Carcinogenesis vol.23 no.6 pp.1071–1077, 2002

Catechol ortho-quinones: the electrophilic compounds that form depurinating DNA adducts and could initiate cancer and other diseases

Ercole L.Cavalieri[1,3], Kai-Ming Li[1], Narayanan Balu[1], Muhammad Saeed[1], Prabu Devanesan[1], Sheila Higginbotham[1], John Zhao[2], Michael L.Gross[2] and Eleanor G.Rogan[1]

[1]Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, 986805 Nebraska Medical Center, Omaha, NE 68198-6805 and [2]Department of Chemistry, Washington University, One Brookings Drive, St Louis, MO 63130, USA

[3]To whom correspondence should be addressed
Email: ecavalie@unmc.edu

Catechol estrogens and catecholamines are metabolized to quinones, and the metabolite catechol (1,2-dihydroxybenzene) of the leukemogenic benzene can also be oxidized to its quinone. We report here that quinones obtained by enzymatic oxidation of catechol and dopamine with horseradish peroxidase, tyrosinase or phenobarbital-induced rat liver microsomes react with DNA by 1,4-Michael addition to form predominantly depurinating adducts at the N-7 of guanine and the N-3 of adenine. These adducts are analogous to the ones formed with DNA by enzymatically oxidized 4-catechol estrogens (Cavalieri,E.L., *et al.* (1997) *Proc. Natl Acad. Sci.*, 94, 10937). The adducts were identified by comparison with standard adducts synthesized by reaction of catechol quinone or dopamine quinone with deoxyguanosine or adenine. We hypothesize that mutations induced by apurinic sites, generated by the depurinating adducts, may initiate cancer by benzene and estrogens, and some neurodegenerative diseases (e.g. Parkinson's disease) by dopamine. These data suggest that there is a unifying molecular mechanism, namely, formation of specific depurinating DNA adducts at the N-7 of guanine and N-3 of adenine, that could initiate many cancers and neurodegenerative diseases.

Introduction

An important pathway in the metabolism of catechol* estrogens (CE) and catecholamines is the oxidation to their respective semiquinones and quinones (1,2). The basis of the biological activity of catechol quinones is related to their ability to act both as oxidants and electrophiles (3). As oxidants, catechol quinones redox cycle with their semiquinones, producing an elevated level of reactive oxygen species, a condition known as oxidative stress (1,2). As electrophiles, catechol quinones can form covalent adducts with cellular macromolecules, including DNA (4). These are stable adducts that remain in DNA unless removed by repair and depurinating ones that are released from DNA by destabilization of the glycosyl bond. Thus, DNA can be damaged by the reactive quinones themselves and by reactive oxygen species (hydroxyl radicals) (1,4,5). The formation of depurinating adducts by CE quinones reacting with DNA may be a major event in the initiation of breast and other human cancers (4,5). The depurinating adducts are released from DNA, leaving apurinic sites in the DNA that can generate mutations leading to cancer (6–8).

An important metabolic pathway of the estrogens, estrone (E_1) and estradiol (E_2), is formation of CE, namely, the hydroxylated estrogens, 4-hydroxyestrone(estradiol) (4-OHE_1(E_2)), which are carcinogenic in animals (9,10), and the isomeric 2-OHE_1(E_2). Oxidation of 4-OHE_1(E_2) to their quinones (E_1(E_2)-3,4-Q) and reaction with DNA form the 4-OHE_1(E_2)-1-N7guanine (Gua) and 4-OHE_1(E_2)-1-N3adenine (Ade) adducts by depurination (Figure 1) (4,5,11).

In this article, we report that the catechol quinone of the leukemogenic benzene and the quinone of the neurotransmitter dopamine (DA) also react with DNA to form depurinating adducts analogous to those formed by E_1(E_2)-3,4-Q. We also postulate that oxidation of catechols to quinones and their reaction with DNA may be a general, unifying mechanism of initiation of some cancers and neurodegenerative diseases.

Benzene

Benzene is carcinogenic and leukemogenic in rats and mice (12–14), and epidemiological studies have established a relationship between exposure to benzene and acute myelogenous leukemia in humans (15,16). Several studies indicate that certain metabolites of benzene are responsible for both its cytotoxic and genotoxic effects (17–20). High levels of peroxidase and a lack of quinone reductase in the bone marrow allow formation of toxic semiquinones and quinones without the possibility of their being reduced (21). Benzene is initially metabolized to phenol in the liver by cytochrome P450 2E1 (19,20,22,23). Other metabolites include catechol (CAT, 1,2-dihydroxybenzene), hydroquinone (1,4-dihydroxybenzene) and muconaldehyde (24–26). Several studies have shown that CAT and hydroquinone accumulate in bone marrow (27,28), where they can be further activated to exert their myelotoxic effects (19). Oxidation of CAT and hydroquinone is catalyzed by peroxidases, including myeloperoxidase and prostaglandin H synthase (29–35), and the resulting quinones can produce DNA adducts (33–35).

We postulate that the leukemogenic effect of benzene may be mainly initiated by formation of CAT quinone and its reaction with DNA to form specific depurinating adducts.

Dopamine

The neurotransmitter DA is formed in the cell bodies of the dopaminergic neurons of the substantia nigra. Degeneration of

Abbreviations: Ade, adenine; *o*-BQ, *ortho*-benzoquinone; CAT, catechol or 1,2-dihydroxybenzene; CE, catechol estrogen(s); CE-Q, catechol estrogen quinone(s); COMT, catechol-*O*-methyltransferase; DA, dopamine; dG, deoxyguanosine; DMF, dimethylformamide; E_1, estrone; E_2, estradiol; E_1(E_2)-3,4-Q, estrone(estradiol)-3,4-quinones or catechol estrogen-3,4-quinones; FAB, fast atom bombardment; Gua, guanine; MS/MS, tandem mass spectrometry; NADA, *N*-acetyldopamine; OHE_1(E_2), hydroxyestrone(estradiol); TFA, trifluoroacetic acid.

*The term catechol refers to an aromatic ring with vicinal hydroxyl substituents. In this article catechol is spelled out when it is used in a general sense. When it refers specifically to the compound 1,2-dihydroxybenzene, normally called catechol, it is abbreviated CAT.

