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Characterization of Drug Metabolism Enzymes in Estrogen-induced Kidney Tumors in Male Syrian Hamsters[1]

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ABSTRACT

In an attempt to characterize metabolism enzymes of the estrogen-induced kidney tumor in male Syrian hamsters, the activities of enzymes involved in drug and glutathione metabolism were determined in tumor tissue. Kidney tumors were induced in male Syrian hamsters by treatment with estradiol for 8 months. Cytochrome P-450 and cytochrome b_5 concentrations in tumors were below detectable levels. However, when cytochrome P-450-mediated oxidation was analyzed by product formation assays, the oxidation of *E*-diethylstilbestrol to diethylstilbestrol-4′,4″-quinone by tumor microsomes was 10–20% of the rate found in control microsomes. In kidney tissue surrounding estrogen-induced tumors, cytochrome P-450 and b_5 contents were 50–60% less than those in untreated kidney. Activities of reducing enzymes of drug metabolism (cytochrome P-450, cytochrome b_5 and NADH:cytochrome *c* reductases), glutathione metabolism enzymes (glutathione peroxidase, glutathione transferase, glutathione reductase, and γ-glutamyl transpeptidase), and free radical scavenging enzymes (superoxide dismutase, catalase, and quinone reductase) in tumor were significantly lower than in untreated kidney tissue. The activities of these enzymes in renal tumor surrounding tissue were between those observed in tumor and control kidney. Glucose-6-phosphate dehydrogenase activity was increased by 50% in surrounding tissue and 430% in tumor compared to values in untreated controls. The decreased enzyme activity levels in hormone-exposed tissue surrounding tumors likely represented an adaptation of this tissue to the neoplastic environment induced by chronic estrogen treatment.

INTRODUCTION

In male Syrian hamsters, chronic administration of estrogens for 6–8 months induces kidney tumors (1). These tumors, like any other renal tumor, are recognized by morphological, biochemical, and physiological changes (2–4) but are not well characterized biochemically. In previous investigations by Llombart-Bosch and Peydro (4, 5) and a more recent histochemical analysis by Hacker *et al.* (6), a number of tumor cell markers were profiled. Activities of glucose-6-phosphate dehydrogenase, adenylate cyclase, and alkaline phosphatase activities were elevated, while those of γ-glutamyl transpeptidase, succinate dehydrogenase, and glucose-6-phosphatase were decreased in estrogen-induced tumor compared to renal tissue. A comprehensive analysis of drug-metabolizing and -detoxifying enzymes has yet to be done in this renal tumor model. Such studies have now been carried out to reveal the response of this neoplasm to cytotoxic agents. Furthermore, mechanistic aspects of carcinogenesis may be recognized by analyses of enzyme activities, since reactive metabolites generated during cytochrome P-450-mediated redox cycling of estrogens have been postulated (7) to be involved in tumorigenesis.

In this report, the activities of some phase I and phase II (*e.g.*, γ-glutamyl transpeptidase, glutathione transferase, and quinone reductase) drug-metabolizing enzymes and free radical-scavenging enzymes are examined in tumor and surrounding renal tissue to elucidate the patterns of metabolism enzyme activities in this neoplasm. In addition, the drug-metabolizing potential of tumor and kidney microsomal enzymes was measured by examining the propensity for oxidation and reduction of stilbene estrogens.

MATERIALS AND METHODS

Materials. 17β-Estradiol, E-DES,[3] cholesterol, 1-chloro-2,4-dinitrobenzene, 1,2-epoxy-3-(*p*-nitrophenoxy)propane, 3,4-dichloronitrobenzene, reduced and oxidized glutathione, glutathione reductase type III from Bakers' yeast, hydrogen peroxide, cumene hydroperoxide, $NADP^+$, NADPH, glucose 6-phosphate, γ-glutamyl-*p*-nitroanilide, xanthine, xanthine oxidase from buttermilk, superoxide dismutase from bovine liver, and ferricytochrome *c* from horse heart were purchased from Sigma Chemical Co., St. Louis, MO. DES Q and *Z,Z*-dienestrol were synthesized as described previously (8). All solvents and common chemicals used were either analytical grade or of highest grade available.

