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Metabolism of ^{3}H-Estradiol in Men with Prostate Cancer*

BARNETT ZUMOFF,†‡ S. Z. FREED,§ J. LEVIN,§ W. F. WHITMORE,|| L. HELLMAN,¶** J. FISHMAN††‡‡ and D. K. FUKUSHIMA††

†*Clinical Research Center*, §*Division of Urology of the Department of Surgery*, ¶*Department of Oncology and* ††*Institute for Steroid Research, Montefiore Hospital and Medical Center, Bronx, New York, U.S.A.*

Abstract—*The metabolism of an i.v. tracer of* ^{3}H-*estradiol was studied in* 5 *men with prostate cancer and* 5 *age-matched normal controls. The percentage of administered radioactivity recovered in urine* (3-day *collection*) *was markedly increased in the cancer patients* (72 *vs* 45%; $P=0.001$); *recovery was proportionately increased in both the glucuronide conjugate fraction* (54 vs 36%; $P<0.005$) *and the nonglucuronide conjugate fraction* (18 vs 10%; $P<0.025$). *Five individual metabolities were quantitated: recovery of radioactivity as estriol was greatly increased* (14 vs. 5.4%, $P=0.05$); *there were no significant differences from normal for estrone, estradiol,* 2-*hydroxyestrone or* 2-*methoxyestrone. The abnormality of estradiol metabolism in prostate cancer is not an effect of nonspecific illness, since it differs from the abnormalities previously observed in female breast cancer, cirrhosis, biliary obstruction, drug-induced cholestasis and dysthyroidism. It does, however, share with the abnormality in male breast cancer the feature of increased conversion of estradiol to estriol. It seems possible that the common clinical behaviour of these two hormone-dependent cancers, i.e., their therapeutic responsiveness to orchiectomy or estrogen administration, may be related to this common hormonal abnormality.*

INTRODUCTION

SOME years ago, we reported [1] that estradiol metabolism was abnormal in male breast cancer, a rare hormone-dependent cancer; the major finding was greatly increased conversion to estriol. This paper reports that estradiol metabolism§§ is also abnormal in prostate cancer, the commonest hormone-dependent cancer in men.

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‡To whom requests for reprints should be addressed.
**Present address: Division of Surgical Immunoendocrinology, Albert Einstein College of Medicine, Bronx, NY, U.S.A.
||Urology Service of the Department of Surgery, Memorial–Sloan–Kettering Medical Centre, New York, NY, U.S.A.
‡‡Present address: Rockefeller University, New York, N.Y., U.S.A.
§§The following abbreviations have been used for estradiol metabolites: E1 = estrone; E2 = estradiol; E3 = estriol; 2-OH-E1 = 2-hydroxyestrone; 2-MeO-E1 = 2-methyoxyestrone.

MATERIALS AND METHODS

Five patients with stage C or D prostate cancer, aged 61–73, and five healthy male volunteers, aged 59–76, gave informed consent for studies involving the administration of tracer amounts of ^{3}H-estradiol and collection of urine for 3 days. One of the five patients had had an orchiectomy, the others were untreated; all had normal liver and kidney function. The five control subjects had negative medical histories and were on no medications.

Each subject studied received approximately 5 μCi of estradiol-6,7-^{3}H by i.v. injection of a weighed amount of a solution of the steroid in freshly redistilled pyrogen-free propylene glycol. A weighed aliquot of propylene glycol solution from which the individual tracer doses were obtained was used for calculation of the administered dose of radioactivity. Complete urine collections as judged from the consistency of creatinine measurements were obtained for 3 days after each injection. The specimens were refrigerated immediately after voiding and at all times until the collection was complete. If not

processed immediately, and this was done in most instances, the entire sample was frozen and kept at −15°C until it was possible to separate the steroid metabolites.

The radioactive estradiol was more than 97% pure by isotope dilution analysis. The carrier steroids used were recrystallized to constant melting point before addition to the extract. Purity of carrier steroids was also checked by thin-layer chromatography and by infra-red spectrometry. All solvents employed were redistilled and of high quality.

In each study the 3-day urine collections were combined and divided into 10, 30 and 60% aliquots, of which the last was frozen and retained. Known amounts (approximately 30 mg) of carrier 2-hydroxyestrone and estradiol were added to the 10% aliquot; no carrier was added to the 30% aliquot. The two aliquots were separately incubated with 300 U of β-glucuronidase per ml of urine at pH 5 with acetate buffer at 38°C for 5 days and then extracted continuously with either for 48 hr. The ether extracts were washed with 9% sodium bicarbonate solution saturated with sodium chloride, with saturated sodium chloride solution and finally with a very small amount of water, and the solvent was removed. The residues were designated the glucuronide extracts.

