Prostacyclin and thromboxane in breast cancer: Relationship between steroid receptor status and medroxyprogesterone acetate

A. Aitokallio-Tallberg\textsuperscript{1}, J. Kärkkäinen\textsuperscript{1}, P. Pantzar\textsuperscript{2}, T. Wahlström\textsuperscript{1,3} & O. Ylikorkala\textsuperscript{1}

1Department of Obstetrics and Gynecology, 2Department of Radiotherapy and Oncology and 3Department of Pathology, University of Helsinki, Finland

Summary To study the production and significance of prostacyclin (PGI\textsubscript{2}) and thromboxane A\textsubscript{2} (TXA\textsubscript{2}) in breast cancer, tissue fragments of breast cancer (n = 23) and mastopathy (n = 10) were superfused in vitro and the release of 6-keto-PGF\textsubscript{1}alpha (a metabolite of PGI\textsubscript{2}) and TXB\textsubscript{2} (a metabolite of TXA\textsubscript{2}) measured by radioimmunoassay. Breast cancer formed more 6-keto-PGF\textsubscript{1}alpha (4.5 ± 0.9 ng min\textsuperscript{-1} g\textsuperscript{-1} of tissue dry weight, mean ± s.e.) and TXB\textsubscript{2} (2.5 ± 0.6 ng min\textsuperscript{-1} g\textsuperscript{-1}) (P < 0.01) than did mastopathic breast (1.4 ± 0.5 and 0.4 ± 0.1 ng min\textsuperscript{-1} g\textsuperscript{-1}, respectively). These productions were similar in steroid receptor positive and negative tumours. Breast cancer metastasized in 15 patients during the follow-up time of 3.7 ± 0.7 years, but the initial prostanoid productions in these patients were not different from those in nonmetastatic patients. Two patients died from metastases, but their initial mammary production of prostanoids was not profoundly different from those in the survivors. In 8 patients (4 with steroid receptor positive and 4 with negative tumour), the cancer tissue was superfused in the presence or absence of medroxyprogesterone acetate (100–5000 ng ml\textsuperscript{-1}), which is commonly used for treatment of breast cancer. This hormone had no effect on mammary PGI\textsubscript{2} and TXA\textsubscript{2} production. We thus conclude that the PGI\textsubscript{2} and TXA\textsubscript{2} productions are increased in mammary cancer but that this may not be of primary significance for metastatic spread.

It is well established that human breast cancer produces increased amounts of classic prostanolphins (PG) belonging to the E and F series (Bennett et al., 1977; Rolland et al., 1980; Karmali et al., 1983). This increase seems to be relative to the cancers tendency to metastasize to the bones (Bennett et al., 1977; Rolland et al., 1980). Moreover, PGE may be responsible for the hypercalcaemia often present in patients with bone metastasis (Tashjian et al., 1978). Taken together, these data suggest a role for classic PGs in the metastasis of breast cancer. On the other hand, circulating platelets may also be a factor in metastatic spread because only the cancer cells which bind platelets to their surface, may form a metastasis (Marcum et al., 1982, Honn et al., 1983). Therefore, antiaggregatory prostacyclin (PGI\textsubscript{2}) and its endogenous antagonist, thromboxane A\textsubscript{2} (TXA\textsubscript{2}), are of great interest in this regard and, indeed, a dominance of PGI\textsubscript{2} over TXA\textsubscript{2} in the circulation seems to decrease the risk of metastasis in experimental animals (Honn et al., 1981; 1982).

Because nothing is known about the significance of the local production of these platelet active prostanoids in breast cancer, we studied the formation of PGI\textsubscript{2} and TXA\textsubscript{2} by metastatic and nonmetastatic breast cancer. Also the effect of medroxyprogesterone acetate (MPA an agent which is commonly used for treatment of advanced breast cancer, on the tumour PGI\textsubscript{2}/TXA\textsubscript{2} was examined.

Materials and methods

Twenty-three patients with ductal adenocarcinoma and ten women with benign mastopathy were studied (Table I). Breast samples taken at surgery were divided into two identical parts. One of them was used for histologic examination of this sample proved to be representative consisting mostly of cancer cells, the other identical sample, which was stored frozen in liquid nitrogen, was later used for biochemical studies. These included the determination of oestrogen and progesterone receptors, as described before (Vihko et al., 1980), and the measurement of PGI\textsubscript{2} and TXA\textsubscript{2} production in vitro with a tissue superfusion method (Mäkilä et al., 1982). Briefly, fragments of breast cancer or noncancerous tissue (some 10–20 mg of tissue dry weight) were gently minced with scissors in Eagle’s medium (Gibco, Biocult 189 G, Paisley, UK.)

