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Hormonal Changes Induced by the Pure Antiandrogen Flutamide in Postmenopausal Women with Advanced Breast Cancer*

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Abstract—*Hormonal changes induced by the pure antiandrogen flutamide were studied in three postmenopausal metastatic breast cancer patients. The drug was administered at a dose of* 250 mg, *three times a day for* 3–6 *months. In each patient a sharp decrease of about* 50% *was observed in the circulating levels of DHT and DHEAS, irrespective of pretreatment values. A concomitant, although less pronounced, reduction in circulating testosterone, androstenedione and estradiol was found. A decrease in circulating steroids was associated with a* 30% *decrease in SHBG concentrations in two patients; in the third patient a* 30% *increase occurred. Androgens in urine, namely testosterone, androstanediol and* 17-*KS, decreased accordingly. In addition, a marked decrease in* 17-*OHCS occurred in two of the patients. These data indicate that flutamide is an effective antiandrogen in women and suggest two possible mechanisms by which the drug exerts its antiandrogenic activity: (a) inhibition of conversion of testosterone into the more active DHT, and (b) inhibition of synthesis of the adrenal precursors of active androgens. Minor variations in circulating LH and FSH were observed. Pretreatment prolactin values, which were higher than normal, dramatically decreased by* 90% *in one patient who had a partial remission of her disease, and they further increased in another patient who relapsed while on therapy.*

INTRODUCTION

FLUTAMIDE [propanamide, 2-methyl-*N*-4-nitro-3-(trifluoromethyl)-phenyl] has been reported to be a pure antiandrogen devoid of any other hormonal or antihormonal activity [1]. The drug has been successfully used in the treatment of prostate cancer. Its effects on hormonal pattern have been studied in males, but there is no similar information for females. Several reports from our laboratory suggest that androgens may play an important role in the development of female breast cancer [2, 3]. To contribute to the available information, we examined the hormonal changes induced by flutamide in three postmenopausal metastatic breast cancer patients. The lack of endocrine effects other than neutralizing the action of endogenous androgens makes the drug suitable for the purpose of this hormonal study.

PATIENTS AND METHODS

Flutamide (supplied by Essex Italia, Milan, Italy) was administered at a dose of 250 mg, three times a day for 3–6 months, to three postmenopausal patients with advanced breast cancer, after informed consent was obtained. The first of them (F1) presented with two small nodules of local recurrence, which had reappeared 1 year after an apparently curative radiotherapy. Partial remission of the nodules occurred spontaneously before starting the flutamide treatment and was complete after 1 month of therapy. The drug was discontinued after 3 months. Six months later, local recurrences appeared again. Hormones in blood and urine were measured before starting flutamide, after 1 month of therapy, and 6 months after discontinuation of the drug, when the patient relapsed. The second patient (F2) presented with diffuse infiltration of the right breast. The nipple was ulcerated and bloody. Thoracic skin under the right breast was infiltrated. Metastatic supraclavicular lymph nodes

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Abbreviations used: DHT, 5-alpha-dihydrotestosterone; androstanediol, 5-alpha-androstan-3-alpha-17-beta-diol; DHEAS, dehydroepiandrosterone sulphate; LH, lutcinizing hormone; FSH, follicle stimulating hormone; SHBG, sex-hormone-binding globulin; 17-KS, 11-deoxy-17-keto-steroids; 17-OHCS, 17-hydroxy-corticoids.

were present at both sides (right, 3.5 × 2.5 cm; left, 0.5 cm in diameter). The disease was stationary for 3 months during flutamide therapy, then relapsed with two small metastatic nodules in the scalp (0.2 cm in diameter). Hormonal measurements were done before and 1 month after starting therapy. The third patient (F3) presented with an infiltrated lumpy area, 3.5 cm in diameter, on the site of the previous mastectomy. Metastatic involvement of contralateral axillary lymph nodes was proven histologically, but its extent was not measurable because of a recent biopsy. There was no mammographic evidence of cancer in the residual breast. An asymptomatic, nonhomogeneous osteosclerotic area was observed upon X-ray examination in the upper third of the right femur. After 1 month of therapy the lumpy area became flat and crusty, and at 6 months there was a partial remission (less than 50%) of the soft tissues. In this patient, hormones were measured before starting the therapy and at 1, 3, 4 and 6 months thereafter.

