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

Ercole L. Cavalieri[a,b,*] and **Eleanor G. Rogan**[a,b]

[a]Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, 986805 Nebraska Medical Center, Omaha, NE 68198-6805, United States

[b]Department of Environmental, Agricultural and Occupational Health, College of Public Health, University of Nebraska Medical Center, 986805 Nebraska Medical Center, Omaha, NE 68198-6805, United States

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.

Keywords

Cancer etiology; Cancer prevention; Catechol estrogen-3,4-quinones; Depurinating estrogen-DNA adducts; Estrogen genotoxicity; Estrogen mutagenicity

*Corresponding author at: Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, 986805 Nebraska Medical Center, Omaha, NE 68198-6805, United States. Tel.: + 1 402 559 7237; fax: + 1 402 559 8068. ecavalie@unmc.edu (E.L. Cavalieri).

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 diolepoxides [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 physicochemical 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 of hydroxyl radicals from redox cycling, because the 2-$OHE_1(E_2)$ and 4-$OHE_1(E_2)$ have the same redox potential [41,42]. Thus, the greater carcinogenic potency of 4-$OHE_1(E_2)$ must be related to the much higher levels of depurinating DNA adducts formed by the 3,4-Q, compared to the 2,3-Q (Fig. 4) [23]. This is due to different mechanisms of adduction. The 3,4-Q react via a proton-assisted 1,4-Michael addition [43], where as the 2,3-Q rearrange to *para*-quinone methides, which react via a 1,6-Michael addition [44].

4. Imbalances in estrogen metabolism

The above paradigm of cancer initiation by estrogens hinges on disruption of homeostatic balance between activating and deactivating pathways of estrogen metabolism (Fig. 5). This homeostasis minimizes formation and reaction of the carcinogenic catechol estrogen quinones with DNA[45,46]. One factor that can help maintain estrogen homeostasis is the feedback inhibition exerted by methoxy estrogens on the expression of CYP1A1 and CYP1B1 [47], which helps regulate the levels of catechol estrogens.

A variety of endogenous and exogenous factors can disrupt estrogen homeostasis. These include diet, environment, lifestyle, aging and genetic factors. The first critical factor is elevation of estrogen levels by excessive synthesis of estrogens due to over-expression of CYP19 (aromatase) [48–50] and/or the presence of unregulated sulfatase that converts excess stored E_1-sulfate to E_1 [51,52]. Breast tissue can synthesize E_2 *in situ*, suggesting that much more E_2 is present in target tissues than would be predicted from plasma concentrations [48]. In fact, the E_2 levels in breast tissue of postmenopausal women are similar to the levels in premenopausal women, although the plasma levels in postmenopausal women are 50–100 times lower [52,53].

A second critical factor unbalancing estrogen homeostasis may be the production of high levels of 4-$OHE_1(E_2)$, due to overexpression of CYP1B1, which converts $E_1(E_2)$ primarily to 4-$OHE_1(E_2)$ (Fig. 5) [38,39,54,55]. High levels of 4-$OHE_1(E_2)$ could result in more oxidation to $E_1(E_2)$-3,4-Q. Anadditional factor could be a lack or low level of COMT activity because of polymorphic variation [56]. Insufficient activity of this enzyme would be translated into low levels of methylation of 4-$OHE_1(E_2)$, which could increase the competitive catalytic oxidation of 4-$OHE_1(E_2)$ to $E_1(E_2)$-3,4-Q (Fig. 5).

Once the $E_1(E_2)$-3,4-Q are formed, they can be inactivated by conjugation with glutathione (GSH) or by reduction back to their catechols by quinone reductase (NQO1 and NQO2) (Fig. 5) [57,58], which can be induced by a variety of compounds [59]. Low cellular levels of GSH could be an additional factor resulting in higher levels of $E_1(E_2)$-3,4-Q. In addition, polymorphisms in NQO1 that decrease conversion of $E_1(E_2)$-3,4-Q to 4-$OHE_1(E_2)$ [60] could also lead to higher levels of $E_1(E_2)$-3,4-Q. This unbalanced metabolism can result in excessive formation of the depurinating estrogen–DNA adducts, 4-$OHE_1(E_2)$-1-N3Ade and 4-$OHE_1(E_2)$-1-N7Gua (Fig. 5). The increased damage to DNA can generate mutations that initiate the series of events leading to breast, prostate and other human cancers.

5. Estrogen–DNA adducts in the etiology of human cancer

Our research has revealed that while most of us metabolize estrogens to products that are easily excreted from the body, others at risk for cancer have a metabolic pathway with increased levels of $E_1(E_2)$-3,4-Q that can react with DNA by proton-assisted 1,4-Michael addition [43] to form specific depurinating adducts (Figs. 3 and 5). These adducts are shed from DNA, and the resultant apurinic sites can be unfaithfully repaired to generate mutations leading to cancer [26,27,29,61]. The depurinating estrogen–DNA adducts travel out of cells and tissues and are excreted in urine, allowing their identification and quantification as biomarkers of risk of developing breast and other human cancers[25,45,46,62,63].

High levels of estrogen–DNA adducts have been seen in analyses of urine and serum from women that are at high risk of breast cancer or have the disease (Fig. 6) [45,46,64]. In these studies one urine and/or one serum sample was collected from women at normal risk for breast cancer, women at high risk for breast cancer (Gail Model score > 1.66% [65]) and women diagnosed with breast cancer. An aliquot of each sample was partially purified by solid phase extraction and 40 estrogen metabolites, conjugates and depurinating DNA adducts were analyzed by using ultraperformance liquid chromatography/tandem mass spectrometry (UPLC–MS/MS).

Risk of developing breast cancer is measured as the ratio of estrogen–DNA adducts to their respective estrogen metabolites and conjugates (Fig. 6), indicating the degree of imbalance in estrogen metabolism. In general, the ratio obtained for women at high risk or diagnosed with breast cancer derives from a high level of adducts and low levels of metabolites and conjugates. In some women, how-ever, the adduct level is not high, but the levels of metabolites and conjugates are very low, suggesting that a high proportion of the metabolites was converted to adducts. In this ratio, the 4-$OHE_1(E_2)$ adducts predominate (97%), and the contribution of the 2-$OHE_1(E_2)$ adducts is minimal (3%) [45,46,64].

Significant differences ($p < 0.001$) in relative levels of estrogen–DNA adducts were observed when urine or serum samples from normal-risk women were compared to those from high-risk women or those with breast cancer (Fig. 6) [45,46,64]. These studies showed that unbalanced estrogen metabolism leading to elevation of estrogen–DNA adduct levels is associated with high risk of developing breast cancer.

Analysis of urine samples from men with and without prostate cancer also showed that men with the disease have relatively high levels of estrogen–DNA adducts in their urine (Fig. 7) [25]. These results were confirmed in a second study of men with and without prostate cancer (Fig. 8) [62]. Men diagnosed with non-Hodgkin lymphoma also have relatively high levels of estrogen–DNA adducts (Fig. 9) [63]. Thus, formation of estrogen–DNA adducts is associated with these types of cancer and could play a critical role in their etiology.

6. Mutagenicity of estrogens

The formation of depurinating estrogen–DNA adducts clearly indicates that $E_1(E_2)$-3,4-Q are, indeed, the predominant carcinogenic metabolites of estrogens. To determine that these quinones play the major role in carcinogenesis, we have investigated their mutagenic activity. Previously, investigators failed to detect estrogen-induced mutations in *in vitro* assays. These findings led to a denial of estrogen genotoxicity [66]. By determining the major pathways of metabolic activation of estrogens, namely, oxidation of catechol estrogens to semiquinones and quinones, and using more sensitive mutagenicity assays, we have succeeded to demonstrate that 4-OHE_2 and E_2-3,4-Q are mutagenic [27–29,61].