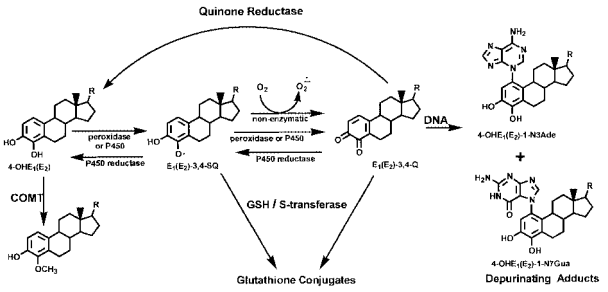

Fig. 1. Metabolism of 4-$OHE_1(E_2)$ and formation of depurinating DNA adducts.

the nigrostriatal dopaminergic neurons and decreased production of DA results in Parkinson's disease. The etiology of Parkinson's disease and its underlying mechanism of loss of DA neurons are unknown. There is evidence, however, that DA is involved in the etiology of this disease, based on the observation by Graham *et al.* (36) that DA is oxidized to the corresponding quinone. Injection of DA into neostriatum generates toxicity to dopaminergic neurons, and the toxicity correlates with protein binding (37,38). Glutathione and ascorbic acid (37–39) diminish the toxicity of protein binding. Covalent binding of DA to DNA occurs upon incubating DA with HL-60 cells or human glioblastoma cell lines (40), by copper-mediated oxidation of DA (41) or by oxidation of DA with prostaglandin H synthase (42,43).

We hypothesize that oxidation of DA to its quinone and subsequent reaction with DNA cause DNA damage via formation of specific depurinating adducts, and the mutations generated by that damage may play a major role in initiating the series of events leading to neurodegenerative disorders such as Parkinson's disease. In general, catecholamine neurotransmitters such as DA can produce semiquinones and quinones via autoxidation, metal ion oxidation, and peroxidative enzyme or cytochrome P450 oxidation (44,45). This oxidative process is similar to the one described above for the benzene metabolite CAT and for the 4-$OHE_1(E_2)$ formed by the metabolism of E_1 and E_2 (4,5).

Materials and methods

Chemicals, reagents and enzymes

CAT was obtained from ICN Pharmaceuticals, Cleveland, OH; Ag_2O, $NaIO_4$, Ade, thymidine, deuteriated acetic acid and trifluoroacetic acid (TFA) were purchased from Aldrich Chemical Co., Milwaukee, WI. 2′-Deoxyguanosine (dG), 2′-deoxyadenosine and 2′-deoxycytidine were purchased from TCI Chemicals. DA, and *N*-acetyldopamine (NADA), horseradish peroxidase (type VI) and mushroom tyrosinase were purchased from Sigma Chemicals, St Louis, MO. Liver microsomes from phenobarbital-induced female Wistar MRC rats (Eppley Colony) were prepared by the previously published method (46).

Instrumentation

UV. The UV spectra were obtained during HPLC by using the photodiode array detector (Waters 996, Milford, MA) for all compounds synthesized. HPLC separations were monitored at 280 nm.

NMR. Proton and homonuclear two-dimensional chemical shift correlation spectroscopy NMR spectra were recorded in DMSO-d_6 with one drop of D_2O and one drop of CD_3COOD on a Varian Unity 500 instrument at 499.835 MHz at 25°C. Chemical shifts are reported relative to DMSO (2.5 p.p.m.).

Mass spectrometry. Exact mass measurements of fast atom bombardment (FAB)-produced ions were carried out on a Kratos MS-50 double focusing mass spectrometer in a peak-match mode. Confirmation of the presence of each adduct was by capillary HPLC coupled via electrospray ionization with a Finnigan LCQ ion trap mass spectrometer operating in the tandem mass spectrometry (MS/MS) mode. The HPLC (Microtech Scientific) made use of a binary gradient of solvent A (0.5% CH_3COOH (v/v) in H_2O) and solvent B (0.5% CH_3COOH (v/v) in CH_3OH) at a flow rate of 40 μl/min with a split of 10:1. The column was 0.3 × 100 Zorbax C18 (Microtech Scientific) with a flow rate on the column of 4 μl/min. The gradient was 95%A/5%B initially for 4 min, then linearly adjusted to 60/40 over 14 min, and held at 60/40 for 20 min.

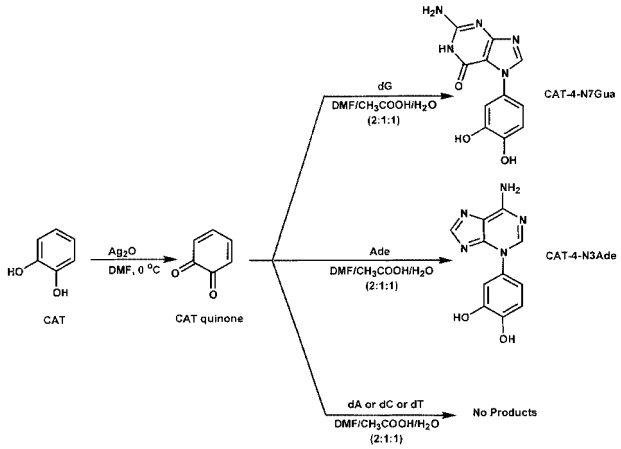

Fig. 2. Synthesis of CAT-4-N7Gua and CAT-4-N3Ade by reaction of CAT quinone with dG or Ade.

HPLC methods for synthetic standards. HPLC was conducted on a Waters (Milford, MA) 600 E system equipped with a Waters 996 photodiode array detector interfaced with an NEC-Powermate computer. Analyses and preparative separations were carried out on reverse-phase C-18, YMC (Morris Plains, NJ) columns (5 μm, 120Δ, ODS-AQ (6 × 250 mm) and ODS-AQ, 5 μm, 120 Δ, (20 × 250 mm), respectively) using specific mobile phases for the different compounds.

Synthesis of standard adducts

Catechol adducts. Because the *ortho*-benzoquinone (*o*-BQ, nascent quinone) is rather unstable, various methods of synthesis were tested to obtain its maximum yield. Oxidation of CAT using Ag_2O in dry dimethylformamide (DMF) was the best method. A solution of CAT (100 mg, 0.91 mmol) in dry DMF (7.5 ml) was stirred with Ag_2O (842 mg, 3.60 mmol) for 30 min at 0°C. The extent of formation of *o*-BQ was followed by HPLC, using a linear analytical gradient from 100% H_2O (0.01% TFA, pH 2.6) to 30% CH_3CN in 60 min at a flow rate of 1 ml/min (monitored by UV absorbance at 300 nm on a Waters 996 photodiode array detector). The yield of *o*-BQ was >95%.

The dark red solution was immediately filtered into a solution of dG (1.20 g, 4.54 mmol) or Ade (613 mg, 4.54 mmol) in DMF/CH_3COOH/H_2O, 7.5 ml each (Figure 2). The reaction mixture was stirred for 8 h at room temperature, filtered and washed with 10 ml of DMF/CH_3COOH/H_2O (2:1:1). The reddish-brown filtrate was directly subjected to HPLC purification, using a linear preparative gradient of 20% CH_3CN in H_2O (0.01% TFA) to 80% CH_3CN in H_2O (0.01% TFA) over 60 min at a flow rate of 9 ml/min with dG or 15% CH_3CN in H_2O (0.01% TFA) to 60% CH_3CN in H_2O (0.01% TFA) over 60 min at a flow rate of 9 ml/min with Ade. The products, isolated under an argon atmosphere and stored at –20° C in 2 ml of DMF/CH_3COOH/H_2O (2:1:1), were CAT-4-N7Gua and CAT-4-N3Ade, the result of a 1,4-Michael addition between dG or Ade and *o*-BQ.