Correspondence: O. Ylikorkala, Departments of Obstetrics and Gynecology, Helsinki University Central Hospital, Haartmaninkatu 2 SF-00290 Helsinki 29, Finland.
Received 19 November 1984; and in revised form, 10 January 1985.
Table I  Clinical characteristics of the study population (mean ± s.e.)

| Diagnosis                  | N  | Age (years) | Follow-up time (years) |
|----------------------------|----|-------------|------------------------|
| Ductal adenocarcinoma      |    |             |                        |
| all                        | 23 | 60.4 ± 2.7  | 3.7 ± 0.7              |
| Receptor positivea         | 11 | 66.7 ± 4.2  | 4.7 ± 1.4              |
| Receptor negativeb         | 12 | 54.4 ± 2.6  | 2.8 ± 0.4              |
| Poorly differentiated      | 16 | 61.5 ± 3.3  | 3.5 ± 0.8              |
| Well differentiated        |  7 | 57.6 ± 3.6  | 2.6 ± 0.2              |
| Metastasis                 | 15 | 58.8 ± 2.9  | 3.7 ± 0.8              |
| No metastasis              |  8 | 60.4 ± 3.2  | 2.2 ± 0.2              |
| Mastopathy                 | 10 | 55.2 ± 5.4  | 2.3 ± 3.0              |

*aBoth oestrogen and progesterone receptors present.  
bBoth oestrogen and progesterone receptors absent.

added with 0.1% of bovine albumin serum. The samples were then perfused with the same medium which was gassed continuously with oxygen (95%) and carbon dioxide (5%) at pH 7.4. After a 2.5 h wash-out period, a 1 h fraction was collected and its contents of 6-keto-PGF1alpha and TxB2, the stable hydration products of PGI2 and TxA2, respectively, were measured by established radioimmunoassays (Yilikorkala & Viinikka, 1982; 1980). The result was expressed as ng of prostanoid min⁻¹ g⁻¹ of tissue dry weight. The storage of tissue in a frozen state did not affect the PGI2 and/or TxA2 release, which was, however, dose-dependently inhibited by the addition of various PG synthesis inhibitors in the perfusion buffer (Mäkilä et al., 1982).

To study the effect of medroxyprogesterone acetate (MPA) on the tumour PGI2 and TxA2 production, tumour samples (4 receptor positive and 4 negative) from 8 patients were cut into 5 identical pieces and perfused in the presence or absence (controls) of MPA (Carlo Erba, Milan, Italy) in concentrations of 100, 500, 1000 and 5000 mg ml⁻¹ for a period of 1 h after a 2.5 L wash-out period. MPA was added into the perfusion medium in ethanol, which was also added into the control perfusion medium. Ethanol did not interfere with the release of these prostanoïds or with the radioimmunoassays of 6-keto-PGF1alpha and TxB2 at the concentrations (0.5%) used in this work. Moreover MPA did not interfere with the radioimmunoassays employed in this study.

After the initial diagnosis the patients were treated according to the guidelines generally approved for the therapy of breast cancer. We were able to follow them up to 3.7 ± 0.7 years (mean ± s.e.) (Table I). During this time 15 patients developed metastasis (axillary lymph nodes in 13, bone in 4, skin in 3, lung in 2, the other breast in 2, brain in 1). Two patients with receptor negative cancer died due to breast cancer during the follow-up time.

The data were subjected after logarithmic transformation to the paired and nonpaired t-test and linear regression analysis.

Results

Breast cancer released more 6-keto-PGF1alpha and TxB2 than did mastopathy (Table II). The rise in TxB2 production was relatively greater than 6-keto-PGF1alpha production, and consequently, the ratio of 6-keto-PGF1alpha to TxB2 tended to be smaller in breast cancer than mastopathy (Table II).

The production of neither 6-keto-PGF1alpha nor TxB2 was related to the steroid receptor status or the subsequent metastasis of the initial tumour (Table III). Prostanoid production was greater in more highly differentiated tumours than in anaplastic ones although the difference reached statistical significance only for TxB2 (Table III).

Two patients died due to the metastatic breast cancer within 5 years of the initial diagnosis. Their

Table II  Production of 6-keto-PGF1alpha and TxB2 (ng min⁻¹ g⁻¹ of dry weight tissue, mean ± s.e.) in breast cancer and mastopathy in vitro.

|                | N  | 6-keto-PGF1alpha | TxB2 | 6-keto-PGF1alpha/TxB2 |
|----------------|----|------------------|------|-----------------------|
| Breast cancer  | 23 | 4.5 ± 0.9        | 2.5  | 2.6 ± 0.4             |
| Mastopathy     | 10 | 1.4 ± 0.5        | 0.4  | 3.6 ± 0.6             |
| Significance   |    | P<0.025          | P<0.0125 | P<0.10 |

