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Advances in Brief

8-Hydroxylation of Guanine Bases in Kidney and Liver DNA of Hamsters Treated with Estradiol: Role of Free Radicals in Estrogen-induced Carcinogenesis[1]

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Abstract

The chronic administration of estradiol induces a high incidence (80–100%) of renal tumors in male Syrian hamsters. As part of our examination of a mechanism of carcinogenesis by free radicals generated during redox cycling of catecholestrogen metabolites, we assayed levels of 8-hydroxy-2′-deoxyguanosine (8-OHdGua), a marker product of hydroxy radical interaction with DNA, in livers and kidneys of hamsters treated with estradiol. Injections of 50 and 100 mg/kg estradiol doubled renal 8-OHdGua levels over controls [10.0 ± 0.1 (SD) and 5.4 ± 0.4 8-OHdGua/10^5dGua, respectively] and raised hepatic 8-OHdGua levels almost 4-fold over control values, respectively. These changes were observed in kidney 4 h and in liver 1 or 2 h after treatment of hamsters with estradiol. Estradiol implants administered to hamsters for 3 days raised renal levels of 8-OHdGua by 50% over control values. Six days after 17β-estradiol implantation, 8-OHdGua levels returned to near-normal values. Liver DNA was not affected by estradiol implants. These data support a mechanism of estrogen-induced carcinogenesis by free radicals generated via redox cycling of catecholestrogen metabolites.

Introduction

Estrogens have been implicated in the etiology of human hormone-associated cancers and are also known to induce tumors in several rodent species (1, 2). The best characterized of these is a kidney tumor induced in almost all male Syrian hamsters treated with the natural hormone E_2[3] or the synthetic estrogen DES for at least 6 months (2). The carcinogenic activities of several synthetic estrogens in the hamster kidney tumor model could not be correlated with their hormonal potencies (3). Therefore, metabolic activation of estrogens, specifically, free radical generation by metabolic redox cycling between quinone and hydroquinone forms of DES or catecholestrogen metabolites has been postulated to be a factor in the initiation of carcinogenesis prior to hormone receptor-mediated cell transformation and tumor growth (4). In the case of the synthetic estrogen DES, free radicals are generated by metabolic redox cycling *in vitro* (4–6). Moreover, metabolic quinone formation, redox cycling, and hydroxy radical action have also been demonstrated in kidneys of DES-treated hamsters (7–9). In the case of steroid estrogens, free radicals are generated by redox cycling between estrone-3,4-quinone and its corresponding hydroquinone in biochemical incubations and in MCF-7 cells in culture, in which this metabolic activity causes DNA single strand breaks (10). However, quinone metabolites may be formed and undergo metabolic redox cycling *in vivo* but they may be too unstable to be detected *in vivo* (11). Therefore, the induction of free radical-mediated DNA damage has been examined in this study in hamsters as an indicator of redox cycling of steroid estrogens. Evidence for free radical action in hamsters treated chronically with E_2 include increased free radical-induced carbonyl content in kidney proteins, as shown by a NaB^3H_4 reduction assay (12). In addition, lipid peroxide levels were increased in kidneys but not in livers of hamsters treated with E_2 (8). Free radical damage to DNA, presumably a factor in the carcinogenic process, has not yet been sufficiently demonstrated in kidneys of hamsters treated with E_2 and has therefore been examined in this study. As an indicator of hydroxy radical action *in vivo*, we initially explored 8-hydroxylation of guanine bases of DNA by varying estrogen doses and exposure times and then assaying 8-OHdGua using high pressure liquid chromatography with electrochemical detection (13, 14). In previous studies, the 8-OHdGua content in kidney DNA of hamsters treated with DES implants for 2 weeks has been shown to increase to twice that of controls, whereas no such effect has been detected in hamsters treated with the natural hormone E_2 for 2 weeks or longer (9). However, the peak in renal lipid peroxide levels within 3 days of estrogen treatment[4] indicated a more rapid onset of free radical action in hamster kidney. Therefore, in this study, we examined 8-hydroxylation of guanine bases in kidney DNA of hamsters treated with E_2 injections and E_2 implants for 3 or 6 days. Values in kidney were compared to those in liver, where tumors do not develop under these conditions.

