Pergamon

0960-0760(95)00017-8

J. Steroid Biochem. Molec. Biol. Vol. 52, No. 6, pp. 541–546, 1995

0960-0760/95 $9.50 + 0.00

Testosterone, Dihydrotestosterone and Oestradiol Levels in Postmenopausal Breast Cancer Tissues

C. Recchione,[1] E. Venturelli,[1] A. Manzari,[2] A. Cavalleri,[1] A. Martinetti[1] and G. Secreto[1]*

[1]*Endocrine Unit and* [2]*Division of Medical Statistics and Biometry, National Cancer Institute, 20133 Milan, Italy*

The ability of breast tumours to synthesize hormones is well recognized, and local production of sex steroids is thought to play a role in breast cancer growth. We measured the intratumour and circulating levels of testosterone, dihydrotestosterone (DHT) and oestradiol in 35 histologically confirmed carcinomatous mammary tissues obtained at breast surgery from 34 postmenopausal patients, age 50–85 years. Intra-tissue steroids were extracted with ethanol:acetone (1:1; v/v), defatted with 70% methanol in water, and extracted with ether. Steroids, from tissue and serum, were separated by partition chromatography on celite columns and were measured by RIA. Intratumour testosterone and DHT concentrations were significantly correlated, after the exclusion of an outlier ($r_s = 0.71$; $P = 0.0001$). No association was found between oestradiol and either of the two androgens. Mean oestradiol and DHT concentrations were significantly higher in tissue than in blood ($P = 0.0001$). Mean testosterone levels in tissues did not significantly differ from those measured in blood. Our data suggest that at least a part of intratissue DHT is produced locally from testosterone. The meaning of high oestradiol and DHT levels in cancer tissue still needs to be defined.

J. Steroid Biochem. Molec. Biol., Vol. 52, No. 6, pp. 541–546, 1995

INTRODUCTION

The ability of breast tumours to synthesize hormones is well recognized, and it is assumed that the total amount of a steroid in breast cancer tissue is provided in part by uptake from plasma and in part by local production. Breast cancer growth is influenced by sex hormones, and hormone levels in cancer tissues are considered to reflect their biological activity better than their levels in blood.

The intratumour concentration of oestrogens has been extensively studied, and particular interest has been addressed to the measurement of adrenal androgens [dehydroepiandrosterone-(sulphate), androstenediol and androstenedione], which can serve as substrates for aromatization to oestrogen [1–8]. Although the presence of androgen receptors in breast tumours [9] indicates a possible role for androgens in regulating cancer growth, only a few papers have dealt with testosterone levels in breast cancer tissues [3, 10], and measurement of dihydrotestosterone (DHT) concentrations has been reported only by Mistry *et al.* [10]. A wide range of testosterone values was found in breast cancer tissue, with a tissue/plasma ratio of 0.05–5.0 [3]. At target tissues, testosterone can act as a full hormone or can serve as a pro-hormone for aromatization to oestradiol by the enzyme aromatase or conversion to DHT by the enzyme 5α-reductase. Aromatase and 5α-reductase activities are present in mammary tumours, and a role of testosterone in the intratumour synthesis of one or the other metabolite can be tentatively inferred. However, whereas testosterone is the obliged precursor of DHT, oestradiol can be produced alternatively by reduction of the ketonic group of oestrone.

In the present report, we measured the intratumoural and the circulating levels of testosterone, DHT and oestradiol in postmenopausal breast cancer patients. The aim of the study was to support and add to previous findings in the literature [3, 10].

*Correspondence to G. Secreto.
Received 15 Jul. 1994; accepted 30 Dec. 1994.

EXPERIMENTAL

Patients and sample preparation and storage

Carcinomatous mammary tissue was obtained at breast surgery from 34 consecutive patients bearing tumours larger than 3 cm, without clinical or radiographic evidence of distant metastases. Two primary carcinomas were found in the same breast of one patient, and they were processed separately as 2 different tumours. All patients were postmenopausal, and their ages ranged between 50 and 85 y (mean $\pm$ SD, 64.4 ± 8.2). None had received any hormonal preparation for the last 6 months, and none had been previously treated with chemotherapy or hormone therapy for their disease.

