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14. ABSTRACT:
Treatment of Noble rats with testosterone plus estradiol (E2) induces prostate carcinomas. We think that estrogens initiate prostate cancer by reaction of catechol estrogen-3,4-quinone (CE-3,4-Q) metabolites with DNA. Formation of depurinating adducts by CE-3,4-Q, which generate apurinic sites in DNA, would be the critical event leading to mutations that initiate prostate cancer. After treatment of rats with CE or CE-3,4-Q, CE metabolites and CE-glutathione (GSH) conjugates were lower in regions where tumors develop and methoxyCE were higher in regions where tumors do not develop. To study the role of CE-Q in initiation of prostate cancer, we are (1) treating rats with E2 and/or testosterone and analyzing the CE metabolites, CE-GSH conjugates and depurinating CE-DNA adducts in the regions of the prostate by HPLC with electrochemical and mass spectrometric detection; (2) studying in the prostate conversion of testosterone into E2 and its metabolism; and (3) determining the expression at the mRNA level of four selected enzymes involved in estrogen activation and deactivation in the prostate of rats treated with E2 and/or testosterone. These studies will provide information critical to understanding the molecular etiology of prostate cancer, identify biomarkers for early detection of susceptibility and lead to development of strategies for prostate cancer prevention.

15. SUBJECT TERMS
estrogens as endogenous tumor initiators; initiation of prostate cancer in Noble rats by estrogens; analysis of estrogen metabolites, conjugates and DNA adducts; expression of estrogen activating and deactivating enzymes

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Introduction

The purpose of this research was to investigate the hypothesis that estradiol (E_2) initiates prostate carcinogenesis and testosterone promotes the process. This was explored in male Noble rats, which develop prostate tumors when treated with E_2 and testosterone [1]. We think that estrogens are involved in the initiation of prostate cancer by a mechanism that involves oxidation of endogenous 4-catechol estrogen (CE) metabolites to CE-3,4-quinones (CE-3,4-Q). Reaction of CE-3,4-Q with DNA results in tumor initiation as the first step in the events leading to prostate cancer. Formation of depurinating DNA adducts by CE-3,4-Q, which generate apurinic sites in DNA, would be the critical event leading to mutations that initiate the cancer [2]. To study the role of CE-Q in the initiation of prostate cancer, we have (1) treated male Noble rats with E_2 by i.p. injection at various doses and for various times, analyzing the estrogen metabolites, estrogen conjugates and depurinating estrogen-DNA adducts and comparing their levels in the various regions of the prostate [3]; (2) treated male Noble rats with 4-hydroxyE_2 (4-OHE_2 and analyzed the estrogen metabolites, estrogen conjugates and depurinating estrogen-DNA adducts in serum and various regions of the prostate; (3) investigated the conversion of testosterone into E_2 in the prostate by analyzing the same compounds in prostate tissues from rats treated with testosterone or testosterone plus the aromatase inhibitor letrozole; and (4) determined the expression of four enzymes involved in the activation and deactivation of estrogens, cytochrome P450 (CYP) 19 (aromatase), CYP1B1, catechol-*O*-methyltransferase (COMT) and quinone oxidoreductase (QOR). The results of these studies provide information on the relationship between estrogen activation and deactivation in relation to tumor initiation in the prostate.

Body

We have followed the objectives of this grant and learned a great deal about estrogen and testosterone metabolism in the prostate. Although our objectives were not changed, we found that we had to modify some experimental parameters. The results we have obtained are reported below in relation to the three objectives of this grant.

<u>Objective #1: Determine the presence and amount of CE metabolites, CE-GSH conjugates and depurinating CE-DNA adducts by HPLC with electrochemical detection and compare their levels in the dorsolateral prostate, periurethral prostate, ventral prostate and anterior prostate of male NBL rats treated with E_2 by intraperitoneal (i.p.) injection at a range of relevant doses and time-points.</u>

Noble rats were treated at NYU with 0, 16, 32 or 48 mg/kg of E_2 by i.p. injection, and after 3 h the prostate tissues were collected and sent to UNMC for analysis. The HPLC analyses with electrochemical and mass spectrometric detection were conducted. Very few metabolites or conjugates were detected at very low levels. One possible explanation of these results is that the treatment with E_2 severely damaged the prostate, greatly inhibiting E_2 metabolism. In fact, implantation of Noble rats with E_2 produces prostate atrophy [1]. Therefore, we have treated rats with 4-OHE_2 instead.

