Possible Relevance of Steroid Availability and Breast Cancer

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INTRODUCTION

Breast cancer is the most frequent female malignancy in Western industrialized countries. Low incidence rates are observed in most Asian and African countries, intermediate rates in South Europe and South America. In high risk countries in Northern Europe and North America the incidence rates increase with age over the entire pre- and postmenopausal age span with some slowing down around the age of menopause. In intermediate risk countries like Greece, postmenopausal incidence rates do not further increase, showing a plateau. In low risk countries such as Japan, declining incidence rates are observed after menopause.[1] Studies of migrants from Japan to Hawaii or the North American continent, and of European compared to Asian and African born Jews migrating to Israël have demonstrated that high incidence rates are not related to genetic, but to environmental factors.[2,3] From these and other observations it appears that the marked differences in breast cancer incidence around the world are related to life style. This is most clearly demonstrated by the differences in age specific incidence rates after menopause. Studies of migrants and their second and third generation descendants have also indicated that breast cancer incidence is significantly influenced by life style factors, provided the latter have been acting at a preadult age. Environmental risk factors may affect both the initiation and the promotion of cancer. The experience with Japanese atomic bomb survivors, and women who underwent diagnostic or therapeutic irradiation involving the breast has shown, that radiation is undoubtedly associated with an increased breast cancer risk. Important is the observation that risk is highest when the breast has been exposed to radiation during the prepubertal to adolescent age period (10 to 19 years), *i.e.*, when breast tissue is still developing.[4,5] Taken together, the available data indicate that breast cancer in general may be initiated before the age of twenty, being modulated thereafter during various decades until clinical manifestation. As modulating factors hormones, reproductive function, which is closely related to hormones, and diet have received the most attention. Among the hormones which seem relevant to human breast cancer, estrogen takes a central position. The role of lactogens such as prolactin which is so obvious in rodent mammary tumor models, is still unresolved in man. The dietary intake of total fat, saturated or unsaturated fat or total energy may be important exogenous factors in human breast cancer.[1,6]

We shall discuss how we came to a working hypothesis which is to clarify the

action of estrogen and to explain how life style, more particularly dietary factors, may act through hormonal mechanisms.

ESTROGENS AND BREAST CANCER

Estradiol (E_2) is indispensable for the growth and development of breast epithelium and stroma. The increase of prepubertal ovarian estradiol production may already occur at the age of 8 years. The age of puberty onset is earlier in the Western industrialized countries, and inversely related to body size and obesity. Breast cancer risk is greater with early menarche or late age at menopause, but less if ovarian function is terminated by ovariectomy. The decrease of risk is greater, the earlier ovarian ablation is done.[7,8] These established risk factors point to the importance of ovarian hormones of which estradiol seems to be the most relevant. It is still unclear how early first full time pregnancy, *i.e.*, before the age of 20 years, or even further pregnancies, which imply exposure to high levels of estrogen (and various other hormones), can reduce breast cancer risk. Our tentative explanation is, that the specific hormonal conditions of pregnancy meet the requirements for differentiation rather than for growth of the breast epithelium, rendering the breast ready for lactation.[9] It is well known from experimental data, that differentiation can markedly reduce the growth potential of cells, including cells progressing to cancer cells. The evidence that estrogens contribute to breast cancer risk is still circumstantial. A major problem has been that estrogen levels in the blood of breast cancer patients or women at risk, have not been found to be unequivocally elevated compared to controls. The estrogen-unopposed-by-progesterone hypothesis put forward by Sherman and Korenman[10] has as yet received little support from objective data, although some studies have shown evidence for a more frequent occurrence of endocrine infertility[11] or luteal insufficiency[12] in women who later developed breast cancer. It has been found that, at least in some studies, postmenopausal patients tend to be obese, and that the amount of peripheral aromatisation of androstenedione to estrone is positively correlated with the amount of adipose tissue.[13,14] These findings, however, do not affect the objective observations that estrone levels in the blood of obese postmenopausal breast cancer patients do not significantly differ from nonobese patients or normal controls.