The mutagenicity of E_2-3,4-Q was demonstrated by treating the dorsal skin of female SENCAR mice [27]. The mice were killed and the treated skin was excised to determine the levels of estrogen–DNA adducts and the H-*ras* mutations generated in the skin. Equal amounts of the depurinating 4-OHE_2-1-N3Ade and 4-OHE_2-1-N7Gua adducts were detected. The observed mutations, however, were A.T to G.C transitions. The same A.T to G.C mutations were predominantly obtained in the H-*ras* gene of mammary tissue of female ACI rats after treatment with E_2-3,4-Q by intramammillary injection [29]. The high levels of mutations at A residues in the H-*ras* gene and only small numbers of mutations at G residues may be due to the rapid depurination of N3Ade adducts and much slower depurination of N7Gua adducts [23,67].

The genotoxicity of 4-OHE_2 and E_2-3,4-Q was also demonstrated in the Big Blue (BB) rat2 embryonic cell line, in which the cells contain approximately 60 copies of the λ-LIZ vector per cell [28]. Treatment of these cells six times with either compound generated statistically significant numbers of mutations. In contrast, no mutagenic activity was detected after treatment of the cells with 2-OHE_2. The spectrum of mutations obtained with 4-OHE_2 contained predominant mutations at A.T base pairs, presumably due to the rapid depurination of N3Ade adducts.

Mutagenicity was further studied in female BB rats, which have approximately 80 copies of the λ-LIZ vector per cell. The rats were implanted with E_2, 4-OHE_2 or both compounds, and, after 20 weeks, mutations in the *cII* gene in the mammary cells were analyzed. Only in the rats treated with 4-OHE_2 (with or without E_2) were A.T to G.C mutations detected [26]. These results demonstrated the mutagenicity of 4-OHE_2 under conditions in which it could be metabolized to E_2-3,4-Q. Thus, 4-OHE_2 and E_2-3,4-Q have been demonstrated to be mutagenic under appropriate assay conditions, whereas 2-OHE_2 was not mutagenic in BB rat2 cells [28]. The spectrum of mutations was consistent with those expected from the DNA

adducts derived from E_2-3,4-Q, providing further support for the mutagenicity of estrogens and the hypothesis that estrogens are carcinogenic through their genotoxicity.

7. Transformation of human breast epithelial cells by estrogens

Studies with cultured human breast epithelial cells have provided further evidence for the initiation of cancer by formation of estrogen–DNA adducts. The MCF-10F cell line is an immortalized, nontransformed estrogen receptor-α-negative cell line. Treatment of these cells with E_2 or 4-OHE_2 generates the depurinating estrogen–DNA adducts [54,68,69]. At doses of 0.007–3.5 nM, treatment with E_2 or 4-OHE_2 leads to transformation of the cells as detected by their ability to form colonies in soft agar [70,71]. These cells are transformed by the estrogens even in the presence of the anti-estrogen tamoxifen or ICI-182,780 [72]. These results indicate that transformation occurs through the genotoxic effects of the estrogen metabolites. 2-OHE_2 induces these changes to a much smaller extent. Implantation of estrogen-transformed MCF-10F cells, selected by their invasiveness, into severely compromised immunodeficient mice generates tumors [73].

These results demonstrate that estrogen receptor-α-negative human breast epithelial cells are transformed by the genotoxic effects of estrogen metabolites, supporting the hypothesis that formation of specific estrogen–DNA adducts is the critical event in the initiation of estrogen-induced cancer.

8. Carcinogenicity of estrogens in animal models

The carcinogenicity of natural and synthetic estrogens was demonstrated by the induction of kidney tumors in Syrian golden hamsters by implantation of E_1, E_2, DES, or hexestrol [32]. The 4-OHE_1(E_2) were carcinogenic in the hamsters, but the 2-OHE_1(E_2) were not [33,34]. The 4-OHE_1(E_2) were also carcinogenic in the CD-1 mouse uterus and the 2-OHE_1(E_2) had borderline carcinogenic activity [35]. The far greater carcinogenic activity of the 4-OHE_1(E_2) compared to the 2-OHE_1(E_2) can be understood from the much greater ability of E_1(E_2)-3,4-Q to form estrogen–DNA adducts compared to E_1(E_2)-2,3-Q [23].

Further important evidence for the role of estrogen genotoxicity in the initiation of cancer comes from studies of transgenic mice with estrogen receptor-α knocked out (ERKO/*wnt-1* mice). The *wnt-1* transgene in female ERKO/*wnt-1* mice induced mammary tumors in 100% of the mice, despite the lack of estrogen receptor-α [74,75]. Both 4-OHE_1(E_2) and GSH conjugates formed by E_1(E_2)-3,4-Q were detected in the mammary tissue of these mice, but no methoxy estrogens were found [76], indicating that the estrogen metabolism was unbalanced toward excess activating pathways and limited deactivating pathways. When the mice were ovariectomized at 15 days of age to remove their major source of estrogens and implanted with E_2, the E_2-treated mice developed mammary tumors in a dose-dependent manner [77,78]. In addition, the mammary tumors developed even when the mice were implanted with both E_2 and the anti-estrogen ICI-182,780 [79]. These results provide further strong evidence for tumor initiation by estrogen-induced genotoxic events.

9. Dopamine

In addition to the estrogens, the other significant aromatic biomolecule in the body is the neurotransmitter dopamine. Analogously to the catechol estrogens, dopamine can be easily oxidized to its quinone, dopamine quinone (DA-Q), by auto-oxidation, metal ion oxidation, or oxidation catalyzed by CYP or peroxidase [80,81].

At neutral pH, DA-Q undergoes intramolecular cyclization by 1,4-Michael addition, followed by oxidation to form leucochrome, and then aminochrome. Polymerization of the aminochrome leads to neuromelanin (Fig. 10). At lower pH, especially between pH 5 and 6, partial protonation of the amino group of dopamine slows down the intramolecular cyclization of DA-Q, rendering competitive the intermolecular reaction of the quinone with DNA to form depurinating N3Ade and N7Gua adducts that are analogous to the depurinating estrogen–DNA adducts (Figs. 2 and 10) [12,82].

Analogously to the depurinating estrogen–DNA adducts, the DA-6-N3Ade adduct depurinates from DNA instantaneously, whereas the DA-6-N7Gua adduct depurinates with a half-life of about 3 h [12]. This common feature is seen not only with the depurinating estrogen [15–17] and dopamine [12,82] adducts, but also with the depurinating adducts of benzene [11,12], naphthalene [13,14], DES [18] and hexestrol [19] (Fig. 2). This common feature may lead to the initiation of cancer or, for dopamine, neurodegenerative disease.

10. Prevention of cancer initiation

The paradigm of cancer initiation in the metabolism of estrogens is related to formation of catechol estrogens, with special emphasis on the 4-$OHE_1(E_2)$. In the oxidative metabolism of estrogens, activating pathways can lead to formation of depurinating estrogen–DNA adducts and deactivating pathways lead to formation of estrogen metabolites and conjugates. This hypothesis has been supported by studies in which the levels of estrogen–DNA adducts in urine samples from women at normal risk for breast cancer are relatively low compared to the much higher levels in women at high risk for breast cancer or diagnosed with the disease (Fig. 6) [45,46]. In contrast, in women at normal risk for breast cancer, the levels of estrogen metabolites and conjugates are high compared to the relatively low levels of metabolites and conjugates in women at high risk or diagnosed with the disease (Fig. 6) [45,46].