We treated rats for three weeks with three different doses of the catechol estrogen 4-OHE_2 using the pellet method of Innovative Research, Inc. (Sarasota, FL), which guarantees a sustained controlled release. In Table 1 and Figures 1 and 2, the most important results of this experiment are summarized for the highest 4-OHE_2 dose which was not toxic or estrogenic (pituitary weights were unchanged). In serum (Figures 1 and 2), not only was there a substantive increase of 4-OHE_2, there were also increases in detoxified methoxy metabolites resulting from activity of catechol-*O*-methyltransferase (COMT), as well as estradiol-17β (E_2). Most significantly, there were detectable serum levels of the depurinating estrogen-DNA adducts 4-hydroxyestrone(4-OHE_1)[or 4-OHE_2)]-1-N7Guanine and 4-OHE_1(E_2)-1-N3Adenine formed by reaction of 4-OHE_1(E_2)-3,4-quinone [E_1(E_2)-3,4-Q] with DNA. In prostate tissue, similar patterns of occurrence of metabolites and estrogen-DNA adducts were found (Table 1). The 4-OHE_1(E_2)-1-N3Ade and 4-OHE_1(E_2)-1-N7Gua adducts were only found in prostate tissue of animals that had been

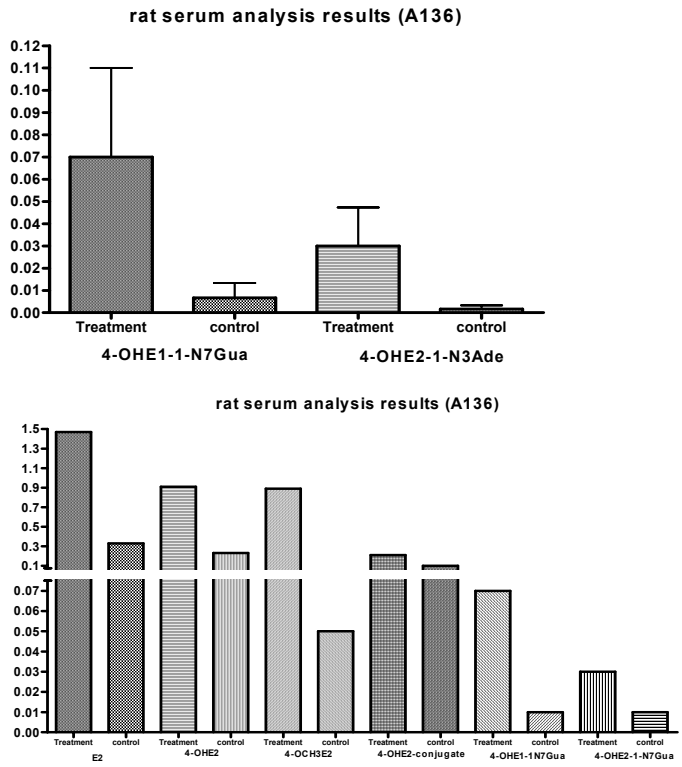

Fig. 1. Rats treated with 4-OHE_2

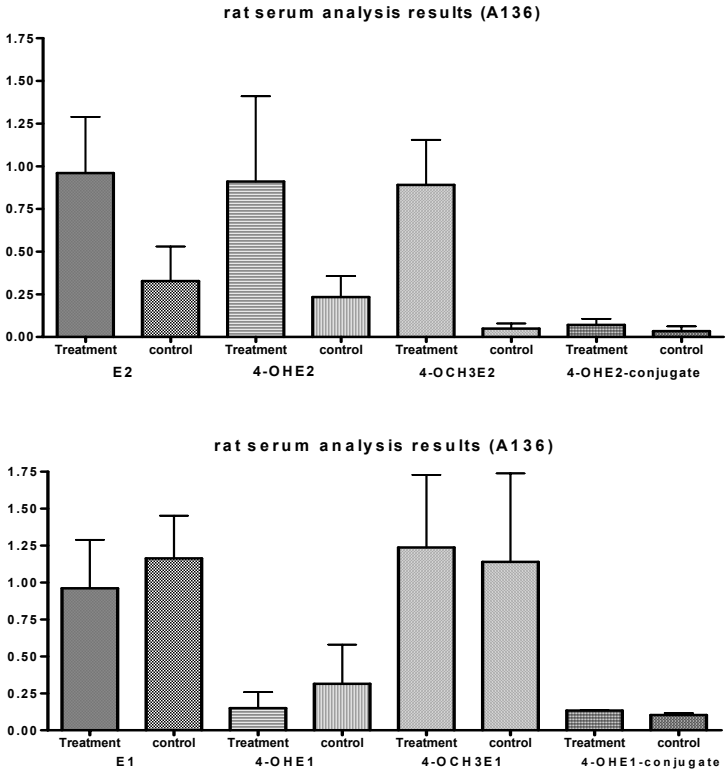

Fig. 2. Rats treated with 4-OHE_2.

treated with 4-OHE_2, with the exception of small amounts of 4-$OHE_1(E_2)$-1-N3Ade found in the anterior prostate (a.k.a. coagulating gland) of untreated control rats. Now that we have identified a non-toxic 4-OHE_2 dose that not only reaches the prostate but also causes DNA adduction in that tissue, we will begin a cancer induction study with groups of Noble rats given 4-OHE_2 (or 2-OHE_2) with and without additional testosterone, supported by other funds. The results will be compared with those from rats given E_2 with testosterone, which is expected to develop a 100% prostate cancer incidence.

Table 1. Estrogen metabolites, conjugates and depurinating DNA adducts in regions of the prostate of Noble rats treated with 4-OHE_2 or untreated.

6/11/2006		CG		DP		LP		CG-4OHE2		DP-4OHE2		LP-4OHE2	