Immediately after removal, a portion of the tumour was cut off, accurately dissected free of fat, pulverized with a micro-dismembrator and stored at $-80°C$ until analysis. The diagnosis of breast cancer was histologically confirmed in the remaining tissue. Blood samples were taken just before surgery, between 8 and 9 a.m. Blood was allowed to clot at room temperature and was centrifuged; the serum was separated and stored at $-30°C$ until the assay.

Tissue steroid assay

Intra-tissue steroid concentrations were measured according to the method of Van Landeghem *et al.* [11]. Aliquots of at least 0.8 g of pulverized tissue were homogenized in Tris-buffer (pH 7.4) and centrifuged at 100,000 *g* for 30 min at 4°C. The cytosols were equilibrated with appropriate tritiated steroid tracers to enable correction of procedural losses. The cytosol samples were extracted with 4 vol of ethanol:acetone (1:1, v/v) and centrifuged at 1100 *g*. The supernatants were evaporated and the residues defatted with 3 ml of 70% methanol in water. After centrifugation, the dried residues were redissolved in 25 μl of ethanol and 1 ml of Tris-buffer. Subsequently, the samples were extracted 3 times with 3 ml of ether and the extracts were dried. The dried residues after ether extraction were resuspended in isooctane saturated with ethylene glycol. Oestradiol, testosterone and DHT were separated by partition chromatography on a celite column, imbued with propylene glycol/ethylene glycol 20:80 (v/v). Steroids were eluted with 3.5 ml of increasing amounts of ethyl acetate in isooctane: 3:97 (v/v) (DHT fraction); 18:82 (v/v) (testosterone fraction); 30:70 (v/v) (oestradiol fraction). The eluates were dried and left at 4°C until the radioimmunoassay.

Serum steroid assay

Three ml aliquots of serum were equilibrated with appropriate tritiated steroid tracers and extracted with 11 ml of ether. The ether extracts were dried and the steroids were separated on celite columns as reported for the tissue extracts.

Radioimmunoassay

Steroids, from tissue and serum, were measured by radioimmunoassay using commercial kits purchased from Biomerieux (Charbonnier le Bains, France). Tissue and serum samples of each patient were determined in the same kit. The sensitivities of the method in sera were 0.02 ng/ml for testosterone, 0.01 ng/ml for DHT, and 3.33 pg/ml for oestradiol. The sensitivities in tissue were 0.02 ng/g for testosterone and DHT and 10.0 pg/g for oestradiol. The practical sensitivities were determined by recoveries and by the quantity of tissue processed. Mean recoveries after the extraction and purification procedures for testosterone, DHT and oestradiol were 51.3, 39.0 and 78.8% in tissue and 80.0, 67.5 and 75.9% in sera, respectively. The intra-assay coefficients of variation (CV) for testosterone, DHT and oestradiol were 7.3, 9.7 and 4.0% in tissue and 7.2, 7.9 and 3.1% in sera, respectively. The inter-assay CV in the sera were 10.0, 11.0 and 8.9% for testosterone, DHT and oestradiol, respectively.

The serum oestradiol sample of one patient was lost during the assay procedure. The testosterone value in one tumour was found unaccountably high (1219 pg/g). It was considered an outlier and was excluded from the calculation.

Statistical analysis

Since the distributional form of hormonal levels in tissue and in blood did not satisfy the normal distribution assumption, the original values were transformed into their natural ranks [12]. The association between hormones was computed by the rank correlation coefficient r_s (Spearman's correlation coefficient). The mean hormonal levels in tissue and in blood were compared by the Wilcoxon signed rank test for matched pairs. The probability values were two tailed.

RESULTS

Oestrogen receptor concentrations were obtained from the clinical records of 30 patients. No correlation was found between oestrogen receptor content and steroid levels measured in tumours. Intratumour concentrations and blood levels of testosterone, DHT and oestradiol are reported in Table 1. Mean oestradiol and DHT concentrations were significantly higher in tissue than in blood ($P < 0.0001$). Testosterone levels in tissues were widely dispersed and did not significantly differ from those measured in blood. The mean tissue/serum ratio for testosterone was close to 1, whereas a mean 9-fold increase in the ratio was observed for oestradiol and a mean 3-fold increase for DHT.