It is generally accepted that the growth of part of the clinically manifest human breast cancers is estrogen dependent. Evidence for this comes from the tumor regression which can be obtained by ovarian ablation, by treatment with antiestrogenic drugs or aromatase inhibitors and also, from the strong correlation between the tumor response and the presence of the estrogen receptor in the tumor. The effects of estrogens and antiestrogens on the growth of human breast cancer cells lines *in vitro* or on nude mouse xenograft models also depend on estrogen receptors. However, the heterogeneous occurrence of "receptor positivity" in fully developed breast cancer is very different from the hard to detect, but indispensable and more homogeneously distributed estrogen receptor at much lower concentration in normal or premalignant breast tissue. Therefore, estrogen receptor positivity of breast cancer seems to represent a receptor concentration which in some cancer cell clones has gotten out of hand, due to disturbed regulation rather than to a prolonged selection in an estrogenic milieu. In most studies looking into a possible connection between obesity or overweight as a presumed hyperestrogenic condition and estrogen receptor concentration in breast cancers, such a correlation could not be found.[15]

ESTROGEN AVAILABILITY AND BREAST CANCER

After the many unsuccessful investigations of urinary metabolites and plasma levels of estrogens the concept of steroid availability for biological action was introduced. Nonprotein bound or free E_2 was considered to be the most bioactive fraction of the plasma total E_2. Preliminary reports by Siiteri *et al.*[16] that free E_2, as a percentage of total E_2, was elevated in postmenopausal breast cancer patients compared to controls, were confirmed in most subsequent studies. The differences found were very small, however. We ourselves could not find an elevated proportion of free E_2 in pre- or postmenopausal breast cancer patients.[17] Neither could we confirm the existence of a difference in binding of E_2 to sex hormone-binding globulin (SHBG) as had been demonstrated by Moore *et al.*[18] Probably more important were the findings that breast cancer patients may have significantly more albumin-bound E_2 and less SHBG-bound E_2 compared to controls.[19–21] Of similar interest is the observation that normal Japanese women had proportionately less albumin-bound E_2, but more SHBG-bound E_2 compared to British women who are at a much greater risk of breast cancer.[22]

The notion that it is solely the nonprotein bound E_2 fraction (1 to 2%) which is bioactive may be incorrect. The much larger fraction (30 to 70%) bound to albumin may also be available. Reasons to believe this are threefold. Firstly, the affinity of binding to albumin is low.[23] Secondly, Pardridge has demonstrated that the fraction of radiolabeled E_2 which enters the rat brain is proportional to the amount of albumin-bound E_2 injected into the carotid artery.[24] Thirdly, we have demonstrated that the binding of E_2 to albumin and to SHBG is strongly influenced by free fatty acids.[25] We have shown that linoleic, linolenic and arachidonic acid (increasingly, in that order) displace E_2 from SHBG, which will result in greater binding of E_2 to the large albumin pool and a tendency to more free E_2, since the polyunsaturated free fatty acids easily displace E_2 also from albumin. The latter process is presumably most important near the endothelial surface. Relatively high FFA concentrations are formed locally when lipoprotein lipase (LPL), attached to the endothelium is activated to degrade triglycerides from chylomicrons and very low density lipoproteins. The resulting free E_2 subsequently enters the interstitial space to reach its target cells. This mechanism could also well explain Pardridge's observation that it is the albumin-bound fraction of E_2 which is proportional to uptake in the brain, as the albumin-bound E_2 as such is not expected to pass the blood brain barrier. The plasma $T_{1/2}$ of FFA is known to be very short, so that mean plasma concentrations are only weakly representative of the fatty acid and free E_2 kinetics at the endothelial level.

We were able to demonstrate that LPL activation by small doses of intravenous heparin does elevate both FFA and free E_2 in women. More physiologically relevant was our observation that the normal rise of FFA during overnight fasting is accompanied by a rise of free E_2. From these results we concluded, that we had found a possible link between estrogen availability and metabolic or dietary factors.[25,26]