N0.	Compound	CG pmole/gm mean, n=2	Standard Deviation ±	DP pmole/gm mean, n=2	Standard Deviation ±	LP pmole/gm mean, n=2	Standard Deviation ±	CG-4OHE2 pmole/gm mean, n=2	Standard Deviation ±	DP-4OHE2 pmole/gm mean, n=2	Standard Deviation ±	LP-4OHE2 pmole/gm mean, n=2	Standard Deviation ±
1	AD	0.17	0.06	0.09	0.03	0.05	0.00	0.13	0.00	0.57	0.00	0.19	0.06
2	Test	0.40	0.11	0.54	0.06	0.19	0.05	0.31	0.06	0.07	0.07	0.12	0.00
3	E2	1.91	0.09	1.36	0.22	12.03	0.07	3.58	1.59	10.05	6.27	4.42	0.33
4	E1	1.68	0.09	1.65	1.08	3.86	0.20	14.55	0.13	12.02	1.22	6.12	0.53
5	E1-Sulfate	0.33	0.00	0.10	0.02	0.12	0.06	0.72	0.32	0.23	0.14	0.20	0.12
6	2-OHE2	1.52	0.14	2.06	0.80	1.51	0.85	4.88	2.25	3.21	1.21	8.67	0.19
7	2-OHE1												
8	4-OHE2	2.64	1.55	0.75	0.03	1.93	1.22	2.51	0.75	4.21	0.07	3.43	0.31
9	4-OHE1			0.03	0.03					0.50	0.36		
10	16a-E2	2.50	1.29			0.00	4.84	6.70	2.99	9.35	2.33	4.74	0.12
11	16a-E1			16.80	0.54			0.00	0.00				
12	2-OCH3E2			1.53	0.57			4.54	1.31				
13	2-OCH3E1	1.96	1.02	2.75	0.97								
14	4-OCH3E2			3.49	2.36					4.90	1.91	1.19	0.24
15	4-OCH3E1	5.18	0.06	3.06	0.26	6.46	1.36	7.39	0.30	7.40	0.82	4.13	0.78
16	2-OH-OCH3E2	0.28	0.00	0.66	0.20	0.25	0.11	2.34	1.62	2.40	1.03	1.50	0.42
17	2-OH-OCH3E1	1.85	0.03										
18	2-OHE2-1-SG			0.04	0.01					0.42	0.14		
19	2-OHE2-4-SG			0.04	0.01					0.49	0.14		
20	2-OHE1-1-SG					0.11	0.02	0.86	0.24	1.25	0.84	0.18	0.00
21	2-OHE1-4-SG					0.10	0.01	0.79	0.24	1.11	0.77	0.18	0.00
22	2-OHE2-1+4-Cys			0.13	0.04								
23	2-OHE1-1-Cys	0.06	0.02	0.04	0.00	0.07	0.00	0.13	0.04			0.13	0.04
24	2-OHE1-4-Cys	0.06	0.02	0.04	0.00	0.07	0.00	0.13	0.04			0.13	0.04
25	2-OHE2-1-NAcCys												
26	2-OHE2-4-NAcCys												
27	2-OHE1-1-NAcCys												
28	2-OHE1-4-NAcCys												
29	4-OHE2-2-SG			0.04	0.01			0.30	0.12	0.17	0.10		
30	4-OHE1-2-SG					0.08	0.01	0.61	0.18	0.90	0.63	0.12	0.00
31	4-OHE2-2-Cys	0.08	0.04	0.63	0.00	0.35	0.02	2.26	0.58	1.06	0.05	0.88	0.09
32	4-OHE1-2-Cys	0.10	0.02	0.08	0.00	0.10	0.00	0.18	0.09			0.22	0.04
33	4-OHE2-2-NAcCys	0.17	0.09							0.09	0.09	0.08	0.08
34	4-OHE1-2-NAcCys					0.06	0.06						
35	4-OHE2-1-N7Gua							0.62	0.54	0.14	0.05	0.08	0.08
36	4-OHE1-1-N7Gua							0.12	0.12				
37	4-OHE2-1-N3Ade	0.22	0.10					0.30	0.21	0.15	0.05		
38	4-OHE1-1-N3Ade							0.30	0.04	0.05	0.05	0.13	0.04
39	2-OHE2-6-N3Ade	0.02	0.02										
40	2-OHE1-6-N3Ade											0.04	0.04