A weak correlation was found between tissue and plasma concentrations of oestradiol, DHT and testosterone (Table 2). The correlation between tissue and blood levels of the 3 steroids are reported in Tables 3

Table 1. Median and min–max for intratumour concentrations and blood levels of testosterone, dihydrotestosterone and oestradiol in 34 postmenopausal breast cancer patients

	Tissue (pg/g)	Blood (pg/ml)	P*
Testosterone			
Median	216	247	0.1513
Min–max	54–645	128–825	
Dihydrotestosterone			
Median	249	86	0.0001
Min–max	110–698	29–222	
Oestradiol			
Median	46	7	0.0001
Min–max	9–211	3–15	

*Wilcoxon signed rank test for matched-pairs.

and 4, respectively. Oestradiol levels were not associated with those of DHT, either in blood or in tissue, whereas they were associated with those of testosterone in blood but not in tissue. The two androgens were correlated in tissue and in blood; correlation coefficients were computed after exclusion of an abnormal tissue testosterone value in one sample.

Sex steroids concentrations in the two tumours from the same patient are reported in Table 5.

DISCUSSION

A major role of oestrogen in the hormonal promotion of breast cancer has long been postulated. Although convincing evidence of hyperoestrogenemia has never been obtained in women with breast cancer [13], a high tissue/plasma ratio of oestradiol has been observed in these patients by several investigators [2–5] and in the present report, which demonstrates that oestrogen level in blood is not representative of the intratumour concentration of the steroid.

Studies from our laboratory have indicated that androgen excess is the endocrine abnormality which characterizes breast cancer patients [14–25], and elevated testosterone levels have been found in breast cancer patients by most of the case-control studies reported in the literature [26–34], though not by all [35–38]. In patients with early breast cancer, hyperandrogenemia was found to be associated with an increased risk of developing metastases [18, 25]. In hyperandrogenic women with metastatic breast cancer, remission of metastases by oophorectomy was associated with normalization of androgen excess [14, 16, 39]. These findings suggest that tumour growth is androgen-dependent in a subgroup of patients. The results of a study from Simon *et al.* [40] support the androgen-dependence of some mammary tumours. The authors observed that testosterone at physiological concentration stimulated cellular growth in 4 out of 7 short-term cultures of mammary cancer cells derived from postmenopausal patients. Cellular growth is also known to be enhanced by androgens in some breast cancer cell lines [41, 42]. Finally, the observation that the mammary gland has the same embryological origin as the apocrine sweat glands of the skin is suggestive for a growth stimulatory effect of androgens on the breast, since apocrine epithelium is under the control of androgens [43, 44]. Women with apocrine cysts of the breast are at increased risk of breast cancer, and apocrine metaplasia is often associated with hyperplastic and neoplastic alterations of breast epithelium [45–47].

Table 2. Spearman correlation coefficient between tissue and blood levels of testosterone, dihydrotestosterone and oestradiol in postmenopausal breast cancer patients

	r_s	P	n
Testosterone	0.37	0.0321	34
Dihydrotestosterone	0.41	0.0148	35
Oestradiol	0.34	0.0467	34

n = number of observations.

Table 3. Spearman correlation coefficient between tissue levels of testosterone (T), dihydrotestosterone (DHT) and oestradiol (E_2) in postmenopausal breast tumours

	r_s	P	n
T vs DHT	0.71	0.0001	34
T vs E_2	0.11	0.5465	34
DHT vs E_2	0.18	0.3104	35

n = number of observations.

Table 4. Spearman correlation coefficient between blood levels of testosterone (T), dihydrotestosterone (DHT) and oestradiol (E_2) in postmenopausal breast tumours

	r_s	P	n
T vs DHT	0.4 4	0.0 080	35
T vs E_2	0.4 9	0.0 035	34
DHT vs E_2	0.2 0	0.2 501	34

n = number of observations.

Table 5. Testosterone (T), dihydrotestosterone (DHT) and oestradiol (E_2) levels in the two tumours from the same patient and in the blood

		T	DHT	E_2
Tumour I	pg/g	520	404	98
Tumour II	pg/g	308	339	17
Blood	pg/ml	179	65	5

Studies on androgen concentrations in mammary tumours have shown that levels of androstenediol, dehydroepiandrosterone and androstenedione were higher in the tumour than in the blood [3, 7, 8, 10], whereas the tissue/plasma ratio of testosterone was found to be close to 1 in the two studies that measured the hormone [3, 10] and in the present report. It should be noted, however, that the tissue/plasma ratio of testosterone varies widely, from 0.05 to 5.0 in the paper of Vermeulen *et al.* [3], and from 0.3 to 4.0 in the present report. This means that testosterone levels in a subgroup of tumours are substantially higher than in the blood but in another subgroup are lower. Whether biological and clinical differences exist between the two subgroups is not known.