For CAT-4-N7Gua, the yield was 59%; UV: λ_{max}, 285 nm. ^{1}H NMR, Δ (p.p.m.): 6.79 (s, 2H, 5-H, 6-H), 7.08 (s, 1H, 3-H), 8.01 (s, 1H, 8-H (Gua)). FAB MS, $(M + H)^+$, $C_{11}H_9N_5O_3$: calcd *m/z* 260.0785; obsd *m/z* 260.0783.

For CAT-4-N3Ade, the yield was 65%; UV: λ_{max}, 279 nm. ^{1}H NMR, Δ (p.p.m.): 6.95 (bd, 2H, 5-H, 6-H), 7.08 (s, 1H, 3-H), 8.52 (s, 1H, 2-H (Ade)), 8.76 (s, 1H, 8-H (Ade)). FAB MS, $(M + H)^+$, $C_{11}H_9N_5O_2$: calcd *m/z* 244.0836; obsd *m/z* 244.0834.

N-Acetyldopamine adducts. A solution of NADA (9 mg, 0.047 mmol) in 1.5 ml of CH_3COOH/ H_2O (1:1) was stirred with $NaIO_4$ (5 mg, 0.023 mmol) for 5 min at room temperature. To the resulting red solution of the NADA quinone was added 5 equivalents of dG (59 mg, 0.23 mmol) in 1.5 ml of CH_3COOH/H_2O (1:1) (Figure 3). The reaction mixture was stirred for 3 h at room temperature and then separated by HPLC, using a 45 min linear preparative gradient from 10% CH_3CN in H_2O (0.01% TFA) to 30% CH_3CN in H_2O (0.01% TFA) at a flow rate of 10 ml/min. The yield of NADA-6-N7Gua was 58%.

The NADA-6-N7Gua adduct was also synthesized following oxidation of

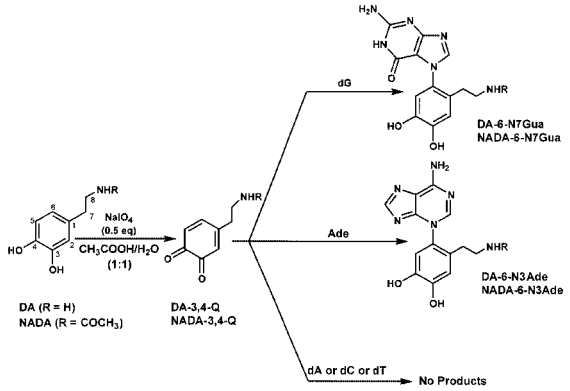

Fig. 3. Synthesis of DA (NADA)-6-N7Gua and DA (NADA)-6-N3Ade by reaction of DA (NADA) quinone with dG or Ade.

NADA by Ag_2O. A solution of NADA (5 mg, 0.023 mmol) in 1 ml of dry DMF was stirred with Ag_2O (43 mg, 0.19 mmol) for 30 min. The suspension was immediately filtered into a solution of dG (29 mg, 0.12 mmol) in DMF/CH_3COOH/H_2O, 1 ml each. The reaction mixture was stirred for 10 h at room temperature, and the product purified by HPLC, yielding 60% NADA-6-N7Gua, UV: λ_{max}, 245, 284 nm. ^1H NMR (p.p.m.): 1.90 (s, 3H, CH_3), 2.25 (t, 2H, J = 6.7 Hz, 7-CH_2), 3.00 (bt, 2H, 8-CH_2), 6.62 (s, 1H, 5-H), 6.66 (s, 1H, 2-H), 7.85 (s, 1H, 8-H (Gua)). FAB MS, $(M + H)^+$, $C_{15}H_{17}N_6O_4$: calcd *m/z* 345.1311; obsd *m/z* 345.1311.

To synthesize the Ade adduct, a solution of NADA (20 mg, 0.094 mmol) in 2 ml of CH_3COOH/H_2O (1:1) was oxidized with $NaIO_4$ (10 mg, 0.047 mmol) and reacted with Ade (63 mg, 0.47 mmol), as described above for the reaction with dG. The product, NADA-6-N3Ade, was purified by HPLC, using a preparative linear gradient from 5% CH_3CN in H_2O (0.01% TFA) over 60 min to 40% CH_3CN in H_2O (0.01% TFA) at a flow rate of 9 ml/min. The yield was 51%; UV: λ_{max}, 275 nm. ^1H NMR (p.p.m.): 1.90 (s, 3H, CH_3), 2.15–2.30 (m, 2H, 7-CH_2), 2.83–3.10 (m, 2H, 8-CH_2), 6.81 (s, 1H, 5-H), 6.85 (s, 1H, 2-H), 8.45 (s, 1H, 2-H (Ade)), 8.65 (s, 1H, 8-H (Ade)). FAB MS, $(M + H)^+$, $C_{15}H_{17}N_6O_3$: calcd *m/z* 329.1361; obsd *m/z* 329.1362.

Dopamine adducts. DA-HCl (50 mg, 0.264 mmol) and dG (622 mg, 2.6 mmol) were dissolved in 13 ml of CH_3COOH/H_2O (1:1). To this mixture a solution of $NaIO_4$ (28 mg, 0.13 mmol) in 2 ml of CH_3COOH/H_2O (1:1) was added dropwise over 10 min (Figure 3). After 3 h at room temperature, the reaction was terminated, and the product, DA-6-N7Gua, was purified by preparative HPLC, using a linear gradient from 10% CH_3CN in H_2O (0.01% TFA) to 30% CH_3CN in H_2O (0.01% TFA) over 60 min, then to 80% CH_3CN in 15 min at a flow rate of 8 ml/min. The colorless semi-solid product was obtained in 46% yield. UV: λ_{max}, 245 (sh), 283 nm. ^1H NMR (p.p.m.): 2.45 (m, 2H, 7-CH_2), 2.75 (m, 2H, 8-CH_2), 6.70 (s, 1H, 5-H), 6.76 (s, 1H, 2-H), 8.15 (s, 1H, 8-H (Gua)). FAB MS, $(M + H)^+$, $C_{13}H_{14}N_6O_3$: calcd *m/z* 303.1207; obsd *m/z* 303.1205.