Objective #2: Determine how well the four regions of the prostate convert testosterone into E_2 and what the effect is of the E_2 formed on estrogen homeostasis in male NBL rats reated with (a) testosterone by i.p. injection of a range of relevant doses and time-points and (b) testosterone plus the aromatase (CYP19) inhibitor letrozole. To this end, the presence and amounts of endogenous estrogens, CE metabolites, CE-GSH conjugates and depurinating CE-DNA adducts will be analyzed by HPLC with electrochemical detection and their levels will be compared in the dorsolateral prostate, periurethral prostate, ventral prostate and anterior prostate.

Male Noble rats were treated with testosterone by implantation for 2 wk or by i.p. injection of 0 or 52 mg/kg for 6 h. The prostate tissues were collected and sent to UNMC for analysis. The HPLC analyses with electrochemical and mass spectrometric detection were conducted. The key result is that E_2 was detected in the prostate of rats injected with testosterone, but not in the untreated rats. This result indicated the presence of aromatase in the rat prostate. Additional experiments with testosterone were then conducted based on the results of this study.

To determine whether inhibition of the aromatase enzyme would eliminate detectable E_2 in the prostate, we embarked on a study with the aromatase inhibitor Letrozole administered by the pellet method of Innovative Research, Inc. (Sarasota, FL). We found a dose-related inhibition of the weight increase caused by testosterone of the dorsolateral prostate (DLP) (Table 2 and Figure 3), but no effects were found in the ventral or anterior prostate or seminal vesicles. We are currently in the process of testing the Letrozole dose required to inhibit the *in vivo* formation of E_2 from testosterone by measuring serum and tissue levels of E_2; it seems that the highest Letrozole dose used in this experiment which was non-toxic, may be an adequate dose. Once this dose has been firmly established, we will conduct, supported by other funds, a cancer induction study with groups of Noble rats given testosterone with and without addition of Letrozole.