Materials and Methods

Chemicals. E_2, 2-OHE_2, 4-OHE_2, nuclease P_1, and *Escherichia coli* alkaline phosphatase were purchased from Sigma Chemical Co. (St. Louis, MO). 8-OHdGua was kindly provided by Dr. Robert A. Floyd, Oklahoma Research Foundation (Oklahoma City, OK). All other reagents and chemicals used were of analytical grade or of highest grade available.

Acute E_2 Treatment. Male Syrian hamsters, 6–8 weeks old, were purchased from Sasco (Omaha, NE). Three hamsters/group were treated with single i.p. injections of either 0, 2, 10, or 50 mg/kg E_2 dissolved in propylene glycol. Animals were sacrificed by decapitation 4 h after E_2 injection. Kidney and liver tissues were excised, frozen immediately on dry ice, and stored at −80°C.

In a second experiment, 3 hamsters/group each received 1 single i.p. injection of 50 mg/kg E_2 dissolved in propylene glycol and were sacrificed 1, 2, 4, or 8 h following injection. Control animals received solvent only. Kidney tissue was excised, frozen on dry ice, and stored at −80°C.

In a third experiment, 3 hamsters/group were treated with single i.p. injections of 100 mg/kg E_2 in propylene glycol and were killed 1, 2, 4, or 8 h after injection. Liver tissues were excised, frozen on dry ice, and stored at −80°C.

In a fourth experiment, 3–4 female hamsters/group were treated with single i.p. injections of 150 mg/kg 2-OHE_2 or 4-OHE_2 and killed 4 h after injection. Liver tissues were excised, frozen on dry ice, and stored at −80°C.

Chronic E_2 Treatment. Hamsters received s.c. implants of 25 mg E_2 containing 10% cholesterol, which were prepared by pellet press (Parr Instrument Co., Moline, IL). Three or 6 days after treatment, kidney and liver tissues were excised, frozen on dry ice, and stored at −80°C.

In a second experiment, 3 hamsters/group received 3 i.p. injections of E_2, 2-OHE_2, or 4-OHE_2 in corn oil (100 μg/animal/day). The animals were killed

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[3] The abbreviations used are: E_2, 17β-estradiol; 2- and 4-OHE_2, 2- and 4-hydroxyestradiol, respectively; DES, diethylstilbestrol; 8-OHdGua, 8-hydroxy-2′-deoxyguanosine; dGua, 2′-deoxyguanosine.

[4] M. Y. Wang and J. G. Liehr, unpublished observations.

4 h after the last injection. Kidney tissues were excised, frozen on dry ice, and stored at −80°C.

8-OHdGua Analysis. DNA was isolated by phenol/chloroform/isoamyl alcohol extraction as described previously (15). DNA was dissolved in 20 mM sodium acetate buffer (pH 4.8) and digested according to the procedure of Kohda *et al.* (13). Briefly, 400 μg DNA were incubated with 20 μg nuclease P_1 at 37°C for 40 min. After adjustment of the pH to 7.5 by addition of 0.1 M Tris-HCl, the DNA was incubated with 100 units of *E. coli* alkaline phosphatase at 37°C for 60 min. dGua and 8-OHdGua concentrations in hydrolysate were determined by high pressure liquid chromatography with UV and electrochemical detectors, respectively, linked in series according to the methods of Floyd *et al.* (14, 16) and Shigenaga *et al.* (17). A Waters (Bedford, MA) solvent delivery system, a UV detector (Waters, model 486), and a Coulochem detector (ESA, Bedford, MA; model 5100A) were used with potentials set at +0.12 and +0.35 V for electrodes 1 and 2, respectively. Compounds were separated on a Microsorb C_{18} (4.6-mm x 25-cm) column under isocratic conditions by a mobile phase containing 12.5 mM citric acid, 25 mM sodium acetate, 30 mM sodium hydroxide, 10 mM acetic acid, and 10% methanol, pH 5.10 (flow rate, 1 ml/min). Concentrations of 8-OHdGua were expressed relative to the concentration of dGua detected by UV absorbance at 260 nm.

The Student *t* test was used for statistical analysis of the data.