A solution of DA-HCl (50 mg, 0.26 mmol) and Ade (356 mg, 2.64 mmol) in 13 ml of CH_3COOH/H_2O (1:1) was treated with a solution of $NaIO_4$ (28 mg, 0.13 mmol) in 2 ml of CH_3COOH/H_2O (1:1) in a manner similar to that used to synthesize DA-6-N7Gua. After 3 h at room temperature, the reaction was terminated. The mixture was subjected to preparative HPLC, using H_2O (0.01% TFA) at a flow rate of 5 ml/min for 20 min, followed by a linear gradient to 80% CH_3CN in H_2O (0.01% TFA) over 40 min at a flow rate of 10 ml/min. DA-6-N3Ade was obtained in 40% yield. UV: λ_{max}, 279 nm. ^1H NMR (p.p.m.): 2.40 (m, 2H, 7-CH_2), 2.82 (m, 2H, 8-CH_2), 6.80 (s, 1H, 5-H), 6.86 (s, 1H, 2-H), 8.05 (s, 1H, 2-H (Ade)), 8.40 (s, 1H, 8-H (Ade)). FAB MS, $(M + H)^+$, $C_{13}H_{14}N_6O_2$: calcd *m/z* 287.1258; obsd *m/z* 287.1256.

Enzymatically-catalyzed covalent binding of catechol and dopamine to DNA

CAT and DA were bound to DNA in 10 ml reaction mixtures containing 3 mM calf thymus DNA in 0.067 M sodium-potassium phosphate (pH 7.0), 0.8 μM CAT or DA in 50 μl DMSO and 1 mg horseradish peroxidase plus 0.5 mM H_2O_2 or 1 mg mushroom tyrosinase. CAT and DA (0.8 μM) were also activated by 10 mg of phenobarbital-induced rat liver microsomes (46) in 150 mM Tris–HCl (pH 7.5), 150 mM KCl, 5 mM $MgCl_2$, 1 mM cumene hydroperoxide and 3 mM DNA. The reactions were incubated for 2 h at 37°C. A 1-ml aliquot was used for analysis of stable DNA adducts by the ^{32}P-postlabeling method with 8 μg of DNA (47). The DNA was precipitated from the remaining reaction mixture with two volumes of ethanol, and the supernatant was used for structure determination of depurinating adducts. After evaporation under vacuum, the residue was dissolved in 1 ml of DMSO/CH_3OH. The CAT adducts were first separated by HPLC on a preparative column with a curvilinear gradient (CV 6) from 100% H_2O (0.01% TFA) to 15% CH_3OH in H_2O (0.01% TFA) in 60 min at a flow rate of 3 ml/min. Fractions at the retention times of CAT-4-N3Ade (34.5 min) and CAT-4-N7Gua (40 min) were collected and analyzed by HPLC, which was eluted with aqueous 50 mM $(NH_4)_3PO_4$, 5 mM sodium dodecyl sulfate, 1% CH_3COOH at a flow rate of 0.5 ml/min. The DA adducts were first separated by HPLC on a preparative column eluted with a curvilinear gradient (CV 6) from 100% aqueous 50 mM $(NH_4)_3PO_4$, 5 mM sodium dodecyl sulfate, 4% CH_3COOH to 100% CH_3CN at a flow rate of 0.5 ml/min. Fractions were collected at 34 min for DA-6-N3Ade and 37 min for DA-6-N7Gua and analyzed by HPLC as described above for the CAT adducts. The remainder of the collected fractions was used to confirm the structures of the adducts by MS.

Results

To demonstrate that the quinones of CAT and DA can react with the nucleobases of DNA, we synthesized standard adducts by reaction of *o*-BQ or DA quinone with dG or Ade. The syntheses provided useful insights into the ability of these electrophilic species to react with nucleophilic groups of deoxyribonucleosides. Furthermore, the adducts served as standards to identify the depurinating adducts formed when CAT and DA were oxidized *in vitro* by various enzymes in the presence of DNA (see below).

Structure elucidation of adducts

Catechol adducts. The reaction of *o*-BQ with the deoxyribonucleoside bases to afford the desired adducts is an acid-assisted 1,4-Michael addition reaction analogous to that of CE quinones with nucleobases (48,49). With *o*-BQ, however, the reaction in CH_3COOH/H_2O (1:1) did not yield any products, due to the instability of *o*-BQ. To render this reaction feasible a compromise was reached by conducting it in DMF/CH_3COOH/H_2O (2:1:1).

Reaction of *o*-BQ with dG afforded CAT-4-N7Gua (Figure 2). The structure was readily determined by both NMR and MS analysis. By MS, the $(M + H)^+$ ion had an *m/z* 260, indicating that deoxyribose had been lost. This implies that Gua is bonded to CAT at the N-7. The NMR resonance of the 5-H and 6-H of the CAT moiety as a singlet at 6.79 p.p.m. and the 3-H as a singlet at 7.08 p.p.m. indicates that the bond to Gua in the CAT aromatic ring occurs at C-4.

For CAT-4-N3Ade, the structure was consistent with the NMR spectrum, showing the aromatic protons 5-H and 6-H as a doublet at 6.95 p.p.m., and the singlet at 7.08 p.p.m. that was assigned as 3-H. Furthermore, the 2-H and 8-H of the Ade moiety were observed at 8.52 and 8.76 p.p.m., respectively. The mass of the FAB-produced ion at *m/z* 244 corroborated the structure of this adduct.

Under the same conditions, *o*-BQ adducts of deoxyadenosine, deoxycytidine and thymidine were not obtained. Furthermore, reaction of the stable 1,4-benzoquinone (*p*-BQ) with dG, Ade, deoxyadenosine, deoxycytidine or thymidine did not afford any detectable adducts.

Dopamine adducts. The oxidation of DA and subsequent reaction with dG or Ade were more difficult to accomplish because the amino group of the DA quinone reacts intramolecularly by a 1,4-Michael addition to produce a dihydroindole, a precursor to neuromelanin biosynthesis. This reaction competes with the intermolecular acid-assisted 1,4-Michael addition of the nucleophilic groups of dG and Ade to the DA quinone. To avoid the competitive cyclization reaction, NADA, in which the nucleophilic amino group of DA is acetylated, was oxidized

to its quinone and reacted with deoxyribonucleosides or nucleobases.

N-Acetyldopamine: The structure of the NADA-6-N7Gua adduct (Figure 3) was consistent with MS results, which showed an *m/z* 345 ion, indicating the loss of the deoxyribose moiety. It was also consistent with the NMR results: the two aromatic protons of the NADA moiety, 5-H and 2-H, resonate at 6.62 and 6.70 p.p.m., respectively, assuming that the reaction was a 1,4-Michael addition. If the reaction occurred by 1,6-addition, however, we should have obtained an adduct at C-2 and/or C-5 of NADA. Reaction at C-2 can be disregarded on the basis that the aromatic protons did not resonate as doublets. The adduct with the Gua-NADA bond at C-5, formed by 1,6-addition, was eliminated from consideration by a nuclear Overhauser enhancement experiment in which the resonance of the 7-CH_2 protons at 2.25 p.p.m. was irradiated. This structure would entail that both the resonance signals corresponding to 2-H and 6-H be enhanced. In fact, only the signals corresponding to the 2-H of NADA at 6.70 p.p.m. and the 8-H of the Gua moiety at 7.85 p.p.m. were enhanced. This result unequivocally assigns the structure of the adduct as NADA-6-N7Gua, proving that the reaction is a 1,4-Michael addition. Following the same approach, the structure of NADA-6-N3Ade was assigned.

