Prostacyclin and thromboxane in benign and malignant breast tumours

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Summary

6-keto-PGF\textsubscript{1\alpha} and thromboxane B\textsubscript{2} were determined by radioimmunoassay in 37 extracts of breast carcinoma, 8 fibroadenoma, 12 sclerocystic-disease specimens and 51 normal breast tissues. More prostanoids were extracted from carcinoma than from normal specimens, fibroadenoma or sclerocystic-disease tissues ($P < 0.05$). The 6-keto-PGF\textsubscript{1\alpha}/TXB\textsubscript{2} ratio was higher in carcinoma than in normal tissues and fibroadenoma ($P < 0.05$) but was not significantly different from the ratio in sclerocystic disease. The prostaglandin levels and the 6-keto-PGF\textsubscript{1\alpha}/TXB\textsubscript{2} ratios from carcinoma did not correlate significantly with age, tumour size, differentiation, lymph node status, nuclear-cytoplasmic ratio, host cell reaction, mast cells, necrosis, elastosis, fibrosis or blood vessel density. Lower nuclear density was associated with lower 6-keto-PGF\textsubscript{1\alpha}/TXB\textsubscript{2} ratios ($P = 0.01$) whereas the latter value was higher when infiltration was lower ($P = 0.03$). There was a positive correlation between mitotic index and the 6-keto-PGF\textsubscript{1\alpha}/TXB\textsubscript{2} ratio ($P = 0.04$). Cumulation of variables revealed lower prostanoid ratios in tumours $>2\text{cm}$ without lymph node metastasis than tumours $<2\text{cm}$ with lymph node metastasis ($P = 0.05$). A first follow-up (14 months) showed a higher 6-keto-PGF\textsubscript{1\alpha}/TXB\textsubscript{2} ratio in patients who developed metastasis ($P = 0.04$). Our study does not confirm the hypothesis that high prostanoid levels are a good prognostic index in breast cancer.

Honn \textit{et al.} (1981) found that prostacyclin (PG\textsubscript{I\alpha}) had a beneficial influence against metastasis of B16 amelanotic melanoma tumours in mice, whereas inhibitors of PG\textsubscript{I\alpha} synthesis enhanced the number of metastases. Other authors tested the antimetastatic potency of acetylsalicylic acid, indomethacin, dipyridamole, flurbiprofen, benorylate, heparin and warfarin (Gasic \textit{et al.}, 1973; Elias \textit{et al.}, 1973; Lione & Bosman, 1978; Bennett, 1982). Most of these studies are done on animals and showed no clearcut results.

Thromboxane (TX) A\textsubscript{2} is often a physiological antagonist of PG\textsubscript{I\alpha} and an imbalance between them can disturb the (anti)haemostatic system. The antiaggregating properties of nonsteroidal anti-inflammatory drugs (NSAID) can be explained by a stronger inhibition of the platelet cyclo-oxygenase in comparison with that of the vessel wall. As a result the release of TX will be lowered and aggregation will be blocked (Bunting \textit{et al.}, 1983). Sloane \textit{et al.} (1981) showed that some tumours are able to release cathepsin B. This enzyme stimulates the synthesis of TX and is produced in a variant of B16 melanoma which has high metastatic activity.

In order to study a possible prognostic value of the PG\textsubscript{I\alpha}/TX ratio in breast cancer, the stable hydrolysis products of PG\textsubscript{I\alpha} (6-keto-PGF\textsubscript{1\alpha}) and TXA\textsubscript{2} (TXB\textsubscript{2}) were determined by RIA. 6-keto-PGF\textsubscript{1\alpha} and TXB\textsubscript{2} levels were examined in relation to the size of the tumour, axillary lymph node status, lymphatic vessel permeation, differentiation of the tumour, mitotic index density of nuclei of tumour cells, and age of the patient.

PG production can be influenced by inflammatory processes (Humes \textit{et al.}, 1977; Brune \textit{et al.}, 1978), and therefore the number of host-derived cells and the amount of necrosis was evaluated. Also the density of blood vessels was estimated as they could be a major source of 6-keto-PGF\textsubscript{1\alpha} (Moncada \textit{et al.}, 1976), and platelets contribute considerably to the amounts of TXB\textsubscript{2} measured (Hamberg \textit{et al.}, 1975).