Kidney Tumor Induction. Kidney tumors were induced in male Syrian hamsters (purchased from Harlan/Sprague-Dawley, Houston, TX, at 8 weeks of age) by s.c. implantation of 1 estradiol pellet (25-mg implant containing 10% cholesterol) every 3 months as described previously (9, 10). After 8 months of chronic estradiol treatment, the hamsters were decapitated. Tumors 3 mm in diameter or larger were separated from surrounding kidney tissue. Kidneys of untreated age-matched hamsters served as controls. Tissues were homogenized in 0.25 M sucrose containing 10 mM EDTA. Microsomes, cytosol, and mitochondria were prepared by differential centrifugation as described previously (11).

Enzyme Activity Measurements. Cytochrome P-450 and cytochrome b_5 were measured in microsomes by the method of Omura and Sato (12). NADPH:cytochrome *c* (cytochrome P-450) reductase was assayed by the method of Dignam and Strobel (13), NADH:cytochrome *c* and cytochrome b_5 reductase were assayed by the method of Sottocasa *et al.* (14). Quinone reductase (DT-diaphorase) was assayed according to the method of Ernster (15) as modified by Benson *et al.* (16). Catalase activity was determined by the method of Claiborne (17), and superoxide dismutase activity was determined by the method of Beyer and Fridovich (18). Glutathione peroxidase was determined according to the procedure of Mohandas *et al.* (19), glutathione reductase by the method of Carlberg and Mannervik (20) as modified by Mohandas *et al.* (19), glutathione transferase by the procedure of Habig *et al.* (21), γ-glutamyl transpeptidase by the method of Tate and Meister (22), and glucose-6-phosphate dehydrogenase by the assay of Baquer and McLean (23). Additional experimental detail for the enzyme assays has been described by Roy and Liehr.[4]

DES Q Formation. Oxidation mixtures consisted of 1 mg/ml microsomal protein, 0.01 M phosphate buffer (pH 7.4), 100 μM E-DES (dissolved in ethanol), and 1.5 mM cumene hydroperoxide. Incubations were carried out at room temperature for 10 min. DES Q formed was analyzed by HPLC using a Waters 5-μm normal phase μBondapak CN column and a tetrahydrofuran:hexane linear gradient from 30 to 50% tetrahydrofuran, 30 min, 1.0 ml/min, with a UV detector setting at 312 nm.[5] The instrumentation consisted of Waters Models 510 and 501 solvent delivery systems, an automated gradient controller, and a Model 490 multiwavelength detector. Data were analyzed by a Waters Model 740 Data Module.

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[3] The abbreviations used are: E-DES, *E*-diethylstilbestrol or *trans*-diethylstilbestrol; Z-DES, *Z*-diethylstilbestrol or *cis*-diethylstilbestrol; DES Q, diethylstilbestrol-4′,4″-quinone; HPLC, high performance liquid chromatography.

[4] D. Roy and J. G. Liehr, submitted for publication.

[5] Conditions for the analysis of DES Q by HPLC have been provided by Dr. Paul Ruehle, Chemsyn Science Laboratories, Lenexa, KS.

Redox Cycling Assay. The analysis of total stilbenes following microsomal oxidations/reductions was carried out by HPLC as described previously (7). Reduction reactions of DES Q were carried out with 1 mg of microsomal protein and 0.01 M phosphate buffer, pH 7.4, in a final volume of 1 ml at room temperature for 10 min.