Twenty-four hour urine collections were obtained by each patient for measurement of testosterone, androstanediol, 17-KS[3] (i.e. dehydroepiandrosterone + androsterone + etiocholanolone), pregnanediol and 17-OHCS. On the same day of the urine collection, 20 ml of blood was drawn by venipuncture between 9 and 11 a.m. for measurement of the circulating levels of testosterone, DHT, androstenedione, DHEAS, estradiol, prolactin, LH, FSH and SHBG. Hormones in urine were measured by gas chromatography: testosterone, androstanediol and 17-KS as previously reported [4, 5], and 17-OHCS and pregnanediol according to the method of Murphy and West [6]. Circulating hormones and SHBG were measured by radioimmunoassay or by immunoradiometric assay using commercial kits purchased from Biomerieux (Charbonnier les Bains, France) for testosterone, DHT and androstenedione, from Sorin (Saluggio, Italy) for estradiol, from Sclavo (Milan, Italy) for DHEAS, from Ares Serono (Milan, Italy) for prolactin, FSH and LH, and from Farmos Group Ltd. (Oulunsalo, Finland) for SHBG. Testosterone, DHT and androstenedione were measured after ether extraction followed by partition chromatography on a celite column. Elution was carried out stepwise using 5 cc of isooctane (androstenedione fraction), followed by 6 cc of 6% ethylacetate in isooctane (DHT fraction), then 6 cc of 20% ethylacetate in isooctane (testosterone fraction).

LH was expressed in international milliunits of the First International Reference Preparation (1st IRP 68/40) and FSH in international milliunits of the Second International Reference Preparation (2nd IRP 78/549). A single kit was used for all measurements of each hormone. The intra-assay variation coefficients for testosterone, DHT, androstenedione, DHEAS, estradiol, prolactin, LH, FSH and SHBG were respectively 9.7%, 7.9%, 6.1%, 6.3%, 7.0%, 1.0%, 1.1%, 3.1% and 3.5%.

RESULTS

For a better comprehension of the hormonal changes observed in the study, the metabolic pathway of steroid hormones is summarized in Fig. 1. Table 1 reports the anamnestic data of the patients. At the dose administered, flutamide was well tolerated and devoid of any side effect. Biochemical parameters, regularly checked, did not show significant variations other than a mild elevation of alkaline phosphatase in patient F3 at 6 months.

Patient F1

Hormones in blood and urine were measured before starting flutamide therapy, after 1 month of therapy, and 6 months after termination of the therapy.

Hormones in blood. After 1 month of therapy, DHT and DHEAS sharply decreased by 66% and 60%, respectively (Table 2A). A concomitant lowering of testosterone (30%), androstenedione (38%), SHBG (28%) and LH (38%) was observed, together with minor reductions in FSH, prolactin and estradiol levels. Testosterone/SHBG and estradiol/SHBG ratios were unchanged; the DHT/SHBG ratio was halved. Six months after the drug was discontinued, DHEAS, LH, FSH, SHBG and estradiol resumed their pretreatment values.

Hormones in urine. Under flutamide therapy, androstanediol and pregnanediol values were halved, 17-OHCS decreased by 80%, testosterone by 40% and 17-KS by 70% (Table 2B). The androsterone/etiocholanolone ratio increased substantially. Six months after the drug was discontinued, testosterone values were twice the basal levels and the other hormones resumed their pretreatment values.

Patient F2

Hormones in blood and urine were measured before starting flutamide therapy and 1 month later.

Hormones in blood. All circulating steroids decreased under flutamide therapy (Table 3A): testosterone by 56%, DHT by 44%, androstenedione by 52%, DHEAS by 47%, estradiol by 63%. A concomitant, although less pronounced, reduction in SHBG (32%) was found. Testosterone/SHBG, DHT/SHBG and estradiol/SHBG ratios decreased accordingly. LH and FSH concentrations were unchanged. Prolactin pretreatment levels were higher than normal and further increased after 1 month of therapy.