Dopamine. Reactions of DA-HCl were set at pH 1.2 in CH_3COOH/H_2O (1:1) to minimize intramolecular 1,4-Michael addition of DA and favor intermolecular 1,4-Michael addition of dG or Ade to the 6 position of DA quinone. Although cyclization was avoided under these conditions, owing to the extensive protonation of the DA amino group, minor side reactions such as dimerization and subsequent oligomerization of the resulting DA quinone could not be eliminated. To minimize these competing reactions and obtain the best yields, we generated the DA *o*-quinone *in situ* by adding a solution of $NaIO_4$ to a mixture of DA-HCl and dG or Ade.

The structures of the adducts obtained by reaction of DA quinone with dG or Ade, DA-6-N7Gua and DA-6-N3Ade (Figure 3), were elucidated following the same criteria adopted for the NADA adducts. Under the above conditions, DA quinone did not react with deoxyadenosine, deoxycytidine or thymidine to form adducts to any measurable extent.

In conclusion, the reaction of CAT quinone and DA quinone with dG or Ade involves the specific nucleophilic sites of the N-7 of Gua and N-3 of Ade in the 1,4-Michael addition. The reactions of $E_1(E_2)$-3,4-Q with dG or Ade by 1,4-Michael addition exhibit the same specificity, forming N7Gua and N3Ade depurinating adducts (11,48).

Enzymatically-catalyzed covalent binding of catechol and dopamine to DNA

Conversion of CAT and DA to their quinones can generally occur by autoxidation, metal-ion oxidation or cytochrome P450 or peroxidase-catalyzed oxidation (44,45). *In vivo* the copper-containing enzyme tyrosinase oxidizes DA to its quinone. To demonstrate binding to DNA *in vitro*, CAT and DA were oxidized in reactions catalyzed by horseradish peroxidase, tyrosinase or phenobarbital-induced rat liver microsomes in the presence of DNA (Table I). All three enzymes catalyzed formation of detectable amounts of the depurinating adducts of DA, DA-6-N3Ade and DA-6-N7Gua, as well as the CAT-4-N7Gua depurinating adduct of CAT. In contrast, the CAT-4-N3Ade adduct was detected only after activation by tyrosinase.

Formation of the stable adducts of DA was low, less than 5% of the total adducts formed with horseradish peroxidase, 4% of the adducts formed with tyrosinase, and 1% of the adducts formed with microsomes. Similarly, with CAT, stable adducts comprised <6% of the total adducts formed with horseradish peroxidase, 0.2% of those formed with tyrosinase, and 0.1% of the adducts formed with microsomes. With DA, the microsomes catalyzed formation of six to seven stable adducts that were separated by the ^{32}P-postlabeling method, whereas tyrosinase and horseradish peroxidase catalyzed formation of the same stable adduct, which appeared to be one of those formed by the microsomes. With CAT, both the microsomes and horseradish peroxidase formed two adducts separated by ^{32}P-postlabeling. One of these adducts was detected with activation by both enzymes. This same adduct was the only stable adduct detected when tyrosinase was used to catalyze the binding of CAT to DNA.

Table I. Catechol- and dopamine-DNA adducts formed *in vitro*

Adduct	μmol adduct/mol DNA-P[a]		
	Horseradish peroxidase	Phenobarbital-induced rat liver microsomes	Tyrosinase
Catechol			
CAT-4-N7Gua	10	32	110
CAT-4-N3Ade	nd[b]	nd	2
Stable adducts	0.64	0.02	0.21
Dopamine			
DA-6-N7Gua	1	23	6
DA-6-N3Ade	5	9	3
Stable adducts	0.30	0.24	0.35

[a]Values are the average of two determinations that varied by 10–20%.
[b]nd, not detected.

Confirmation of the presence of each depurinating adduct reported in Table I was by capillary HPLC/tandem mass spectrometry. We required that the unknowns have identical HPLC retention times as the standards and give product-ion spectra of the $(M + H)^+$ ions containing the same two or three intense signals as those of the standards. The product-ion spectrum of the two modified guanine $(M + H)^+$ ions showed that losses of 17 (NH_3) and 42 (NC-NH_2) occurred for both. The CAT-modified Gua showed an additional loss of 24 (possible via formation of an ion-molecule product in the trap), whereas the DA-modified Gua underwent a loss of 35 (NH_3 and H_2O). The modified bases isolated from the *in vitro* experiments showed these same ions. The $(M + H)^+$ ions of the adenines modified with CAT or DA fragmented by losses of 17 and 46, and the unknowns also showed signals for these processes. The product-ion spectra of all the *in vitro* adducts showed other comparable or weaker signals owing to coeluting interferences from the reaction mixture.

In summary, enzymatic oxidation of CAT or DA in the presence of DNA resulted in the formation of 94–99.9% depurinating CAT adducts or 95–99% depurinating DA adducts.

Discussion

The catechol *o*-quinones derived from benzene and DA undergo 1,4-Michael addition with the N-7 and N-3 nucleophilic sites of Gua and Ade in DNA, respectively, to form predominantly depurinating adducts analogous to those formed by the $E_1(E_2)$-3,4-Q (Figure 1) (4,11). These depurinating adducts are by far

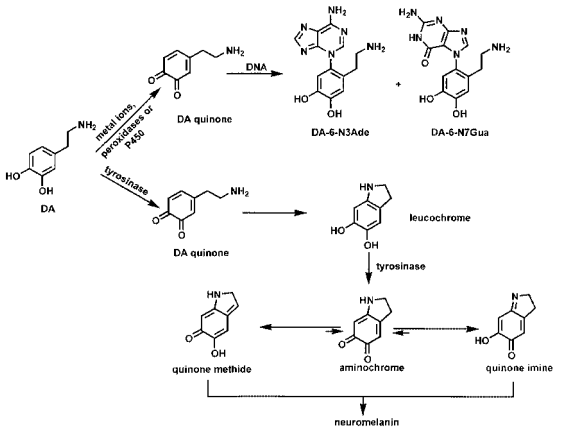

Fig. 4. Metabolism of DA to form neuromelanin or depurinating DNA adducts.

the major products (94–99.9%) when the two *o*-quinones are enzymatically obtained from the corresponding catechols, CAT and DA, in the presence of DNA (Table I).

