Published in final edited form as:

Free Radic Biol Med. 2010 January 15; 48(2): 318. doi:10.1016/j.freeradbiomed.2009.11.002.

Benzene and dopamine catechol quinones could initiate cancer or neurogenic disease

Muhammad Zahid¹, Muhammad Saeed¹, Eleanor G. Rogan^{1,2}, and Ercole L. Cavalieri^{1,*}

¹Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha, NE.

²Department of Environmental, Agricultural and Occupational Health, College of Public Health, University of Nebraska Medical Center, Omaha, NE.

Abstract

Catechol quinones of estrogens react with DNA by 1,4-Michael addition to form depurinating N3Ade and N7Gua adducts. Loss of these adducts from DNA creates apurinic sites that can generate mutations leading to cancer initiation. We compared reaction of the catechol quinones of the leukemogenic benzene (CAT-Q) and *N*-acetyldopamine (NADA-Q) with dG or DNA. NADA was used to prevent intramolecular cyclization of dopamine quinone. Reaction of CAT-Q or NADA-Q with dG at pH 4 afforded CAT-4-N7dG or NADA-6-N7dG, which lost deoxyribose with a half-life of 3 h to form CAT-4-N7Gua or 4 h to form NADA-6-N7Gua. When CAT-Q or NADA-Q was reacted with DNA, N3Ade adducts were formed and lost from DNA instantaneously, whereas N7Gua adducts were lost over several hours. The maximum yield of adducts in the reaction of CAT-Q or NADA-Q with DNA at pH 4 to 7 was at pH 4. When tyrosinase-activated CAT or NADA was reacted with DNA at pH 5 to 8, adduct levels were much higher (10–15-fold), and the highest yield was at pH 5. Reaction of catechol quinones of natural and synthetic estrogens, benzene, naphthalene and dopamine with DNA to form depurinating adducts is a common feature that may lead to initiation of cancer or neurodegenerative disease.

Keywords

1,4-Michael addition; catechol quinones; depurinating DNA adducts; kinetics of depurination

Introduction

Experiments on estrogen metabolism, formation of DNA adducts, mutagenicity, cell transformation and carcinogenicity led to and support the hypothesis that reaction of specific metabolites, mostly catechol estrogen-3,4-quinones, with DNA forms small amounts of stable adducts (< 1%) and predominant amounts of depurinating adducts (>99%) [1,2]. This reaction occurs by a proton-assisted 1,4-Michael addition [3]. The depurinating adducts obtained are 4-hydroxyestrone(estradiol)-1-N3Ade [4-OHE₁(E₂)-1-N3Ade] and 4-OHE₁(E₂)-1-N7Gua.

© 2009 Elsevier Inc. All rights reserved.

*Ercole L. Cavalieri, Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, 986805 Nebraska Medical Center, Omaha, NE 68198-6805, Telephone: 402-559-7237, Fax: 402-559-8068, ecavalie@unmc.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The stable adducts remain in DNA unless removed by repair, whereas the depurinating adducts detach from DNA, leaving behind apurinic sites. Errors in the repair of these sites can lead to the critical mutations initiating breast, prostate and other human cancers [1,2,4,5].

Strong evidence has also been obtained that the synthetic estrogen hexestrol and the human carcinogen diethylstilbestrol are metabolized predominantly to their respective catechols. When the catechols are oxidized to quinones, they also react with DNA by 1,4-Michael addition to form depurinating N3Ade and N7Gua adducts that are analogous to those formed by the catechol estrogen-3,4-quinones [6–8]. Similarly, the catechol quinone of naphthalene reacts with DNA *in vitro* and *in vivo* to form analogous depurinating N3Ade and N7Gua adducts [9,10].

We have evidence that the *ortho*-quinones of the leukemogenic benzene and the neurotransmitter dopamine can also react with DNA by 1,4-Michael addition to form depurinating N3Ade and N7Gua adducts [11] analogous to those formed by the natural and synthetic estrogens, as well as naphthalene. In this article, we report a further study of the benzene catechol (1,2-dihydroxybenzene, CAT) and *N*-acetyldopamine (NADA), which is itself a catechol (Fig. 1). Benzene is metabolized to phenol in the liver by cytochrome (450 2E1 [12,13]. Other metabolites include CAT and hydroquinone (1,4-dihydroxybenzene) [14]. Oxidation of CAT and hydroquinone is catalyzed by peroxidases, forming quinones that can exert myelotoxic effects [15] and produce stable [16,17] and depurinating [11] DNA adducts. In fact, higher levels of peroxidases and the lack of quinone reductase in the bone marrow allow formation of toxic quinones without the possibility of their being reduced [18].

