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# Increased Synthesis of Prostacyclin and Thromboxane in Human Ovarian Malignancy[1]

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## ABSTRACT

**To study the production of antiaggregatory prostacyclin ($PGI_2$) and proaggregatory thromboxane ($TxA_2$) by ovarian tumors, we incubated pieces of benign and malignant ovarian tissue *in vitro*, and measured by radioimmunoassay the release of 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) (a hydration product of $PGI_2$) and thromboxane $B_2$ ($TxB_2$) (a hydration product of $TxA_2$). Healthy ovary ($n = 10$) produced both 6-keto-$PGF_{1\alpha}$ [mean, 8.9; 95% confidence interval (CI) from 5.4 to 15.2 ng/mg protein/min] and $TxB_2$ (mean, 1.9 ng/mg protein/min; 95% CI from 1.0 to 3.7 ng/mg protein/min). The production of 6-keto-$PGF_{1\alpha}$ (mean, 12.2; 95% CI from 7.7 to 19.3 ng/mg protein/min) and that of $TxB_2$ (mean, 4.8; 95% CI from 2.1 to 11.9 ng/mg protein/min) by benign cystic tumors ($n = 12$) was normal. Ovarian anaplastic cancer and adenocarcinoma ($n = 12$) produced 6-keto-$PGF_{1\alpha}$ on average 11.6-fold (95% CI from 5.2 to 26.0) 6-keto-$PGF_{1\alpha}$ and $TxB_2$ on average 30.0-fold (95% CI from 13.5 to 66.7) over production by healthy ovaries, and the ratio of 6-keto-$PGF_{1\alpha}$ to $TxB_2$ shifted to the dominance of $TxB_2$. Similarly, ovarian metastases of breast cancer, tubal cancer, and colon cancer produced increasingly 6-keto-$PGF_{1\alpha}$ (mean, 20.7 ng/mg protein/min) and $TxB_2$ (5.1 ng/mg protein/min). The dominance of $TxA_2$ in human ovarian cancer may contribute to the aggressing growth and spreading of this tumor.**

## INTRODUCTION

Substantial experimental evidence links PG[3] with cancer promotion, growth, and metastasis (1–3). In this regard, the antiaggregatory $PGI_2$ and its endogenous antagonist, $TxA_2$, are most interesting, because their endogenous balance regulates the interaction between the circulating platelets and vascular endothelium (4), a function which may be a factor in metastatic spread (5, 6). This has led to the hypothesis that a dominance of $PGI_2$ over $TxA_2$ in the circulation and/or in tumor itself could reduce the potentiality of cancer to spread. The hypothesis is supported by some researchers (7, 8), but disputed by the others (9) employing rather similar *in vitro* or animal models.

It is known that breast cancer produces increased amounts of classic PGs (10). Also the production of $PGI_2$ and $TxA_2$ by breast cancer were approximately three to six times higher than that by mastopathic breast (11). It is further known that the ovarian cells produces $PGI_2$, as evident from cultured rat (12, 13) and human granulosa cells (14), or from experiments using other ovarian components than granulosa cells (15, 16). It was recently reported that experimental mice ovarian reticulosarcoma, a tumor metastazing rapidly to the liver and uterus, produces approximately five times more $TxA_2$ than $PGI_2$ (17). Also the human ovarian cancer grows rapidly and sends out metastasis into the peritoneal cavity and/or through bloodstream at early stages (18). Because no data exist on the production of $PGI_2$ and $TxA_2$ by human ovarian cancer, we assessed these productions by healthy ovaries and by benign and malignant ovarian tumors.

## PATIENTS AND METHODS

Thirty-seven patients were studied with the approval of the local committee on ethics (Table 1). Ten women (six premenopausal, four postmenopausal) underwent hysterectomy for uterine fibroids, and their macroscopically and histologically healthy ovaries were removed as a prophylactic measure. Another 12 women had benign ovarian tumors (serous cystadenoma, five patients; mucinous cystadenoma, four patients; functional cyst, one patient; ovarian struma, one patient; and hyperthecosis, one patient). Twelve additional patients underwent surgery because of ovarian cancer, which was histologically anaplastic ($n = 5$), or adenocarcinoma ($n = 7$). Tumor had spread to clinical Stage I in four, to Stage II in three, to Stage III in one, and to Stage IV in four patients at the time of surgery. In addition, we studied three patients who had ovarian metastasis either from breast, tubal or colonic cancer (Table 1). All the study subjects denied the use of PG synthesis inhibitors within 7 days before the operation. After surgery all patients with ovarian cancer were treated with routine cytostatic regimens. At second-look laparotomy done 6 months later in seven of patients, five had residual tumor and/or metastasis. During the follow-up time of 10 to 40 months, five patients died of ovarian cancer.