Materials and methods

We obtained 108 specimens from 67 patients who underwent surgery for a breast lump. Each specimen was divided into two representative parts and prepared as described earlier by Vergote \textit{et al.} (1985). The tissues were immediately immersed either in acetone cooled by solid CO\textsubscript{2} ($-70^\circ\text{C}$) for 6-keto-PGF\textsubscript{1\alpha} or TXB\textsubscript{2} analysis, or in Bouin's liquid for histopathological examination. The tissue samples for prostanoid investigation were stored at $-30^\circ\text{C}$ until radioimmunoassay was performed.
Thirty-five tumours were diagnosed as primary breast cancer (patient age range: 36–82; mean 64). The patients were classified according to the pathological TNM system (UICC, Livre de Poche), pT1aNo 9; pT1aN1a 1; pT1aN1b 4; pT1aNx 2; pT2aNo 4; pT2aN1a 2; pT2aN1b 4; pT2aNx 2; pT3aN1 2; pT4bNx 1. No patient had overt metastases at the time of surgery and none was under current treatment with non-steroidal inflammatory drugs or corticosteroids. Five local relapses (ductal carcinoma) and one cellular intracanicular fibroadenoma were included. Twenty tumours were benign: 8 fibroadenoma (age range: 17–47, mean: 34) and 12 sclerocystic-disease specimens (age range: 37–70, mean: 49). We investigated also histologically confirmed normal breast tissue of patients with malignant tumours and fibroadenoma. Furthermore, 12 specimens of patients who had neither benign nor malignant tumours were studied. The age of the patients and the tumour size at anatomopathological examination were recorded. The amounts of 6-keto-PGF₁α and TXB₂ were expressed as ng mg⁻¹ protein and from these values the 6-keto-PGF₁α/TXB₂ ratio was calculated.

### Histopathology

The slides were independently reviewed by two of the authors and re-evaluated by a senior pathologist. In case of discordance, the results were not included in this study.

Tumours were classified according to the methodology used earlier (Vergote et al., 1985). Subdivision of histopathological variables is shown in Table III.

### Radioimmunoassay (RIA)

For the extraction of prostaglandins and the protein measurement the procedure of Vergote et al. (1985) was used. Acetone was evaporated under nitrogen and the weight of the tissue determined. Tris buffer (50 mM, pH = 8.0 at 25°C) was added (3 ml g⁻¹ tissue) and sonicated for 90 min (Bransonic). Ice was regularly added to the bath fluid. The supernatant was separated from the tissue after centrifugation at 10,000 g (Eppendorf centrifuge). RIA was performed directly on the supernatant according to Granström and Kindahl (1978). The antisera were raised in rabbits. Cross reactivities on the 50% binding level of the curve were: for the 6-keto-PGF₁α-antiserum: PGF₁α, 1%; 15-HETE (hydroxyeicosatetraenoic acid), 0.01%; 15-HPETE (hydroperoxycosatetraenoic acid), 0.01%; PGE₂, 15-keto-PGE₂, TXB₂, and AA (arachidonic acid) <0.01%; for the TXB₂ antiserum: PGD₂, 8.9%; PGF₂α, 1%; PGE₂, 0.9%; 6-keto-PGF₁α, 0.1%; 15-keto-13,14-dihydro-PGF₂α, AA, 15-HETE and 15-HPETE, <0.01%.

The extraction recoveries for 6-keto-PGF₁α were 112±10% (mean ± SE: n = 3) and for TXB₂ 97±16 (mean ± SE; n = 3). The intra-assay variation coefficient for RIA of 6-keto-PGF₁α and TXB₂ were 15±1% (n = 103) and 13±1% (n = 111) respectively.

### Reagents

TXB₂ and 6-keto-PGF₁α (Upjohn), (3H)-radio-labelled 6-keto-PGF₁α and (3H)-TXB₂ (NEN). Tris buffer was made with trizma base (Sigma) and HCl (Merk p.a.).

### Statistical analysis

Nonparametric statistical analysis was used to compare two (Wilcoxon test) or more groups (Kruskal & Wallis test; Sokal & Rohlf, 1981). Correlation coefficients were calculated by linear regression.