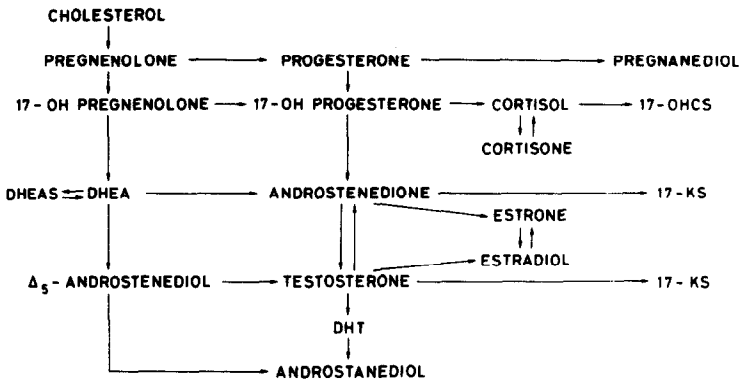

Fig. 1. Metabolic pathway of steroid hormones.

Table 1. Anamnestic data in the three patients with advanced breast cancer submitted to flutamide treatment (750 mg/day)

Parameter	F1	F2	F3
Age (years)	57	46	58
Age of menopause	52	44	52
Type of breast surgery performed	Radical right mastectomy (Nov '81)	Quadrantectomy right breast (Jan '85)	Radical right mastectomy + RT (1976)
Site of recurrence before flutamide treatment	Local (May '86)	Local + soft tissues (May '86)	Local + soft tissues + bone (June '86)
Clinical response to flutamide	Not evaluable	Progression at 3 months	Partial remission (<50%) at 6 months
Comments	Thyroidectomy for papillary adenocarcinoma (1976) Local recurrence (May '85) Local recurrence (Dec '86)		Hysterectomy + bilateral oophorectomy for uterine fibroma (July '85)

Hormones in urine. Testosterone and 17-KS decreased by about 30% (Table 3B). The androsterone/etiocholanolone ratio increased substantially because of the contemporary reduction in etiocholanolone and increase in androsterone concentrations. Androstanediol and 17/OHCS were unchanged. Dehydroepiandrosterone and pregnanediol levels increased.

Patient F3

Hormones in blood and urine were measured before starting flutamide therapy and 1, 3, 4 and 6 months thereafter.

Hormones in blood. Testosterone, DHT, androstenedione and estradiol pretreatment values were very low, possibly because of the previous oophorectomy (Table 4A). Testosterone, androstenedione and estradiol levels were unaffected by the drug, whereas DHT concentrations further were reduced by 38% at 1 month and by 49% at 4 months. A concomitant progressive decrease in DHEAS from 33% to 50% of the basal value was observed in subsequent measurements. In contrast to the other patients, SHBG increased stepwise from 23% to 38% of the basal value, thus resulting in a net decrease in the testosterone/SHBG, DHT/SHBG and estradiol/SHBG ratios. Minor variations were found in LH and FSH levels. Prolactin concentrations, whose pretreatment values were above normal, dramatically decreased by 90% at 1 month and remained low at subsequent measurements.

Hormones in urine. Large fluctuations were seen in subsequent measurements of testosterone, pregnanediol, etiocholanolone, androsterone and in the androsterone/etiocholanolone ratio (Table 4B). Androstanediol decreased by 40–60% of pretreatment values. Similarly, 17-OHCS was reduced by 44–56%.

