Eur J Cancer Clin Oncol, Vol. 21, No. 9, pp. 1051-1055, 1985.
Printed in Great Britain.

0277-5379/85$3.00+0.00

Prostaglandins, Steroids and Human Mammary Cancer

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Abstract—*PGE_2 and $PGF_{2\alpha}$ secretion* in vitro *were measured from tissues of patients with breast tumours and examined in relation to oestrogen receptor status. Prostaglandin secretion was significantly greater from malignant breast tumours than from 'normal' breast tissue or benign tumours. Synthesis of PGE_2 by oestrogen-positive and malignant tumours was significantly higher than by oestrogen-negative tumours, suggesting a correlation between prostaglandin E_2 and receptor status. Synthesis of $PGF_{2\alpha}$ by the malignant tumour tissues also occurred, but only at relatively low levels and with no significant difference between oestrogen-positive and negative tissues. Exogenous oestrogen* in vitro *had a significant stimulatory effect on both PGE_2 and $PGF_{2\alpha}$ secretion by oestrogen-positive malignant tumour tissues but did not influence oestrogen-negative tumour tissues. Progesterone, on the other hand, had no statistically significant effect on PG secretion by either type of tumour tissue, although the increased levels of PGE_2 secreted by oestrogen-positive tumour tissue in the presence of progesterone are unlikely to have arisen by chance. The results support the concept of steroidal influence on excess production of prostaglandins by human malignant breast tumour tissues.*

INTRODUCTION

SUBSTANTIAL evidence has been presented demonstrating increased arachidonate metabolism in many human and animal tumours and in experimentally induced tumours. Although the bulk of this evidence relates to increased prostaglandin E_2 (PGE_2) secretion by tumours, and in particular by breast tumours, it now seems clear that other eicosanoids such as prostacyclin (PGI_2) and thromboxane A_2 (TXA_2) probably also play a role in tumour growth and development.

Elevated levels of PGE_2 have been demonstrated in malignant breast tumours [1-4] and in experimental mammary tumours induced by dimethylbenz(a)anthracene [5], and these high levels appear in many instances to be associated with tumours which demonstrate histological evidence of invasiveness [7, 8], bone metastasis [1] and hypercalcaemia [9]. The prostaglandin may contribute to the development of metastatic disease [7] and, indeed, the survival time after mastectomy has been found to correlate inversely with amounts of prostaglandin-like material extracted from human mammary cancers [10].

More recently it has been demonstrated that PGI_2 and TXA_2 may also be associated with tumour growth and metastasis [4, 11, 12] and, indeed, selective inhibitors of TXA_2 or analogues of PGE_2 reduced the incidence of lung metastases in an animal tumour system. This implies that a wider range of arachidonate metabolites are involved in the disease and it has been suggested that many of the physiological and pathological effects previously attributed to PGE_2 may in fact be due in part to the action of these other metabolites.

Oestrogen receptor (ER) status has been used for some time to select breast cancer patients likely to respond to oestrogen therapy. Recent evidence suggests that oestrogen-positive breast tumours preferentially metastasise to bone [13] and, since this is also a property of tumours capable of producing high levels of PGE_2 [1], the possibility

Accepted 16 April 1985.

exists of some degree of correlation between oestrogen receptor and prostaglandin synthesising capacity. Further, biosynthesis of prostaglandins in reproductive tissues has been described as oestrogen-directed [14]. The purpose of the present investigation was to determine whether any correlation exists in human malignant breast tumours between oestrogen receptor status and capacity for prostaglandin synthesis *in vitro* and to determine whether exogenous steroids affect this synthetic capacity.

MATERIALS AND METHODS

All chemicals were of analytical grade and solvents were further purified by distillation in an all-glass system [5, 6, 8, 11, 12, 14, 15(*n*)-^{3}H]prostaglandin E_2 (sp. act. 5.92 TBq/mmol) and [5, 6, 8, 11, 12, 14, 15(*n*)-^{3}H]prostaglandin $F_{2\alpha}$ (sp. act. 6.66 TBq/mmol) were purchased from Amersham International, U.K. Antibody to PGE_2 was purchased from the Pasteur Institute, Paris, while antibody to $PGF_{2\alpha}$ was a gift from Dr N. L. Poyser, Department of Pharmacology, University of Edinburgh. Indomethacin, oestradiol-17β and progesterone were purchased from Sigma, U.K.