### Results

PG levels in relation to the histopathological groups

6-keto-PGF₁α levels were higher in carcinomata (CA) than in normal breast tissue (N), fibroadenoma (FA) and sclerocystic disease (SCD) (P = 0.0003, Kruskal & Wallis). In FA, N and SCD the levels did not differ significantly (P = 0.17, Kruskal & Wallis). CA-TXB₂ levels were significantly higher in comparison with the other groups (P = 0.05, Kruskal & Wallis). The differences between N, FA and SCD were not significant (P = 0.83, Kruskal & Wallis). The 6-keto-PGF₁α/TXB₂ ratio in CA was higher than in N and FA (P = 0.002, Kruskal & Wallis) which were similar (P = 0.39). SCD and CA also gave similar ratios (P = 0.67). These results are summarized in Table I and Figure 1. When local relapses (n = 5) were calculated separately, 6-keto-PGF₁α, TXB₂ and the 6-keto-PGF₁α/TXB₂ ratio were respectively, median (limit values): 12.7 (0.6–15.0) ng mg⁻¹ protein, 2.5 (1.8–3.4) ng mg⁻¹ protein and 4.0 (0.3–7.3). They were similar to the ductal carcinomata (P = 0.95; P = 0.40 and P = 0.28 respectively).

### Histological type and differentiation

The infiltrating ductal carcinomata composed the substantial group. Statistical comparison between all the groups was difficult because some contained very few cases (Table II). We divided the tumours into two groups: undifferentiated and some degree of differentiation (small, moderate or high). Both
Table I  PG-levels in relation to the pathology

| Pathology | n  | ng 6-keto-PGF$_{1α}$ mg$^{-1}$ protein median (semiquartiles) | ng TXB$_2$ mg$^{-1}$ protein median (semiquartiles) | 6-keto-PGF$_{1α}$/TXB$_2$ median (semiquartiles) |
|-----------|----|-------------------------------------------------------------|--------------------------------------------------|-----------------------------------------------|
| CA        | 37 | 4.4 (1.7–14.3)                                              | 0.6 (0.4–2.8)                                    | 4.6 (3.2–9.2)                                 |
| N         | 51 | 1.2 (0.4–3.3)                                               | 0.4 (0.2–0.8)                                    | 2.6 (1.4–5.2)                                 |
| FA        | 8  | 0.3 (0.2–1.8)                                               | 0.4 (0.2–0.5)                                    | 1.8 (0.7–4.9)                                 |
| SCD       | 12 | 1.7 (0.9–3.5)                                               | 0.4 (0.2–1.0)                                    | 4.3 (2.7–8.7)                                 |

CA = carcinomata; N = normals; FA = fibroadenomata; SCD = sclerocystic disease.

Figure 1  Individual 6-keto-PGF$_{1α}$/TXB$_2$ values in the pathological groups: CA (n=37; carcinomata), N (n=51; normal breast tissue), FA (n=8; fibroadenomata), SCD (n=12; sclerocystic disease). Medians and semiquartiles are indicated.

Table II  PG-levels in relation to the histological type

| Type                  | n  | ng 6-keto-PGF$_{1α}$ mg$^{-1}$ protein | ng TXB$_2$ mg$^{-1}$ protein | 6-keto-PGF$_{1α}$/TXB$_2$ |
|-----------------------|----|---------------------------------------|----------------------------|--------------------------|
| Infiltrating ductal   | 23 | 4.4 (2.3–16.6)*                      | 1.0 (0.3–3.4)*              | 4.6 (3.3–9.6)*           |
| carcinoma             |    |                                       |                            |                          |
| Lobular               | 2  | 5.1–8.1                               | 0.4–0.5                    | 12.8–15.2                |
| Comedo                | 4  | 0.4–2.2b                              | 0.1–0.7b                   | 0.6–8.33b                |
| Medullary             | 1  | 1.4                                   | 0.4                        | 4.0                      |
| Mucoid                | 1  | 7.7                                   | 3.6                        | 2.1                      |

*Median (semiquartiles); bLimit values
groups showed similar 6-keto-PGF$_{1a}$, TXB$_2$ and 6-keto-PGF$_{1a}$/TXB$_2$ levels (Table III).