DISCUSSION

To our knowledge, this is the first report dealing with the endocrine effects of flutamide in women. Our data suggest that flutamide is highly effective in inhibiting the testosterone → DHT conversion,

Table 2A. Patient F1. Hormones in blood measured before (0) *starting flutamide, after* 1 *month of therapy* (1), *and* 6 *months after therapy was discontinued* (6)

Hormonal parameter	0	1	6
Testosterone (T), ng/ml	0.196	0.118	ND
DHT, ng/ml	0.257	0.087	ND
Androstenedione, ng/ml	0.437	0.271	ND
DHEAS, ng/ml	418.4	166.8	339.9
SHBG, nmol/l	35.38	25.54	31.96
Estradiol (E2), pg/ml	5.2	3.9	4.9
Prolactin, ng/ml	4.6	3.8	ND
LH, mIU/ml	38.4	23.7	35.0
FSH, mIU/ml	81.6	65.5	72.6
T/SHBG × 100	0.55	0.46	ND
DHT/SHBG × 100	0.73	0.34	ND
E2/SHBG	0.147	0.153	0.153

ND, not determined.

Table 2B. Patient F1. Hormones in urine measured before (0) *starting flutamide, after* 1 *month of therapy* (1), *and* 6 *months after therapy was discontinued* (6)

Hormonal parameter	0	1	6
Testosterone, μg/24 h	3.5	2.1	7.2
Androstanediol, μg/24 h	32.9	16.1	36.3
Dehydroepiandrosterone (D), mg/24 h	0.010	0.010	0.008
Etiocholanolone (E), mg/24 h	0.238	0.022	0.204
Androsterone (A), mg/24 h	0.235	0.118	0.243
17-Ks (D + E + A), mg/24 h	0.483	0.150	0.455
A/E ratio	0.99	5.36	1.19
17-OHCS, mg/24 h	5.6	1.1	5.7
Pregnanediol, mg/24 h	0.8	0.4	0.7

as shown by the sharp decrease in circulating DHT levels observed in each patient while on therapy, irrespective of pretreatment values. This finding is in accord with the reported ability of flutamide to inhibit DHT synthesis *in vitro* [1], whereas it is in contrast with studies on men, in which DHT levels were shown to be unaffected by the drug [7, 8]. A reduction in circulating DHT in our patients was not associated with an increase in testosterone, androstenedione or estradiol levels, which, on the contrary, also decreased. This finding is not in accord with those in men, where significant increases in circulating testosterone [7, 8] and estradiol [8] while on flutamide therapy were reported. According to our data, a decrease in circulating levels of androstenedione, testosterone, DHT, estradiol and the corresponding urinary excretion of testosterone and androstanediol may be explained by the simultaneous marked decrease in DHEAS concentrations observed in each patient.

DHEAS, although virtually devoid of androgenic activity, is an important precursor for the biosynthesis of active androgens. On this basis, we suggest that flutamide is effective in inhibiting androgen precursor biosynthesis at the adrenal level. Whether the inhibitory effect of the drug is directed to the gland or whether it is mediated through the adrenal–pituitary axis cannot be stated from our data. In a previous study [7], DHEAS concentrations were shown to be unaffected by flutamide in men, but a recent report by Labrie *et al.* [9] strongly supports our finding. A sharp reduction in urinary excretion of 17-OHCS was found in two of our patients. This finding is in accord with the hypothesis of an inhibitory effect of flutamide on the first steps of the steroid biosynthetic pathway in adrenal glands. A decrease in urinary corticosteroid excretion has been observed in prostate cancer patients treated with flutamide [10]. However, circulating cortisol concentrations did not change under therapy [9, 10]. Fukushima *et al.* [10] demonstrated that flutamide markedly reduced the production rate and increased the plasma half-life of cortisol, thus explaining differences in the concentrations of the hormone in plasma and urine.

We would like to stress the hormonal differences between patient F2, who relapsed while on flutamide therapy, and patient F3, who had a partial remission after 6 months of therapy. In the latter case, androstanediol and 17-OHCS halved, prolac-

Table 3A. Patient F2. Hormones in blood measured before (0) *and* 1 *month after starting flutamide therapy* (1)

Hormonal parameter	0	1
Testosterone (T), ng/ml	0.258	0.115
DHT, *ng/ml*	0.115	0.064
Androstenedione, ng/ml	1.010	0.480
DHEAS, ng/ml	1123.0	592.8
SHBG, nmol/l	38.48	26.16
Estradiol (E2), pg/ml	9.2	3.4
Prolactin, ng/ml	26.0	37.0
LH, mIU/ml	40.43	44.64
FSH, mIU/ml	119.0	102.3
T/SHBG × 100	0.67	0.44
DHT/SHBG × 100	0.30	0.24
E2/SHBG	0.239	0.130