The relative levels of estrogen metabolites, conjugates and depurinating DNA adducts can be related to the expression of five key estrogen-metabolizing enzymes in breast tissue [83]. The first activating enzyme is CYP19 (aromatase), which converts androgens to estrogens (Fig. 5). A case–control study of 2018 women found that women who consumed mushrooms in their diet had a 50% lower incidence of breast cancer than women who did not consume mushrooms. In addition, women consuming both mushrooms and green tea had a 90% lower incidence of breast cancer [84]. The mushrooms contain phytochemicals that inhibit aromatase activity. These effects have been studied in cell culture, and the anti-aromatase activity of the phytochemicals has been suggested to be responsible for the reduced incidence of breast cancer [85,86]. The second estrogen-activating enzyme is CYP1B1, which converts $E_1(E_2)$ almost exclusively to 4-$OHE_1(E_2)$ (Fig. 5). Further oxidation of 4-

$OHE_1(E_2)$ leads to $E_1(E_2)$-3,4-Q, the predominant metabolites in the initiation of cancer by estrogens.

Two protective phase II enzymes are COMT and quinone reductase. The former catalyzes the methylation of catechol estrogens, thereby preventing their conversion to semiquinones and quinones (Fig. 5), whereas the latter, NQO1 and NQO2, reduce catechol estrogen quinones back to catechol estrogens [57,58,87] (Fig. 5).

Breast tissue from women who do not have breast cancer has been found to have high levels of the protective enzymes COMT and NQO1, and low levels of expression of the activating enzymes CYP19 and CYP1B1 [83]. In contrast, non-tumor breast tissue from women diagnosed with breast cancer has high levels of the activating enzymes CYP19 and CYP1B1, with low levels of COMT and NQO1 [83]. An additional protective enzyme is glutathione-*S*-transferase (GST), which facilitates the reaction between catechol estrogen quinones and GSH (Fig. 5).

Homeostasis in estrogen metabolism minimizes reaction of the electrophilic catechol estrogen quinones with DNA, thereby reducing the amount of DNA damage and resulting risk of cancer initiation. This balance is naturally maintained by the enzyme COMT, which methylates catechol estrogens, impeding their further oxidation to semiquinones and quinones (Fig. 5) [54,69]. The ubiquitous antioxidant GSH, which reacts non-enzymatically with the catechol estrogen quinones or, more efficiently, with the catalytic activation of GST (Fig. 5), also helps maintain estrogen homeostasis. A third deactivating phase II enzyme is NQO1 (and NQO2) [57,58]. These enzymes limit the reaction of catechol estrogen quinones with DNA by reduction of the quinones to catechol estrogens [57,58,87].

Unbalanced estrogen homeostasis can be mitigated by the use of specific antioxidant compounds. N-Acetylcysteine (NAcCys) and resveratrol are particularly effective in inhibiting formation of estrogen–DNA adducts [88]. NAcCys is the acetyl derivative of the amino acid cysteine, which is one component in the antioxidant tripeptide GSH. Resveratrol, 3,5,4′-hydroxystilbene, is a natural antioxidant present in grapes and many other plants [89] and has anticarcinogenic effects in diverse *in vitro* and *in vivo* systems [90,91].

The human breast epithelial cell line MCF-10F (estrogen receptor-α-negative and aryl hydrocarbon receptor-positive) was used to study the effect of resveratrol [55,92] and NAcCys [93] on estrogen metabolism and formation of estrogen–DNA adducts. The combined antioxidant activity of NAcCys and resveratrol was also determined in MCF-10F cells [94]. In addition, the capacity of NAcCys to inhibit transformation of E6 mouse mammary cells [95] and resveratrol to inhibit transformation of MCF-10F cells [55] has been seen.

The antioxidant effect of NAcCys in reducing formation of estrogen–DNA adducts in MCF-10F cells treated with 4-OHE_2 (Figs. 5 and 11) [93] is due to the reaction of NAcCys with E_2-3,4-Q and the reduction of semiquinones to 4-OHE_2 [88,96]. Resveratrol similarly reduces semiquinones to 4-OHE_2 [55,88,92]. Furthermore, resveratrol induces NQO1, which catalyzes the reduction of E_2-3,4-Q to 4-OHE_2 [57], thereby limiting reaction of

E_2-3,4-Q with DNA. Another important action of resveratrol is to modulate the expression of CYP1B1 if it is overexpressed [55].

When NAcCys and resveratrol are mixed together, they have an additive effect in reducing formation of estrogen–DNA adducts in MCF-10F cells (Fig. 11) [94]. Low concentrations of NAcCys and resveratrol inhibit estrogen–DNA adduct formation similarly, but at higher doses, the effect of resveratrol is about 50% greater than that of NAcCys [94].

Use of these antioxidants is the logical preventive strategy to re-establish and/or maintain the homeostatic balance of estrogen metabolism, thereby reducing DNA damage, the resulting mutations and risk of initiating cancer.

11. Conclusions

Metabolism of estrogens is characterized by a homeostatic set of activating and deactivating pathways. The homeostasis minimizes formation of the catechol estrogen quinones, the ultimate carcinogenic metabolites of estrogens, and their reaction with DNA. When homeostasis is disrupted, excessive oxidation of catechol estrogens to semiquinones and quinones occurs. The quinones can react with DNA to form predominantly the depurinating adducts 4-$OHE_1(E_2)$-1-N3Ade and 4-$OHE_1(E_2)$-1-N7Gua. These adducts generate apurinic sites leading to the mutations that can initiate breast, prostate and other prevalent types of cancer. A similar mechanism of metabolic activation occurs for the carcinogens benzene, naphthalene, diethylstilbestrol and hexestrol (Fig. 2).

Substantial evidence for the genotoxicity of the endogenous estrogens has been obtained in studies conducted *in vitro*, in cell culture and in laboratory animals. In addition, women at high risk for breast cancer or diagnosed with the disease (Fig. 6), men with prostate cancer (Figs. 7 and 8), and men with non-Hodgkin lymphoma (Fig. 9) form relatively high levels of depurinating estrogen–DNA adducts compared to control subjects.

The initiating step of cancer by estrogens can be blocked by the antioxidants *N*-acetylcysteine and resveratrol (Fig. 11). By preventing the initiation of the disease, promotion, progression and development of cancer are eliminated. This strategy of prevention does not require knowledge of the genes involved or the complex series of events that occur after cancer initiation.

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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-αknocked 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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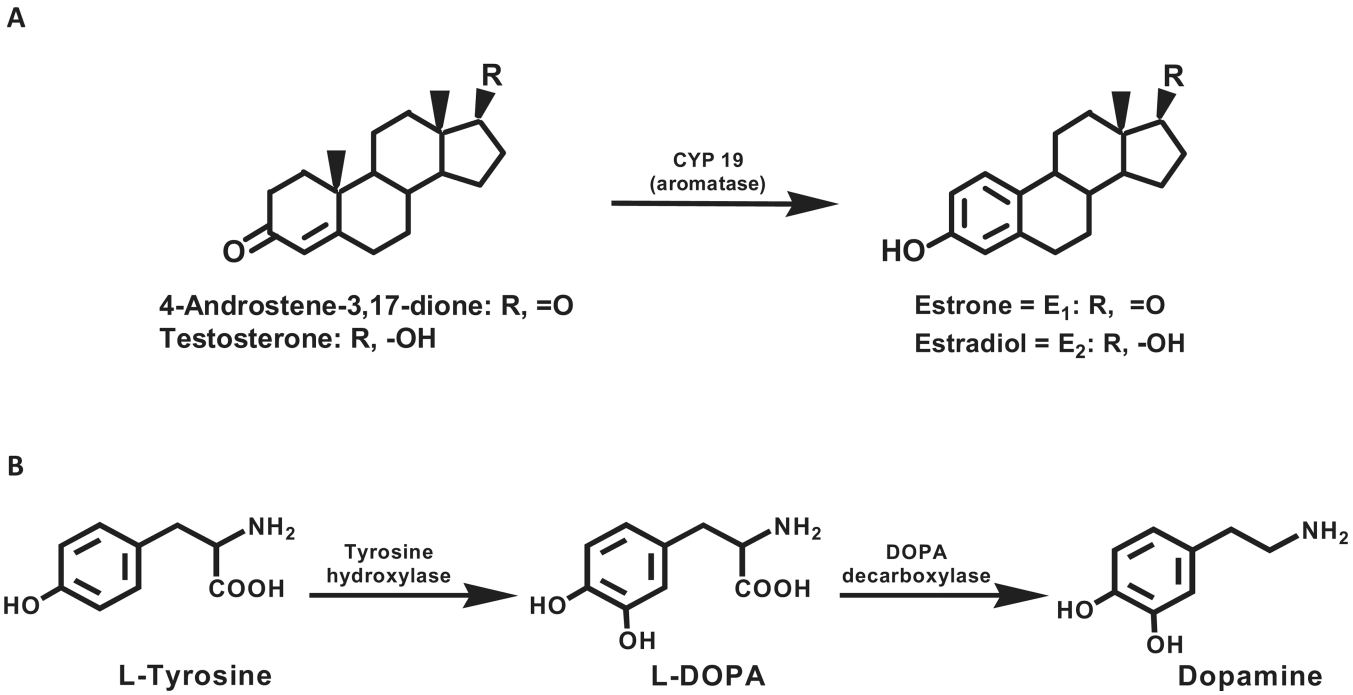