**Lymph node metastasis and lymphatic vessel permeation**

The median amount of 6-keto-PGF$_{1a}$ was slightly higher in tumour extracts from patients without lymph node metastasis ($P=0.23$). Median TXB$_2$ amounts and the 6-keto-PGF$_{1a}$/TXB$_2$ ratio were comparable.

The groups without lymphatic vessel permeation had more 6-keto-PGF$_{1a}$ ($P=0.04$), but the TXB$_2$ values and the 6-keto-PGF$_{1a}$/TXB$_2$ ratio were similar (Table III).

**Size and density of nuclei of carcinoma cells**

Tumours with moderate density had significantly less 6-keto-PGF$_{1a}$ ($P=0.04$) and TXB$_2$ ($P=0.01$) than those with low or high density. Higher 6-keto-PGF$_{1a}$/TXB$_2$ ratios were found in moderate and high density groups ($P=0.01$; Table III).

Carcinomata with large nuclei tended to yield less TXB$_2$ than those having moderate size nuclei ($P=0.18$; Table III).

**Mitotic index**

There was at most a weak correlation between the number of mitoses per high power field (HPF) and the 6-keto-PGF$_{1a}$ or TXB$_2$ levels ($r=-0.167$, $P=0.18$ and $-0.212$, $P=0.13$ respectively), but a positive correlation occurred with the 6-keto-PGF$_{1a}$/TXB$_2$ ratio ($r=0.33$, $P=0.04$).

**Nuclear and cellular polymorphism and the nuclear cytoplasmic ratio**

Tumours with a low nuclear and cellular polymorphism showed at most a weak tendency to higher 6-keto-PGF$_{1a}$ levels ($P=0.19$) no other relationships were seen.

**Host cell reaction, necrosis and mast cells**

Host cell reaction, necrosis and presence of mast cells did not correlate with the amounts of extracted prostanoids.

**Elastosis, fibrosis and infiltration**

No significant tendencies were observed between PG-levels and elastosis or fibrosis. Infiltration was inversely related to the 6-keto-PGF$_{1a}$/TXB$_2$ ratio ($P=0.03$), but no significant differences were seen for the 6-keto-PGF$_{1a}$ or TXB$_2$ (Table III).

**Blood vessel density**

Presence of blood vessels did not correlate with prostanoid yields in the tumour biopsies. the 6-keto-PGF$_{1a}$/TXB$_2$ ratio even tended to be lower when more blood vessels were present ($P=0.18$; Table III).

**Age**

No correlations between prostanoids and age were seen: 6-keto-PGF$_{1a}$, $r=-0.023$ ($P=0.45$), TXB$_2$, $r=0.157$ ($P=0.20$), 6-keto-PGF$_{1a}$/TXB$_2$ $r=0.179$ ($P=0.20$).

**Cumulation of variables and follow up**

Tumour size showed little or no relationship to tissue prostanoids: 6-keto-PGF$_{1a}$, $r=-0.182$ ($P=0.16$), TXB$_2$, $r=-0.126$ ($P=0.41$), 6-keto-PGF$_{1a}$/TXB$_2$, $r=-0.038$ ($P=0.42$). Tumours with strong metastatic potential (<2 cm and lymph node metastasis, n = 5) had a median 6-keto-PGF$_{1a}$/TXB$_2$ ratio of 3.8 (2.0–9.4), which was higher ($P<0.05$) than with tumours having a relatively weak metastatic potential (>2 cm and no lymph node metastasis, n = 7; 2.8 (0.5–4.6)).

A preliminary analysis of 19 patients with a follow-up of 14 months revealed metastases in 5 patients. These 5 patients had a median 6-keto-PGF$_{1a}$/TXB$_2$ ratio of 9.2 (4.0–15.3) which was higher than the ratio of nonmetastatic patients 4.8 (1.6–13.5) ($P=0.04$).

**Discussion**

Little is known about the role of PGI$_2$ and TX in human malignant tumours, and only 2 studies on extracted breast tissues have been published.

Karmali et al. (1983) measured 6-keto-PGF$_{1a}$ and TXB$_2$ in 24 breast tumours and expressed their results as log ng g$^{-1}$ wet weight. They obtained more TXB$_2$ from large tumours and those with lymph node metastasis. These results were interpreted as supporting the findings of Honn (1981) that a higher TXA$_2$/PGI$_2$ ratio has a worse prognosis in terms of metastasis.

