Journal of Steroid Biochemistry, 1976. Vol. 7, pp. 605–609. Pergamon Press. Printed in Great Britain

PLASMA ESTROGENS AND ANDROGENS IN MALE BREAST CANCER

E. CALABRESI, G. DE GIULI, A. BECCIOLINI, P. GIANNOTTI, G. LOMBARDI and M. SERIO
Endocrinology Unit and Department of Radiotherapy, University of Florence, Italy

(*Received* 30 *October* 1975)

SUMMARY

Androstenedione, estrone, estradiol-17β, estriol and testosterone concentrations have been measured by radioimmunoassay in plasma of 17 human males affected by breast cancer. The mean values of estrone, estradiol-17β, and estriol in male breast cancer were significantly higher than in normal controls of comparable age. Androstendione and testosterone were in normal range.

Orchidectomy, performed in 3 subjects, decreased the levels of estradiol-17β, but to a lesser extent those of estrone and estriol.

INTRODUCTION

The relationship between estrogens and breast cancer in human males has been discussed for many years. The pathological and clinical course of breast cancer in the male approximates that in the female, but the incidence comprises about 1% of all mammary cancer.

In Egypt[1] and in certain tropical regions[2, 3] the male–female ratio of breast cancer is quite high: ranging from 3 to 10%. This high incidence of male breast cancer has been attributed by several authors [1–3] to an impairment of hepatic metabolism of estrogens due to the fact that liver damage occurs frequently in these countries.

In 1968 Symmers[4] made a remarkable report on the development of breast cancer in two transvestite men who underwent orchidectomy and subsequent treatment with estrogens given both orally and by implantation. This paper suggests some causal relationships between estrogens and breast cancer. The results on urinary estrogen excretion in male breast cancer are rather contradictory.

De Giuli and De Giuli[5] reported that in 29 cases of male breast cancer they found 24 patients with total urinary estrogens higher than in normal controls.

Dao *et al.*[6] found that the values of urinary estrone, estriol and estradiol-17β in seven males affected by breast cancer, were significantly higher than in normal controls. The estrogens decreased markedly after orchiedectomy which was performed on two patients. On the other hand, Scheike *et al.*[7] did not find any increase of urinary estrogens in male breast cancer.

The availability of very sensitive and specific radioimmunoassays for measurements of estrogens in human male plasma allowed the study of plasma levels of estrone, estradiol-17β, estriol and their androgen precursors (androstenedione and testosterone) in male breast cancer. The results are reported in this paper.

EXPERIMENTAL

Clinical material. The investigations were performed on 17 men, aged between 31 and 75 years with histologically proved mammary carcinoma, being treated in the department of Radiotheraphy at the University of Florence (Table 1). The primary treatment of these patients was radical mastectomy with postoperative radiotherapy. Two patients were treated again for local recurrences, five patients had metastases and three of these had bilateral orchidectomy. The remaining patients were free of metastases at the time of this report.

Men of comparable age, all healthy and free of any evidence of disease or history of chronic disease, were studied as controls. Samples of peripheral blood from the cubital vein were collected into heparinized syringes by needle aspiration (8–10 a.m.). Blood plasma was obtained by centrifugation and stored at −20°C until analyzed for androstenedione, estrone, estradiol-17β, estriol and testosterone.

METHODS

Plasma estrone, estradiol-17β, estriol, androstenedione and testosterone were measured by radioimmunoassay. The details of the androstenedione and testosterone methods and their reliability criteria have been previously described in precedent publications[8, 9]. Studies on their accuracy performed by recovery experiments and by studying the effects of plasma dilutions showed absence of systematic errors.

The within assay coefficients of variation were 8.9% for testosterone and 5.6% for androstenedione. The between assay coefficients of variation were 9.6% for testosterone and 7.7% for androstenedione.

