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Perspectives and Commentaries

Prolactin and Breast Cancer

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(A COMMENT ON: Wang DY, Hampson S, Kwa HG *et al.* Serum prolactin levels in women with breast cancer and their relationship to survival. *Eur J Cancer Clin Oncol* 1986, **22**, 487–492.)

THE PAPER by Wang *et al.* [1] addressed the debate on the possible role of prolactin (PRL) in human breast cancer. In rodents, the central role of this hormone in the initiation and maintenance of induced, as well as spontaneous, mammary tumours is well documented. It seems therefore likely that PRL is also involved in human breast carcinogenesis and everyone accepts the participation of PRL in the genesis of breast cancer. However, evidence against this hypothesis came from negative results in a phase II clinical therapeutic trial with the PRL inhibition by bromocryptine [2]. It is possible that oral bromocryptine did not suppress PRL levels down to zero concentration. Furthermore, bromocryptine produces a mild stimulation of the secretion of growth hormone (GH), another hormone of the lactogenic family which binds to the lactogenic receptors in mammary tissue and in human breast tumours. Thus, an activation of lactogenic receptors in mammary tumor may have been caused by an elevation of GH secretion. The fact that hyperprolactinaemia (as induced by pituitary stalk transsection or by treatment with massive doses of oestrogens) cannot prevent tumour regression in 'endocrine-sensitive' cases, seems also quite inconsistent with a major role of PRL in human breast cancer. It should be recalled that, in some animal models, PRL has been shown to be, on the contrary, beneficial, preventing carcinogen-induced neoplastic processes or inducing differentiation of the neoplastic mammary epithelium and inhibition of its growth [3]. Furthermore, even in the case of the DMBA-induced mammary cancer, advanced stages appear to be PRL-independent. There exists thus the possibility that PRL might be involved in two separate processes, that of carcinogenesis on the one hand and, on the other hand, that of maintenance (or possibly growth stimulation) of an established breast cancer.

Measurement of PRL concentrations in women with breast cancer has yielded conflicting results which can partly be caused by the difficulties in assessing PRL secretion, because of the numerous factors known to influence its secretion. These include the oestroprogestative endocrine balance, the time of the day or night, stress effects, drug effects and diet. Furthermore, PRL is secreted in a pulsatile episodic fashion, with a short half-life of about 20 min. It is thus not surprising that not all studies found a slight but significant elevation of PRL levels in women with familial risk for breast cancer [4]. It should be emphasized that in studies with positive findings, the reported elevations of PRL concentration were quite minimal. Since the development of human breast cancer takes many years, it is, however, only those combined epidemiological and endocrine studies which could shed any light on this matter. There is no evidence that long-term use of neuroleptics in psychiatric patients have increased the risk of breast cancer [5], despite the well documented, systematic and consistent, induction of hyperprolactinaemia by neuroleptics. The argument that PRL secretion induced by neuroleptics may be biologically inactive does not hold because menstrual cycle disturbances and galactorrhoea are often observed in these patients. Therefore, one has to question whether an increased PRL secretion might be the cause of (or an adjuvant factor to) breast carcinogenesis or, on the contrary, be the result of the

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factor(s) responsible for such carcinogenesis. Taking into account the latter hypothesis, oestrogens could be a good candidate since it is well established that PRL secretion is directly related to the level of oestrogenic stimulation. However, the bulk of epidemiologic evidence is largely against any association between long-term use of oral oestro-progestative contraceptives and development of breast cancer. Similarly, after reviewing the literature relating the relative risk of breast cancer to hormonal replacement therapy after menopause [6], we concluded, from the prospective studies available, that moderate doses of oestrogens actually reduced the relative risk of developing breast cancer; this effect was even more pronounced when oestro-progestative preparations were utilized.