In the present report, a 3-fold increase in intratumour DHT concentrations over circulating levels was observed. This finding corroborates previous data of Mistry *et al.* [10], who found significantly higher levels of DHT in breast cancer tissue than in blood. Evidence of 5α-reductase activity in mammary tumours [48] suggests that at least a portion of intratumour DHT arises from testosterone conversion within the breast. Testosterone is the immediate and obliged precursor of DHT in the biosynthetic pathway of androgens, and a correlation between these two steroids can be expected in tissues where the conversion of testosterone to DHT occurs.

Mistry *et al.* [10] reported a highly significant positive correlation between intratumour concentrations of testosterone and DHT, and, in the present study, a similar strong linear correlation between the two androgens could be observed after the exclusion of an outlier. The ability of breast tumours to metabolize testosterone in 5α-reduced metabolites depends on the degree of apocrine differentiation of the tumour, and apocrine differentiation is frequently present in breast tumours [48].

The reported evidence indicates that large amounts of androgens are present in breast cancer tissue and suggests that androgens may play a role in the regulation of cancer growth. The route by which androgen influences growth of mammary cancer is not known, but several mechanisms can be assumed. Binding to androgen receptors may be important in stimulating growth of androgen-dependent tumours. Androgen receptors were found in 50–90% of breast tumours [9, 49], and were associated with longer survival in operable disease and with favourable response to hormonal therapy in advanced disease [49]. Interestingly, a strong positive correlation has been reported among the presence of androgen, oestrogen and progesterone receptors in breast cancer tissue [50, 51], suggesting that sex steroids act in concert in the growth regulation of mammary tumours. In addition to the physiological linkage to androgen receptor, there is evidence that androgen, at a high concentration, competes for oestrogen receptors in human breast cancer [52], in breast cancer cell lines [53] and in DMBA-induced rat mammary carcinoma [54], thus eliciting oestrogenic effects on tumour growth. A third mechanism is local conversion to oestrogen. *In situ* aromatization of androgen is thought to be an important source of intratumour oestrogen production [55, 56], although some authors have emphasized local sulphatase activity as the major determinant of intratumoural oestrogen levels [3, 57]. The aromatase enzyme complex has been detected in 50–70% of human breast cancers [55, 56], and aromatase activity was found to be higher in mammary tumours than in normal breast tissue [3] and in the adipose tissue around the tumour [58].

In conclusion, a regulatory effect of androgens on breast cancer growth is conceivable, whatever the mechanism of action. We suggest that androgen and oestrogen act in concert in the control of tumour growth, and, in our opinion, further studies on this topic should be developed to investigate the occurrence of androgen/oestrogen imbalance in breast cancer.