Table 1. Clinical information on the male breast cancer patients studied

Subject	Age	Follow-up
A.E.	31	Radical mastectomy; no metastasis
M.B.	36	Radical mastectomy; local recurrence; metastasis; bilateral orchidectomy
F.O.	42	Radical mastectomy; no metastasis
B.U.	48	Radical mastectomy; metastasis
A.M.	51	Radical mastectomy; local recurrence
G.S.	52	Radical mastectomy; metastasis
C.F.	53	Radical mastectomy; bilateral orchidectomy for diffuse metastasis
B.G.	56	Radical mastectomy; no metastasis
B.O.	58	Radical mastectomy; no metastasis
M.P.	60	Metastasis
M.A.	60	Radical mastectomy; no metastasis
S.A.	60	Radical mastectomy; no metastasis
V.D.	62	Radical mastectomy; no metastasis
M.V.	63	
A.G.	68	Radical mastectomy; bilateral orchidectomy for diffuse metastasis
S.R.	74	Radical mastectomy; no metastasis
B.L.	75	No metastasis

Estriol was measured by radioimmunoassay after extraction (three times) of 2 ml of human male plasma with 8 ml of diethyl ether. Diethyl ether and ethanol (Merck) were purified as previously described[10]. The extracts were dried and redissolved in 1 ml of ethanol; 0.2 ml (= 0.4 ml of male plasma) in duplicate were used for radioimmunoassay and 0.2 ml were used for calculation of recovery.

2,4,6,7-^{3}H Estriol-(NEN GmbH), S.A. 110 Ci/mmol purified by paper chromatography in benzene: hexane:methanol:water (3:1:2:1 by vol.) was used as a radioactive steriod. Chemically pure estriol was used as supplied by Vister. The antiserum used was obtained by injecting a mixture of estriol-2CMO-BSA and estriol-4CMO-BSA conjugate into rabbits. This antiserum was a kind gift from Dr. Kunzig and its characteristics have been previously reported [11] (see also Table 2).

A mixture of charcoal-Dextran 10:1 v/v (0.5 mg per tube) was used to separate the bound from the free fraction. The recovery of radioactive estriol (N = 60) was 80.0% ± 4.8 (mean ± S.D.) The blank values (N = 10) were 0.5 ± 1.1 pg per tube.

However, estriol was not measured in amounts less than 5 pg. The within assay coefficient of variation (N = 8) was 5.2% and the between assay coefficient of variation (N = 8) was 13.6%. The accuracy was studied in two different ways:

(a) adding different amounts (0,20; 50 pg) (N = 15) of estriol to "steroid free" plasma and measuring estriol by the present method; a significant correlation was observed between expected and measured values ($y = 0.9747\ x + 0.2895$).

(b) measuring estriol concentration in increasing amounts of human male plasma (0.2, 0.4, 0.6 ml) (N = 18); a good linearity was observed ($y = 1.2500\ x - 1.5667$).

Estrone and estradiol-17β were measured simultaneously by a radioimmunoassay after extraction of 1 vol of male plasma (usually 2 ml) with 10 vol. of hexane–ethyl acetate mixture (3:1 v/v); 0.5 ml of carbonate buffer (pH 9.5)[12] was added to male plasma before the extraction. Hexane and ethyl acetate (Merck) were distilled just before use[10].

[6-7-^{3}H]-Estrone (S.A. 45 Ci/mmol) and [6,7-^{3}H]-estradiol-17β (S.A. 44.6 Ci/mmol) (NEN chemicals GmbH), repurified on arrival by paper chromatography, were used as specific radioactive steroids, respectively for measurement of estrone and estradiol-17β. Chemically pure estrone and estradiol-17β were used as supplied by Vister. The antiserum (N°E 17–94 Endocrine Science) was obtained by injecting estradiol-17β-17-CMO-BSA conjugate into rabbits. The cross-reactivity of antiserum N°E-17-94 is reported in Table 3. Paper chromatography on Bush B_3 system[13] was used to separate estrone from estradiol-17β.

Whatman paper No. 2 and glassware were prepared as described by Arnold and James[14] to avoid specific interferences. After scanning paper eluates were dried and then redissolved in 1 ml of pure ethanol[10] 0.2 ml (= 0.4 ml of male plasma) in duplicate, they were used for radioimmunoassay and 0.2 ml were used for calculation of recovery. Dextran coated charcoal (0.5 mg per tube) was used to separate bound from the free fraction.