However, Karmali et al. (1983) did not give any data about the 6-keto-PGF$_{1a}$/TXB$_2$ ratio which is important in the regulation of blood platelet aggregation. Furthermore, the size of the tumour is not necessarily an indication of the metastatic potential. In addition, Karmali et al. (1983) observed no correlation between TXB$_2$ levels and metastasis. Aitokallio-Tallberg et al. (1985) studied the *in vitro* production of 6-keto-PGF$_{1a}$ and TXB$_2$ by 23 breast tumour tissues, but as their investigations were directed towards steroid receptor status...
Table III  Prostanoid levels and anatomopathological variables

| Variable (n)                      | ng 6-keto-PGF<sub>1α</sub> mg<sup>-1</sup> prot. median (limit values) | ng TXB<sub>2</sub> mg<sup>-1</sup> prot. median (limit values) | 6-keto-PGF<sub>1α</sub> TXB<sub>2</sub> median (limit values) | P    |
|-----------------------------------|------------------------------------------------------------------------|---------------------------------------------------------------|-------------------------------------------------------------|------|
| Differentiation                   |                                                                        |                                                              |                                                             |      |
| no diff. (15)                     | 5.0 (0.6-27.4)                                                         | 0.44 (0.1-17.0)                                               | 0.71 (0.3-17.5)                                             | 0.68 |
| diff. (16)                        | 4.4 (0.9-62.9)                                                         | 0.8 (0.1-12.8)                                               | 5.0 (2.4-14.4)                                             |      |
| Lymph node metastasis             |                                                                        |                                                              |                                                             |      |
| positive (11)                     | 3.9 (0.7-45.3)                                                         | 0.23 (0.3-6.5)                                               | 0.70 (2.8-10.0)                                            | 0.89 |
| negative (15)                     | 5.4 (0.5-62.9)                                                         | 0.7 (0.1-12.8)                                               | 4.3 (1.8-9.2)                                             |      |
| Lymphatic vessel permeation        |                                                                        |                                                              |                                                             |      |
| positive (23)                     | 4.0 (0.6-62.9)                                                         | 0.04* (0.1-17.0)                                            | 0.17 (0.3-17.5)                                            | 0.53 |
| negative (8)                      | 9.3 (4.3-29.9)                                                         | 2.1 (0.3-9.6)                                                | 5.2 (3.1-15.2)                                            |      |
| Density of nuclei of carcinoma cells |                                                                        |                                                              |                                                             |      |
| low (8)                           | 12.7 (1.4-62.9)                                                        | 0.04* (0.4-12.8)                                            | 0.01* (2.1-4.6)                                            | 0.01*|
| moderate (16)                     | 4.0 (0.6-16.7)                                                         | 0.4 (0.1-2.7)                                                | 6.1 (0.3-17.5)                                            |      |
| high (7)                          | 7.7 (2.2-27.4)                                                         | 0.8 (0.3-17.0)                                               | 7.0 (1.6-14.4)                                            |      |
| Size of nuclei of carcinoma cells |                                                                        |                                                              |                                                             |      |
| small (3)                         | 8.1 (0.6-14.3)                                                         | 0.44 (0.2-1.8)                                               | 0.18 (0.3-15.2)                                            | 0.34 |
| moderate (23)                     | 5.0 (0.9-27.4)                                                         | 0.8 (0.1-17.0)                                               | 4.4 (1.6-17.5)                                            |      |
| large (5)                         | 4.0 (1.2-8.3)                                                         | 0.4 (0.3-0.9)                                                | 9.7 (4.6-13.4)                                            |      |
| Nuclear and cellular polymorphism |                                                                        |                                                              |                                                             |      |
| low (8)                           | 11.2 (0.6-62.9)                                                        | 0.19 (0.2-17.0)                                              | 0.44 (0.3-15.2)                                            | 0.98 |
| moderate (15)                     | 4.4 (0.9-29.9)                                                         | 0.8 (0.1-11.2)                                               | 4.4 (2.4-17.5)                                            |      |
| high (8)                          | 3.1 (1.2-8.3)                                                         | 0.4 (0.3-3.6)                                                | 4.9 (2.1-13.4)                                            |      |
| Nuclear cytoplasmic ratio         |                                                                        |                                                              |                                                             |      |
| low (4)                           | 5.0 (1.2-16.6)                                                         | 0.93 (0.3-3.6)                                               | 0.74 (2.1-6.1)                                            | 0.77 |