Furthermore, a recent study [7] has demonstrated that, after having been treated for breast carcinoma, a subsequent pregnancy did not alter the prognosis of the disease. Pregnancy is not only characterized by hyperprolactinaemia but also by massively increased secretions of oestrogens, progestogens, human placental lactogen (another lactogenic hormone with high GH immunologic but little GH biologic activity) and other factors possibly involved in breast cancer. However, Musey *et al.* [8] reported long-term decreased basal and perphenazine-stimulated PRL levels after pregnancy. They concluded that the known protective effect against subsequent breast cancer of an early first full term pregnancy may be mediated by this long-term depression of PRL secretion. Thus, it appears that PRL *per se* cannot play a unique role in breast carcinogenesis and that its effect, if any, would be slight and mostly of a modulatory nature and/or perhaps at crucial sensitive periods. Nevertheless, a variety of epidemiologic risk factors have been identified, which imply a deficient oestro-progestative balance with oestrogenic dominance. Also, high dietary fat intake induces a greater PRL secretion [9], and such a diet is associated with a greater risk for breast cancer than a low fat diet. However, there is no evidence that these factors would increase the risk of breast cancer through increased PRL secretion.

The effects of prolactin could depend on the sensitivity of the mammary epithelium and/or on its state of development. Prolactin could be protective in some instances (e.g. in full term pregnancy) but contribute to breast cancer during puberty and adolescence, because of some mitogenic activity of PRL, besides its major role on differentiation for lactogenesis.

One major issue is that most of the work presently available, as reported above, has been performed using radioimmunoassays for human prolactin. Immunoreactive PRL may not be identical to PRL that is biologically active, especially with relevance to the growth of human breast epithelium. As extensively reviewed recently by Subramanian and Gala [10], circulating PRL is quite heterogeneous: besides the classical, monomeric, 'little' PRL, there are two other circulating forms: the 'big' and the 'big-big' PRL. These high molecular weight forms of prolactin are apparently polymers of little PRL, that constitute a true secretory product of the pituitary gland and not an artefact of peripheral alteration. In most instances, the predominant form in serum is little PRL but shifts in the proportions of PRL heterogeneity have been reported under a number of physiological, pathological and experimental conditions: pregnancy, sleep-induced rise in PRL, TRH-induced plasma PRL increase, prolactinoma or acromegaly patients, glucose tolerance test, galactorrhoea despite normal prolactin levels, normal menstrual and gonadal function in presence of high PRL levels. These PRL variants generally exhibit comparable activity in classical radioimmunoassays, with parallel inhibition curves. Thus, shifts in PRL heterogeneity would remain undetected using routine radioimmunoassays. Although it results from some studies that the various forms of PRL exhibit comparable binding affinity to membrane receptors, most of the evidence is in favour of less bioactivity for the large molecular weight variants of PRL, especially in studies using a true bioassay (Nb_2 lymphoma cells).

In animals, PRL size heterogeneity has been correlated with high or low incidence of spontaneous mammary tumours, and with high or low susceptibility to mammary tumourigenesis by chemical carcinogens. It is striking that the same heterogeneity shift—namely a greater percentage of high molecular weight forms—has been found in subjects with breast cancer [10] and in mice strains with a high incidence of mammary tumours.

In addition, smaller fragments of the PRL molecule can also be found in the circulation. In the rat, a 16 K polypeptide cleaved PRL was reported to exhibit a significant *in vivo* stimulatory effect upon DNA synthesis and cell division of mammary epithelial cells [12]. Therefore, efforts should be placed upon confirmation of its existence and of its specific mitogenic activity in the human, as well as on the development of a specific (radioimmuno)assay for this fragment. It is not yet known whether such a 16 K PRL fragment is active or not in the new bioassay for lactogenic hormones, based on the Nb_2 lymphoma cells culture. Nevertheless, using this assay, Love and Rose [13] found elevated bioactive 'PRL', up to 15 times the RIA levels, in eight women who had at least

two first-degree relatives with breast cancer and a history of familial breast cancer. Their conclusion, that a mitogenic 'PRL' species not recognized by RIA is elevated in a homogeneous subset of women at risk for familial breast cancer, strongly supports a possible promotional or permissive role for 'PRL' in the induction of breast cancer, at least in sensitive subjects and perhaps at privileged periods of life. These data need, however, confirmation by others, as well as extension to other risk factors and to patients with actual breast cancer.