Tissues

Samples of human malignant and benign tumour tissues were obtained from the Victoria Infirmary, Glasgow, courtesy of Mr D. C. Smith. In a number of instances there were viable amounts of surrounding tissue, which was also investigated and was classed as 'normal'. Patients' ages ranged from 26 to 82 yr and most of the tumours studied were primary invasive carcinomas. No attempt was made to correlate PG production with tumour size or stage of breast malignancy as Karmali *et al.* [4] reported that there were no significant trends in adjusted microsomal PG synthesis *in vitro* with increasing stage of breast malignancy. Further, TXB_2 was the only arachidonate metabolite showing a significant relationship with tumour size and number of positive nodes. The tissue samples were transported to the laboratory in chilled medium or saline and were used within 1-2 hr of surgery. Tumour tissue was trimmed free of excess fat and sliced to a thickness of 0.4 mm using a hand microtome. The first and last slices were rejected and the remainder were cut into finer pieces before being incubated as described below using 20-30 mg per incubation. 'Normal' tissue, when available, was trimmed of excess fat and chopped into fine pieces with a blade.

Incubation

All incubations were set up in triplicate in Medium 199 (3 ml, pH 7.2-7.4) for 3 hr at 37°C with shaking. Test substances, where appropriate, were present in the medium at a concentration of 1 μg/ml (oestradiol, progesterone or indomethacin). After incubation aliquots of the medium were either rapidly frozen and stored overnight at -20°C prior to extraction for prostaglandin analysis or acidified and extracted with solvent, which was then stored at -20°C to await analysis for prostaglandin.

Analysis of samples

Aliquots (1 ml) of each incubation medium were acidified with citric acid buffer (0.5 ml, Ph 4) and extracted with ethyl acetate (5 ml). Recovery of prostaglandins using this extraction technique was always in excess of 90%. Appropriate aliquots of the ethyl acetate extracts were used for PGE_2 and $PGF_{2\alpha}$ analysis by standard radioimmunoassay techniques using highly specific antibodies [maximum cross-reactivities, (a) PGE_2: 15-oxo-PGE_2, 13.2%; PGE_1, 10.7%; 13,14-dihydro-PGE_2, 2.1%; PGA_2, 0.3%: (b) $PGF_{2\alpha}$: $PGF_{1\alpha}$, 28%; TXB_2, 0.6%; PGE_1, 0.5%; 15-oxo-$PGF_{2\alpha}$, 0.4%]. The final results were obtained by subtracting values of blank incubations (medium alone with no tissue) from control and experimental incubations.

Nuclear (ER_n) and cytosol (ER_c) oestrogen receptor data were provided by Dr R. E. Leake, Biochemistry Department, University of Glasgow, who also obtained tissue samples at the time of surgery.

Statistical treatment

The calculated arithmetic means were analysed for their level of significance where appropriate by Student's *t*-test.

RESULTS

The production of PGE_2 and $PGF_{2\alpha}$ during a 3-hr incubation of human breast tissues *in vitro* is shown in Table 1 for 'normal' breast, benign tumour and both oestrogen-positive and negative malignant tumour tissues. Although relatively few samples of 'normal' breast and benign tumours were available it is obvious that these produced minimal levels of prostaglandins.

The malignant tumours, on the other hand, synthesised considerable amounts of PGE_2 as well as small amounts of $PGF_{2\alpha}$. In addition, there was a significantly greater synthesis of PGE_2 by those with oestrogen-positive receptor tissues. That the increase in prostaglandin secretion was due to *de novo* synthesis in the tissue is demonstrated by the much lower levels of PGE_2 present in the incubation medium when it contained indomethacin. Further, in the presence of indomethacin the relatively higher concentrations of PGE_2 present with oestrogen-positive tissue

Table 1. Prostaglandin secretion (ng/100 mg *tissue*/3 hr) in vitro *by tissues from human breast in the presence and absence of indomethacin and steroids*

Tissue	Treatment	PGF_{2a}	PGE_2	*n*
'Normal'	control	3.1 ± 0.59	15.1 ± 6.62	6
Benign	control	3.7 ± 1.40	2.6 ± 1.6	5
Malignant*	control	12.7 ± 1.69†	239.4 ± 29.9†‡	20
ER+	indomethacin (1 μg/ml)	4.6 ± 1.34	115.6 ± 22.9	20
	oestradiol (1 μg/ml)	21.5 ± 3.47§	738.8 ± 205§	20
	progesterone (1 μg/ml)	19.2 = 4.07	495.1 ± 211	12
Malignant	control	10.0 ± 1.07	80.7 ± 19.8†	22
ER-	indomethacin (1 μg/ml)	4.1 ± 1.14	41.5 ± 14.1	22
	oestradiol (1 μg/ml)	15.7 ± 3.04	95.4 ± 29.0	22
	progesterone (1 μg/ml)	13.8 ± 3.35	169.7 ± 104	12

Each value represents the mean ± S.E.M. value of prostaglandins from triplicate incubations of the numbers of samples indicated.

*ER_C 141.5 ± 40.2 fmol/mg protein; ER_N 1083 ± 175 fmol/mg DNA.

†$P < 0.01$ relative to 'normal', benign.