Ovarian samples taken at surgery were divided into two identical sections. One of these was used for careful histopathological examination to ascertain the character of the tissue, and the adjacent one was frozen in liquid nitrogen and assessed later for $PGI_2$ and $TxA_2$ production *in vitro*. Briefly, pieces of histologically confirmed healthy and diseased (benign or malignant) ovary (approximately 20–50 mg dry weight) were incubated in physiological saline (0.154 M NaCl) buffered with phosphate (0.1 M) at 37°C, pH 7.4. To prevent the microbial growth, incubation buffer contained sodium azido (0.03 M), and this did not affect the PG production. Because the tissue trauma induces a huge release of PGs, the medium after the first 15-min incubation was discarded, and incubation was continued in fresh medium for 30 min, when a true production capacity could be assessed. The test incubation was halted by the addition of indomethacin (0.1 mmol/liter, final concentration). Release of $PGI_2$ and $TxA_2$ was evaluated by measuring concentrations of 6-keto-$PGF_{1\alpha}$ (a hydration product of $PGI_2$) and $TxB_2$ (a hydration product of $TxA_2$) in the incubation fluid by radioimmunoassay (19, 20). The protein content of the sample was measured by the method of Lowry (21) after homogenization of the tissue. Prostanoid production is expressed as nanograms of PG per milligram of protein per minute. The presence of indomethacin (0.1 mmol/liter) from the beginning of the test incubation inhibited release of 6-keto-$PGF_{1\alpha}$ by 90.6 ± 8.4% and that of $TxB_2$ by 92.4 ± 7.2% (mean ± SD, $n = 12$). The recovery of 6-keto-$PGF_{1\alpha}$ and $TxB_2$ put into the incubation tubes were 105 ± 11% and 91 ± 8%, respectively (mean ± SD, $n = 10$). Four samples were studied immediately and then after 3 months following continuous storage in frozen state (−70°C). No difference was seen in production, confirming previous data that a tissue's capacity to synthesize $PGI_2$ and $TxA_2$ is maintained at −70°C (22). From six cancer patients, we succeeded to collect preoperative urine samples. They were assayed for 6-keto-$PGF_{1\alpha}$, as described before (23), and this output, as expressed as picomoles of 6-keto-$PGF_{1\alpha}$ per mmol creatinine, was related to the 6-keto-$PGF_{1\alpha}$ production by the tumor.

The data were transformed logarithmically and are given as means and 95% confidence intervals.

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[3] The abbreviations used are: PG, prostanoids; $PGI_2$, prostacyclin; $TxA_2$, thromboxane $A_2$.

## RESULTS

Healthy ovary produced approximately four times more 6-keto-$PGF_{1a}$ than $TxB_2$ (Table 2, Figs. 1 and 2). Production of 6-keto-$PGF_{1a}$ and $TxB_2$ by six premenopausal women and four postmenopausal women exhibited no significant differences.

Benign tumors produced similar amounts of 6-keto-$PGF_{1a}$ and $TxB_2$ to those of healthy ovarian tissue, although $TxB_2$ production in five benign tumors exceeded the normal range (Figs. 1 and 2 and Tables 2 and 3). The ratio of 6-keto-$PGF_{1a}$ to $TxB_2$ was normal (Tables 2 and 3).