Results

Acute E_2 Treatment. Estrogen doses and exposure times were varied to explore the conditions of optimal 8-hydroxylation of guanine bases induced by estrogen. A single injection of 50 mg/kg E_2 elevated renal 8-OHdGua concentrations to almost twice the control values, whereas hepatic 8-hydroxylation of guanine bases of DNA was not affected (Table 1, Experiment 1). Moreover, this doubling of renal 8-OHdGua concentrations occurred 4 h after injection of E_2 but not earlier or later (Table 1, Experiments 1 and 2). In contrast, hepatic 8-OHdGua concentrations increased almost 4-fold over control values 1 and 2 h after injection of 100 mg/kg E_2. Values at later times after injection of this dose were not different from controls (Table 1, Experiment 3). After a single injection of a 150-mg/kg dose of either 2-OHE_2 or 4-OHE_2, only 4-OHE_2 raised hepatic 8-OHdGua concentrations significantly over control values (Fig. 1). These data demonstrate that single large doses of either E_2 or 4-OHE_2 elevate 8-OHdGua concentrations in liver or kidney DNA of hamsters.

Chronic E_2 Treatment. The chronic treatment of hamsters with E_2 implants for 3 days raised renal 8-OHdGua concentrations by almost 50% over control values, whereas levels in liver remained unaffected (Fig. 2). In contrast, chronic treatment with E_2 implants for 6 days did not affect 8-OHdGua levels in livers or kidneys. Renal 8-OHdGua concentrations were not significantly different in hamsters treated for 3 days with injections of E_2 or 2- or 4-OHE_2 (100 μg/animal/day) over control values (Table 2). These data demonstrate an increase in 8-OHdGua levels specifically in kidney, the target organ of E_2-induced carcinogenesis, within 3 days of chronic exposure to estrogen.

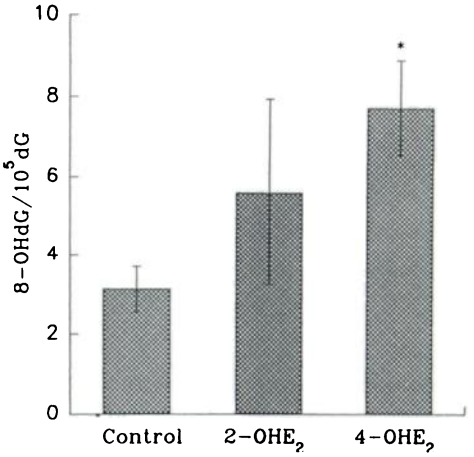

Fig. 1. Effect of catecholestrogens on hepatic 8-OHdGua (*8-OHdG*) concentrations in female Syrian hamsters. Animals were treated with a single i.p. injection of 150 mg/kg 2- or 4-OHE_2. Control animals received only solvent. Animals were killed 4 h after treatment. Values are expressed as means ± SD (*bars*) (n = 3–4). *, significantly different from controls ($P < 0.05$).

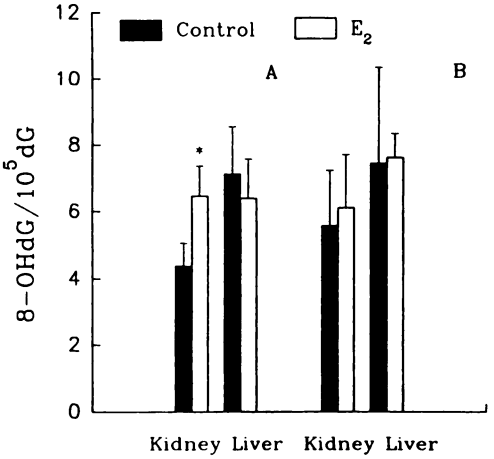

Fig. 2. Effect of chronic treatment of male Syrian hamsters with E_2 on renal and hepatic 8-OHdGua (*8-OHdG*) concentrations. Animals were treated with one 25-mg implant of E_2 for 3 days (*A*) or 6 days (*B*). Control animals remained untreated. Values are expressed as means ± SD (n = 9 and n = 3 for values in *A* and *B*, respectively). *, significantly different from controls ($P < 0.05$).

Table 1 *Effect of E_2 on kidney and liver DNA of male Syrian hamsters*

Animals were treated with one i.p. injection of an indicated dose of E_2 and killed at the indicated time after treatment. Values are expressed as means ± SD (N = 3).