RESULTS

Activities of Drug-metabolizing Enzymes. The contents of cytochrome P-450 and cytochrome b_5 in primary tumor and kidney tissues were measured by the method of Omura and Sato (12) (Table 1). The tumor did not contain any levels of cytochromes P-450 or b_5 detectable by this assay. In the surrounding tissue, the levels of these enzymes were less than one-half of concentrations observed in age-matched untreated hamster kidneys. A decrease in various microsomal P-450 forms in male hamster kidneys in response to chronic (3 months) estrogen treatment was observed previously (24) and supports the decrease in total P-450 content obtained after 8 months of treatment (Table 1). The activities of microsomal oxidation enzymes were further analyzed by cytochrome P-450-mediated oxidation of E-DES to DES Q (7). In incubations with kidney microsomes from untreated hamsters, E-DES was oxidized to DES Q [45.0 ± 6.5 (SD) nmol product formed/mg protein/10 min]. This rate of DES Q formation was lowered to 8.1 ± 0.6 ($P < 0.005$) with surrounding tissue and 3.9 ± 0.01 ($P < 0.001$) with tumor microsomes, respectively ($N = 3$–4 reactions).

Statistically insignificant decreases in activities of cytochrome P-450 reductase, NADH:cytochrome *c* reductase, and cytochrome b_5 reductase were observed in surrounding tissue when compared to age-matched untreated control kidney (Table 1). Activities of cytochrome P-450 reductase in chronically (3 months) estrogen-treated and control hamster kidney had been reported previously (24) and are in agreement with those presented here. In primary tumor tissue, activities of cytochrome P-450 reductase were reduced by approximately one third, of NADH:cytochrome *c* and cytochrome b_5 reductases by approximately two-thirds from levels observed in untreated control tissue. These measurements demonstrate that the capability to metabolize drugs in estradiol-induced tumor tissue was markedly lowered when compared to surrounding tissue or untreated kidney.

The microsomal oxidation and reduction potential was analyzed by a redox cycling assay developed previously (7). When all stilbenes extracted were analyzed by HPLC after DES Q was allowed to rearrange to *Z,Z*-dienestrol, the product distribution (Table 2) was comparable to DES Q formation reported above. The decreased oxidation of E-DES to DES Q and, subsequent to rearrangement, to *Z,Z*-dienestrol by tumor microsomes compared to kidney microsomes confirmed the measurements of cytochrome P-450 content reported in Table 1. The NADPH-dependent reductions of DES Q to Z-DES (Table 2) confirmed the lower activity of cytochrome P-450 reductase in tumor compared to kidney (Table 1). Unreacted DES Q substrate rearranged to *Z,Z*-dienestrol prior to analysis. Tumor microsomes formed only 28% Z-DES compared to 40% Z-DES by control kidney microsomes.

Activities of Enzymes Detoxifying Free Radicals. Since redox cycling of estrogens (7) and free radical formation from redox cycling *in vitro* (11) had been demonstrated previously, activities of enzymes controlling free radical formation were examined in tumor and control tissues. Activities of catalase, superoxide dismutase, and quinone reductase were highest in untreated hamster kidney tissue (Table 3). Activities of the latter enzyme in estrogen-treated (4 months) hamster kidneys and untreated control kidneys had been reported previously (11) and are in close agreement with measurements obtained after 8 months of exposure to hormone. In tumor tissue the corresponding activities of all three enzymes assayed were decreased to approximately one-third to one-fourth of levels observed in untreated kidney. In tissue surrounding tumors, enzyme activity levels were between those found in untreated kidney and tumors (Table 3).

Activities of Glutathione Metabolism Enzymes. Glutathione metabolism enzymes were assayed because of their involvement in the detoxification of reactive drug intermediates and peroxides. In the hamster tissues examined in this study, the highest levels of glutathione peroxidase I and II activities were found in untreated kidney (Table 3). In tumor, these activities were decreased by approximately 90%; in tissue surrounding tumor, they were decreased by 25 and 45%, respectively. Comparable relationships between the three tissues examined were found for glutathione reductase, glutathione transferase, and mitochondrial or microsomal γ-glutamyl transpeptidase. Activities of these enzymes in tumor tissue ranged from 45% (mitochondrial) to 15% (microsomal γ-glutamyl transpeptidase) of levels observed in untreated kidney. Significant but less marked decreases in activities were measured in surrounding tissue.