The role of estrogens in causing DNA damage (4,5,50) is better understood than that of CAT and DA. The estrogens E_1 and E_2, which are biochemically interconvertible, are metabolized via two major pathways: formation of CE and, to a lesser extent, 16α-hydroxylation. In general, estrogens and CE are inactivated by conjugating reactions, such as glucuronidation and sulfation, especially in the liver. The most common pathway of CE conjugation in extrahepatic tissues is *O*-methylation catalyzed by the ubiquitous catechol-*O*-methyltransferase (COMT, Figure 1). Relatively high levels of cytochrome P450 1B1 and other 4-hydroxylases could cause the 4-$OHE_1(E_2)$, which are usually minor metabolites, to be the major ones, rendering conjugation of 4-$OHE_1(E_2)$ via methylation in extrahepatic tissues insufficient. In this case, competitive catalytic oxidation of CE to CE quinones could occur (Figure 1). Redox cycling generated by reduction of CE-Q to CE semiquinones, catalyzed by cytochrome P450 reductase, and subsequent oxidation back to CE-Q by molecular oxygen causes formation of superoxide anion radicals and, subsequently, hydroxyl radicals (not shown in Figure 1) (1). This process, which also occurs with the quinones of CAT and DA, may constitute a significant source of reactive oxygen species. Hydroxyl radicals can also react with DNA and contribute to total DNA damage.

CE-Q can be inactivated by conjugation with glutathione (Figure 1). A second inactivating pathway for CE-Q is their reduction to CE by quinone reductase and/or cytochrome P450 reductase (Figure 1) (51,52). If the two inactivating processes are insufficient, CE-Q may react with DNA to form predominantly stable adducts for the 2-$OHE_1(E_2)$ (not shown in Figure 1) (4,48,53) and predominantly depurinating adducts for the 4-$OHE_1(E_2)$ (Figure 1) (4,11,48). The depurinating adducts generate apurinic sites that may lead to oncogenic mutations (6–8), thereby initiating a variety of human cancers, including breast and prostate. In support of this hypothesis, a burst of apurinic sites leads to mutations in the H-*ras* gene of mouse skin treated with E_2-3,4-Q (8).

The initiating mechanism of carcinogenesis for the synthetic estrogen hexestrol may have a similar explanation. This compound, which is carcinogenic in the kidney of Syrian golden hamsters (54,55), also has catechol as a major metabolite (55,56), which can be metabolically converted to catechol quinone. The catechol quinone of hexestrol has chemical properties similar to those of $E_1(E_2)$-3,4-Q, namely, it specifically forms an N7Gua adduct by 1,4-Michael addition after reaction with dG or DNA (49).

The formation of depurinating adducts specifically at the N-7 of Gua and N-3 of Ade by 1,4-Michael addition to CAT quinone, analogously to those formed by $E_1(E_2)$-3,4-Q, leads us to hypothesize that the metabolite CAT may play a major role in tumor initiation by benzene. In fact, CAT is carcinogenic in mice and rats, inducing glandular stomach tumors in these animals (57). The overall leukemogenicity of benzene could result from a synergistic genotoxic response to CAT quinone, which predominantly produces depurinating DNA adducts, and 1,4-benzoquinone, which produces only stable DNA adducts (33,34,58,59).

One of the functions of the neurotransmitter DA or its precursor, L-Dopa, is the synthesis of neuromelanin (60). This occurs by oxidation of DA to its *o*-quinone, followed by intramolecular cyclization of the nucleophilic amino group via a 1,4-Michael addition (Figure 4). The product, leucochrome, is further oxidized to aminochrome, which, after tautomerization to its quinone methide and quinone imine, polymerizes to neuromelanin, the pigment of the substantia nigra. Disregulation of DA compartmentalization may lead to DA quinone formation by various oxidants (42–45). Under these circumstances, intermolecular 1,4-Michael addition of the N-7 of Gua or N-3 of Ade in DNA to DA quinone could compete successfully with the intramolecular cyclization of DA quinone that leads to dihydroindole derivatives (Figure 4). In fact, DA cyclizes at a slower rate than L-Dopa and epinephrine (61). Thus, if oxidation of DA to its quinone does not occur in a properly controlled environment, we hypothesize that the quinone might react with DNA to form depurinating DNA adducts, generating mutations that could initiate neurodegenerative disorders such as Parkinson's disease.

Summary

The *o*-benzoquinones formed in the metabolism of natural and synthetic estrogens, benzene, and DA react with DNA via 1,4-Michael addition to form specific depurinating adducts that we hypothesize may lead to critical mutations responsible for initiating many cancers and neurodegenerative diseases. Recognition of this proposed unifying mechanism in the etiology of these diseases may provide unique opportunities to develop strategies to assess risk and to prevent diseases.

Acknowledgements

This research was supported by U.S. Public Health Service grants R01 CA49917 and P01 CA49210 from the National Cancer Institute. Core support in the Eppley Institute is provided by grant P30 CA36727 from the National Cancer Institute. The Washington University Mass Spectrometry Laboratory is supported by grant P41 RR00954 from the National Center for Research Resources of the National Institutes of Health.

References

1. Liehr,J.G. and Roy,D. (1990) Free radical generation by redox cycling of estrogens. *Free Radic. Biol. Med.*, **8**, 415–423.
2. Cavalieri,E.L. and Rogan,E.G. (2000) The key role of catechol estrogen-3,4-quinones in tumor initiation. In Creveling,C.R. (ed.) *Role of Catechol Quinone Species in Cellular Toxicity.* F.P.Graham Publishers, Johnson City, TN, pp. 247–260.
3. Finley,K.T. (1993) Quinones: the present state of addition and substitution chemistry. In Patai,S. (ed.) *The Chemistry of Hydroxyl, Ether and Peroxide Groups.* John Wiley, Chichester, Suppl. E, pp. 1027–1134.