One of the major metabolic pathways of the neurotransmitter dopamine is oxidation to its catechol quinone, which at neutral pH regularly undergoes intramolecular cyclization by 1,4-Michael addition, followed by oxidation to form leukochrome and then aminochrome. Polymerization of the aminochrome leads to neuromelanin (Fig. 2, top). At lower pH, however, partial protonation of the amino group of dopamine slows down the intramolecular cyclization of the dopamine quinone, rendering competitive the reaction of the quinone with DNA to form depurinating N3Ade and N7Gua adducts (Fig. 2, bottom). Although the two depurinating adducts were obtained from dopamine activated by tyrosinase [11], NADA was used in this study to protect the amino group of dopamine and totally avoid the pathway of intramolecular cyclization after formation of the quinone. In this way we could compare the ability of the catechol quinone of benzene and the dopamine quinone to react intermolecularly via 1,4-Michael addition at various pH.

Materials and Methods

Chemicals and reagents

Catechol (1,2-dihydroxybenzene, CAT), Ag₂O, potassium tetraborate, acetic anhydride, formic acid, acetic acid, trifluoroacetic acid (TFA), dimethylformamide (DMF) and dimethylsulfoxide (DMSO)-*d*₆ were purchased from Aldrich Chemical Co. (Milwaukee, WI) and used as such without further purification. 2'-Deoxyguanosine (dG) and calf thymus DNA were purchased from USB (Cleveland, OH). Dopamine (DA) hydrochloride and mushroom tyrosinase were purchased from Sigma Chemical (St. Louis, MO). The standard depurinating adducts of CAT, i.e., CAT-4-N7Gua and CAT-4-N3Ade, and those of NADA, NADA-6-N7Gua and NADA-6-N3Ade, were synthesized according to the procedure reported earlier [11].

Instrumentation

UV—UV spectra were obtained during HPLC by using a Waters (Milford, MA) 996 or 2996 photodiode array detector for all compounds synthesized. HPLC separations were monitored at 290 nm for both CAT and NADA.

HPLC—Analytical HPLC was conducted on a Waters 2690 Separations Module equipped with a Waters 996 or 2996 photodiode array detector and a reverse-phase Phenomenex Luna-2 C-18 column (250×4.6 mm, 5 μm; 120 Å, Torrance, CA). For CAT, the column was eluted starting with 8% CH₃CN in H₂O (0.25% TFA) and continuing with a linear gradient to 10% CH₃CN in 25 min. For NADA, the column was eluted starting with 6% CH₃CN in H₂O (0.25% TFA) and then with a linear gradient to 8% CH₃CN in 20 min at a flow rate of 1 ml/min.

Mass Spectrometry

ESI-MS: All experiments were performed on a Waters Quattro Micro triple quadrupole mass spectrometer by using electrospray ionization (ESI) in the positive ion (PI) mode, with an ESI-MS capillary voltage of 3.0 kV, an extractor cone voltage of 2 V, and a detector voltage of 650 V. Desolvation gas flow was maintained at 400 l/h. Cone gas flow was set at 60 l/h. Desolvation temperature and source temperature were set to 200 and 100 °C, respectively. The acquisition range was 50–700 Da. The sample was introduced to the source at a flow rate of 10 μl/min by using an inbuilt pump.

UPLC-MS/MS: Samples were analyzed on an Acquity UPLC system attached to a MicroMass QuattroMicro triple quadrupole mass spectrometer. The 10-μl injections were made to a Waters Acquity UPLC BEHC₁₈ column (1.7 μm, 1×100 mm). The instrument was operated in the positive ESI mode. All aspects of system operation, data acquisition and processing were controlled using QuanLynx v4.0 software (Waters). The column was eluted with 5% CH₃CN in H₂O (0.1% formic acid) for 10 min at a flow rate of 150 μl/min. Ionization was achieved using the following settings: capillary voltage 3 kV; cone voltage 15–60 V; source block temperature 100 °C; desolvation temperature 200 °C, with a nitrogen flow of 400 l/h. Nitrogen was used as both the desolvation and auxiliary gas. Argon was used as the collision gas. Three-point calibration curves covering the range of analytes detected were run for each standard.