REFERENCES

1. Bonney R. C., Reed M. J., Davidson K., Beranek P. A. and James V. H. T.: The relationship between 17-hydroxysteroid dehydrogenase activity and oestrogen concentrations in human breast tumours and in normal breast tissue. *Clin. Endocr.* **19** (1983) 727–739.
2. Van Landeghem A. A. J., Poortman J., Nabuurs M. and Thijssen J. H. H.: Endogenous concentration and subcellular distribution of estrogens in normal and malignant human breast tissue. *Cancer Res.* **45** (1985) 2900–2906.
3. Vermeulen A., Deslypere J. P., Paridaens R., Leclercq G., Roy F. and Heuson J. C.: Aromatase, 17β-hydroxysteroid dehydrogenase and intratissular sex hormone concentrations in cancerous and normal glandular breast tissue in postmenopausal women. *Eur. J. Cancer Clin. Oncol.* **22** (1986) 515–525.
4. Santen R. J.: Determination of tissue oestradiol in human breast cancer. *Cancer Surveys* **5** (1986) 597–616.
5. Thijssen J. H. H. and Blankenstein M. A.: Endogenous oestrogens and androgens in normal and malignant endometrial and mammary tissues. *Eur. J. Cancer Clin. Oncol.* **25** (1989) 1953–1959.
6. Miller W. R.: Aromatase activity in breast tissue. *J. Steroid Biochem. Molec. Biol.* **39** (1991) 783–790.
7. Blankestein M. A., Maitimu-Smeele I., Donker G. H., Daroszewski J., Milewicz A. and Thijssen J. H. H.: Tissue androgen and the endocrine autonomy of breast cancer. *J. Steroid Biochem. Molec. Biol.* **43** (1992) 167–171.
8. Pasqualini J. R.: Role of androgens in breast cancer. *J. Steroid Biochem. Molec. Biol.* **45** (1993) 167–172.
9. Soreide J. A., Lea O. A., Varhaug J. E., Skarstein A. and Kvinnsland S.: Androgen receptors in operable breast cancer: relation to other steroid hormone receptors, correlations to prognostic factors and predictive value for effect of adjuvant tamoxifen treatment. *Eur. J. Surg. Oncol.* **18** (1992) 112–118.
10. Mistry P., Griffiths K. and Maynard P. V.: Endogenous C_{19}-steroids and oestradiol levels in human primary breast tumour tissues and their correlation with androgen and oestrogen receptors. *J. Steroid Biochem.* **24** (1986) 1117–1125.
11. Van Landeghem A. A. J., Poortman J., Helmond-Agema A. and Thijssen J. H. H.: Measurement of endogenous subcellular concentration of steroids in tissue. *J. Steroid Biochem.* **20** (1984) 639–644.
12. Conover W. J. and Iman R. L.: Rank transformations as a bridge between parametric and non parametric statistics. *Am. Stat.* **35** (1981) 124–133.
13. Zumoff B.; Hormonal profiles in women with breast cancer. *Anticancer Res.* **8** (1988) 627–636.