LIFE STYLE AND ESTROGEN AVAILABILITY

One common denominator of the life style in Western industrialized countries is the affluent diet rich in fat, energy and protein. Another one is a more sedentary behaviour, which in combination with the affluent diet tends to render people less muscular and more obese. It is known that obese individuals tend to have higher

levels of VLDL, which constitute the main source of triglycerides from which fatty acids can be split off by lipase activity. Many studies have been undertaken to relate weight, weight for height, ideal weight or body mass index (kg/m^2) and breast cancer risk. One series of investigations, which demonstrated a relation between weight, body size and postmenopausal risk was done in the Netherlands by De Waard *et al.*[27-30] These investigators have postulated that the geographic differences in age-adjusted incidence rates which are so evident after menopause may be explained by differences in body weight or size. However, the results of some other investigators remain negative on this point.[31] A similar controversy has existed on the question whether cardiovascular risk and overweight are related. Recent observations seem to have presented a clue to this problem. When body fat distribution rather than obesity, defined in one way or another, was taken into account, it was found that coronary risk in middle-aged men is related to the accumulation of intraabdominal fat or central obesity, characterized by a high waist girth/hip girth ratio (W/H ratio).[32,33] Interesting hormonal and metabolic differences were observed between men and women having high compared to low W/H ratios.[34-37] A high W/H ratio is commonly observed in the Western over-40 year-old male and the postmenopausal female. A low W/H ratio is characteristic for the typical female fat distribution with fat accumulation in the gluteofemoral region. For the latter contours to develop in females an active endocrine ovarian function is required, although important individual and probably genetically determined differences exist. The gluteofemoral fat seems to be preserved as an energy store for the lactation period in the female, with a low metabolic turnover rate till then. The abdominal fat store on the other hand has a high metabolic turnover rate, yielding a high flux of triglycerides and free fatty acids towards the liver through the portal vein. As one of the consequences, the liver of individuals with a high W/H ratio produces much VLDL.[36,37]

Seidell *et al.* (unpublished results) have observed significant partial correlations of W/H ratio in women with VLDL-cholesterol and IDL-cholesterol in fasting serum. These lipid levels did not correlate anymore with body mass index or age after adjustment for W/H ratio. It was found that, in general, intraabdominal fat estimates correlate more strongly with serum lipids than estimates of subcutaneous fat do.

We have postulated now, that especially abdominal fat accumulation, which is phenotypically associated with a relatively high W/H ratio and which is metabolically associated with relatively high plasma levels of triglycerides, may cause greater availability of estradiol and testosterone.[26] When present at prepubertal age, this mechanism may enhance puberty onset. After puberty, and in particular after menopause when this type of fat distribution is so common in the Western world, more available E_2 can contribute to the promotion of cancers of the breast and the endometrium. Our hypothesis may also help to explain why very lean, physically very active athletic girls and ballet dancers have a late puberty onset and poor breast development.[38-41] That greater physical activity at college age reduces the risk of breast and other endocrine-related cancers later in life has been recently reported by Frisch *et al.*[42] This observation underlines again that the pubertal and adolescent age period is already very important for the modulation of cancer through metabolic and hormonal factors, which seem to be closely related.

To test our hypothesis we currently perform a number of investigations. In a case-control study we compare body fat distribution, serum lipids and estrogen availability, *i.e.*, binding to serum proteins. This study involving 675 women is being carried out in the Netherlands Cancer Institute in cooperation with the Preventicon Cancer Screening Centre in Utrecht and the Department of Radiotherapy of the University Hospital Utrecht. Preliminary results from this study clearly demonstrate that W/H ratio and body mass index are not correlated at all, indicating that W/H ratio is indeed a new way of looking at adipose tissue or obesity in this category of women.

We compared the serum SHBG levels with height, body weight, body mass index and waist/hip ratio by linear regression analysis in 42 apparently healthy postmenopausal women (mean age ± SD 57.5 ± 1.3 years). The mean SHBG concentration, determined by IRMA (Farmos Diagnostics, Oulunsalo, Finland), was 46.6 ± 22.0 nM (range 21 to 121 nM). No correlation was found between SHBG and height or weight (mean ± SD 71.2 ± 11.2 kg). A significant correlation existed between SHBG and body mass index ($r -0.31$; $p < 0.05$). However, the strongest correlation was observed between SHBG and waist/hip ratio ($r -0.52$; $p < 0.001$). One possible explanation for the decrease of SHBG related to central obesity may be the following. The increased flux of triglycerides and free fatty acids to the liver may exert a decreased sex steroid to protein binding, *i.e.*, greater availability. The change in androgen/estrogen signal may then result in a decrease of hepatic SHBG production. These preliminary findings support our hypothesis that the metabolic consequences of central fat accumulation are related to sex steroid availability.