The recovery of radioactive estrone was 78.9 ± 5.5% (mean ± S.D.) (N = 80), the recovery of radioactive estradiol-17β was 80.1 ± 4.8 (mean ± S.D.) (N = 80). The blank values were 0.5 ± 0.6 pg

Table 2. Specifity of the estriol antiserum

Compound	Cross reaction %
Estrone	<0.1
Estradiol-17β	<0.1
Estriol	100
16-Epiestriol	0.7
17-Epiestriol	16.8
16,17-Epiestriol	1.1
Progesterone	<0.1
Testosterone	<0.1
Androstenedione	<0.1

Table 3. Steroid cross-reaction with antiserum No. E 17-94

	[6,7^{3}H]-Estrone	[6,7^{3}H]-Estradiol-17β
Estrone	100%	130%
Estradiol-17β	60%	100%
Estriol	14%	25%
Dihydrotestosterone	<0.1	<0.1
Progesterone	<0.1	<0.1
17-hydroxy-progesterone	<0.1	<0.1
Testosterone	<0.1	<0.1
Cortisol	<0.1	<0.1
Cortisone	<0.1	<0.1
Androstenedione	<0.1	<0.1
Etiocholanolone	<0.1	<0.1
Androsterone	<0.1	<0.1

Table 4. Control of specificity after paper chromatography on Bush B_3 system

Steroid added	Estrone measured (pg) (mean of two determinations)	Estradiol-17β measured (pg) (mean of two determinations)
Blank value	0	0
Estrone (20 pg)	19.6	0
Estradiol-17β (20 pg)	0	19.7
Estriol (1000 pg)	0	0
Dehydroepiandrosterone (1000 pg)	0	0
Androstenedione (1000 pg)	0	0
Dihydrotestosterone (1000 pg)	0	0
Progesterone (1000 pg)	0	0
17-hydroxy-progesterone (1000 pg)	0	0
Testosterone (1000 pg)	0	0
Cortisol (1000 pg)	0	0

* 0.2 ml fractions of ether extracted plasma were loaded with several steroids and measured as estrone and estradiol-17β by the present method.

per tube (mean $\pm$ S.D.) (N = 16) for estrone and 0.6 ± 0.6 pg per tube (mean $\pm$ S.D.) (N = 10) for estradiol-17β. However, estrone and estradiol-17β were not measured in amounts less than 5 pg.

The within assay coefficients of variation were 10.5% for estrone (N = 8) and 5.6% for estradiol-17β (N = 8). The between assay coefficients of variation were 16.0% for estrone (N = 8) and 8.5% for estradiol-17β (N = 8).

The accuracy was studied in two different ways: (a) adding different amounts (0,10,20,30,50 pg) (N = 50) of cold estrone and estradiol-17β to "steroid free" plasma and measuring these steroids by the present method; significant correlation was observed between expected and measured values: $y = 1.0615\,x - 0.0996$ for estrone, $y = 1.0385\,x - 0.6483$ for estradiol-17β.

(b) measuring estrone and estradiol-17β in increasing amounts of male plasma (0.2,0.4,0.6 ml) (N = 12; a good linearity was observed: $y = 1.2237\,x - 1.300$ for estrone, $y = 1.1267\,x - 0.900$ for estradiol-17β.

Lastly, the capacity of the chromatographic system to separate estrone and estradiol-17β from other interfering steroids is reported in Table 4.

Table 5. Plasma steroids in male breast cancer

Subject	Age	Estrone ng/100 ml	Estradiol-17β ng/100 ml	Estriol ng/100 ml	Androstenedione ng/100 ml	Testosterone ng/100 ml
A.E.	31	2.50	0.95	3.00	204.0	761.0
M.B.	36	2.03	2.23	—	145.0	422.0
F.O.	42	2.60	1.90	4.20	94.4	312.0
B.V.	48	1.68	0.54	3.80	—	—
A.N.	51	4.84	2.46	—	—	—
G.S.	52	1.15	0.76	6.10	129.4	150.4
C.F.	53	3.90	2.13	5.80	63.6	162.0
B.G.	56	1.73	0.67	2.45	127.4	357.0
B.O.	58	4.72	3.65	3.00	31.4	384.3
M.P.	60	2.50	1.80	3.50	94.3	505.0
M.A.	60	2.42	1.22	5.80	—	—
S.A.	60	1.43	0.64	2.90	176.8	—
V.D.	62	4.31	2.30	8.40	114.6	473.0
M.V.	63	3.48	3.00	10.75	114.4	515.8
A.G.	68	2.96	2.20	1.53	78.0	629.0
S.R.	74	6.73	4.00	4.50	205.0	510.0
B.L.	75	2.05	2.36	0.40	—	—
Mean	55	3.00*	1.93†	4.40‡	121.3	431.7
$\pm$ S.D.	$\pm$11	$\pm$1.48	$\pm$1.03	$\pm$2.65	$\pm$51.9	$\pm$176.2
Normal controls		(N = 15)	(N = 15)	(N = 18)	(N = 15)	(N = 31)
Mean		1.85	1.01	1.93	104.5	478.0
$\pm$ S.D.		$\pm$1.05	$\pm$0.81	$\pm$1.04	$\pm$36.0	$\pm$232.0
Range of age		30–79	30–79	25–75	30–74	25–80
Mean $\pm$ S.D.		50 $\pm$ 11	50 $\pm$ 11	56 $\pm$ 23	50 $\pm$ 17	53 $\pm$ 12