Fig. 1.
Biosynthesis of (A) estrogens and (B) dopamine.

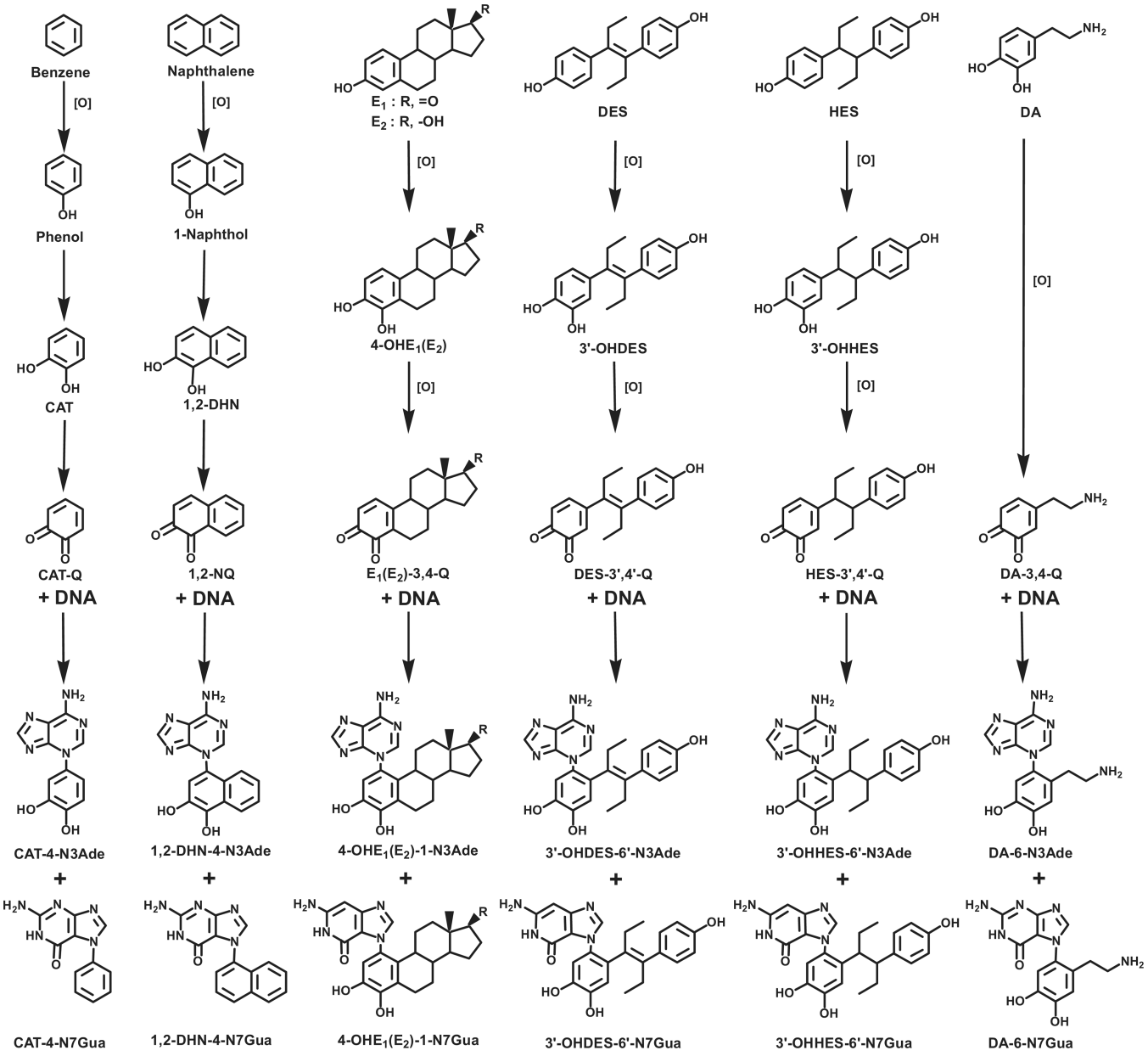

Fig. 2.

Common mechanism of metabolic activation to form depurinating DNA adducts for benzene, naphthalene, estrone/estradiol, diethylstilbestrol, hexestrol and dopamine. 1,2-DHN, 1,2-dihydroxynaphthalene; 1,2-NQ, 1,2-naphthoquinone; HES, hexestrol; DA, dopamine.