Table 3B. Patient F2. Hormones in urine measured before (0) *and* 1 *month after starting flutamide therapy* (1)

Hormonal parameter	0	1
Testosterone, μg/24 h	4.5	3.0
Androstanediol, μg/24 h	29.2	32.1
Dehydroepiandrosterone (D), mg/24 h	0.004	0.015
Etiocholanolone (E), mg/24 h	0.572	0.126
Androsterone (A), mg/24 h	0.271	0.465
17-KS, (D + E + A), mg/24 h	0.847	0.597
A/E ratio	0.47	3.69
17-OHCS, mg/24 h	3.4	3.2
Pregnanediol, mg/24 h	0.7	1.6

Table 4A. Patient F3. Hormones in blood measured before (0) *and* 1, 3, 4 *and* 6 *months after starting flutamide therapy* (1, 3, 4 *and* 6)

Hormonal parameter	0	1	3	4	6
Testosterone (T), ng/ml	0.126	0.118	0.105	0.125	ND
DHT, ng/ml	0.063	0.039	0.039	0.032	ND
Androstenedione, ng/ml	0.360	0.326	0.367	0.300	ND
DHEAS, ng/ml	450.2	299.9	259.1	257.4	227.0
SHBG, nmol/l	34.99	46.89	45.40	45.99	56.46
Estradiol (E2), pg/ml	4.8	3.7	2.7	4.3	4.1
Prolactin, ng/ml	28.3	2.9	4.7	3.8	3.7
LH, mIU/ml	8.24	8.22	9.54	7.93	9.20
FSH, mIU/ml	16.22	16.61	21.11	21.14	22.96
T/SHBG × 100	0.360	0.250	0.230	0.270	ND
DHT/SHBG × 100	0.180	0.083	0.086	0.069	ND
E2/SHBG	0.137	0.079	0.059	0.077	0.073

ND, not determined.

tin dramatically reduced, and SHBG increased. In the former, androstanediol and 17-OHCS did not change, prolactin was persistently high, and SHBG decreased. In addition, concentrations of circulating androgens and the testosterone/SHBG, DHT/SHBG and estradiol/SHBG ratios reached much lower values under flutamide therapy in patient F3 than in patient F2. Whether the differences observed are merely fortuitous or whether they are indicative of different responses to the drug and could thus have prognostic significance is still unknown. Studies on larger series are needed for an answer to this question.

In conclusion, our data indicate that flutamide is

Table 4B. Patient F3. Hormones in urine measured before (0) *and* 1, 3, 4 *and* 6 *months after starting flutamide therapy* (1, 3, 4 *and* 6)

Hormonal parameter	0	1	3	4	6
Testosterone, μg/24 h	3.7	0.4	4.4	0.9	2.9
Androstanediol, μg/24 h	22.2	13.4	10.6	11.4	9.2
Dehydroepiandrosterone (D), mg/24 h	0.006	0.004	0.009	0.006	0.010
Etiocholanolone (E), mg/24 h	0.103	0.085	0.064	0.111	0.042
Androsterone (A), mg/24 h	0.131	0.075	0.113	0.012	0.105
17-KS (D + E + A), mg/24 h	0.240	0.164	0.186	0.129	0.157
A/E ratio	1.27	0.88	1.76	0.11	2.5
17-OHCS, mg/24 h	3.4	1.6	1.5	1.9	1.6
Pregnanediol, mg/24 h	0.4	0.2	0.7	0.8	0.0

an effective antiandrogen in women and suggest two possible mechanisms by which the drug exerts its antiandrogenic activity: (a) inhibition of conversion of testosterone into the more active DHT, and (b) selective inhibition in synthesis of the adrenal precursors of active androgens. In our opinion, these effects of flutamide, if confirmed, may be usefully employed in the treatment of female breast cancer.

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