4. Cavalieri,E.L., Stack,D.E., Devanesan,P.D., *et al.* (1997) Molecular origin of cancer: Catechol estrogen-3,4-quinones as endogenous tumor initiators. *Proc. Natl Acad. Sci. USA*, **94**, 10937–10942.
5. Cavalieri,E., Frenkel,K., Liehr,J.G., Rogan,E. and Roy,D. (2000) Estrogens as endogenous genotoxic agents: DNA adducts and mutations. In Cavalieri,E. and Rogan,E. (eds) *JNCI Monograph: Estrogens as Endogenous Carcinogens in the Breast and Prostate.* Oxford University Press, pp. 75–93.
6. Chakravarti,D., Pelling,J.C., Cavalieri,E.L. and Rogan,E.G. (1995) Relating aromatic hydrocarbon-induced DNA adducts and c-Harvey-*ras* mutations in mouse skin papillomas: The role of apurinic sites. *Proc. Natl Acad. Sci. USA*, **92**, 10422–10426
7. Chakravarti,D., Mailander,P.C., Cavalieri,E.L. and Rogan, E.G. (2000) Evidence that error-prone DNA repair converts dibenzo[*a,l*]pyrene-induced depurinating lesions into mutations: Formation, clonal proliferation and regression of initiated cells carrying H-*ras* oncogene mutations in early preneoplasia. *Mutat. Res.*, **456**, 17–32.
8. Chakravarti,D., Mailander,P., Li,K.-M., Higginbotham,S., Zhang,H., Gross,M., Meza,J.L., Cavalieri,E. and Rogan,E. (2001) Evidence that a burst of DNA depurination in SENCAR mouse skin induces error-prone repair and forms mutations in the H-*ras* gene. *Oncogene*, **20**, 7945–7953.
9. Liehr,J.G., Fang,W.F., Sirbasku,D.A. and Ulubelen,A.A. (1986) Carcinogenicity of catecholestrogens in Syrian hamsters. *J. Steroid Biochem.*, **24**, 353–356.
10. Li,J.J. and Li,S.A. (1987) Estrogen carcinogenesis in Syrian hamster tissue: role of metabolism. *Fed. Proc.*, **46**, 1858–1863.
11. Li,K.-M., Devanesan,P.D., Rogan,E.G. and Cavalieri,E.L. (1998) Formation of the depurinating 4-hydroxyestradiol (4-OHE_2)-1-N7Gua and 4-OHE_2-1-N3Ade adducts by reaction of E_2-3,4-quinone with DNA. *Proc. Am. Assoc. Cancer Res.*, **39**, 636.
12. Cronkite,E.P., Drew,R.T., Inoue,T., Hirabayashi,Y. and Bullis,J.E. (1989) Hematotoxicity and carcinogenicity of inhaled benzene. *Environ. Health Perspect.*, **82**, 97–108.
13. Maltoni,C., Ciliberti,A., Cotti,G., Conti,B. and Belpoggi,F. (1989) Benzene: an experimental multipotential carcinogen: results of the long-term bioassays performed at the Bologna Institute of Oncology. *Environ. Health Perspect.*, **82**, 109–124.
14. Huff,J.E., Haseman,J.K., DeMarini,D.M., *et al.* (1989) Multiple-site carcinogenicity of benzene in Fischer 344 rats and $B6C3F_1$ mice. *Environ. Health Perspect.*, **82**, 125–163.
15. IARC (1982) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans* **29**, 93–148.
16. Rinsky,R.A., Smith,A.B., Hornung,R., Filloon,T.G., Young,R.J., Okun,A.H. and Landrigan, P.J. (1987) Benzene and leukemia. An epidemiologic risk assessment. *N. Engl. J. Med.*, **316**, 1044–1050.
17. Andrews,L.S., Lee,E.W., Witmer,C.M., Kocsis,J.J. and Snyder,R. (1977) Effects of toluene on the metabolism, disposition and hemopoietic toxicity of [^{3}H] benzene. *Biochem. Pharmacol.*, **26**, 293–300.
18. Sammett,D., Lee,E.W., Kocsis,J.J. and Snyder,R. (1979) Partial hepatectomy reduces both metabolism and toxicity of benzene. *J. Toxicol. Environ. Health*, **5**, 785–792.
19. Kalf,G.F. (1987) Recent advances in the metabolism and toxicity of benzene. *CRC Crit. Rev. Toxicol.*, **18**, 141–159.
20. Snyder,R. and Kalf,G.F. (1994) A perspective on benzene leukemogenesis. *CRC Crit. Rev. Toxicol.*, **24**, 177–209.
21. Twerdok,L.E. and Trush,M.A. (1990) Differences in quinone reductase activity in primary bone marrow stromal cells derived from C57BL/6 and DBA/2 mice. *Res. Commun. Chem. Pathol. Pharmacol.*, **67**, 375–386.
22. Koop,D.R., Laethem,C.L. and Schneir,G.G. (1989) Identification of ethanol-inducible P450 isozyme 3a. P450 II E1 as a benzene and phenol hydroxylase. *Toxicol. Appl. Pharmacol.*, **98**, 278–288.
23. Guengerich,F.P., Kim,D.H. and Iwasaki,M. (1991) Role of human cytochrome P-450 II E1 in the oxidation of many low molecular weight cancer suspects. *Chem. Res. Toxicol.*, **4**, 168–179.
24. Sabourin,P.J., Bechtold,W.E., Birnbaum,L.S., Lucier,G.W. and Henderson,R.F. (1989) Effect of exposure concentration, exposure rate and route of administration on metabolism of benzene by Fischer 344 rats and $B6C3F_1$ mice. *Toxicol. Appl. Pharmacol.*, **99**, 421–444.
25. Latriano,L., Goldstein,B.D. and Witz,G. (1986) Formation of muconaldehyde, an open ring metabolite of benzene in mouse liver microsomes: an additional pathway for toxic metabolites. *Proc. Natl Acad. Sci. USA*, **83**, 8356–8360.
26. Schlosser,P.M., Bond,J.A. and Medinsky,M.A. (1993) Benzene and phenol metabolism by mouse and rat liver microsomes. *Carcinogenesis*, **14**, 2477–2486.
27. Rickert,D.E., Edgar,T.S., Barrow,C.S., Bus,J. and Irons,R.D. (1979) Benzene absorption in rats after inhalation exposure. *Toxicol. Appl. Pharmacol.*, **49**, 417–423.
28. Greenlee,W.F., Gross,E.A. and Irons,R.D. (1981) Relationship between benzene toxicity and the disposition of ^{14}C-labeled benzene metabolites in the rat. *Chem. Biol. Interact.*, **33**, 285–299.
29. Eastmond,D.A., Smith,M.T., Ruzo,O. and Ross,D. (1986) Metabolic activation of phenol by human myeloperoxidase and horseradish peroxidase. *Mol. Pharmacol.*, **30**, 674–679.
30. Subrahmanyam,V.V., Kolachana,P. and Smith,M.T. (1991) Metabolism of hydroquinone by human myeloperoxidase: mechanism of stimulation by other phenolic compounds. *Arch. Biochem. Biophys.*, **286**, 76–84.
31. Sadler,A., Subrahmanyam,V.V. and Ross,D. (1988) Oxidation of catechol by horseradish peroxidase and human leukocyte peroxidase: reactions of *o*-benzoquinone and *o*-benzosemiquinone. *Toxicol. Appl. Pharmacol.*, **93**, 62–71.
32. Schlosser,M.J., Shurina,R.D. and Kalf,G.F. (1990) Prostaglandin H synthase catalyzed oxidation of hydroquinone to a sulfhydryl-binding and DNA damaging metabolite. *Chem. Res. Toxicol.*, **3**, 333–339.
33. Levay,G., Pongraz,K. and Bodell,W.J. (1991) Detection of DNA adducts in HL-60 cells treated with hydroquinone and *p*-benzoquinone by ^{32}P-postlabeling. *Carcinogenesis*, **12**, 1181–1186.
34. Levay,G. and Bodell,W.J. (1992) Potentiation of DNA adduct formation in HL-60 cells by combination of benzene metabolites. *Proc. Natl Acad. Sci. USA*, **89**, 7105–7109.
35. Levay,G., Ross,D. and Bodell,W.J. (1993) Peroxidase activation of hydroquinone results in the formation of DNA adducts in HL-60 cells, mouse bone marrow macrophages and human bone marrow. *Carcinogenesis*, **14**, 2329–2334.
36. Graham,D.G., Tiffany,S.M., Bell,W.R., Jr. and Gutknecht,W.F. (1978) Autoxidation versus covalent binding of quinones as the mechanism of toxicity of dopamine, 6-hydroxydopamine, and related compounds toward C1300 neuroblastoma cells in vitro. *Mol. Pharmacol.*, **14**, 644–653.
37. Hastings,T.G., Lewis,D.A. and Zigmond,M.J. (1996) Role of oxidation in the neurotoxic effects of intrastriatal dopamine injections. *Proc. Natl Acad. Sci. USA*, **93**, 1956–1961.
38. Filloux,F. and Townsend,J.J. (1993) Pre- and postsynaptic neurotoxic effects of dopamine demonstrated by intrastitial injection. *Exp. Neurol.*, **119**, 79–88.
39. Hastings,T.G. and Zigmond,M.J. (1994) Identification of catechol-protein conjugates in neostriatal slices incubated with [^{3}H]dopamine: impact of ascorbic acid and glutathione. *J. Neurochem.*, **63**, 1126–1132.
40. Levay,G. and Bodell,W.J. (1993) Detection of dopamine-DNA adducts: potential role in Parkinson's disease. *Carcinogenesis*, **14**, 1241–1245.
41. Levay,G., Ye,Q. and Bodell,W.J. (1997) Formation of DNA adducts and oxidative base damage by copper-mediated oxidation of dopamine and 6-hydroxydopamine. *Exp. Neurol.*, **146**, 570–574.
42. Hastings,T.G. (1995) Enzymatic oxidation of dopamine: the role of prostaglandin H synthase. *J. Neurochem.*, **64**, 919–924.
43. Mattammal,M.B., Strong,R., Lakshmi,V.M., Chung,H.D. and Stephenson,A.H. (1995) Prostaglandin H synthease-mediated metabolism of dopamine: implication for Parkinson's disease. *J. Neurochem.*, **64**, 1845–1854.
44. Kalyanaraman,B., Felix,C.C. and Sealy,R.C. (1985) Semiquinone anion radicals of catechol(amine)s, catechol estrogens, and their metal ion complexes. *Environ. Health Perspect*., **64**, 185–194.
45. Kalyanaraman,B., Felix,C.C. and Sealy,R.C. (1984) Peroxidatic oxidation of catecholamines: a kinetic electron spin resonance investigation using the spin stabilization approach. *J. Biol. Chem.*, **259**, 7584–7589.
46. Wong,A.K.L., Cavalieri,E. and Rogan,E. (1986) Dependence of benzo[*a*]pyrene metabolic profile on the concentration of cumene hydroperoxide with uninduced and induced rat liver microsomes. *Biochem. Pharmacol.*, **35**, 1583–1588.
47. Bodell,W.J., Devanesan,P.D., Rogan,E.G. and Cavalieri,E.L. (1989) ^{32}P-Postlabeling analysis of benzo[*a*]pyrene-DNA adducts formed *in vitro* and *in vivo*. *Chem. Res. Toxicol.*, **2**, 312–315.
48. Stack,D., Byun,J., Gross,M.L., Rogan,E.G. and Cavalieri,E. (1996) Molecular characteristics of catechol estrogen quinones in reactions with deoxyribonucleosides. *Chem. Res. Toxicol.*, **9**, 851–859.
49. Jan,S.-T., Devanesan,P.D., Stack,D.E., Ramanathan,R., Byun,J., Gross,M.L., Rogan,E.G. and Cavalieri,E.L. (1998) Metabolic activation and formation of DNA adducts of hexestrol, a synthetic non-steroidal carcinogenic estrogen. *Chem. Res. Toxicol.*, **11**, 412–419.
50. Service, R. (1998) New role for estrogen in cancer? *Science*, **279**, 1631–1633.