Table 2. Mean relative weights of select tissues from male Noble rats treated with testosterone alone or testosterone + letrozole for three weeks

Treatment	Mean relative organ weights (mg/100 g body weight)						
	DLP	VP	SV (w/o fluid)	CG	Urethra	Pituitary	Liver (whole)
T + 0 µg L	[b]129.65 ± 29.140	[b]136.32 ± 13.449	[b]123.31 ± 21.312	[b]68.22 ± 4.458	[b]68.96 ± 11.780	3.42 ± 0.609	[b]4312.30 ± 153.652
T + 700 µg L	[b]125.24 ± 18.292	[b]138.61 ± 1.484	[b]122.37 ± 9.220	[a,b]55.54 ± 4.925	[b]63.77 ± 6.347	3.11 ± 0.553	[b]4243.89 ± 78.782
T + 1400 µg L	[b]113.87 ± 5.427	130.33 ± 27.567	117.84 ± 55.320	[b]55.32 ± 7.741	[b]63.11 ± 4.721	2.89 ± 0.251	4321.57 ± 249.117
T + 2800 µg L	[a,b]91.38 ± 12.844	[b]139.96 ± 7.441	[b]125.55 ± 14.460	[b]62.63 ± 8.486	[b]68.90 ± 10.204	3.03 ± 0.718	[a]4733.14 ± 88.526
Sham-operated control	[a]64.92 ± 4.145	[a]70.31 ± 7.842	[a]76.25 ± 2.770	[a]34.00 ± 7.057	[a]43.88 ± 3.438	3.20 ± 0.603	[a]4688.68 ± 223.716

T: testosterone; L: letrozole. [a]Value is significantly different from T alone (T + 0 µg L) (T-test, $p<0.05$); [b]Value is significantly different from untreated (sham operated) control (T-test, $p<0.05$).

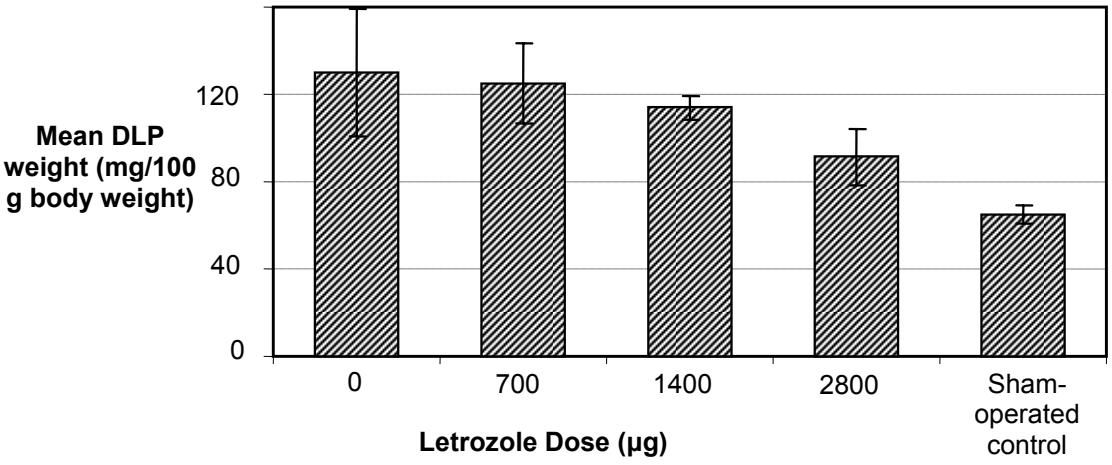

Fig. 3. Effect of letrozole on the dorsolateral prostate.

Objective #3: Determine the expression of several enzymes involved in estrogen activation and deactivation: CYP1A1, CYP1B1, aromatase (CYP19), COMT and QOR in the dorsolateral prostate, periurethral prostate, ventral prostate and anterior prostate of untreated rats, and determine the possible induction or inhibition of these enzymes in the four regions of the prostate by treatment of rats with E_2 or testosterone.

Analysis of the four enzymes, CYP19, CYP1B1, COMT and QOR, in control rats was conducted by using prostate tissues from the animals in the experiments described above, control rats injected with solvent or implanted with an empty implant. The normal levels of expression in the prostate of the four enzymes at the mRNA level are shown in Table 3.