Using the classical RIA for PRL, Wang *et al.* [1] have reported in patients with operable breast cancer highly significantly elevated PRL levels 10 days after mastectomy, as compared to preoperative levels, with normalization at 3–12 months postoperatively. The increased PRL level at 10 days cannot be attributed either to stress at surgery or to the drugs used for premedication, anaesthesia and analgesia. The likely cause of this increase has been clearly missed and even not discussed by Wang *et al.* [1]. The normalization of PRL levels at 3 months and up to 12 months, suggests, however, that it was the surgical procedure itself and not the disease which was in some way responsible. As is well documented, any kind of chest wall injury (surgical, traumatic or viral) can induce transiently the occurrence of galactorrhoea which is due to a transient hyperprolactinaemia. This hyperprolactinaemia is thought to be induced by stimulation of the neurogenic component of the suckling-induced mechanism leading to increased PRL secretion during lactation. This matter was recently reviewed by Holtkamp *et al.* [14], who clearly demonstrated such elevated PRL levels, up to 30 days after mastectomy but not after tumourectomy (even for a malignant breast lesion). Nevertheless, Wang *et al.* [1] identified subgroups of patients in which either preoperative and/or postoperative PRL levels were significantly correlated to survival: in all cases, the least favourable prognosis was associated with the highest PRL levels. This general trend has been confirmed by Dowsett *et al.* [15] in early disease, as well as in advanced disease, by Dowsett *et al.* [16] and by Holtkamp *et al.* [17]. The higher the PRL level prior to treatment, the less probability of response to endocrine treatment or even to chemotherapy; furthermore, hyperprolactinaemic patients experiencing remission after chemotherapy exhibited a return of their PRL to normal. All these data would thus indicate either that PRL might be significant in the support of breast cancer growth *in vivo* (and for example enhance the survival of cells disseminated at operation) and/or conversely that hyperprolactinaemia might be only a consequence, by an unknown mechanism, of the progression of the disease. The possibilities should therefore be explored that aggressive breast cancer would be associated with the secretion of an unknown factor acting at the hypothalamo-pituitary level to enhance PRL secretion, or perhaps that the tumour itself secretes PRL.

The hypothesis that PRL might support the growth of breast cancer *in vivo* is indeed supported by *in vitro* experiments, as reviewed and confirmed by Peyrat *et al.* [18]: physiological concentrations of PRL can promote the growth as well as DNA synthesis of human breast cancer cells in a small proportion (15–16%) of cases. The prerequisite for action of a peptidic hormone is to bind to a specific membrane receptor: such PRL-receptors are indeed present in about 19% of the cases of human breast cancer [19]. Furthermore Peyrat *et al.* [18] reported that *in vitro* stimulation of DNA synthesis by PRL never occurred in the absence of PRL-receptors. The whole picture fits well with the report by Waseda *et al.* [20], that PRL-receptor positive patients had a significantly reduced survival rate than the PRL-receptor negative group.

Therefore further investigations to show a significant clinical role of PRL in established breast cancer should involve new therapeutic trials. They would require the combined administration (preferably as injectable or implantable long-acting formulas) of a dopaminergic drug to inhibit PRL and of a somatostatin analog to suppress simultaneously GH secretion. The latter hormone, which possess biological lactogenic properties, has been shown to be elevated in 40% of 42 breast cancer patients [21] and to bind to PRL-receptors. Ideally the analysis of such trials should take into account at least PRL secretion (pre-, peri- and post-operative levels as well as before, during and after therapy) and the PRL-receptor status. One might discuss the opportunity to add or not tamoxifen to this anti-hormonal regimen, for example in case of positive oestrogen-receptors. In addition of being used in advanced disease, it would also be worth testing such regimen around mastectomy and perhaps as adjuvant therapy and—why not?—in subjects with familial risk. The hope is to identify at least a subgroup, perhaps small, which would benefit of a new 'endocrine' treatment.

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