A nested case-control study is part of a prospective investigation involving 19,000 women in the Guttman Breast Diagnostic Institute in New York (directed by Dr. Ph. Strax) in collaboration with the Department of Environmental Medicine of the New York University Medical Center (B. S. Pasternack, R. E. Shore *et al.*) and the Department of Endocrinology of the ICRF in London (J. W. Moore, D. Y. Wang *et al.*). Pubertal development, body fat distribution, serum lipoproteins and sex steroid protein binding are currently prospectively investigated in 8 to 14 year-old schoolchildren in Utrecht, in collaboration with the Department of Exercise and Sports Physiology of the University of Utrecht (W. Erich, Chr. de Ridder *et al.*) and the Laboratory of Endocrinology of the University Hospital Utrecht (J. H. Thijssen, M. A. Blankenstein). Mensink and Katan[42] have recently reported a decrease of serum triglycerides by 0.06 mM ($p < 0.001$) after a change from a Western diet, high in saturated (20 energy %) and total fat (38 energy %), to an isocaloric olive-oil-rich diet (containing oleic acid 24 energy %, total fat 41 energy %). Within the same intervention study, performed at the Department of Human Nutrition of the Agricultural University in Wageningen, the Netherlands, we have made the preliminary observation that the olive-oil-rich diet also lead to a significant reduction of serum linoleic acid levels. This could not be ascribed to a different intake of polyunsaturated fat, since the polyunsaturated fat content was similar (4 to 5 energy %) for both diets. These findings may have consequences for a decreased availability of E_2 which may be reflected by the intermediate breast cancer incidence rate in countries such as Greece.

The average per caput consumption of calories, fat or meat protein shows a linear correlation with mortality from breast, endometrium and prostatic cancer in many countries.[43,44] It is generally accepted that endogenous and exogenous estrogens increase the risk of endometrial cancer, which is also clearly related to obesity.[46,47] The development of prostatic carcinoma is dependent on testosterone. A high incidence of invasive cancer of the prostate in American Japanese or blacks was related to a relatively high fat intake, compared to Japanese living in Japan[48] or blacks in Nigeria.[49] The incidence rates of latent prostatic cancer were similar.

CONCLUSIONS

Complex relationships exist between steroid availability and life style factors, such as dietary intake and physical activity. The metabolic consequences of body composition and especially body fat distribution appear to influence the binding of sex steroids

to SHBG and albumin. Further steroid protein binding studies may contribute to the understanding of the promoter role of sex steroids in the etiology of cancers of the breast, the endometrium and the prostate.

SUMMARY

The as yet circumstantial evidence for a central role of estrogens in the promotion of human breast cancer is supported by many data. However, it has not been possible to identify breast cancer patients or women at risk by abnormally elevated estrogen levels in plasma. The concept of available, *i.e.*, non-SHBG bound sex steroid seems to offer a better understanding than total serum steroid levels do. We demonstrated that sex steroid protein binding is decreased by free fatty acids. This finding may help to explain how the affluent Western diet and sedentary life style is related to high incidence rates of breast cancer. We have postulated that it is especially the central (abdominal) type of obesity which may increase sex steroid availability. This mechanism could be important already at the age of breast development when the sensitivity to promotion seems relatively great. It may also explain the increased incidence rates which are observed in Western industrialized countries after menopause. It seems likely that other endocrine-related cancer, such as endometrial or prostatic carcinomas are influenced in an analogous way.

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the "PRL reserve capacity" of the pituitary gland. The basic mechanism of this investigation is the following. The PRL secretion is regulated by two hypothalamo-hypophyseotrophic messengers, a PRL releasing factor (PRF) and an inhibiting factor (PIF).[23] In humans, regulation of PRL secretion is predominantly based on the inhibitory effect of the hypothalamus which secretes and delivers PIF via the hypophyseal portal circulation. PIF is supposed to be dopamine (DA). The PIF secretion is under dopaminergic control, so most of the PRL-stimulating agents act by blocking DA synthesis or DA receptors. Although TRH does not seem to be involved in the physiological control of PRL release, a single intravenous injection of 400 μg of TRH is followed by a rapid rise of PRL with peak values recorded at 15 min. Therefore, TRH seems to be suitable for measuring the "PRL reserve capacity" of the adenohypophysis. Presumably it has a direct action upon the pituitary gland. Mechanisms blocking the DA/PIF release elevate serum PRL. Accordingly, this physiologic system can be considered as a normal stimulation test to control PRL dynamics. At the same time, measurement of the TRH induced PRL release during the administration of different antiestrogen drugs can provide information about the PRL synthesizing ability of the hypophysis under the influence of these compounds.