Table 3. Expression of CYP19, CYP1B1, COMT and QOR at the mRNA level in regions of the prostate of control rats

	Enzyme, copies of mRNA/μg of total RNA			
Enzyme	CYP19	CYP1B1	COMT	QOR
Dorsolateral prostate	1.60×10^{6a}	0.71×10^{7a}	4.42×10^{11a}	$1.36\ 10^{7} \pm 0.85 \times 10^{7}$
Urethra	$4.31 \times 10^{6} \pm 2.28 \times 10^{6}$	2.00×10^{7a}	$1.74 \times 10^{11} \pm 1.24 \times 10^{11}$	$1.93 \times 10^{7} \pm 1.41 \times 10^{7}$
Ventral prostate	0.98×10^{6a}	2.50×10^{7a}	$0.54 \times 10^{11} \pm 0.46 \times 10^{11}$	$0.23 \times 10^{7} \pm 0.14 \times 10^{7}$

[a]Multiple determinations were made on only two different samples.

Each of the enzymes was expressed at the mRNA level at similar levels in the three areas of the prostate. It is noteworthy that COMT is expressed at much higher levels than the other three enzymes. Expression of the CYP19 and CYP1B1 proteins was determined by the western blot method. Both proteins were detected in the ventral prostate at about twice the levels found in the dorsolateral prostate and urethra. Analysis of the four enzymes, CYP19, CYP1B1, COMT and QOR, in rats treated with E_2 or testosterone was conducted. The levels of expression of the four enzymes at the mRNA level are shown in Tables 4 and 5.

Table 4. Expression of CYP19, CYP1B1, COMT and QOR at the mRNA level in regions of the prostate of rats treated with testosterone

	Enzyme, copies of mRNA/μg of total RNA[a]			
Enzyme	CYP19	CYP1B1	COMT	QOR
Implantation (2 wk)				
Dorsolateral prostate	2.66×10^{6}	4.22×10^{7}	2.64×10^{10}	6.57×10^{6}
Urethra	2.11×10^{6}	2.26×10^{7}	2.93×10^{10}	5.44×10^{6}
Ventral prostate	0.64×10^{6}	0.78×10^{7}	1.72×10^{10}	1.68×10^{6}
Injection (6 h)				
Dorsolateral prostate	1.14×10^{6}	1.37×10^{7}	2.40×10^{10}	5.66×10^{6}
Urethra	0.40×10^{6}	0.83×10^{7}	0.25×10^{10}	0.73×10^{6}
Ventral prostate	1.54×10^{6}	0.78×10^{7}	1.03×10^{10}	0.96×10^{6}

[a]Multiple determinations were made on two different samples.

Table 5. Expression of CYP19, CYP1B1, COMT and QOR at the mRNA level in regions of the prostate of rats injected with E_2

	Enzyme, copies of mRNA/μg total RNA[a]			
Enzyme	CYP19	CYP1B1	COMT	QOR
16 mg E_2/kg				
Dorsolateral prostate	0.80×10^8	4.12×10^8	4.96×10^{10}	6.46×10^7
Urethra	4.63×10^8	-[b]	10.6×10^{10}	12.9×10^7
Ventral prostate	1.96×10^8	2.39×10^8	2.36×10^{10}	2.46×10^7
32 mg E_2/kg				
Dorsolateral prostate	1.48×10^8	7.96×10^8	6.28×10^{10}	4.99×10^7
Urethra	1.55×10^8	6.22×10^8	1.33×10^{10}	1.67×10^7
Ventral prostate	1.11×10^8	2.28×10^8	2.63×10^{10}	1.76×10^7
48 mg E_2/kg				
Dorsolateral prostate	0.36×10^8	1.02×10^8	2.12×10^{10}	2.62×10^7
Urethra	5.79×10^8	3.66×10^8	11.6×10^{10}	20.8×10^7
Ventral prostate	2.69×10^8	6.04×10^8	1.16×10^{10}	1.40×10^7

[a]Multiple determinations from two different samples.
[b]Data were not obtained from this sample.

Implantation of testosterone for two weeks had minimal effects on the expression of the four enzymes at the mRNA level (Table 4), except that CYP19 and CYP1B1 appeared to be induced in the dorsolateral prostate and the level of COMT was consistently reduced. As could be anticipated, expression of the enzymes had changed little 6 h after injection of testosterone, except that the level of COMT was greatly reduced. Treatment with testosterone had no effect on the levels of the CYP19 and CYP1B1 proteins, except that both enzymes seemed to be increased in the ventral prostate two weeks after implantation. The short-term (3 h) effects of injection with E_2 on the expression of the enzymes (Table 5) were questionable. Once again, the levels of COMT were several orders of magnitude greater than that of the other three enzymes, but lower than in the control tissues (Table 3). The other three enzymes appeared to be increased, but this result would have to be repeated for validation.