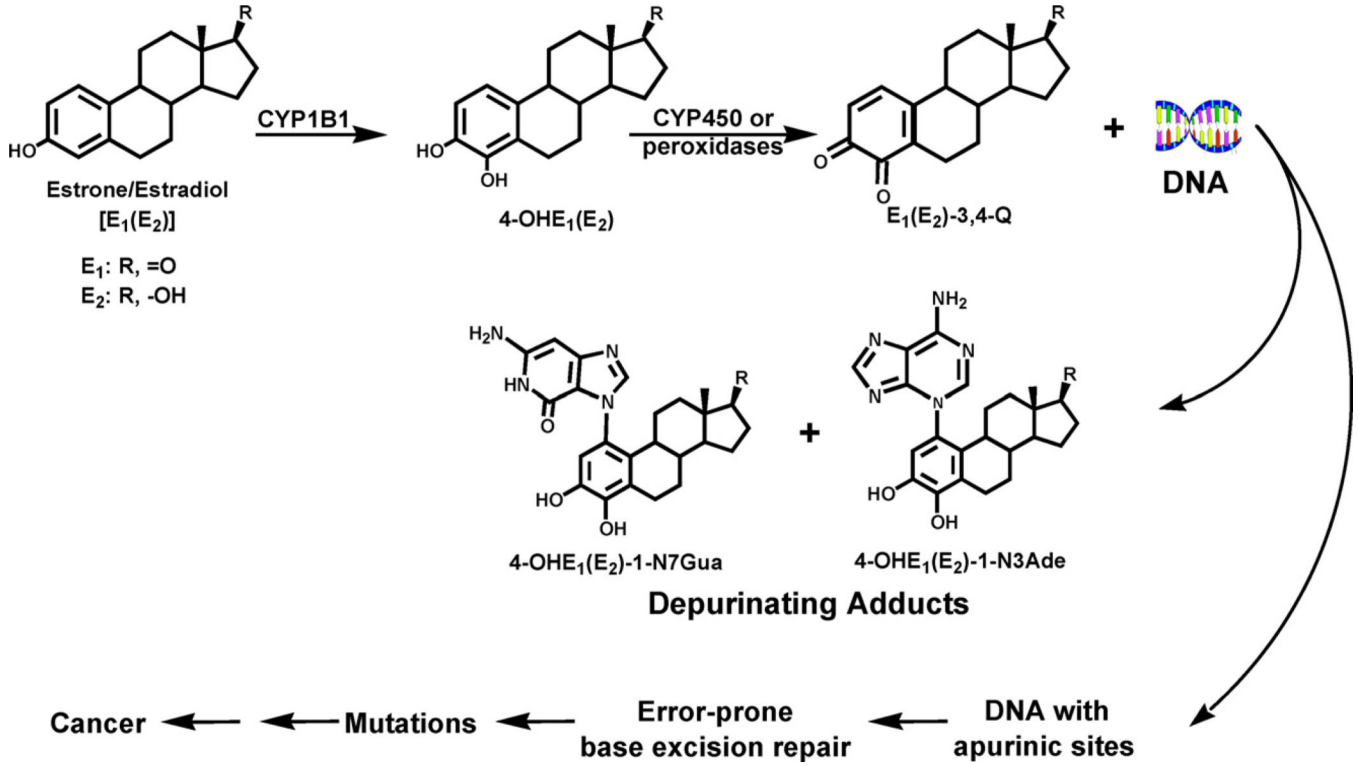

Fig. 3.
Major metabolic pathway in cancer initiation by estrogens.

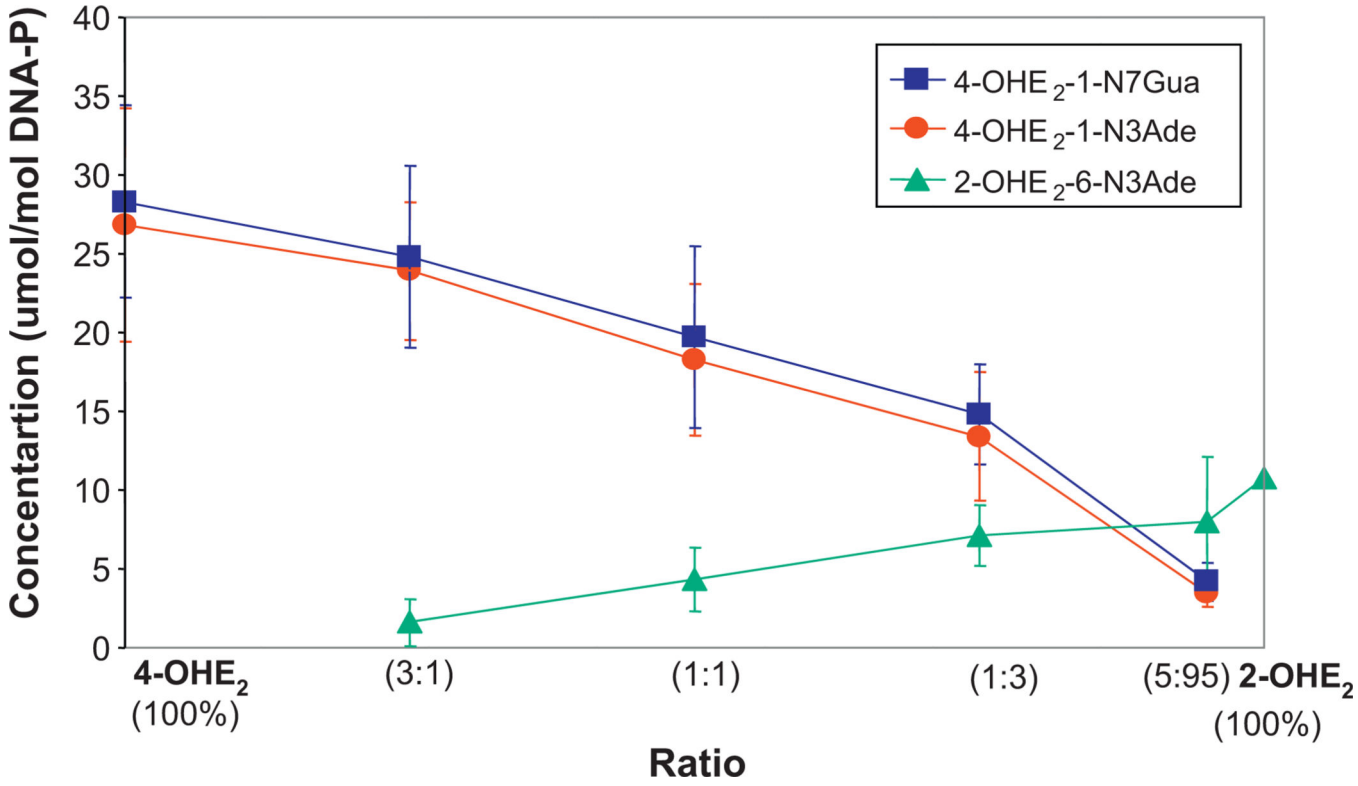

Fig. 4.

Depurinating adducts formed in the prostaglandin H synthase-catalyzed reaction of mixtures of 4-OHE_2 and 2-OHE_2 at different ratios after 10 h of incubation with DNA [23].

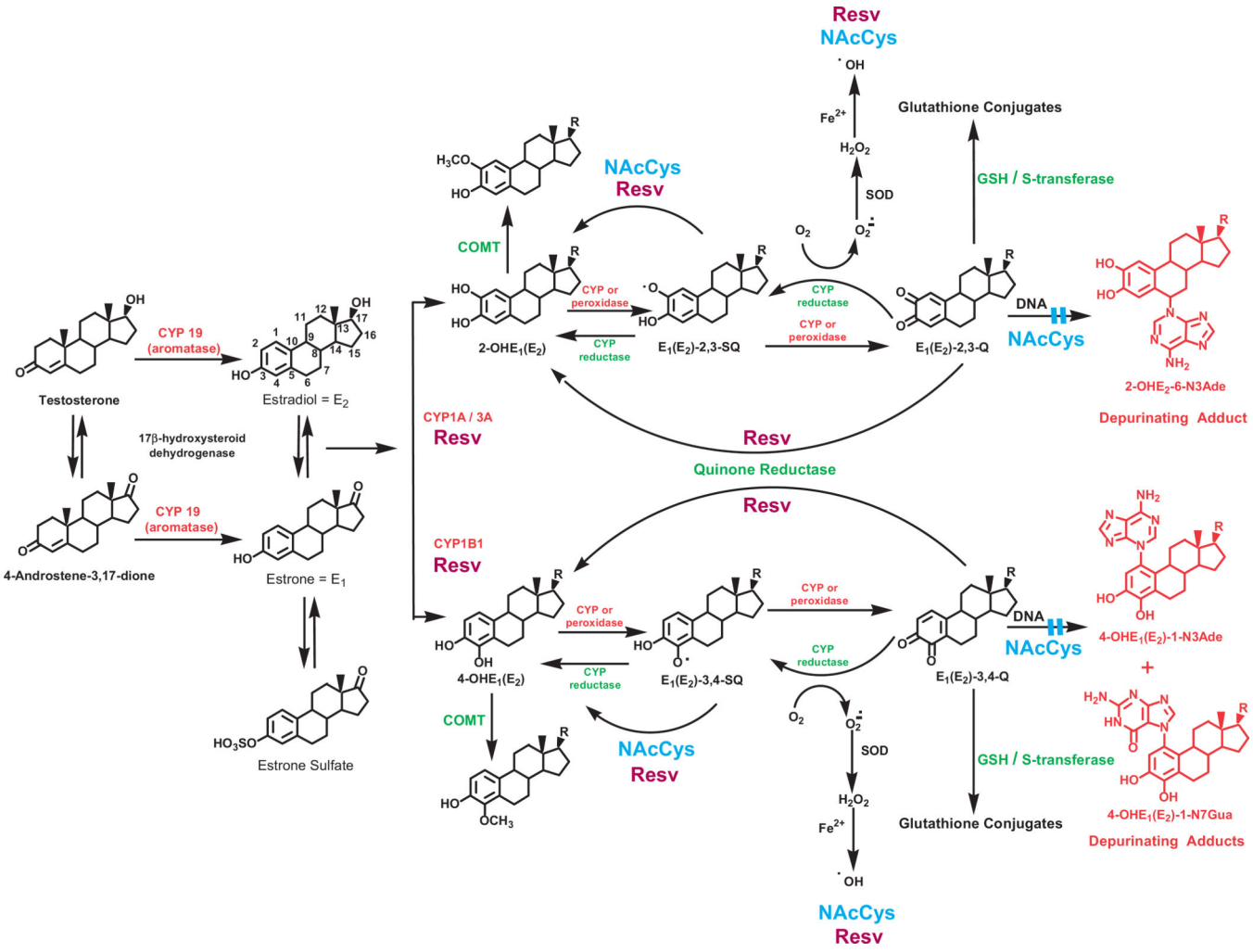

Fig. 5.