Table III  Production of 6-keto-PGF1alpha and TxB2 (ng min⁻¹ g⁻¹ tissue dry weight, mean ± s.e.) in various subgroups of patients with breast cancer

| Subgroups         | N  | 6-keto-PGF1alpha | TxB2 | 6-keto-PGF1alpha/TxB2 |
|-------------------|----|------------------|------|-----------------------|
| Metastasis        | 15 | 4.8 ± 1.3        | 2.9  | 1.6 ± 1.5             |
| No metastasis     |  8 | 4.1 ± 0.8        | 1.7  | 2.3 ± 0.6             |
| Poorly differ.    | 16 | 3.8 ± 0.9        | 1.8  | 2.6 ± 0.4             |
| Well differentiated|  7 | 6.2 ± 2.0        | 4.1  | 1.4 ± 1.4             |
| Ster. rec. posit. |  9 | 3.8 ± 0.8        | 2.6  | 2.5 ± 0.5             |
| Ster. rec. negat. | 12 | 5.2 ± 1.5        | 2.4  | 2.7 ± 0.6             |

aP<0.05 in comparison with poorly differentiated.
mammary productions of 6-keto-PGF1alpha were 2.6 and 12.7 ng min⁻¹ g⁻¹ and those of TxB2 1.4 and 11.7 g min⁻¹ g⁻¹, respectively.

MPA had no effect on 6-keto-PGF1alpha and TxB2 production by the steroid receptor positive and/or negative cancer (Figure 1).

Discussion

Substantial evidence suggests that the balance between the systemic antiaggregatory prostacyclin (PGI2) and proaggregatory thromboxane (TxA2) is significant in tumour metastasis (Honn et al., 1981; 1983). This balance may also be an important determinant inside the cancer cells, because only the malignant cells which can bind platelets onto their surface in the circulation, may form metastases (Honn et al., 1983). Ductal breast cancer metastasizes readily, and therefore we studied its production of PGI2 and TxA2. It is very difficult to obtain samples of normal ductal epithelium therefore we used benign mastopathy as a control tissue.

It is clear from our data that breast cancer tissue produces more PGI2 and TxA2 than does mastopathic tissue. Relatively, the rise in TxA2 production was greater than that of 6-keto-PGF1alpha, and therefore, the ratio of 6-keto-PGF1alpha to TxB2 was decreased in breast cancer. Our findings obtained by the tissue superfusion method are in general agreement with the data of Karmali et al. (1983) who extracted 6-keto-PGF1alpha and TxB2 from breast cancer and who also demonstrated the synthesis of those prostanoids in the microsomal fraction of breast cancer cells. Bearing in mind the overall stimulation in prostaglandin synthesis by cancer cells including breast cancer (Bennett, 1979) we may speculate that the cancer cells themselves released increased amounts of 6-keto-PGF1alpha and TxB2 in superfusion, although a contribution by the inflammatory cells unavoidably associated with cancer cells cannot be excluded.

It has been proposed that the elevated production of various classic prostaglandins could be used as a marker of the high metastatic potential for neoplastic cells in breast cancer (Bennett et al., 1977; 1979; Rolland et al., 1980). In this regard, PGI2 and TxA2 with their potent but opposing effects on platelets, may be theoretically more promising indices of metastatic capacity of the primary cancer (Honn et al., 1981; 1983). We followed our patients for a sufficiently long period to assess the prognostic significance of PGI2 and TxA2 production. There was no difference in the production between patients with and without subsequent metastasis. Moreover, the production was similar in cancers with and without steroid receptors, although the presence of these receptors indicates a more favourable prognosis (Martin et al., 1979), as is also evident from the present series. These data may imply that the measurement of the production of PGI2 and/or TxA2 by breast cancer cannot be used as an indicator of the metastatic potential of breast cancer.

High doses of MPA can be effective in the treatment of advanced breast cancer, even in cases which have failed to respond to other hormonal or cytostatic agents (Mannes et al., 1976; Brunner et al., 1977; Pannuti et al., 1978). The mechanism of this action of MPA is not understood. In view of the possible role of prostaglandins in cancer (Bennett, 1979), we speculated that MPA could change prostanoid production by the breast cancer tissue and thereby reduce pain and improve the outcome of patients with advanced disease (Mannes et al., 1976; Brunner et al., 1977; Pannuti et al., 1978). However, MPA at clinically achievable (Hesselius & Johansson, 1981) or higher concentrations did not change the endogenous synthesis of 6-keto-PGF1alpha and TxB2 by receptor positive or negative breast cancer. Therefore it seems likely that the antitumour effect of MPA is not mediated through prostanoids.