Synthesis of NADA—NADA was synthesized from DA with a slight modification of the reported method [19]. The 0.8 g of DA hydrochloride was dissolved in 40 ml of 10% potassium tetraborate that had been purged with nitrogen. Then 0.44 ml of 99% acetic anhydride was slowly added over a period of 30 min to the DA hydrochloride solution under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 8 h, and then 3 ml of concentrated formic acid was added to precipitate boric acid. After filtration, the supernatant was extracted with ethyl acetate (3×50 ml). The combined organic layers were concentrated and purified by column chromatography on silica gel using CHCl₃-CH₃OH (1:1) as the solvent system. Fractions containing common TLC spots were pooled, and concentrated to obtain pure NADA (540 mg, 66%). The NMR and MS data for the compound matched exactly those already reported in the literature [19].

Reaction of CAT-Q or NADA-Q with dG and loss of deoxyribose—CAT (11 mg, 0.1 mmol) or NADA (20 mg, 0.1 mmol) was dissolved in 2 ml of DMF and stirred at 0 °C. To this solution was added Ag₂O (140 mg, 0.6 mmol) portion-wise and the reaction mixture was stirred at 0 °C for 30 min. A dark-colored solution of CAT-Q or NADA-Q was filtered quickly through a syringe filter into a solution of dG (142 mg, 0.5 mmol) in H₂O/CH₃COOH (2 ml, 1:1) and the pH of the solution was adjusted to 4. The reaction mixture was stirred at room temperature and aliquots at different time intervals (0.33, 0.66, 1, 2, 3, 4, 5, 7, 8, 12, 16, 20 and 24 h) were analyzed by using analytical HPLC monitored at 290 nm. The CAT-4-N7dG and

CAT-4-N7Gua peaks were identified and showed R_f at 9.5 and 10.2 min, respectively. The NADA-6-N7dG and NADA-6-N7Gua peaks were eluted at 8.8 and 10.4 min, respectively. The compounds were analyzed and quantified at 290 nm by using Empower software after comparing the response of samples with the calibration curves made with the standard depurinating adduct, i.e., CAT-4-N7Gua and NADA-6-N7Gua. The rationale for using the N7Gua adducts for quantitation of the N7dG adducts derives from their similar UV spectra. The reactions were conducted and analyzed in triplicate.

Kinetics of depurinating adduct formation after reaction of DNA with CAT-Q or NADA-Q—CAT (19.2 mg, 174 μ mol) or NADA (33.9 mg, 174 μ mol) was dissolved in 1 ml of acetone and stirred at 0 °C. Then Ag₂O (242.2 mg, 6 eq) was slowly added. After 30 min, the solution was filtered through a Gelman acrodisc. Five μ l of the freshly prepared quinone (8.7 μ mol of CAT-Q or NADA-Q) in acetone was reacted with 3 mM DNA in a 10-ml reaction mixture at pH 4. At different time points (1, 2, 3, 4, 5, 6, 7, and 10 h), aliquots of the mixtures were treated quickly with 2 volumes of ethanol to remove DNA and the supernatants were analyzed for the formation of depurinating adducts, as described above. The reactions were performed in triplicate and control experiments were conducted with 5 μ l of acetone in place of the quinone solution.

Covalent binding of CAT-Q or NADA-Q to DNA at different pHs—Five 5 μ l (87 μ M final concentration) of a stock solution of CAT-Q or NADA-Q was mixed with DNA in a total 10-ml reaction mixture containing 3 mM calf thymus DNA in 0.1 M sodium acetate (for pH 4 or 5) or 0.1 M sodium-potassium phosphate (pH 6 or 7). After incubation at 37 °C for 10 h, the DNA was precipitated with two volumes of ethanol; the supernatant was evaporated and dissolved in methanol (1 ml), lyophilized and re-dissolved in 50 μ l of methanol/water (1:1). The final solution was passed through a 5,000 M.W. cut-off filter (Millipore, Billerica, MA) and 10 μ l of each sample was analyzed for depurinating adducts by UPLC-MS/MS. Control reactions were carried out under identical conditions using 50 μ l of acetone in place of the quinone solution.

Covalent binding of tyrosinase-activated CAT or NADA to DNA at different pHs—Stock solutions of CAT (0.96 mg) or NADA (1.69 mg) were made in 50 μ l of acetone and 5 μ l of each solution was mixed with DNA in a total 10-ml reaction mixture containing 3 mM calf thymus DNA in 0.1 M sodium acetate (pH 5) or 0.1 M sodium-potassium phosphate (pH 6, 7 or 8) and 1 mg of mushroom tyrosinase (2577 units). The reaction mixture was incubated at 37 °C in the presence of air for 10 h. After precipitation of DNA with ethanol, the supernatant was used for analysis of depurinating adducts, as described above. Control reactions were carried out under identical conditions using 50 μ l of acetone in place of the CAT or NADA solution.