| moderate (20)                     | 4.3 (0.9-62.9)                                                         | 0.7 (0.1-17.0)                                               | 4.2 (1.6-17.5)                                            |      |
| high (6)                          | 6.6 (0.6-24.6)                                                         | 0.4 (0.2-5.3)                                                | 5.1 (0.3-15.2)                                            |      |
| Host cell reaction                |                                                                        |                                                              |                                                             |      |
| low (19)                          | 5.0 (0.6-62.9)                                                         | 0.59 (0.1-12.8)                                              | 0.39 (0.3-17.5)                                            | 0.67 |
| moderate (11)                     | 4.2 (1.0-27.4)                                                         | 0.5 (0.2-17.0)                                               | 5.1 (1.6-13.4)                                            |      |
| Mast cells                        |                                                                        |                                                              |                                                             |      |
| positive (11)                     | 4.1 (1.2-62.9)                                                         | 0.56 (0.3-12.8)                                              | 0.77 (2.1-13.4)                                            | 0.34 |
| negative (20)                     | 5.4 (0.6-29.9)                                                         | 0.6 (0.1-17.0)                                               | 5.1 (0.3-17.5)                                            |      |
| Necrosis                          |                                                                        |                                                              |                                                             |      |
| positive (15)                     | 4.0 (1.2-26.5)                                                         | 0.21 (0.1-11.2)                                              | 0.59 (2.1-17.5)                                            | 0.97 |
| negative (16)                     | 7.9 (0.6-62.9)                                                         | 0.8 (0.1-17.0)                                               | 4.4 (0.3-15.2)                                            |      |
| Elastosis                         |                                                                        |                                                              |                                                             |      |
| positive (21)                     | 5.4 (1.4-62.9)                                                         | 0.32 (0.2-12.8)                                              | 0.57 (0.3-17.5)                                            | 0.13 |
| negative (10)                     | 4.3 (0.6-27.4)                                                         | 0.6 (0.1-17.0)                                               | 3.2 (2.4-13.4)                                            |      |
| Fibrosis                          |                                                                        |                                                              |                                                             |      |
| negative (2)                      | 10.4-15.0                                                              | 0.52 (2.9-3.4)                                               | 0.40 (3.6-4.4)                                            | 0.55 |
| low (6)                           | 5.9 (0.6-8.3)                                                         | 0.7 (0.3-1.8)                                                | 9.4 (0.3-15.2)                                            |      |
| moderate (16)                     | 4.4 (1.2-62.9)                                                         | 0.7 (0.1-17.0)                                               | 4.6 (1.6-17.5)                                            |      |
| high (7)                          | 4.2 (0.9-29.9)                                                         | 0.5 (0.1-9.6)                                                | 8.1 (2.1-10.1)                                            |      |
| Infiltration                      |                                                                        |                                                              |                                                             |      |
| low (3)                           | 10.4 (1.7-15.0)                                                        | 0.92 (0.4-3.4)                                               | 0.49 (3.6-4.6)                                            | 0.03*|
| moderate (14)                     | 4.4 (0.9-26.5)                                                         | 0.5 (0.1-11.2)                                               | 8.0 (2.4-17.5)                                            |      |
| high (14)                         | 5.0 (0.6-26.9)                                                         | 1.0 (0.2-17.0)                                               | 3.2 (0.3-10.0)                                            |      |
| Blood vessel density              |                                                                        |                                                              |                                                             |      |
| low (14)                          | 5.3 (0.9-29.9)                                                         | 0.49 (0.1-9.6)                                               | 0.38 (2.1-17.5)                                            | 0.18 |
| moderate (8)                      | 6.0 (1.2-27.4)                                                         | 0.9 (0.3-17.0)                                               | 3.7 (1.6-15.2)                                            |      |
| high (7)                          | 2.2 (0.6-62.9)                                                         | 0.4 (0.2-12.8)                                               | 4.8 (0.3-13.4)                                            |      |

*Significantly different
they did not include control tissue superfusion in their protocol. The prostanoid yields were similar from metastatized and non-metastatized cancers (follow-up of at least 3 years).