Based on these results, we have begun a follow-up study to discover whether (1) the treatment with E_2 is destroying the prostate and (2) simultaneous treatment with testosterone can reverse this effect. In this study rats are being treated with E_2 alone for 3 or 6 h, implanted testosterone plus E_2 for 3 or 6 h, implanted testosterone or vehicle alone. The estrogen metabolites, estrogen conjugates or estrogen-DNA adducts will be analyzed. In addition, the expression of CYP19, CYP1B1, COMT and QOR will be determined in the vehicle and implanted testosterone groups. Following this experiment, the effects of testosterone plus the aromatase inhibitor letrozole will be compared to treatment with testosterone alone.

Following in part our success in this grant, we have analyzed urine samples from men with and without prostate cancer (primarily supported by our program project grant from the National Cancer Institute). We found that the depurinating DNA adduct 4-$OHE_1(E_2)$-1-N3Ade is present in the urine of men with prostate cancer or other urological conditions in statistically higher amounts than in urine from healthy control men (Fig. 4). These results suggest that the depurinating adducts could be biomarkers useful for early detection of prostate cancer.

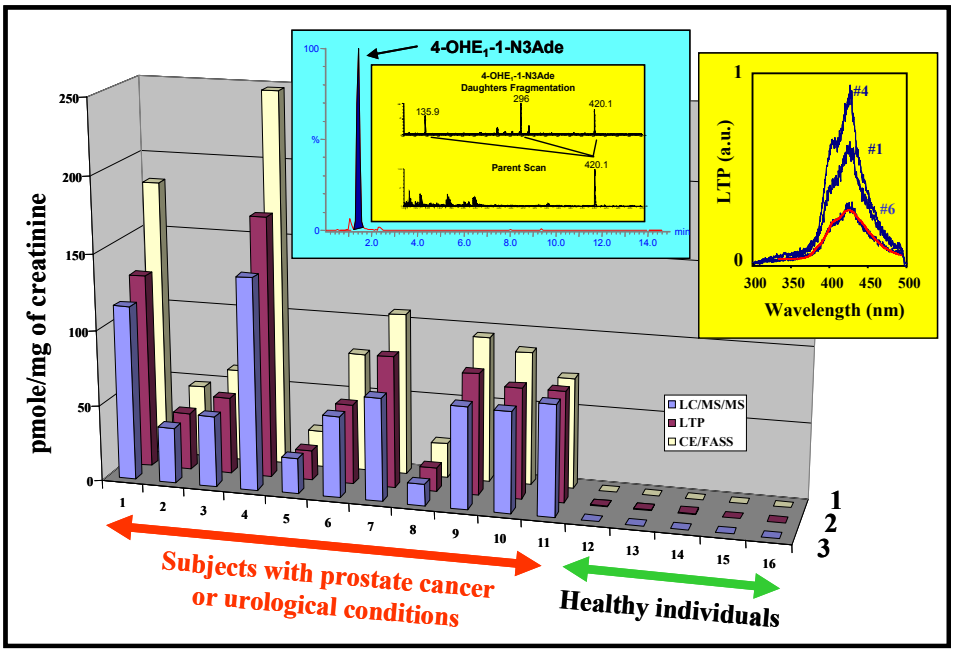

Fig. 4. Identification of the 4-OHE_1-1-N3Ade adduct in human urine samples from men with prostate cancer or urological conditions and healthy men as controls. Right inset: The spectra labeled 1, 4 and 6 refer to individual samples 1, 4 and 6, respectively; the red spectrum is that of the standard adduct. Left inset: Identification of 4-OHE_1-1-N3Ade by LC/MS/MS. The *m/z* 420.1 corresponds to the molecular weight of the parent compound and *m/z* 135.9 and 296 are the fragmentation daughters selected for the unequivocal identification of the adduct.

Key Research Accomplishments

1. Treatment with E_2 at sufficiently high doses to allow analysis for estrogen metabolites, estrogen conjugates and estrogen-DNA adducts has not been found to be feasible thus far.