Results

Formation of CAT-4-N7dG and NADA-6-N7dG adducts

The reaction of CAT-Q with dG at pH 4.0 (Fig. 1) afforded initially a labile adduct as observed by HPLC analysis (retention time 9.5 min, data not shown). After 1 h an aliquot of the reaction mixture was directly infused into the mass spectrometer (Fig. 3) to afford the molecular ions $[M]^+$ at m/z 376, 267, 260 and 152, corresponding to CAT-4-N7dG ($C_{16}H_{18}N_5O_6^+$), dG, CAT-4-N7Gua and Gua, respectively. MS/MS analysis of the peak at m/z 376 afforded a daughter ion at m/z 260, corresponding to CAT-4-N7Gua. Attempts to isolate and record the NMR spectrum of the CAT-4-N7dG adduct were not successful, because the compound decomposed during the removal of the solvent.

Analogously, the reaction of NADA-Q with dG at pH 4.0 yielded initially a labile adduct, NADA-6-N7dG, which slowly converted to the depurinating NADA-6-N7Gua adduct. The reaction mixture was analyzed after 3 h by direct infusion into the mass spectrometer. A molecular ion $[M]^+$ peak at $m/z = 461$ (Fig. 4) showed the formation of NADA-6-N7dG adduct that was selectively fragmented to its daughter ion at m/z 345, corresponding to the depurinating NADA-6-N7Gua adduct, m/z 303, corresponding to NADA-6-N7Gua minus an acetyl group, and m/z 152, corresponding to the released Gua moiety.

Kinetics of loss of the deoxyribose moiety

Loss of deoxyribose from CAT-4-N7dG—The rate of loss of the deoxyribose moiety was studied by reacting CAT-Q with dG and analyzing aliquots of the reaction mixture at different time points. The time course of this reaction is represented in Figure 5 by plotting the concentrations of CAT-4-N7dG and CAT-4-N7Gua over time. The maximum formation (6.4 ± 1.1 mM) of the CAT-4-N7dG adduct was obtained within 20 min, indicating that most of the CAT-Q has reacted during this time. This adduct subsequently released the deoxyribose moiety and was converted to CAT-4-N7Gua. The concentration of CAT-4-N7Gua was minimal (1.6 ± 0.3 mM) at 20 min, but slowly increased with time, indicating the slow depurination of CAT-4-N7dG. The concentrations of the CAT-4-N7dG and CAT-4-N7Gua were almost equal (4.4 ± 0.6 mM and 4.3 ± 0.8 mM, respectively) at 2 h. By 12 h, almost complete conversion of CAT-4-N7dG to CAT-4-N7Gua (9.5 ± 1.4 mM) was observed, and after that the amount of the depurinating adduct remained constant. The half-life of CAT-4-N7dG conversion to CAT-4-N7Gua was approximately 3 h (Fig. 5).

Loss of deoxyribose from NADA-6-N7dG—In the reaction between NADA-Q and dG, the concentration of NADA-Q decreased rapidly, with the concomitant formation of NADA-6-N7dG. This adduct subsequently released the deoxyribose moiety and was converted to the depurinating NADA-6-N7Gua adduct. The concentration of the N7dG adduct increased initially and reached its highest level of 1.8 ± 0.6 mM at 2 h and then decreased continuously until it reached a trace amount of < 0.1 mM at 12 h (Fig. 6). On the other hand, the amount of the N7Gua adduct was minimal (0.2 ± 0.2 mM) at 30 min and then increased continuously until it reached the maximum amount of 2.9 ± 0.7 mM at 12 h. After 12 h, the adduct concentrations remained constant as shown in Figure 6. The half-life of NADA-6-N7dG conversion to NADA-6-N7Gua was approximately 4 h (Fig. 6).

Time course of the formation of depurinating adducts of CAT and NADA in DNA

After finding the optimum conditions for the formation of depurinating adducts, a time course study was conducted by reacting CAT-Q or NADA-Q with DNA at pH 4. Aliquots of the reaction mixture were analyzed at various time points. As shown in Figure 7, depurination of the N3Ade adduct (26.0 ± 4.4 μ mol/mol DNA-P) was almost complete within 1 h of incubation time and its level remained constant after that. On the other hand, depurination of the N7Gua adduct was not instantaneous. A gradual increase in the level of the N7Gua adduct formed was observed starting initially with 3.4 ± 0.1 μ mol/mol DNA-P at 1 h and reaching a maximum level of 12.1 ± 1.4 μ mol/mol DNA-P at about 6 h.