E_2 (mg/kg)	Exposure time (h)	8-OHdGua/10^5 dGua	
		Kidney	Liver
Experiment 1			
0	4	5.4 ± 0.7	10.4 ± 1.8
2	4	8.0 ± 2.7	ND[a]
10	4	6.8 ± 3.6	11.4 ± 3.2
50	4	10.0 ± 0.2[b]	10.3 ± 3.2
150	4	ND	10.3 ± 2.5
Experiment 2			
0	8	4.2 ± 0.4	ND
50	1	5.1 ± 0.4	ND
50	2	5.2 ± 2.1	ND
50	4	8.2 ± 4.9[b]	ND
50	8	4.3 ± 0.8	ND
Experiment 3			
0	8	ND	13.1 ± 12.1
100	1	ND	50.3 ± 19.8[b]
100	2	ND	50.6 ± 12.9[b]
100	4	ND	33.7 ± 14.2
100	8	ND	17.0 ± 16.6

[a] ND, not determined.
[b] Significantly different from controls ($P < 0.05$).

Table 2 *8-OHdGua levels in kidney DNA of male Syrian hamsters treated with a single i.p. injection/day of various estrogens for 3 days (100 μg/animal/day)*

Values are expressed as means ± SD (N = 9).

Estrogen	8-OHdGua/10^5 dGua	P
Control	3.6 ± 1.2	
E_2	4.1 ± 1.1	0.22
2-OHE_2	3.8 ± 1.6	0.39
4-OHE_2	4.0 ± 0.9	0.22

Discussion

Single injections of large doses of E_2 or 4-OHE_2 into hamsters increase concentrations of 8-OHdGua in both liver and kidney DNA. In contrast, chronic estrogen administration by implant raises 8-OHdGua levels only in kidney, but not in liver. These data are consistent with previous results obtained with the synthetic estrogen DES, which induced 8-hydroxylation of DNA either in liver or in kidney when hamsters were treated with either injections of large doses or DES implants for 15 days, respectively (9). In that previous study, renal 8-OHdGua levels were not affected in hamsters treated with implants of the steroid hormone E_2 for 8 or 15 days or 3 or 5 months. However, treatment of hamsters with implants for 3 days raised 8-OHdGua concentrations in kidney by 50%, as shown in this study.

The increase in renal 8-OHdGua concentrations after 3 days of chronic exposure of hamsters to E_2 has confirmed the expected correlation between renal lipid peroxidation in response to E_2 implants and hydroxy radical damage to DNA. This correlation exists because organic hydroperoxides are necessary cofactors for the cytochrome P450 1A1-mediated oxidation of catecholestrogens to quinones, the oxidative step of the redox cycle, which is the source of free radicals (18). After 6 days or more of exposure to E_2, renal 8-OHdGua concentrations may have returned to near control values, because renal lipid peroxide concentrations have fallen off their peak concentration[4] and because the supply of catecholestrogen substrates may no longer be sufficient for maximal redox cycling. The renal activity of estradiol 2-hydroxylase remains near control levels only within 2–3 days of E_2 treatment and then decreases to approach 25% of control levels after 5–7 days of E_2 treatment (19). Thus, catechol substrates for redox cycling and free radical generation may be available in optimal quantity only during the initial days of estrogen exposure. In contrast, hepatic estradiol 2- and 4-hydroxylase activity is much higher than that in kidney (19). However, hepatic cytochrome P450 1A1 activity, necessary for the oxidative step of the redox cycle (18), does not exceed that in kidney (18). Therefore, hepatic formation of free radicals may be comparatively low and their concentrations in liver may be kept at minimal levels by detoxifying enzymes during chronic administration of low amounts of E_2 to hamsters. Only injections of very large doses of E_2 may significantly elevate hepatic 8-OHdGua levels as shown in this study. These data demonstrate that kidneys are particularly vulnerable to free radical damage either induced by redox cycling of catecholestrogen substrates as outlined in this study or induced by toxic chemicals as demonstrated by others (20).

Our data are consistent with a postulated mechanism of estrogen-induced carcinogenesis by conversion of estrogens to catechol metabolites, which may generate free radicals by metabolic redox cycling (3, 4). This postulated mechanism is further supported by observed DNA single strand breaks in kidney but not in liver of hamsters chronically treated with either E_2 or 4-OHE_2 (21) and by increases in lipid hydroperoxide (8, 19) and in lipid-hydroperoxide-induced DNA adducts in kidneys of estrogen-treated hamsters compared to controls.[4]

The increases in renal DNA single strand breaks or 8-OHdGua levels induced by estrogens are significant yet moderate when compared to DNA damage by other common carcinogens (20). In this context it is important to realize that our studies with estrogen-induced DNA alterations were carried out using kidney homogenate. It is possible that severalfold increases in 8-OHdGua levels are induced by estrogen but may occur only in the cell type, in which kidney tumors arise. This possibility will have to be examined in future studies.

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