Table 1 *Clinical characteristics of study populations*

| Diagnosis | n | Age (mean ± SD) |
|---|---|---|
| Healthy ovaries | 10 | 58.0 ± 3.3 |
| Benign ovarian tumor | 12 | 42.3 ± 5.9 |
| Ovarian malignancy | | |
| Primary | 12 | 50.0 ± 3.5 |
| Secondary | 3 | 58.0 ± 12.8 |

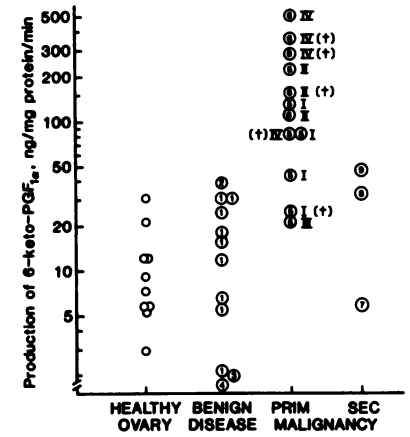


Fig. 1. Production of 6-keto-$PGF_{1a}$ in healthy ovary and in benign and malignant ovarian lesions: *1*, cystadenoma; *2*, functional cyst; *3*, ovarian struma; *4*, hyperthecosis; *5*, adenocarcinoma; *6*, anaplastic carcinoma; metastases from breast (*7*), tubal (*8*), or colonic cancer (*9*). *Roman numbers*, clinical staging at surgery; †, death within 10 to 40 months. Note the logarithmic scale for 6-keto-$PGF_{1a}$.

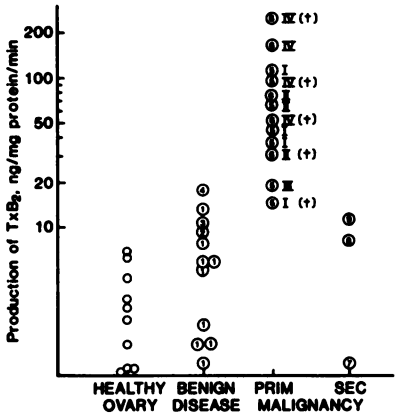


Fig. 2. Production of thromboxane $B_2$ in healthy ovary and in benign and malignant ovarian lesions. See Fig. 1, *legend*, for symbols.

The mean 6-keto-$PGF_{1a}$ production in the cancer group was 11.6 times higher than that of the controls (Fig. 1 and Tables 2 and 3). All ovarian cancers released more $TxB_2$ than did healthy ovary (Fig. 2); on average $TxB_2$ release was increased 30-fold (Tables 2 and 3). The 6-keto-$PGF_{1a}$/$TxB_2$ ratio was lower in the cancer group (Tables 2 and 3). Anaplastic cancer tended to be accompanied by higher 6-keto-$PGF_{1a}$ and $TxB_2$ release than adenocarcinomas, but no statistical significance was seen in this regard (Figs. 1 and 2). Prostanoid production was correlated neither to clinical staging at surgery nor the prognosis of patients in this series (Figs. 1 and 2), but in six patients with preoperative urine sampling, the urinary 6-keto-$PGF_{1a}$ output (range from 25 to 92 pmol/mmol creatinine) correlated with the production of 6-keto-$PGF_{1a}$ by the tumor (range from 21 to 353 ng/mg/min) ($r = 0.94$; $p < 0.01$).

Ovarian metastases of breast cancer, tubal cancer, and colonic cancer produced less 6-keto-$PGF_{1a}$ and $TxB_2$ than did primary ovarian malignancies, and their ratio was normal (Figs. 1 and 2, Table 2).

## DISCUSSION

Ovarian cancer grows and spreads rapidly along the peritoneal surfaces into the peritoneal cavity. This must be partly due to the topography of the ovaries which allows symptomless enlargement of the ovaries and easy spreading of cancer cells, and partly to biochemical tumor characteristics which may facilitate its spread. Ovarian cancer can also spread via the bloodstream at the early stage of the disease (18).