The enzyme supplying the necessary cofactor for glutathione metabolism, glucose-6-phosphate dehydrogenase, was also assayed. This enzyme activity was increased by 50% in surrounding tissue and 430% in tumor *versus* untreated hamster kidney.

In summary, glucose-6-phosphate dehydrogenase was the only enzyme for which activity was found to be increased in tumor or surrounding tissue compared to control kidney. All other enzymes measured, enzymes of drug metabolism, glutathione metabolism, and free radical detoxification were decreased in activity in kidney tissue surrounding tumors and decreased even further in estrogen-induced renal tumors.

Table 1 *Cytochrome P-450 and b_5 contents and activities of reductase enzymes of drug metabolism in estradiol-induced hamster kidney tumor*

Primary hamster kidney tumors were induced by estradiol treatment for 8 months. Microsomes from tumor, surrounding kidney tissue, and untreated age-matched controls were prepared, and microsomal reductases were measured. Microsomal cytochrome P-450 and b_5 contents were determined by the method of Omura and Sato (12). Values represent averages and standard deviation ($N = 6$).

Enzyme activities	Untreated kidney	Surrounding tissue	Tumor
Cytochrome P-450[a]	0.17 ± 0.02[b]	0.08 ± 0.01[c]	<0.02
Cytochrome b_5[a]	0.22 ± 0.05	0.09 ± 0.01[c]	<0.02
Cytochrome P-450 reductase[d]	32 ± 4	28 ± 5	22 ± 1[e]
NADH:cytochrome *c* reductase[d]	300 ± 19	238 ± 11[e]	81 ± 7[f]
Cytochrome b_5 reductase[d]	3590 ± 125	3210 ± 149	1205 ± 85[f]

[a] nmol/mg protein.
[b] Mean ± SD.
[c] $P < 0.005$.
[d] nmol/mg protein/min.
[e] $P < 0.05$.
[f] $P < 0.001$.

DISCUSSION

Enzyme activities in untreated hamster kidneys reported in Tables 1–3 are in agreement with values reported previously (6, 7, 11, 24–26). With the exception of glucose-6-phosphate dehydrogenase, activities of the xenobiotic and glutathione metabolism enzymes examined here were lower in tissue surrounding tumor than those in untreated kidney. It is possible that surrounding tissue may have contained various amounts of microscopic tumors. In that case, enzyme activities would be expected to be between those in normal and neoplastic cells. A

Table 2 *Product analysis of incubation of stilbene estrogens and microsomal enzymes*

The incubations were carried out as described previously (7). Amounts of products are expressed as percentage of total stilbenes extracted (N = 3–4). DES Q spontaneously rearranged to *Z,Z*-dienestrol prior to analysis.

Source of microsomes	Substrate	Cofactor	E-DES	Z-DES	*Z,Z*-Dienestrol
Untreated	E-DES	Cumene hydroperoxide	49.5	28.0	22.5
Surrounding tissue	E-DES	Cumene hydroperoxide	56.3	31.2	12.5
Tumor	E-DES	Cumene hydroperoxide	64.9	30.5	4.6
—[a]	DES Q	NADPH	0.6	0.8	98.6
Untreated	DES Q	NADPH	8.5	40.0	51.5
Surrounding tissue	DES Q	NADPH	8.8	34.9	56.3
Tumor	DES Q	NADPH	10.0	28.0	62.0

[a] This control reaction was carried out without microsomal protein.