400 μg of TRH injection (Institute for Drug Research, Budapest, Hungary and Ferring AB, Malmö, Sweden) was given intravenously to the patients. For the measurement of PRL level blood samples were taken at 0, 15, 30 and 120 min after TRH administration. The TRH provocation test was performed before TAM or TOR treatment and in the 2nd, 8th and 12th week after the therapy. The TRH-induced PRL release of 10 postmenopausal healthy women served as normal control for TAM and the PRL secretion of 8 volunteers was the control for the TOR study.

Patients' Response to the Treatment

The clinical response of the patients investigated was assessed using WHO criteria. (Complete response: CR, partial response: PR, no change: NC, progression: PROGR). After 12 weeks, TAM treatment was considered successful in 18 out of 30 patients. The other 12 patients failed to respond to the therapy. The investigation of TOR is an ongoing study; clinical evaluation is not yet finished.

RESULTS

Tamoxifen

Serum Hormones

The E_2 levels of cancer patients were above the normal postmenopausal range (normal: 36.7-146.8 pmol/litre; patients: 175.8-250.4 pmol/litre). The E_2 concentrations of responders continuously increased during the treatment reaching a peak value at the 2nd week (375 ± 50 pmol/litre). Then it decreased to the initial value. This transient elevation could not be observed in nonresponders (FIGURE 1). The

levels of PROG, TE, FSH, LH and basal PRL did not show any significant changes either in responders or in nonresponders. The hormone levels were within the normal postmenopausal range.[22]

Serum SHBG

The SHBG binding capacity (mean ± SE) was significantly ($p < 0.01$) higher after an 8-week treatment period than the mean initial value (27.08 ± 15 nmol/litre vs. 44.24 ± 15 nmol/litre). However, this elevation was not in correlation with the clinical response of the patients (FIGURE 2).

TRH Functional Test

The TRH-inducible PRL release of breast cancer patients was higher (1,800 ± 245 mIU/litre) than that of the normal control group (810 ± 150 mIU/litre). Maximal PRL stimulation occurred at the 15th min after TRH injection: 2,213 ± 372 mIU/litre in healthy women and 5,600 ± 800 mIU/litre in cancer patients. After an 8-week TAM treatment there was a significantly ($p < 0.01$) suppressed PRL

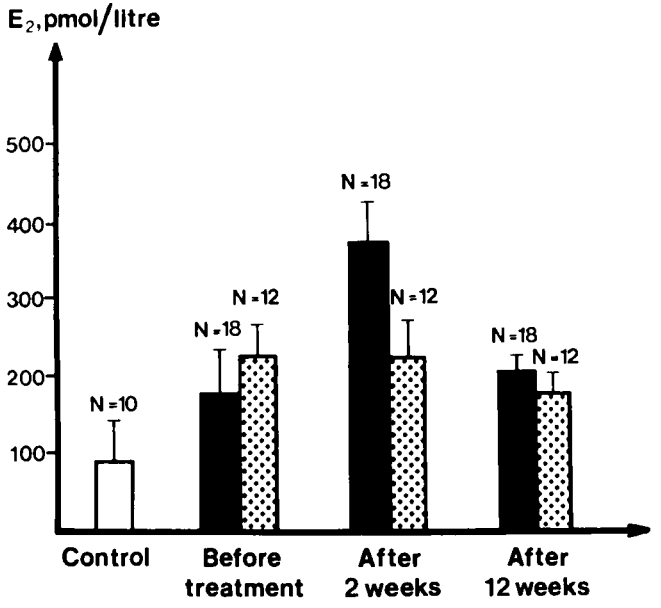

FIGURE 1. Serum E_2 concentrations (mean ± SE) in healthy women (□) and in breast cancer patients treated with TAM. E_2 level of responders (CR, PR, ■) showed a transient elevation at the 2nd week of the therapy. (Nonresponders: NC, PROGR, ▦).