We discovered profoundly increased $PGI_2$ and $TxA_2$ production in ovarian malignancies. This stimulation can have several explanations. Prostanoids are formed when the precursor agent, arachidonic acid, is metabolized to different biologically active endproducts including $PGI_2$ and $TxA_2$. Thus, increased $PGI_2$ and $TxA_2$ production may merely reflect increases in all metabolic events in cancer cells. This hypothesis is, however, unlikely since $TxA_2$ synthesis was increased almost three times more than was $PGI_2$ synthesis. If PG stimulation were unspecific, the synthesis of both $PGI_2$ and $TxA_2$ should have risen similarly. Moreover, we related the production of prostanoids to protein content of the tissue. If a rise in prostanoid production were just a reflection of overall metabolic stimulation including arachidonic acid, no increase of PG/protein ratio would have been seen. Another explanation could be that $PGI_2$ and $TxA_2$ rise may originate from lymphocytes, other leukocytes, and platelets which accumulate in cancer tissue and which are capable of $PGI_2$ and $TxA_2$ synthesis (24–26). These cells probably release their prostanoids soon after the start of the incubation and therefore, they may not have significantly contributed to the final PG production, because we discarded the first

Table 2 *6-Keto-$PGF_{1a}$ and thromboxane $B_2$ production (ng/mg protein/min) and their relation in healthy and diseased human ovary (mean, 95% confidence intervals)*

| | 6-Keto-$PGF_{1a}$ | $TxB_2$ | 6-Keto-$PGF_{1a}$/$TxB_2$ |
|---|---|---|---|
| Healthy ovary | 8.9 (5.4–15.2) | 1.9 (1.0–3.7) | 3.7 (2.6–5.4) |
| Benign ovarian disease | 12.2 (7.7–19.3) | 4.8 (2.1–11.9) | 3.4 (1.7–6.8) |
| Malignant ovarian disease | | | |
| Primary | 102.5 (51.4–192.5) | 56.8 (32.1–92.8) | 1.8 (1.2–2.5) |
| Secondary | 20.7 (1.4–298.9) | 5.1 (0.28–94.5) | 4.1 (3.1–5.3) |

Table 3 *Differences (mean ± 95% confidence intervals) in 6-Keto-$PGF_{1\alpha}$ and $TxB_2$ production (ng/mg protein/min) between healthy and diseased ovarian tissue*

| | 6-Keto-$PGF_{1\alpha}$ | $TxB_2$ |
|---|---|---|
| Benign *vs.* healthy | 1.4 (−0.6–3.2) | 2.6 (−0.9–7.5) |
| Malignant *vs.* healthy | 11.6 (5.2–26.0) | 30.0 (13.5–66.7) |
| Malignant *vs.* benign | 8.4 (3.5–20.6) | 11.4 (4.5–28.8) |

15-min incubation media. Furthermore, primary ovarian cancers produced much more $PGI_2$ and $TxA_2$ than did the ovarian metastasis, and there is no reason to believe that cancer metastases should contain fewer blood cells than do primary ovarian cancers. Thus we believe that $PGI_2$ and $TxA_2$ stimulation is a specific event of ovarian cancer. In effect, tumor $PGI_2$ stimulation may explain the rise of 6-keto-$PGF_{1\alpha}$ output in these patients (23). This hypothesis gains further support from a strong correlation between 6-keto-$PGF_{1\alpha}$ output and production in our six patients.

Of the large family of prostanoids, $TxA_2$ has been most often connected to the tumor growth and spread (17, 27). Thus, $TxA_2$-mimicking agents increased the proliferation of B16 amelanotic melanoma cells *in vitro* and this could be prevented by $TxA_2$ synthesis inhibitors (27). Chiabrando *et al.* (17) demonstrated that highly metastazing ovarian reticulosarcoma metabolized arachidonic acid to $PGF_{2\alpha}$, $PGD_2$, $PGE_2$ 6-keto-$PGF_{1\alpha}$, and, most of all, to $TxA_2$. In this experiment, however, $TxA_2$ inhibitors did not reduce tumor growth (17). We found that $TxA_2$ stimulation by human ovarian malignancy was almost three times larger than that of $PGI_2$. The same tendency towards $TxA_2$ excess was seen in breast cancer (11), although not so markedly as in ovarian cancer in the present work. Whether this $TxA_2$ dominance is present in the early phase of malignant transformation and perhaps contributes to the cell proliferation (27) or is only a secondary alternation remains unanswered in our study. Ovarian cancer spreads aggressively along peritoneal surfaces but can also send out metastases through blood circulation (18). In both events $TxA_2$ dominance may be of importance.

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