In conclusion, although human breast cancer produces increased amounts of PG12 and TxA2, these do not seem to reflect the future tumour behaviour.

This work was supported by the Finnish Cancer Research Foundation and the Sigrid Juselius Foundation.

Figure 1  The production of 6-keto-PGF1alpha (□) and thromboxane B2 (□) in the presence of various concentrations of medroxyprogesterone acetate (MPA) as percentages (mean±s.e.) from the control values (n for each=8) (The production of 6-keto-PGF1alpha was 5.5±2.1 ng min⁻¹ g⁻¹ and that of TxB2 3.5±0.3 ng min⁻¹ g⁻¹ in the control perfusion.)

PROSTANOIDS IN BREAST CANCER 673
References

BENNETT, A., CHARLIER, E.M., MCDONALD, A.M., SIMPSON, J.S., STAMFORD, I.F. & ZERBO, T. (1977). Prostaglandins and breast cancer. *Lancet*, ii, 624.

BENNET, A. (1979). Prostaglandins and cancer. In *Practical Application of Prostaglandins and their Synthesis Inhibitors*, pp. 149. (Ed. Karim.), MTP Press Ltd, Lancaster.

BRUNNER, K.W., SONNTAG, R.W., ALBERTO, P. & 4 others. (1977). Combined chemotherapeutic and hormonal therapy in advanced breast cancer. *Cancer*, 39, 2923.

HESSELIUS, I., JOHANSSON, E.D.B. (1981). Medroxyprogesterone acetate (MPA) plasma levels after oral and intramuscular administration in a long term study. *Acta Obstet. Gynecol. Scand. Suppl.* 101, 65.

HONN, K.V., BOCKMAN, R.S. & MARNETT, L.J. (1983). Prostaglandins and cancer: A review of tumor initiation through tumor metastasis. *Prostaglandins* 21, 833.

HONN, K.V., CICONE, B. & SKOFF, A. (1981). Prostacyclin: A potent antimetastatic agent. *Science*, 212, 1270.

KARMALI, R.A., WELT, S., THALER, H.T. & LEFEVRE, F. (1983). Prostaglandins and breast cancer: Relationship to disease stage and hormone status. *Br. J. Cancer*, 48, 689.

MANNES, P., DERRICKS, R., MOENS, R., LAURENT, C. & DALQ, J.M. (1976). Multidisciplinary curative treatment for disseminated carcinoma of the breast. *Cancer Treat. Rep.*, 60, 85.

MARCUM, J.M., MCGILL, M., BASTIDA, E., ORDINAS, A. & JAMIESON, G.A. (1980). The interaction of platelets, tumor cells and vascular subendothelium. *J. Lab. Clin. Med.*, 96, 1046.

MARTIN, P.M., ROLLAND, P.H., JACQUEMIER, J., ROLLAND, A.M. & TOGA, M. (1979). Multiple steroid receptors in human breast cancer. II. Estrogen and progestin receptors in 672 primary tumours. *Cancer Chemother. Pharmacol.*, 2, 107.

MÄKILÄ, U.-M., WAHLBERG, L., VIINIKKA, L. & YLIKORKALA, O. (1982). Regulation of prostacyclin and thromboxane production by human umbilical vessels: the effect of estradiol and progesterone in a superfusion model. *Prostagl. Leukotrienes Med.*, 8, 115.

PANNUTI, F., MARTONI, A., LENAZ, G.R., PIANA, E. & NANNI, P. (1978). A possible new approach to the treatment of metastatic breast cancer: Massive dose of medroxyprogesterone acetate. *Cancer Treat. Rep.*, 62, 499.

ROLLAND, P.H., MARTIN, P.M., JACQUEMIER, J., ROLLAND, A.M. & TOGA, M. (1980). Prostaglandin in human breast cancer: Evidence suggesting that an elevated prostaglandin production is a marker of high metastatic potential for neoplastic cells. *J. Natl Cancer Inst.* 64, 1061.

TASHIJAN, A.H. (1978). Role of prostaglandins in the production of hypercalcemia by tumors. *Cancer Res.*, 38, 4138.

VIHKO, R., JÄNNE, O., KONTULA, K. & SYRJÄLÄ, P. (1980). Female sex steroid receptor status in primary and metastatic breast carcinoma and its relationship to serum steroid and peptide hormone levels. *Int. J. Cancer*, 26, 13.

VIINIKKA, L. & YLIKORKALA, O. (1980). Measurement of thromboxane in human plasma or serum by radioimmunoassay. *Prostaglandins*, 20, 759.

YLIKORKALA, O. & VIINIKKA, L. (1981). Measurement of 6-keto-prostaglandin Flα in plasma with radioimmunoassay: Effect of prostacyclin infusion. *Prostagl. Med.*, 6, 427.