* $P < 0.02$ in comparison to normal controls.
† $P < 0.01$ in comparison to normal controls.
‡ $P < 0.005$ in comparison to normal controls.

Table 6. Effects of orchidectomy on plasma steroid levels (in brackets the basal values)

Case	Time after orchidectomy	Estrone ng/100 ml	Estradiol-17β ng/100 ml	Estriol ng/100 ml	Androstenedione ng/100 ml	Testosterone ng/100 ml
C.F.		(3.90)	(2.13)	(5.80)	(63.6)	(162.0)
	1 week	1.70	N.D.	2.50	59.2	10.7
	3 months	2.50	0.03	3.80	40.6	14.0
	9 months	1.24	0.33	—	14.8	18.7
A.G.		(2.96)	(2.20)	(1.50)	(78)	(629)
	4 days	1.80	0.60	N.D.	59.4	19.7
	5 days	1.70	0.40	0.40	64.2	—
	8 days	1.00	0.30	0.50	50.8	7.5
	4 months	1.00	0.30	1.00	—	—
	9 months	2.29	0.80	—	41.8	8.1
	1 year	1.28	0.55	—	34.0	9.1
M.B.		(2.03)	(2.23)	—	(145)	(422)
	5 days	0.94	0.78	—	—	—
	6 weeks	1.42	1.56	—	15.6	3.4

RESULTS

The mean values of estrone, estradiol-17β and estriol in patients affected by breast cancer are significantly higher than in normal controls ($E_1 = P < 0.02$; $E_2 < 0.01$; $E_3 = P < 0.005$) (see Table 5). On the other hand, androstenedione and testosterone concentrations in these patients are not different from normal controls. The effects of orchidectomy performed in three patients is reported in Table 6.

DISCUSSION

The results of our study confirm previous data obtained by measuring urinary estrogens[5] and conclusively indicate the existence of an abnormality in estrogen production in men with breast cancer. The studies on metabolism of estradiol-17β in male breast cancer are rather contradictory because Zumoff *et al.*[15] found a marked increase in estriol formation from administered estradiol-17β, while Scheike *et al.*[7] did not find any significant difference between males with breast cancer and normal controls of comparable age. Anyway, to elucidate the increase of plasma estrogen levels in male breast cancer, it seems more important to study the source of these steroids.

In normal males estrone and estradiol-17β are partly secreted directly by the testis, and partly produced by transformation of their androgen precursors: androstenedione and testosterone. Using published data,[16–20] we calculated that the daily production of estradiol-17β in man should be about 40–60 μg (50% from testicular secretion, 50% from peripheral transformation of testosterone); only 2–3 μg per day is produced from circulating estrone[16]. The estrone production is more complicated because about 70% comes from peripheral transformation of androstenedione[18], 10% from testicular secretion[19, 20] and about 15–20% from peripheral transformation of estradiol-17β[16]. Thus we can conclude that circulating estradiol-17β is entirely of testicular origin, while circulating estrone is partly (about 50%) of testicular origin (from direct secretion, from peripheral transformation of estradiol-17β and from peripheral transformation of androstenedione produced by the testis) [8]and partly of adrenal origin. Therefore, the high estrogen production in male breast cancer may be due either to a major secretion from the testis or to an increased peripheral transformation of precursors. Increased estrogen production of extraglandular origin has been clearly demonstrated in essential gynaecomastia[21], in cirrhosis of the liver[22] and in Klinefelter's syndrome[23].