In the reaction between NADA-Q and DNA, instantaneous depurination of the N3Ade adduct was observed and its level of 14.8 ± 0.7 μ mol/mol DNA-P remained unchanged after 1 h (Fig. 8). Depurination of the N7Gua adduct was slow. At 1 h, a minimal amount, 1.2 ± 0.1 μ mol/mol DNA-P of the N7Gua adduct was observed in the reaction mixture, which continuously increased until it reached its maximum level of 15.2 ± 1.2 μ mol/mol DNA-P at 5 h. The adduct level remained unchanged afterwards (Fig. 8).

The half-lives of CAT-4-N7dG and NADA-6-N7dG in DNA were approximately 3 h.

Formation of depurinating adducts of CAT and NADA at different pH

The effect of pH on the formation of depurinating adducts was studied by reacting CAT-Q (or NADA-Q) or enzymatically activated CAT (or NADA) with DNA. The level of the depurinating adducts was measured by using UPLC-MS/MS. For CAT, the highest level (29.0 ± 4.5 $\mu\text{mol/mol}$ DNA-P for N3Ade and 15.4 ± 2.6 $\mu\text{mol/mol}$ DNA for N7Gua) of depurinating adducts was detected at pH 4 (Fig. 9A). When the pH of the reaction mixture was increased to 5, the level of the N3Ade adduct was reduced to 10.1 ± 2.1 $\mu\text{mol/mol}$ DNA-P and that of the N7Gua adduct to 3.3 ± 0.3 $\mu\text{mol/mol}$ DNA-P. At pH 6, the level of CAT-4-N3Ade was 3 ± 0.5 $\mu\text{mol/mol}$ DNA-P, and CAT-4-N7Gua, 1.5 ± 0.4 $\mu\text{mol/mol}$ DNA-P. At neutral pH, a sharp reduction in the level of depurinating adducts was observed, giving a level of 1.1 ± 0.3 $\mu\text{mol/mol}$ DNA-P for the N3Ade adduct and 0.4 ± 0.1 $\mu\text{mol/mol}$ DNA-P for N7Gua. Quite surprisingly, the N3Ade adduct was formed in much higher amounts than the corresponding N7Gua adduct (Fig. 9A). When tyrosinase-activated CAT was reacted with DNA at various pH (5–8, Fig. 9B), the highest level of depurinating adducts was observed at pH 5, 95 ± 15 $\mu\text{mol/mol}$ DNA-P for N3Ade and 114 ± 18 $\mu\text{mol/mol}$ DNA-P for N7Gua. Again, at higher pH (6–8), the depurinating adduct levels were reduced. However, slightly more Gua than Ade adducts were detected at pH 5 and 6, which is reversed for pH 7 and 8.

For NADA, the reaction between NADA-Q and DNA at pH 4 was found to be optimum with respect to the formation of depurinating adducts (NADA-6-N7Gua and NADA-6-N3Ade, Fig. 10A). Both the N3Ade and N7Gua adducts were detected in almost similar amounts, 16.4 ± 3.2 and 13.8 ± 2.7 $\mu\text{mol/mol}$ DNA-P, respectively. By increasing the pH to 5, the amounts of the depurinating adducts were lowered to half, with the formation of 7.2 ± 0.4 $\mu\text{mol/mol}$ DNA-P for the N3Ade adduct and 7.7 ± 0.3 $\mu\text{mol/mol}$ DNA-P for the N7Gua adduct. At neutral pH, the amount of the N3Ade adduct was found to be 2.0 ± 0.3 $\mu\text{mol/mol}$ DNA and that of the N7Gua adduct was 1.8 ± 0.3 $\mu\text{mol/mol}$ DNA-P. A similar trend was seen when NADA was activated with tyrosinase in the presence of DNA and the reaction was conducted at different pH (5–8, Fig. 10B).

Discussion

Reaction of catechol quinones with nucleophiles occurs via a proton-assisted 1,4-Michael addition [3]. In this article, we have compared the reaction of the catechol quinone of benzene, CAT-Q, with dG or DNA to that of the catechol quinone of NADA, NADA-Q.

Reaction of the quinones with dG forms N7dG adducts that slowly lose the deoxyribose moiety to form N7Gua adducts. We have investigated the kinetics of this loss of the deoxyribose moiety for both CAT-4-N7dG and NADA-6-N7dG at pH 4 (Fig. 5 and Fig. 6) and observed the rate of disappearance of the N7dG adducts with formation of the N7Gua adducts. The conversion of the CAT-4-N7dG adduct to the N7Gua adduct had a half-life of approximately 3 h (Fig. 5), while the conversion of the NADA-6-N7dG adduct had a half-life of approximately 4 h (Fig. 6).