We studied the PG-levels in the tumours at the time of resection. Treatment with acetone at −70°C inactivated the tumour enzymes and stopped the conversion of arachidonic acid to prostaglandins (Vergote et al., 1985). Prostanoids were measured in the tumour extracts, but the recoveries were about 100%, we can consider theRIA-results as reflecting the prostanoid production at the time of resection. Since the surgical manipulation might induce PG12 and TXA2 production, we also analyzed normal breast tissue taken from the neighbourhood of the cancer. The tumour specimen was always taken first, so that artefactual prostanoid might be higher in the normal tissue because of the longer trauma or lower because of prostanoid metabolism. For example, 6,15-diketo 13,14-dihydro PGF1α is formed from PG12 in plasma and vascular tissue (Peskar et al., 1980).

However, continued enzymatic conversion would be blocked by the acetone treatment.

Non-malignant sources of the tumour prostanoids are the host cells, but no correlation was found between the amounts of the prostanoids and the host cell reaction or the presence of mast cells. Furthermore, the 6-keto-PGF1α and TXB2 tissue levels did not correlate with the density of blood vessels.

We preferred to express the prostanoid yields in ng mg⁻¹ protein, rather than to use ng g⁻¹ wet or dry weight, in an attempt to reduce the variation of the results. One of the main conclusions from our study is that carcinomata had higher median 6-keto-PGF1α and TXB2 levels than N, FA or SCD, but even so, the ranges overlapped considerably.

As can be seen from Table III, most of the differences were not significant, but a high metastatic potential and mitotic index correlated with higher 6-keto-PGF1α/TXB2 ratios. Furthermore, with 42 comparisons, 2 would be expected by chance to show P values <0.05 when no difference really exists.

No firm conclusions can yet be made, although follow-up over 14 months revealed a higher 6-keto-PGF1α/TXB2 ratio in patients with metastasis. These data should be interpreted very cautiously, as the number of patients evaluated is low and the follow-up period is limited. Nevertheless, the histopathology results do not support the suggestion of Karmali et al. (1983), for a protective action of prostacyclin against metastasis in human breast cancer, or the hypothesis of Honn (1981) that TXA2 promotes metastasis.

A common observation by several authors is the greater prostaglandin production by malignant breast tumours compared with normal breast tissues. This conclusion is independent of the prostaglandin measured or the ways of performing the incubations, extractions or measurements (Bennett et al., 1975; Kibbey et al., 1979; Greaves et al., 1980; Rolland et al., 1980; Malachi et al., 1981; Campbell et al., 1983; Karmali et al., 1983). The relationship between high prostaglandin levels and malignancy of breast tumours could lead to the systematic incorporation of cyclo-oxidase inhibitors (NSAID) in cancer therapy. However, when histopathological prognostic variables of malignant tissues are examined, the prognosis seems to be better when the PGF2α levels were higher (Vergote et al., 1985). Although this was not found for 6-keto-PGF1α and TXB2 in our present study, the mitotic index, prognosis and the first follow-up results indicate that tumour 6-keto-PGF1α at the time of surgery was often higher in patients with bad prognosis or metastasis. Using NSAID could theoretically worsen the prognosis, by depressing the 6-keto-PGF1α levels, but we do not know what effect this would have on the 6-keto-PGF1α/TXB2 ratio. Therefore we can neither support nor recommend the use of NSAID in the treatment of breast cancer, particularly since other actions of prostaglandins, e.g. in immunological functions, may also be affected.

The authors wish to thank Dr J. Vanderheyden, Dr G. Albertyn, Dr J. Verkinderen, Dr E. Schattenman, Dr P. Meulyzer, Dr H. Wauters, Dr P. Dalemans and Dr J. Van Wiemeersch of the Department of Gynaecology and Obstetrics, St. Camillus Hospital, University of Antwerp for kindly providing the clinical material; Miss A. Van Hoydonck for technical assistance and Miss L. Van den Eynde for the typing.

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