14. Grattarola R.: Androgen in breast cancer. I. Atypical endometrial hyperplasia and breast cancer in married premenopausal women. *Am. J. Obstet. Gynecol.* **116** (1973) 423–428.
15. Grattarola R., Secreto G., Recchione C. and Castellini W.: Androgens in breast cancer. II. Endometrial adenocarcinoma and breast cancer in married postmenopausal women. *Am. J. Obstet. Gynecol.* **118** (1974) 173–181.
16. Grattarola R.: Ovariectomy alone or in combination with dexamethasone in patients with advanced breast cancer and high levels of testosterone excretion. *J. Natn. Cancer Inst.* **56** (1976) 11–19.
17. Secreto G., Recchione C., Cavalleri A., Miraglia M., and Dati V.: Circulating levels of testosterone, 17-oestradiol, luteinising hormone and prolactin in postmenopausal breast cancer patients. *Br. J. Cancer* **47** (1983) 269–275.
18. Secreto G. and Zumoff B.: Paradoxical effects associated with supranormal urinary testosterone excretion in premenopausal women with breast cancer: increased risk of postmastectomy recurrence and higher remission rate after ovariectomy. *Cancer Res.* **43** (1983) 3408–3412.
19. Secreto G., Fariselli G., Bandieramonte G., Recchione C., Dati V. and Di Pietro S.: Androgen excretion in women with a family history of breast cancer or with epithelial hyperplasia or cancer of the breast. *Eur. J. Cancer Clin. Oncol.* **19** (1983) 5–10.
20. Secreto G., Oriana S. and Recchione C.: Ovariectomy alone or in combination with dexamethasone in patients with advanced breast cancer and high levels of testosterone or androstanediol excretion. *Rev. Endocrine-Related Cancer.* **Suppl. 14** (1984) 55–58.
21. Secreto G., Recchione C., Fariselli G. and Di Pietro, S.: High testosterone and low progesterone circulating levels in premenopausal patients with hyperplasia and cancer of the breast. *Cancer Res.* **44** (1984) 841–844.
22. Secreto G., Toniolo P., Berrino F., Recchione C., Di Pietro S., Fariselli G. and De Carli A.: Increased androgenic activity and breast cancer risk in premenopausal women *Cancer Res.* **44** (1984) 5902–5905.
23. Secreto G., Toniolo P., Pisani P., Recchione C., Cavalleri A., Fariselli G., Totis A., Di Pierto S. and Berrino F.: Androgens and breast cancer in premenopausal women. *Cancer Res.* **49** (1989) 471–476.
24. Secreto G., Toniolo P., Berrino F., Recchione C., Cavalleri A., Pisani P., Totis A., Fariselli G. and Di Pietro S.: Serum and urinary androgens and risk of breast cancer in postmenopausal women. *Cancer Res.* **51** (1991) 2572–2576.
25. Ballerini P., Oriana S., Duca P., Martinetti A., Venturelli E., Ferrari L., Dolci S. and Secreto G.: Urinary testosterone as a marker of risk of recurrence in operable breast cancer. *Breast Cancer Res. Treat.* **26** (1993) 1–6.
26. McFadyen I. J., Forrest A. P. M., Prescott R. J., Groom G. V., Golder M. P. and Fahmy D. R.: Circulating hormone concentrations in women with breast cancer. *Lancet* **1** (1976) 1100–1102.
27. Malarkey W. B., Schroeder L. L., Stevens V. C., James A. G. and Lanese R. R.: Twenty-four hour preoperative endocrine profiles in women with benign and malignant breast disease. *Cancer Res.* **37** (1977) 4655–4659.
28. Adami H. O., Johansson E. D. B., Vegelius J. and Victor A.: Serum concentrations of estrone, androstenedione and testosterone and sex hormone-binding globulin in postmenopausal women with breast cancer and in age matched controls. *Upsala J. Med. Sci.* **84** (1979) 259–275.
29. Chen F., Wang S., Dai Q., Xing L., Lu W., Yang W. and Wang Z.: A study on plasma sex hormone levels in patients with breast cancer. *Chin. Med. J. (Engl. Ed.)* **98** (1985) 507–510.
30. Hill P., Garbaczewski L. and Kasumi F.: Plasma testosterone and breast cancer. *Eur. J. Cancer Clin. Oncol.* **21** (1985) 1265–1266.
31. Takatani O., Nosano H., Okumoto T., Akamatsu K., Tamakuma S. and Hiraide H.: Distribution of estradiol and percentage of free testosterone in sera of Japanese women: preoperative breast cancer patients and normal controls. *J. Natn. Cancer Inst.* **79** (1987) 1199–1204.
32. Adlercreutz H., Hamalainen E., Gorbach S. L., Goldin B. R., Wood M. N. and Dwyer J. T.: Diet and plasma androgens in postmenopausal vegetarian and omnivorous women and postmenopausal women with breast cancer. *Am. J. Clin. Nutr.* **49** (1989) 433–442.
33. Key T. J. A., Chen J., Wang D. Y., Pike M. C. and Boreham J.: Sex hormones in women in rural China and in Britain. *Br. J. Cancer* **62** (1990) 631–636.
34. Wang D. Y., Key T. J. A., Pike M. C., Boreham J. and Chen J.: Serum hormone levels in British and rural Chinese females. *Breast Cancer Res. Treat.* **18** (1991) S41–S45.