Table 3 *Activities of enzymes regulating glutathione metabolism and of enzymes detoxifying free radicals in estradiol-induced hamster kidney tumor*

Primary hamster kidney tumors were induced by estradiol treatment for 8 months. Mitochondria, microsomes, and cytosol from tumors, surrounding tissue, and untreated age-matched controls were prepared and enzyme activities were assayed as described previously (15–23). Values represent averages and standard deviation (N = 6).

Enzyme activities (nmol/mg protein/min)	Untreated kidney	Surrounding tissue	Tumor
Catalase	257 ± 29[a]	145 ± 16[b]	65 ± 9[c]
Superoxide dismutase[d]	22 ± 1	13 ± 1[b]	6 ± 1[c]
Quinone reductase	148 ± 12	115 ± 8[e]	50 ± 7[c]
Glutathione peroxidase I	141 ± 9	107 ± 11[e]	13 ± 1[f]
Glutathione peroxidase II	69 ± 8	30 ± 5[c]	8 ± 1[c]
Glutathione reductase	40 ± 5	19 ± 2[b]	10 ± 2[c]
Glucose-6-phosphate dehydrogenase	12 ± 2	18 ± 2[e]	64 ± 8[c]
Glutathione transferase	205 ± 17	141 ± 10[b]	60 ± 5[f]
γ-Glutamyl transpeptidase			
Mitochondrial	836 ± 85	965 ± 105	380 ± 21[f]
Microsomal	2415 ± 225	1770 ± 87[b]	362 ± 55[f]

[a] Mean ± SD.
[b] $P < 0.03$.
[c] $P < 0.005$.
[d] Units/mg protein.
[e] $P < 0.05$.
[f] $P < 0.001$.

more likely explanation for the lowered enzyme activities in surrounding tissue is the acquisition of an altered enzyme pattern in adaptation to the neoplastic environment induced by chronic exposure to estrogen. A histochemical analysis carried out previously (6) also indicated such an adaptation of enzyme patterns in surrounding tissue to those prevalent in tumor. In hepatic cells in the vicinity of ciprofibrate-induced hepatocellular carcinoma, decreases in activities of phase I and phase II metabolism enzymes were also observed (27). An adaptation of metabolic patterns (28) rather than a summation of enzyme activities of normal and tumor cell mixtures is therefore the more likely explanation of enzyme activity profiles in hormone-exposed kidney.

Activities of enzymes regulating xenobiotic and glutathione metabolism in tumors were very low except for that of glucose-6-phosphate dehydrogenase (Tables 1–3). The high activity of the latter enzyme and also of enzymes involved in glycolysis had been demonstrated previously by histochemical analysis (4, 6). This enzyme pattern of estrogen-induced renal tumor cells may be the result of reprogramming (28) of the metabolism to support almost exclusively proliferation of this rapidly growing estrogen-dependent neoplasm. The estrogen receptor content of the hamster kidney tumor is known (29) to be elevated approximately 4-fold in tumor *versus* control kidney. This increase in receptors in combination with decreased metabolic conversion of estrogen by tumor (Table 1) may result in increased growth stimulus of this estrogen-dependent tumor and thus supports the above mentioned concept of reprogramming (28).

The enzyme profile of estrogen-induced hamster kidney tumors described above and in previous work (4, 6) bears similarities to that of hepatic carcinoma induced by peroxisome proliferators, especially with respect to decreased activities of phase I and phase II enzymes (27, 30). Increased drug metabolism enzymes may be found in some tumors induced by cytotoxic carcinogens (31), but not in those induced by peroxisome proliferators (27, 30) or estrogenic hormones. The enzyme patterns described for the latter two tumor types may also reflect a possible common mechanism of action. A prooxidant state produced by peroxisome proliferators has been postulated (30) to play a role in the initiation of hepatocarcinogenesis. Likewise, redox cycling of estrogens (7) and the formation of superoxide radicals, demonstrated *in vitro* (11), has also been postulated to be involved in hormonal carcinogenesis in the hamster kidney.

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