On the other hand, the decrease in estrogen levels after orchiedectomy does not prove the testicular origin of increased estrogen production as maintained by Dao *et al.*[6]. The surgical intervention, in fact, drastically reduces testosterone and estradiol-17β, but to a lesser extent androstenedione, estrone and estriol (see Table 5).

Furthermore, the reduction of estradiol-17β in one case was less than expected in relation to data on hormone production in the normal male (see Table 5).

Moreover, even reduction of estrone may be partly due to decrease of peripheral transformation from androstenedione (40% of testicular origin)[24] and from estradiol-17β.

In conclusion, the data presented in this paper are in agreement with previous results obtained from studying steroid excretion in urine[5], and indicate that men with breast cancer produce significantly higher amounts of endogenous estrogens. The origin of these estrogens (glandular secretion or peripheral transformation from precursors) and their importance in the pathogenesis of male breast cancer remains to be clarified.

REFERENCES

1. El-Gazayerli M. M. and Abdel-Azi A. S.: *Br. J. Cancer* **17** (1963) 566–571.
2. Davies J. N. P.: *Lancet* **2** (1958) 474–475.
3. Davies J. N. P.: *Brit. Med. J.* **2** (1949) 676–679.
4. Symmers W. St. C.: *Brit. Med. J.* **2** (1968) 83–86.

5. De Giuli G. and De Giuli S.: In *Characterization of Human Tumors* (Edited by W. Davis and C. Maltoni). Excerpta Medica, Amsterdam (1973) pp. 213–217.
6. Dao T. L., Morreal C. and Nemoto T.: *New Engl. J. Med.* **298** (1973) 138–140.
7. Scheike O., Sventrup B. and Frandsen *J. steroid Biochem.* **4** (1973) 489–501.
8. Forti G., Pazzagli M., Calabresi E., Fiorelli G. and Serio M.: *Clin. endocr.* **3** (1974) 5–17.
9. Fiorelli G., Borrelli D., Forti G., Gonnelli P., Pazzagli M. and Serio M.: *J. steroid Biochem.* **7** (1976) 113–116.
10. Serio M., Forti G., Fiorelli G. and Pazzagli M.: In *The Endocrine Function of the Human Testis* (Edited by V. H. T. James, M. Serio and L. Martini). Academic Press, London-New York **1** (1973) 15–39.
11. Kunzig H. J. and Geiger W.: *Archs. Gynak.* **216** (1974) 378–394.
12. Cameron E. H. D. and James D. A.: *Steroids* **20** (1972) 737–801.
13. Bush I. E.: *Biochem. J.* **50** (1952) 370–378.
14. Arnold M. L. and James V. H. T.: *Steroids* **18** (1971) 789–801.
15. Zumoff B., Fichermann J., Cassouto J., Hellman L. and Gallagher T. F. *J. clin. Endocr. Metab.* **26** (1966) 960–966.
16. Kirschner M. A. and Taylor J. P.: *J. clin. Endocr. Metab.* **35** (1972) 513–521.
17. Longcope C., Layne D. S. and Tait J. F.: *J. clin. Invest.* **47** (1968) 93–106.
18. Mac Donald P. C., Rombaut R. P. and Siiteri P. K.: *J. clin. Endocr. Metab.* **27** (1967) 1103–1111.
19. Baird D. T., Galbraith A., Fraser I. S. and Newsan J. E.: *J. Endocr.* **57** (1973) 285–288.
20. Weinstein R. L., Kelch R. P., Jenner M. R., Kaplans L. and Grumbach M. M.: *J. clin. Invest.* **53** (1974) 1–6.
21. Siiteri P. K. and Mac Donald P. C.: In *Handbook of Physiology-Section 7 Vol. II: Female Reproductive System, part 1* (Edited by R. O. Greep). The Williams & Wilkins Company, Baltimore (1973) 615–629.
22. Vermeulen A.: In *The Endocrine Function of the Human Testis* (Edited by V. H. T. James, M. Serio and L. Martini). Academic Press, London-New York **1** (1974) pp. 181–194.
23. Wang C., Baker H. G., De Kretser D. M. and Hudson B.: *Clin. Endocr.* **4** (1975) 399–411.
24. Hudson R., Coghean J. P. and Dulmanis A.: In *Colloquia on Endocrinology: The Testis* (Edited by G. E. W. Wolstenholme and M. O'Conner). Churchill, London Vol. **16** (1967) pp. 140–155.