35. Wang D. J., Hayward J. C. and Bulbrook R. D.: Testosterone levels in the plasma of normal women and patients with benign breast disease or with breast cancer. *Eur. J. Cancer* **2** (1966) 373–376.
36. Wysowski D. K., Comstock G. W., Helsing K. J. and Lan H. L.: Sex hormone levels in serum in relation to development of breast cancer. *Am. J. Epidemiol.* **125** (1987) 791–799.
37. Pearce S., Dowsett M. and McKinna J. A.: Albumin-bound and non-protein-bound oestradiol and testosterone in postmenopausal breast disease. *Eur. J. Cancer* **27** (1991) 259–263.
38. Langer M., Kubista E., Scheinger M. and Spona J.: Androgen receptors, serum androgen levels and survival of breast cancer patients. *Arch. Gynecol. Obstet.* **247** (1990) 203–209.
39. Secreto G., Boracchi P., Buzzoni R., Venturelli E., Cavalleri A. and Dolci S.: Testosterone levels as a marker of prognosis to goserelin treatment in metastatic breast cancer. *Eur. J. Cancer* **30** (1994) 1629–1631.
40. Simon W. E., Albrecht M., Trams G., Dietel M. and Hölzel F.: *In vitro* growth promotion of human mammary carcinoma cells by steroid hormones, tamoxifen and prolactin. *J. Natn. Cancer Inst.* **73** (1984) 313–321.
41. Marugo M., Bernasconi D., Miglietta L., Fazzuoli L., Ravera F., Cassulo S. and Giordano G.: Effects of dihydrotestosterone and hydroxyflutamide on androgen receptors in cultured human breast cancer cells (EVSA-T). *J. Steroid Biochem. Molec. Biol.* **42** (1992) 547–554.
42. Hall R. E., Birrell S. N., Tilley W. D. and Sutherland R. L.: MDA-MB-453, an androgen-responsive human breast carcinoma cell line with high level androgen receptor expression. *Eur. J. Cancer* **30** (1994) 484–490.
43. Wales N. A. M. and Ebling F. J.: The control of apocrine glands of the rabbit by steroid hormones. *J. Endocr.* **51** (1971) 763–770.
44. Barthe P. L., Bullock L. P., Mowszowicz I., Bardin W. C. and Orth D. N.: Submaxillary gland epidermal growth factor: a sensitive index of biologic androgen activity. *Endocrinology* **95** (1974) 1019–1025.
45. Mazoujian G., Pinkus G. S., Davis S. and Haagensen D. E.: Immunochemistry of a gross cystic disease fluid protein (GCDFP-15) of the breast. *Am. J. Pathol.* **110** (1983) 105–112.
46. Wellings S. R. and Alpers C. E.: Apocrine cystic metaplasia: subgross pathology and prevalence in cancer. *Hum. Pathol.* **18** (1987) 381–386.
47. Dixon J. M., Lumdsen A. B. and Miller W. R.: The relationship of cyst type to risk factors for breast cancer and the subsequent development of breast cancer in patients with breast cystic disease. *Eur. J. Cancer* **21** (1985) 1047–1050.
48. Miller W. R., Telford J., Dixon J. M. and Shivas A. A.: Androgen metabolism and apocrine differentiation in human breast cancer. *Breast Cancer Res. Treat.* **5** (1985) 67–73.
49. Bryan R. M., Mercer R. J., Rennie G. C., Lie T. H. and Morgan F. J.: Androgen receptors in breast cancer. *Cancer* **54** (1984) 2436–2440.
50. Miller W. R., Telfors J., Dixon J. M. and Hawkins R. A.: Androgen receptor activity in human breast cancer and its relationship with oestrogen and progestogen receptor activity. *Eur. J. Cancer Clin. Oncol.* **21** (1985) 539–542.
51. Valyani S. H., Roberts J. V., Colletta A. A. and Iqbal J. M.: Sex hormone receptors in human breast cancer. Incidence and interrelationship. *Anticancer Res.* **7** (1987) 1281–1284.
52. Hahnel R. and Twaddle E.: The steroid specificity of the estrogen receptor of human breast carcinoma. *J. Steroid Biochem.* **5** (1974) 119–122.
53. Zava D. T. and McGuire W. L.: Androgen action through estrogen receptor in human breast cancer cell line. *Endocrinology* **103** (1978) 624–631.
54. Nicholson R. L., Davies P. and Griffiths K.: Interaction of androgens with oestradiol 17β receptor proteins in DMBA induced mammary tumours; a possible oncolytic mechanism. *Eur. J. Cancer* **14** (1978) 439–445.
55. Reed M. J.: The role of aromatase in breast tumours. *Breast Cancer Res. Treat.* **30** (1994) 7–17.

56. Bolufer P., Ricart E., Lluch A., Vazquez C., Rodriguez A., Ruiz A., Llopis F., Garcia-Conde J. and Romero R.: Aromatase activity in human breast cancer: its relationship to estradiol and epidermal growth factor receptors and to tumor-node-metastasis staging. *J. Clin. Oncol.* **10** (1992) 438–446.
57. Pasqualini J. R., Schatz B., Varin C. and Nguyen B. L.: Recent data on estrogen sulfatases and sulfotransferases activities in human breast cancer. *J. Steroid Biochem. Molec. Biol.* 41 (1992) 323–329.
58. O'Neill J. S., Elton R. A. and Miller W. R.: Aromatase activity in adipose tissue from breast quadrants: a link with tumour site. *Br. J. Cancer* **296** (1988) 741–743.