A further comparison of CAT-Q and NADA-Q was obtained by reaction of the quinones with DNA at pH 4. With DNA, it was observed that the CAT-4-N3Ade and NADA-6-N3Ade adducts were formed and lost from DNA instantaneously, whereas the N7Gua adducts were lost more slowly, with half-lives of approximately 3 h (Fig. 7 and Fig. 8). With CAT-Q, the amount of the N3Ade adducts was more than twice that of the N7Gua adduct after 10 h (Fig. 7). In contrast, NADA-Q formed almost equal amounts of the N3Ade and N7Gua adducts in 10 h (Fig. 8).

Finally, the formation of N3Ade and N7Gua adducts by reaction of CAT-Q with DNA was studied at pH 4 to 7 (Fig. 9A). The maximum yield of both adducts was observed at pH 4,

whereas at pH 7 the yield was negligible. In contrast, when tyrosinase-activated CAT was reacted with DNA at pH 5 to 8 (Fig. 9B), the amounts of the N3Ade and N7Gua adducts were about the same. The highest yield was at pH 5 and the yield decreased as the pH was higher. The amounts of the adducts were much larger than those obtained from the reaction of the CAT-Q with DNA. For example, at pH 5, the amounts of the adducts obtained from tyrosine-activated CAT were at least ten-times higher than those obtained with CAT-Q (Fig. 9A and B). This is presumably due to the instability of the quinone and the efficient reaction of the catechol after intercalation in the DNA and activation to quinone by tyrosinase. This mechanism has been demonstrated with the catechol of diethylstilbestrol [8].

When formation of N3Ade and N7Gua adducts by reaction of NADA-Q with DNA was studied at pH 4 to 7 (Fig. 10A), the maximum yield of both adducts was observed at pH 4 and decreased as the pH rose from 5 to 7. Both adducts were observed at similar levels at all pH. When NADA was activated by tyrosinase to react with DNA at pH 5 to 8, the amounts of adducts were at least 15-times higher than following the direct reaction of NADA-Q with DNA (Fig. 10B). Again, this is presumably due to the instability of NADA-Q and the efficient reaction of NADA after intercalation in the DNA and activation to NADA-Q by tyrosinase. The levels of the N3Ade and N7Gua adducts were not significantly different from each other.

The catechol quinones of natural [20–22] and synthetic [6–8] estrogens, benzene [11], naphthalene [9,10] and dopamine [11] react with DNA by 1,4-Michael addition to form predominantly depurinating N3Ade and N7Gua adducts. With all of these compounds, the N3Ade adduct depurinates instantaneously from DNA, whereas the N7Gua adduct depurinates slowly, with a half-time of a few hours. These common features may lead to the initiation of cancer or neurodegenerative disease.

Abbreviations

Ade	adenine
CAT	1,2-dihydroxybenzene
CAT-Q	catechol-1,2-quinone
DA	dopamine
dG	2'-deoxyguanosine
DMF	dimethylformamide
DMSO	dimethylsulfoxide
ESI	electrospray ionization
Gua	guanine
NADA	<i>N</i> -acetyldopamine
NADA-Q	<i>N</i> -acetyldopamine-3,4-quinone
OHE ₁ (E ₂)	hydroxyestrone(estradiol)
PI	positive ion
Q	quinone
TFA	trifluoroacetic acid
UPLC-MS/MS	ultraperformance liquid chromatography/tandem mass spectrometry

Acknowledgments

This research was supported by grants P01 CA49210 from the National Cancer Institute and DAMD17-03-10229 from the Department of Defense Breast Cancer Research Program and by Prevention LLC. Core support at the Eppley Institute was provided by grant P30 CA36727 from the National Cancer Institute.