Formation, metabolism and DNA adducts of estrogens. Activating enzymes and depurinating DNA adducts are in red and protective enzymes are in green. *N*-Acetylcysteine (NAcCys, shown in blue) and resveratrol (Resv, burgundy) indicate the various points where NAcCys and Resv could improve the balance of estrogen metabolism and minimize formation of depurinating estrogen–DNA adducts.

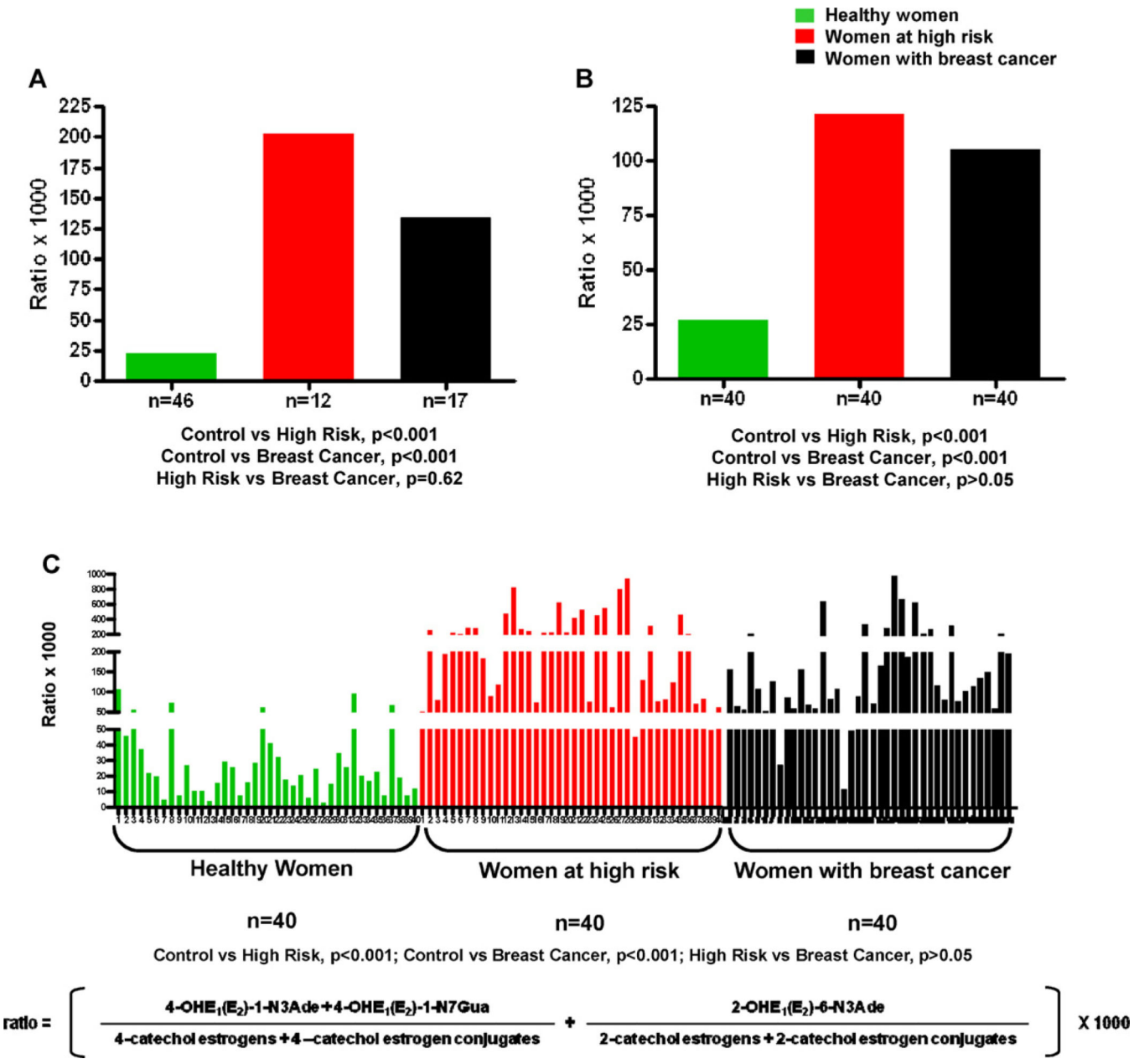

Fig. 6.

Depurinating estrogen–DNA adducts in (A) median level in urine of healthy women, high-risk women and women with breast cancer – first study [45]; (B) median level in urine of healthy women, high-risk women and women with breast cancer – second study [46]; and (C) serum of healthy women, high-risk women and women with breast cancer [64].

