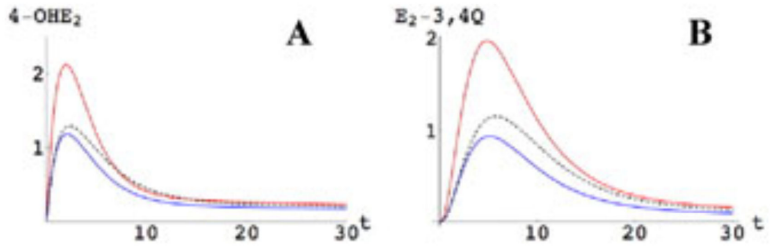

Figure 3.

Kinetic–genomic modeling of catechol estrogen (**A**) 4-OHE_2 and estrogen quinone (**B**) E_2-3,4-Q using rate constants for wild-type and variant CYP1A1, CYP1B1, and COMT. The Area Under the Curve = AUC represents the metabolite production over time. Only the highest, lowest, and wild-type (dotted line) AUCs are shown.[23]

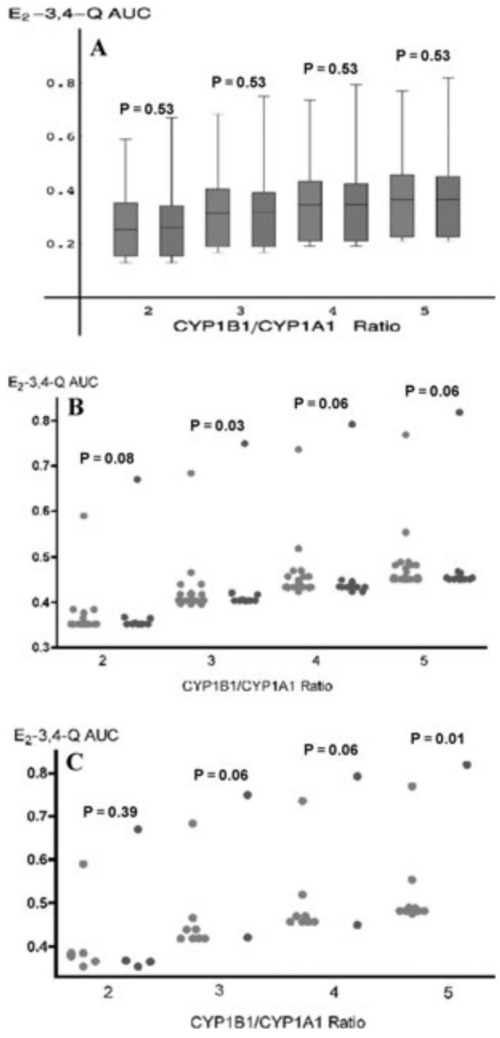

Figure 4.

Correlation of E_2-3,4-Q AUC with CYP1B1/CYP1A1 ratio for cases and controls.[23] (**A**) Box and whisker graph of E_2-3,4-Q AUCs for entire population of 221 cases (red) and 217 controls (blue). Each box includes 84% of the respective group while the whiskers represent the top and bottom 8 percentiles. As indicated by the medians (center line in each box), the AUCs for cases and controls rise with increasing CYP ratio. However, there are no significant differences between case and control medians at any CYP ratio tested (see *P*-values). (**B**) Column scatter graph of E_2-3,4-Q AUCs for top 8 percentile (35 subjects) of entire study population. Each dot represents an individual case (red) or control (blue). Subjects with the same composite CYP1A1-CYP1B1-COMT enzyme haplotype have the same E_2-3,4-Q AUC. As the CYP ratio increases, their E_2-3,4-Q AUC changes in the same manner. However, subjects with different composite enzyme haplotypes may yield different E_2-3,4-Q AUC values, resulting in a change in their ranking with increasing CYP ratio. (**C**) Column scatter graph of E_2-3,4-Q AUCs for top 2 percentile (10 subjects) of entire study population. There are significantly more cases (red) than controls (blue) ($P = 0.01$ at CYP1B1/CYP1A1 = 5).

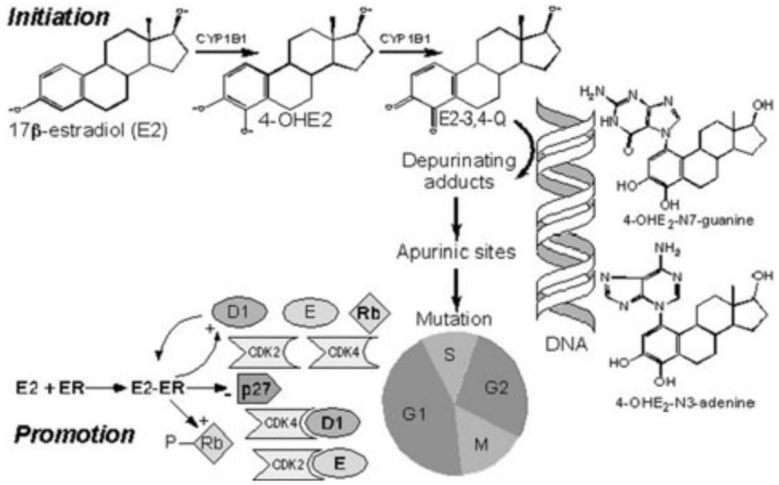

Figure 5.

Overview of estrogen carcinogenesis as a two-step process beginning with genotoxic effects (initiation) followed by enhanced cell proliferation (promotion). In the breast, E_2 is a substrate for CYP1B1 and a ligand for the estrogen receptor. The main enzyme, CYP1B1, oxidizes E_2 to catechol estrogens and further to quinones, such as 4-OHE_2 and E_2-3,4-Q. The highly reactive E_2-3,4-Q forms Michael addition products with deoxyribonucleosides. The resulting depurinating adducts, such as 4-OHE_2-N7-guanine and 4-OHE_2-N3-adenine, leave apurinic sites in the DNA. Unless repaired during G1 of the cell cycle, DNA replication during the S phase may lead to mutations that can be propagated into daughter cells during the M phase. Studies of ER-positive breast cancer cell lines demonstrated that E_2 increased the rate of cell proliferation by two mechanisms, i.e., by recruiting noncycling cells from a quiescent G0 state into the cell cycle and by shortening the overall cell cycle time due predominantly to a reduction in length of the G1 phase.[48] Binding of E_2 to ER stimulates progression of G1 by increasing the concentration of cyclin D1, which, in turn, enhances ER-mediated transcription. E_2 also causes a decrease in the concentration of CDK inhibitor p27 and a rise in cyclin D1-CDK4 and cyclin E-CDK2 activities accompanied by hyperphosphorylation of Rb.