References

1. Cavalieri E, Chakravarti D, Guttenplan J, Hart E, Ingle J, Jankowiak R, Muti P, Rogan E, Russo J, Santen R, Sutter T. Catechol estrogen quinones as initiators of breast and other human cancers: implications for biomarkers of susceptibility and cancer prevention. *Biochim. Biophys. Acta* 2006;1766:63–78. [PubMed: 16675129]
2. Cavalieri E, Rogan E. Catechol quinones of estrogens in the initiation of breast, prostate, and other human cancers: Keynote lecture. *Ann. N. Y. Acad. Sci* 2006;1089:286–301. [PubMed: 17261777]
3. Stack DE, Li G, Hill A, Hoffman N. Mechanistic insights into the Michael addition of deoxyguanosine to catechol estrogen-3,4-quinones. *Chem. Res. Toxicol* 2008;21:1415–1425. [PubMed: 18547067]
4. Chakravarti D, Mailander PC, Li KM, Higginbotham S, Zhang HL, Gross ML, Meza JL, Cavalieri EL, Rogan EG. Evidence that a burst of DNA depurination in SENCAR mouse skin induces error-prone repair and forms mutations in the H-ras gene. *Oncogene* 2001;20:7945–7953. [PubMed: 11753677]
5. Mailander PC, Meza JL, Higginbotham S, Chakravarti D. Induction of A.T to G.C mutations by erroneous repair of depurinated DNA following estrogen treatment of the mammary gland of ACI rats. *J. Steroid Biochem. Mol. Biol* 2006;101:204–215. [PubMed: 16982187]
6. Saeed M, Zahid M, Gunselman SJ, Rogan E, Cavalieri E. Slow loss of deoxyribose from the N7deoxyguanosine adducts of estradiol-3,4-quinone and hexestrol-3',4'-quinone. Implications for mutagenic activity. *Steroids* 2005;70:29–35. [PubMed: 15610894]
7. Saeed M, Gunselman SJ, Higginbotham S, Rogan E, Cavalieri E. Formation of the depurinating N3adenine and N7guanine adducts by reaction of DNA with hexestrol-3',4'-quinone or enzyme-activated 3'-hydroxyhexestrol. Implications for a unifying mechanism of tumor initiation by natural and synthetic estrogens. *Steroids* 2005;70:37–45. [PubMed: 15610895]
8. Saeed M, Rogan E, Cavalieri E. Mechanism of metabolic activation and DNA adduct formation by the human carcinogen diethylstilbestrol: the defining link to natural estrogens. *Int. J. Cancer* 2009;124:1276–1284. [PubMed: 19089919]
9. Saeed M, Higginbotham S, Rogan E, Cavalieri E. Formation of depurinating N3adenine and N7guanine adducts after reaction of 1,2-naphthoquinone or enzyme-activated 1,2-dihydroxynaphthalene with DNA. Implications for the mechanism of tumor initiation by naphthalene. *Chem. Biol. Interact* 2007;165:175–188. [PubMed: 17224140]
10. Saeed M, Higginbotham S, Gaikwad N, Chakravarti D, Rogan E, Cavalieri E. Depurinating naphthalene-DNA adducts in mouse skin related to cancer initiation. *Free Radic. Biol. Med* 2009;47:1075–1081. [PubMed: 19619639]
11. Cavalieri EL, Li KM, Balu N, Saeed M, Devanesan P, Higginbotham S, Zhao J, Gross ML, Rogan EG. Catechol ortho-quinones: The electrophilic compounds that form depurinating DNA adducts and could initiate cancer and other diseases. *Carcinogenesis* 2002;23:1071–1077. [PubMed: 12082031]
12. Snyder R, Kalf GF. A perspective on benzene leukemogenesis. *Crit. Rev. Toxicol* 1994;24:177–209. [PubMed: 7945890]
13. Guengerich FP, Kim DH, Iwasaki M. Role of human cytochrome P-450 IIE1 in the oxidation of many low molecular weight cancer suspects. *Chem. Res. Toxicol* 1991;4:168–179. [PubMed: 1664256]
14. Sabourin PJ, Bechtold WE, Griffith WC, Birnbaum LS, Lucier G, Henderson RF. Effect of exposure concentration, exposure rate, and route of administration on metabolism of benzene by F344 rats and B6C3F1 mice. *Toxicol. Appl. Pharmacol* 1989;99:421–444. [PubMed: 2749731]
15. Kalf GF. Recent advances in the metabolism and toxicity of benzene. *Crit. Rev. Toxicol* 1987;18:141–159. [PubMed: 3311642]
16. Levay G, Pongracz K, Bodell WJ. Detection of DNA adducts in HL-60 cells treated with hydroquinone and p-benzoquinone by 32P-postlabeling. *Carcinogenesis* 1991;12:1181–1186. [PubMed: 2070482]