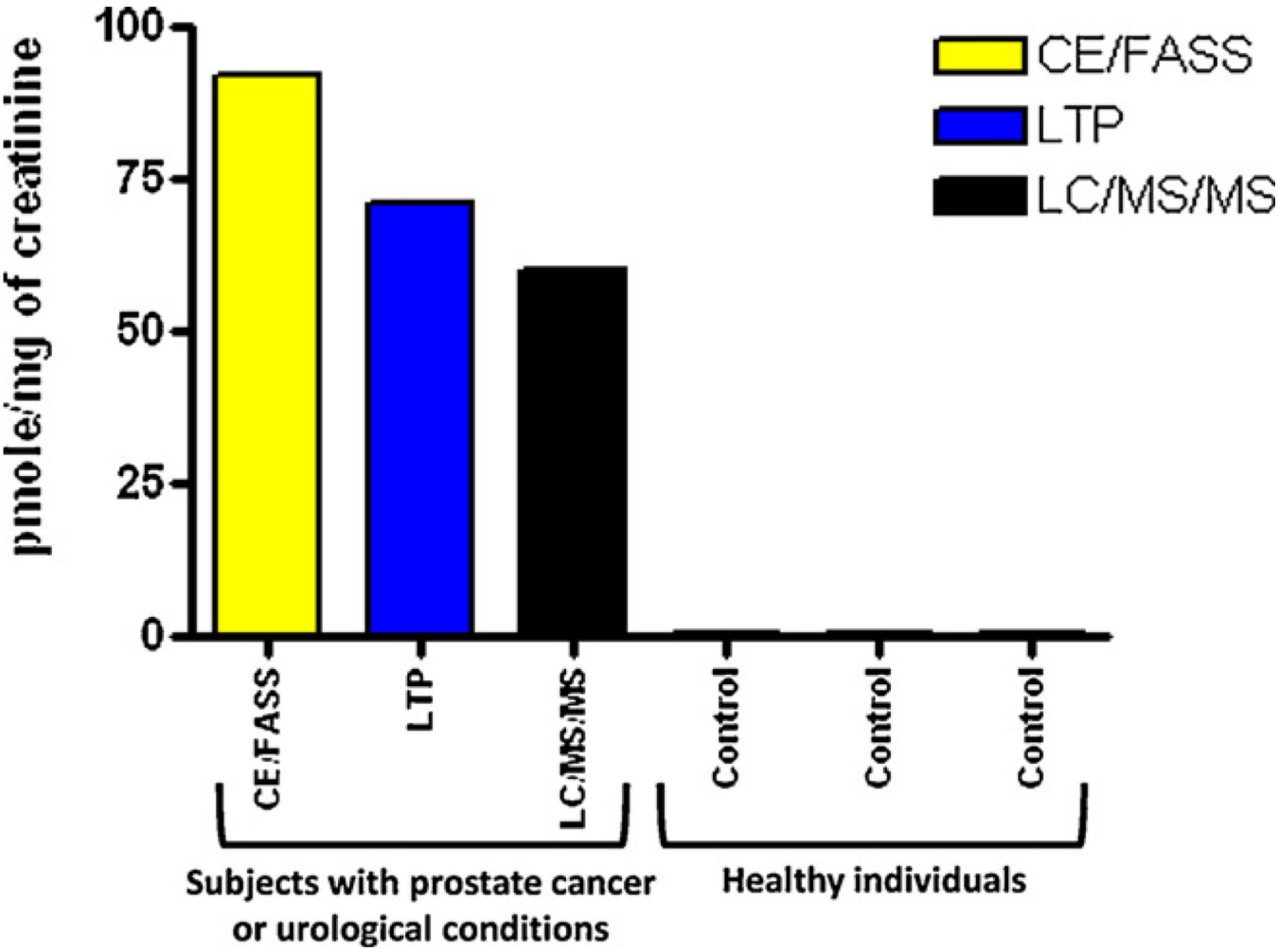

Fig. 7.

Median level of the 4-OHE$_1$-1-N3Ade adduct in human urine from men with prostate cancer or urological conditions and healthy men [25].

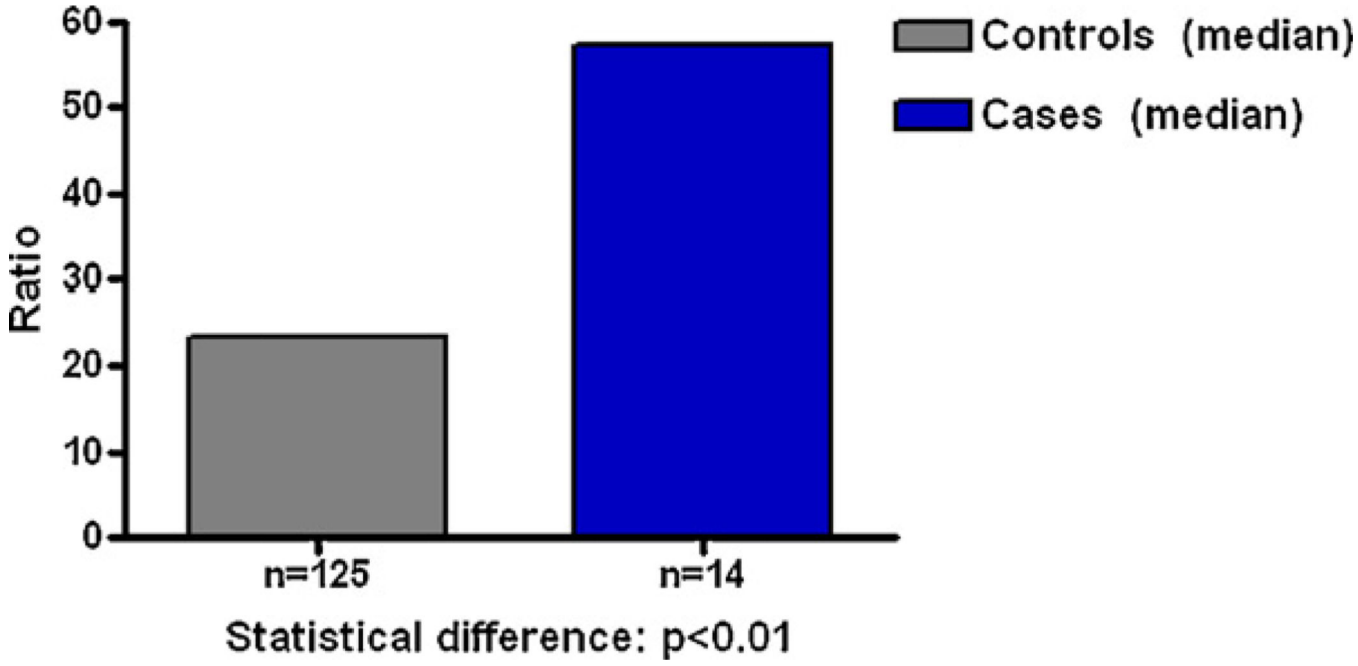

Fig. 8.

Average levels of estrogen–DNA adducts in urine samples from men with and without prostate cancer, $p < 0.001$ [62].

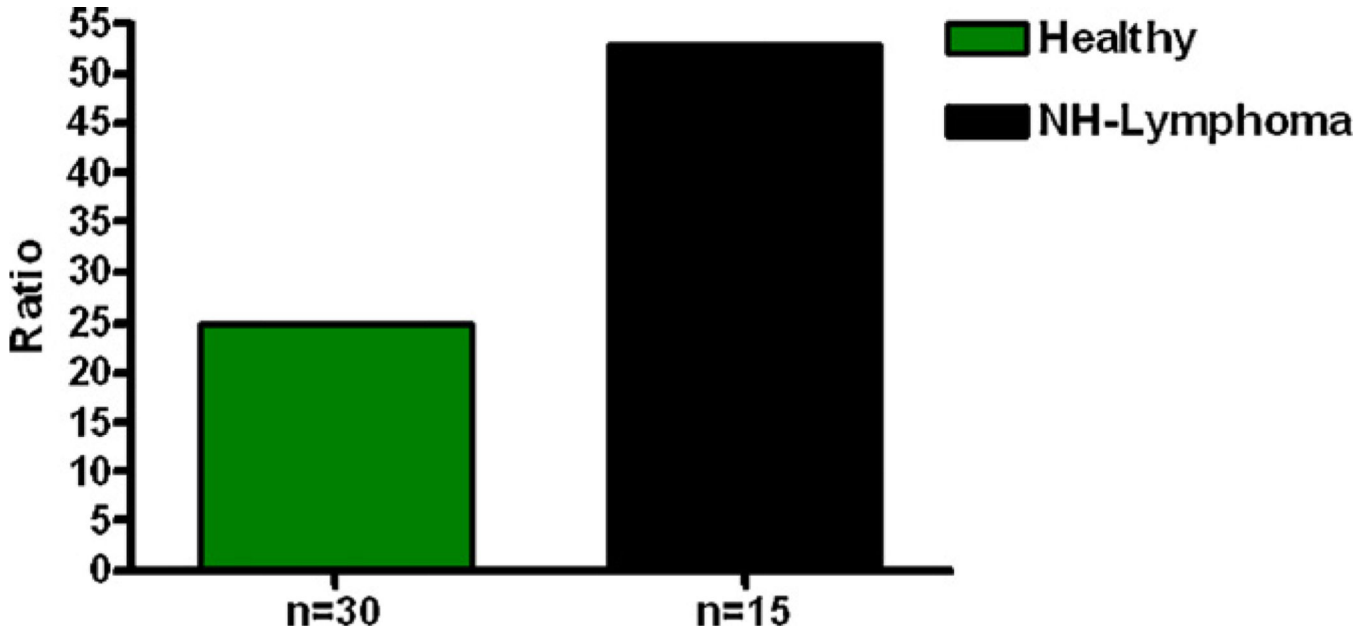

Fig. 9.