‡$P < 0.01$ relative to ER- control.

§$P < 0.05$ relative to ER+ control.

compared with oestrogen-negative tissues tends to confirm the higher tissue content of PGE_2 in the former as the PG is unlikely to be due to *de novo* synthesis but rather to diffusion from the tissue.

In incubations where oestradiol was added to the incubation medium it can be seen that there was a marginally significant increase ($P < 0.5$) in PGE_2 secretion from oestrogen-positive but not oestrogen-negative tumours. A similar marginally significant increase was observed for $PGF_{2\alpha}$ secretion from oestrogen-positive tumour tissues, even though the control values had not been significantly different from those of oestrogen-negative tumours.

Addition of progesterone to the incubations gave no statistically significant effect on prostaglandin secretion from either type of malignant tumour, although in the case of the oestrogen-positive tumour tissue the increased secretion of PGE_2 in the presence of progesterone is unlikely to have arisen by chance.

DISCUSSION

Many previous studies have demonstrated that tumour tissues contain high levels of prostaglandin and also appear to have a greater capacity for synthesis of PG-like material than normal tissues. Only a few of these reports, however, have linked this increased PG activity with other metabolic variables [4, 7, 16]. The present results indicate that there is a significant correlation between the capacity of malignant human breast tumour tissues to synthesise PGE_2 *in vitro* and their oestrogen receptor status, with oestrogen-positive tumours having greater PG synthesising potential than oestrogen-negative tumours. These results agree with the data of Campbell *et al.* [15], who also demonstrated an association between oestrogen receptor status and PG synthetic capacity in epithelial cell preparations from human primary breast cancer tumours.

Karmali *et al.* [4], using a microsomal breast tumour preparation, noted increased activity of PG synthetase in favour of PGE_2 in oestrogen-positive relative to oestrogen-negative tumours, although their results just failed to reach conventional statistical significance. These authors did, however, report significantly greater PGE_2 synthesis by progesterone-positive tumours, as well as significantly decreased TXB_2 production by both oestrogen-positive and progesterone-positive breast tumours. Increased PG activity in progesterone-positive tumours was predictable and is consistent with the present results and those of Campbell *et al.* [15], as generally patients with both soluble and nuclear oestrogen receptor almost always have soluble progesterone receptor.

Wilson *et al.* [16] have reported that tumour prostaglandins and oestrogen receptors are independent variables which should be taken into account in assessing prognosis. This view is supported by Watson *et al.* [17], who found no significant correlation between oestrogen receptor status and 'basal' prostaglandin content of human breast tumour tissues.

The apparent differences in the results from the above experiments by various groups of workers may well be attributable more to the different experimental techniques used by each group than

to different metabolic activities in the tissues. Whether any of the three techniques quoted, prostaglandin synthetic capacity, enzyme activity or 'basal' prostaglandin levels of the tumour tissue, more accurately reflects the events occurring *in vivo* compared with the others remains the subject of debate.

High levels of prostaglandin activity in tumour tissues with positive oestrogen receptor would be consistent with the fact that both these variables have been reported as a characteristic of breast cancers which preferentially metastasise to bone [1, 13, 15]. More recently, however, Bennett *et al.* [18] have presented evidence which casts doubt on the connection between increased prostaglandins and bone metastases, although they do suggest that there is substantial support for the hypothesis that high prostaglandin production, at least in some stage of tumour development, carries a poor prognosis. The poor prognosis associated with high PG activity in breast tumours is inconsistent with ER status in that patients with ER-negative cancers fare worse than those whose tumours are ER-positive. Neither ER status nor PG production are ideal prognostic factors and further study is essential to understand interrelationships between these two variables.

Oestrogen-directed synthesis of PGE_2 has been proposed by Rolland *et al.* [7]. Such a concept of steroid involvement in the production of excess PG by breast tumour cells would be in keeping with known effects in reproductive tissues (ovary, uterus) of a number of animal species [14] and is supported by the increasecd synthesis of PG in response to oestradiol (and possibly progesterone, although non-significantly) observed in the present experiments.

To date the reasons for the observed increase in arachidonate metabolism in breast cancer tissues is not clearly understood. A number of mechanisms are, however, possible, including increased availability of PG substrate, increased PG enzyme activity, decreased catabolic activity and a breakdown in the negative feedback controls which normally regulate PG formation. Of these, the increased availability of substrate appears most likely, while the possibility of increased enzyme activity has been ruled out by Kibbey *et al.* [19] and factors involved in negative feedback have been discussed by Horrobin [20].

It is clear from the present results with PGE_2 and those of Karmali *et al.* [4] with TXB_2 that specific arachidonate metabolism does appear to occur in particular tumour types and continued studies with more refined cell culture methods are essential and may offer great potential as diagnostic factors and in the management of cancer.

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