2. Tissues from the E_2 and testosterone experiments were analyzed for expression of the estrogen-metabolizing enzymes CYP19 (aromatase), and CYP1B1 at the protein level. These enzymes are present at both the mRNA and protein levels, consistent with previous findings that suggested activity of these in enzymes in the Noble rat prostate [3].

3. Treatment with 4-OHE_2 led to detectable estrogen metabolites, conjugates and depurinating DNA adducts in both the serum and regions of the prostate.

4. Treatment with testosterone led to detectable estrogen metabolites, conjugates and depurinating DNA adducts in regions of the prostate, but Letrozole prevented formation of estrogen.

5. Depurinating estrogen-DNA adducts were detected in the urine of men with prostate cancer or other urological conditions at significantly higher levels than in urine from healthy control men.

Reportable Research Accomplishments

Singh, S., Bosland, M.C., Cavalieri, E.L. and Rogan, E.L. Effect of treatment with estradiol or testosterone on the expression of CYP19, CYP1B1, COMT and NQO1 in the prostate of male Noble rats. *Proc. Amer. Assoc. Cancer Res*., #14, 2004.

Singh, S., Bosland, M.C., Cavalieri, E.L. and Rogan, E.L. Effect of treatment with estradiol or testosterone on the expression of CYP19, CYP1B1, COMT and NQO1 in the prostate of male Noble rats. Manuscript in preparation.

Markushin, Y., Gaikwad, N., Zhang, H., Rogan, E., Cavalieri, E., Trock, B., Pavlovich, C. and Jankowiak, R. Potential biomarker for early risk assessment of prostate cancer. *Prostate*, in press, 2006.

Conclusions

We previously analyzed CE metabolites and conjugates in different regions of the prostate of Noble (NBL) rats which were treated with the 4-OHE_2 or E_2-3,4-Q [3]; these regions of the prostate differ in susceptibility to carcinoma formation following treatment with testosterone and E_2 [1] . Following treatment of rats with 4-OHE_2, the non-susceptible ventral (VP) and anterior prostate (AP) had higher levels of 4-methoxyCE and glutathione (GSH) conjugates than the susceptible dorsolateral prostate (DLP) and periurethral prostate (PUP). After treatment with E_2-3,4-Q, the VP and AP contained more GSH conjugates, 4-CE and 4-methoxyCE than the susceptible DLP and PUP. These results suggest that prostate areas susceptible to carcinoma induction have less protection by COMT, GSH, and quinone reductase and/or cytochrome P450 reductase, favoring reaction of CE-3,4-Q with DNA, presumably to initiate cancer.

Furthermore, we have analyzed estrogen metabolites, estrogen conjugates and depurinating estrogen-DNA adducts in the regions of the NBL rat prostate after treatment with E_2 or testosterone. We showed that following treatment with testosterone, the prostate contains significant amounts of E_2, which is not present in the prostates of untreated rats. We have also determined the expression of four selected estrogen-metabolizing enzymes, CYP19, CYP1B1, COMT and QOR, in the regions of the prostate from control rats and rats treated with E_2 or testosterone, and we showed that all of these enzymes are, indeed, present in the rat prostate.

We have not been able to detect estrogen-DNA adducts in the prostate tissue from NBL rats treated with E_2, perhaps because of the limits of detection of the measurement techniques we used. However, using new methodologies, we have detected estrogen-DNA adducts in the prostate tissue and even in serum from rats treated with a non-toxic dose of 4-OHE_2. Armed with this information, we are embarking on a study (funded elsewhere) to determine whether treatment with 4-OHE_2, with or without additional testosterone, causes prostate cancer in NBL rats.

We have begun studies (funded elsewhere) to determine whether inhibition of E_2 formation from administered testosterone by an aromatase inhibitor can prevent the induction of prostate cancer in NBL rats given only testosterone. We showed in a pilot study that a non-toxic dose of the aromatase inhibitor Letrozole inhibits the weight increase caused by testosterone selectively in the dorsolateral prostate. Using this dose of Letrozole we are beginning a study to determine whether treatment with this aromatase inhibitor will inhibit the development of prostate tumors in rats treated with testosterone only.

The depurinating estrogen-DNA adducts may serve as biomarkers for early detection of prostate cancer in men.

References

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