One half of the glucuronide extract from the 30% aliquot was submitted to gradient-elution partition chromatography on an acid-washed Celite column, as described by Engel *et al.* [2] and used in this laboratory routinely [3]. This procedure yields well-defined, homogeneous and specific peaks for 2-methoxyestrone, estrone and estriol, and the values for these compounds were directly determined by combining the pertinent fractions and measuring their radioactivity. Estradiol and 2-hydroxyestrone are eluted together and therefore a separate method was used for their quantitation.

The dried glucuronide extract from the 10% aliquot was acetylated by pyridine and acetic anhydride at room temperature overnight. Excess reagent was removed under a nitrogen stream on a water bath, and the acetates were chromatographed on 8 g of acid-washed alumina. Elution with 3:2 (v/v) benzene–petroleum ether (boiling range 58–62°C) gave estradiol diacetate, which was recrystallized to constant specific activity from petroleum ether–acetone. Elution with 1:9 (v/v) ethyl ether–benzene gave 2-hydroxyestrone diacetate, which was recrystallized to constant specific activity from petroleum ether–acetone. The total radioactivity of each compound was then calculated from the specific activity and the weight of carrier added. The values were expressed as the free steroid (Table 1).

Radioactivity was measured in a Packard liquid-scintillation spectrometer, Model 3375, with appropriate corrections for quenching.

Table 1. ^{3}H-*Estradiol metabolites in the urine of men with prostate cancer and normal men*

	Percentage of injected radioactivity							
Subjects	Total urine (3 Days)	Glucuronides	Non-glucuronide conjugates	E1	E2	E3	2-OHE1	2MeO-E1
Normal								
1	42	34	8	8.8	1.4	5.1	7.1	1.6
2	41	34	7	7.8	1.9	2.5	7.8	1.8
3	48	36	12	8.6	2.3	10	2.7	0.6
4	45	36	9	11	4.3	4.3	9.0	1.3
5	51	38	13	9.7	2.4	5.3	9.1	1.4
(Mean)	(45)	(36)	(10)	(9.2)	(2.5)	(5.4)	(7.1)	(1.3)
Prostate Cancer								
1	63	46	17	5.6	1.7	13	7.2	1.8
2	88	71	17	8.4	4.0	31	3.0	0.9
3	76	52	24	17	5.9	9.8	7.7	3.3
4	72	50	22	9.0	3.4	10	9.3	4.2
5	61	51	10	5.9	2.1	7.3	9.6	3.1
(Mean)	(72)	(54)	(18)	(9.2)	(3.4)	(14)	(7.4)	(2.7)
t	5.01	4.14	3.04	0	1.07	*	0.13	2.14
P	0.001	<0.005	<0.025	NS	NS	0.05	NS	NS

*Because of the wide variations in both normals and cancer patients, the values for E3 were compared by means of the nonparametric Mann–Whitney U-test. This yielded $P=0.05$.

RESULTS

Total urinary recovery of radioactivity in the patients with prostate cancer was markedly increased over that of normal men (Table 1) and was comparable to that seen in normal women [3]. Both glucuronides and nonglucuronide conjugates were increased proportionately in the cancer patients, however, whereas the higher values in women than men are entirely accounted for by increased nonglucuronide conjugates, with glucuronides being the same as in men.

Two of the individual metabolites, estriol and 2-methoxyestrone, showed increased excretion in the cancer patients; the increase in estriol was statistically significant, but the increase in 2-methoxyestrone was not.

DISCUSSION

The pattern of abnormality of estradiol metabolism we have now observed in men with prostate cancer is different from that of patients with female breast cancer [4], cirrhosis of the liver [5], biliary obstruction [6], drug-induced cholestasis [7] and dysthyroidism [8]. It is, therefore, not a nonspecific illness effect. Although the abnormality of estradiol metabolism in male breast cancer [1] differs somewhat in its totality from that in prostate cancer, it is intriguing and thought-provoking that these two diseases share the common feature of increased conversion of estradiol to estriol. It is well known that both diseases are responsive to orchiectomy and/or estrogen therapy; the present findings suggest that this common clinical behavior may have a basis in a common hormonal abnormality.

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