51. Ernester,L., Estabrook,R.W., Hochstein,P. and Orrenius,S. (eds) (1987) DT diaphorase – a quinone reductase with special functions in cell metabolism and detoxication. *Chem. Script.*, 27A.
52. Roy,D. and Liehr,J.G. (1988) Temporary decrease in renal quinone reductase activity induced by chronic administration of estradiol to male Syrian hamsters. *J. Biol. Chem.*, **263**, 3646–3651.
53. Dwivedy,I., Devanesan,P., Cremonesi,P., Rogan,E. and Cavalieri,E. (1992) Synthesis and characterization of estrogen 2,3- and 3,4-quinones. Comparison of DNA adducts formed by the quinones *versus* horseradish peroxidase-activated catechol estrogens. *Chem. Res. Toxicol.*, **5**, 828–833.
54. Li,J.J., Li,S.A., Klicka,J.K., Parsons,J.A. and Lam,L.K.T. (1983) Relative carcinogenic activity of various synthetic and natural estrogens in the Syrian hamster kidney. *Cancer Res.*, **43**, 5200–5204.
55. Liehr,J.G., Ballatore,A.M., Dague,B.B. and Ululeben,A.A. (1985) Carcinogenicity and metabolic activation of hexestrol. *Chem. Biol. Interact.*, **55**, 157–176.
56. Metzler,M. and McLachlan,J.A. (1981) Oxidative metabolism of the synthetic estrogens hexestrol and dienestrol indicates reactive intermediates. *Adv. Exp. Med. Biol.*, **136A**, 829–837.
57. Hirose,M., Fukushima,S., Tanaka,H., Asakawa,E., Takahashi,S. and Ito,N. (1993) Carcinogenicity of catechol in F344 rats and B6C3F$_1$ mice. *Carcinogenesis*, **14**, 525–529.
58. Robertson,M., Eastmond,D. and Smith,M.T. (1990) Two benzene metabolites, catechol and hydroquinone, produce a synergistic genotoxic response in cultured human lymphocytes. *Mutat. Res.*, **249**, 201–209.
59. Smith,M.T. (1996) The mechanism of benzene-induced leukemia: A hypothesis and speculations on the causes of leukemia. *Environ. Health Perspect.*, **104** (Suppl. 6), 1219–1225.
60. Smit,N.P.M., Pavel,S. and Riley,P.A. (2000) Mechanisms of control of the cytotoxicity of orthoquinone intermediates of melanogenesis. In Creveling,C.R. (ed.) *Role of Catechol Quinone Species in Cellular Toxicity.* F.P.Graham Publishing, Johnson City, TN, pp. 191–245.
61. Pelizzetti,E., Mentasti,E. and Pramauro,E. (1976) Kinetics and mechanism of oxidation pathways of some catecholamines with periodic acid. *J. Chem. Soc. Perkins II*, 1651–1655.

Received December 14, 2001; revised January 29, 2002; accepted March 26, 2002