Median levels of depurinating estrogen–DNA adducts in urine of healthy control men and men with NHL. Healthy controls vs. NHL, $p < 0.007$ [63].

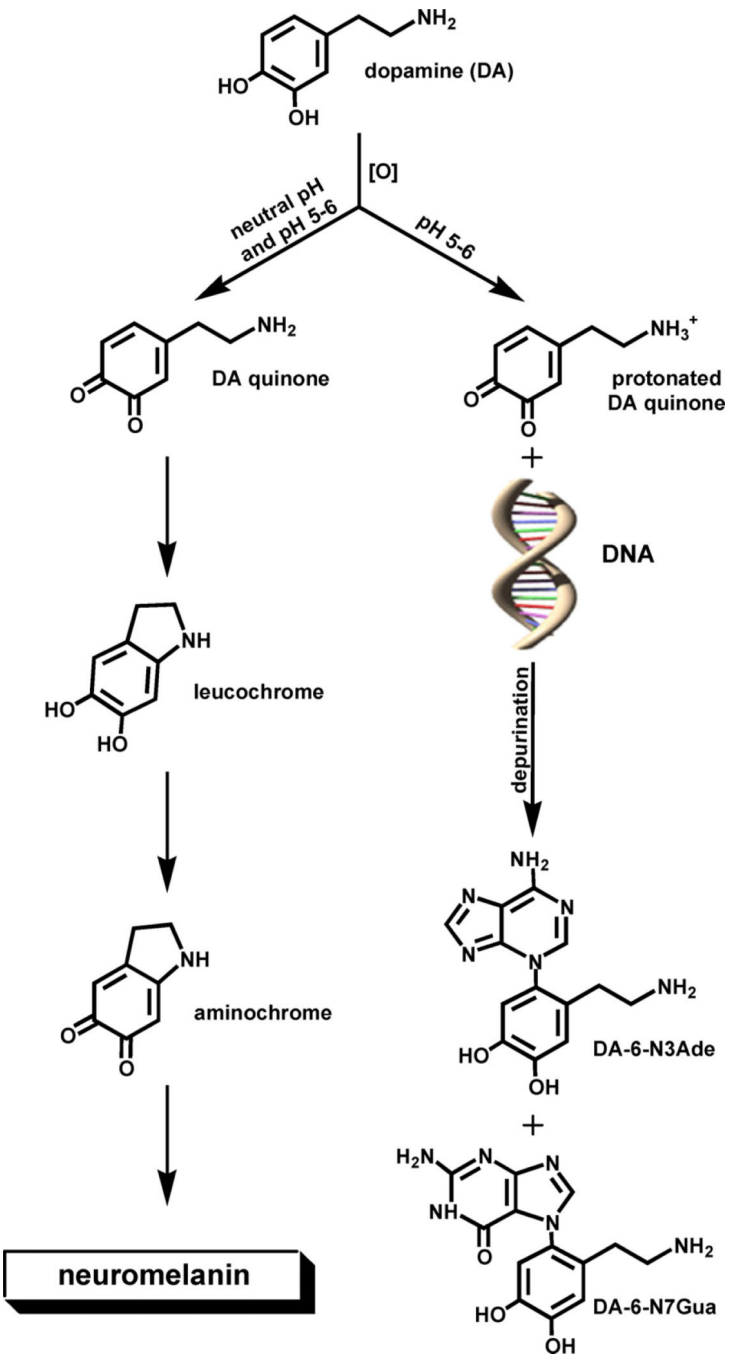

Fig. 10.

Formation of neuromelanin from dopamine quinone at pH 7 and competitive formation of DA-6-N3Ade, DA-6-N7Gua and neuromelanin from dopamine quinone at pH 5–6.

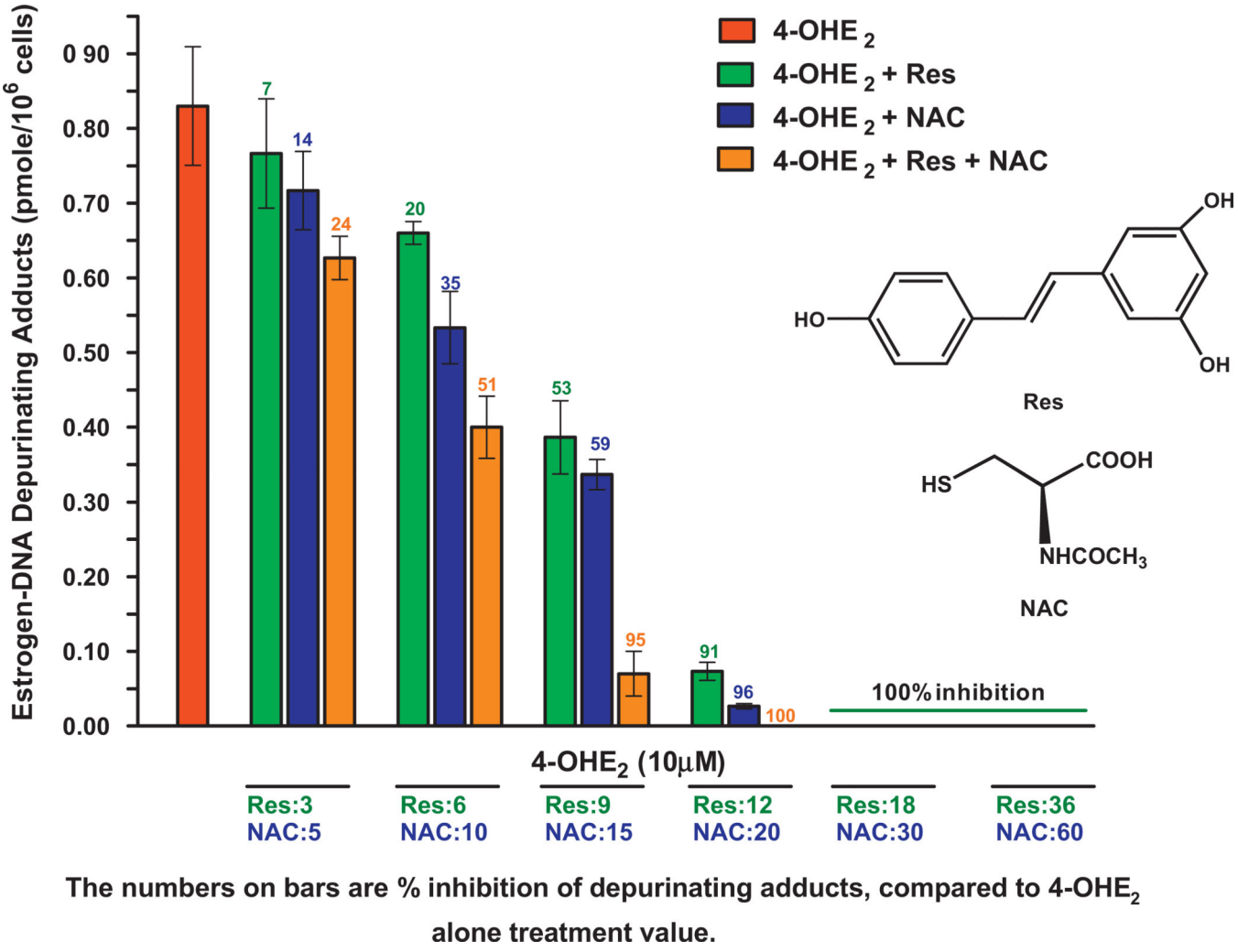

Fig. 11.

Effects of NAcCys, resveratrol, or NAcCys + resveratrol on the formation of depurinating estrogen–DNA adducts in MCF-10F cells treated with 4-OHE_2. NAC, NAcCys; Res, resveratrol [94].