17. Levay G, Ross D, Bodell WJ. Peroxidase activation of hydroquinone results in the formation of DNA adducts in HL-60 cells, mouse bone marrow macrophages and human bone marrow. *Carcinogenesis* 1993;14:2329–2334. [PubMed: 8242863]
18. Twerdok LE, Trush MA. Differences in quinone reductase activity in primary bone marrow stromal cells derived from C57BL/6 and DBA/2 mice. *Res. Commun. Chem. Pathol. Pharmacol* 1990;67:375–386. [PubMed: 2343185]
19. Huang X, Xu R, Hawley MD, Kramer KJ. Model insect cuticle sclerotization: Reactions of catecholamine quinones with the nitrogen-centered nucleophiles imidazole and N-acetylhistidine. *Bioorg. Chem* 1997;25:179–202.
20. Cavalieri EL, Stack DE, Devanesan PD, Todorovic R, Dwivedy I, Higginbotham S, Johansson SL, Patil KD, Gross ML, Gooden JK, Ramanathan R, Cerny RL, Rogan EG. Molecular origin of cancer: Catechol estrogen-3,4-quinones as endogenous tumor initiators. *Proc. Natl. Acad. Sci. U. S. A* 1997;94:10937–10942. [PubMed: 9380738]
21. Li KM, Todorovic R, Devanesan P, Higginbotham S, Kofeler H, Ramanathan R, Gross ML, Rogan EG, Cavalieri EL. Metabolism and DNA binding studies of 4-hydroxyestradiol and estradiol-3,4-quinone in vitro and in female ACI rat mammary gland in vivo. *Carcinogenesis* 2004;25:289–297. [PubMed: 14578156]
22. Zahid M, Kohli E, Saeed M, Rogan E, Cavalieri E. The greater reactivity of estradiol-3,4-quinone vs estradiol-2,3-quinone with DNA in the formation of depurinating adducts: implications for tumor-initiating activity. *Chem. Res. Toxicol* 2006;19:164–172. [PubMed: 16411670]

Figure 1. Oxidation CAT and NADA to their respective quinones and reaction of the quinones with Ade or dG to form N3Ade and N7Gua adducts.

Figure 2.
Metabolism of dopamine to form neuromelanin and depurinating DNA adducts.

Figure 3.

Mass spectrum of the reaction mixture of CAT-Q with dG after 1 h at 22 °C. Inset: MS/MS of m/z 376, CAT-4-N7dG, showing the daughter ion at m/z 260, CAT-4-N7Gua.

Figure 4.

Mass spectrum of the reaction mixture of NADA-Q with dG after 3 h at 22 °C. Inset: Right, MS/MS of m/z 461, NADA-6-N7dG, showing the daughter ion at m/z 345, NADA-6-N7Gua, and m/z 152, Gua. Left, MS/MS of m/z 345, NADA-6-N7Gua, showing the daughter ions at m/z 303, NADA-6-N7Gua minus the acetyl group, and m/z 152, Gua.

Figure 5.

Time course of the reaction of CAT-Q with dG to form CAT-4-N7dG and its conversion to CAT-4-N7Gua. CAT (0.05M) was oxidized to CAT-Q with 3M Ag₂O in 2 ml of DMF and filtered in to 0.25M dG in 2 ml of H₂O:CH₃COOH (1:1). The pH was adjusted to 4 and the reaction continued at room temperature.

Figure 6. Time course of the reaction of NADA-Q with dG to form NADA-6-N7dG and its conversion to NADA-6-N7Gua. The same reaction conditions were used as in Figure 5, except that NADA was used instead of CAT.

Figure 7.

Time course of the formation of CAT-4-N3Ade and CAT-4-N7Gua after the reaction of CAT-Q with DNA at pH 4. CAT-Q ($87 \mu\text{M}$) was reacted with 3mM DNA at pH 4 and room temperature.

Figure 8.

Time course of the formation of NADA-6-N3Ade and NADA-6-N7Gua after the reaction of NADA-Q with DNA at pH 4. The same reaction conditions were used as in Figure 7, except that NADA-Q was used instead of CAT-Q.

Figure 9.

Effect of pH on formation of the depurinating CAT-4-N3Ade and CAT-4-N7Gua adducts by (A) reaction of CAT-Q with DNA for 10 h and (B) tyrosinase-activated CAT with DNA for 10 h. (A) CAT-Q (87 μ M) was reacted with 3 mM DNA at the indicated pH and 37° C, or (B) 87 μ M CAT was reacted with 3 mM DNA in the presence of 2577 units of mushroom tyrosinase at the indicated pH and 37° C.

Figure 10.

Effect of pH on formation of the depurinating NADA-6-N3Ade and NADA-6-N7Gua adducts by reaction of (A) NADA-Q with DNA for 10 h, and (B) tyrosinase-activated NADA with DNA for 10 h. The same reaction conditions were used as in Figure 9, except that (A) NADA-Q or (B) NADA was used instead of CAT-Q or CAT.