Prostaglandin and Thromboxane Synthesis by Human Intracranial Tumors

Maria Grazia Castelli, Chiara Chiabrando, Roberto Fanelli, Luciana Martelli, Giorgio Butti, Paolo Gaetani, and Pietro Paoletti


ABSTRACT

Prostaglandin (PG) and thromboxane (TX) production by homogenates of human intracranial tumors (33 gliomas, 32 meningiomas, six brain metastases) and "normal" brain (n = 26) from tumor-bearing patients was studied. PGF2α, PGE2, PGD2, 6-keto-PGF1α (the hydrolysis product of PGI2) and TXB2 (the hydrolysis product of TXA2) were determined by high-resolution gas chromatography-mass spectrometry after ex vivo metabolism of endogenous arachidonic acid. Prostaglandin profiles (relative abundance of each metabolite) were different for gliomas and meningiomas, but similar for gliomas and their non-tumoral counterpart, i.e., "normal" brain. Mean overall prostanooid production was significantly higher in gliomas (539 ± 95) and meningiomas (523 ± 69) than in "normal" brain (198 ± 23). Prostaglandin synthesis significantly increased with anaplastic grade (glioblastomas > anaplastic astrocytomas > slow-growing astrocytomas). Tumors showed no marked prevalence of any particular metabolite and no major differences between histological subgroups. All brain metastases from different carcinomas (n = 5) showed a prevalence of TXB2 and PGE2, and very low PGD2 synthesis.

INTRODUCTION

Several experimental and human tumors synthesize prostanooids (1–5), which can be increasingly produced during tumor development (6–9). These cyclooxygenase metabolites of AA (PGF2α, PGE2, PGD2, PGI2, and TXA2) may influence physiological processes related to tumor development and dissemination (e.g., cell proliferation, migration and adherence, host immunity, angiogenesis, hemostatic mechanisms) (1–5, 10, 11). Cyclooxygenase or thromboxane synthetase inhibitors may reduce tumor growth and metastasis, although there are controversial reports on this issue (1, 2, 7, 12–16).

The capacity of tumors to grow, disseminate, and influence host homeostasis has in some cases been related to the production of elevated amounts of some prostanooids. In human cancer, high content or synthesis capacity of selected prostanooids have been by some authors related to high metastatic potential (17), tumor size and metastatic spread (18), and irresponsiveness to chemotherapy (19). Others found that although prostanooid synthesis of selected products was higher in malignant than in benign lesions, this did not correlate either with metastatic spread (20), or with clinical staging and prognosis (21), and was not associated with favorable prognosis (22).

Data is scant regarding prostanooid production by human intracranial tumors. Elevated amounts of prostanooids, especially TXB2 (the hydrolysis product of TXA2) and PGE2, are produced by short-term cell cultures of human meningiomas and gliomas (23). Using a highly selective method such as high resolution gas chromatography-mass spectrometry to measure the five stable cyclooxygenase metabolites of AA, we have preliminarily reported on prostanooid production by homogenates of human meningiomas and gliomas (24). In that study we found high synthesis capacity and characteristic metabolic profiles for each tumor, as we had already shown for murine tumors such as Lewis lung carcinoma and M5076 ovarian reticulosarcoma (6, 7). We have now extended our observation to a larger number of patients with gliomas, meningiomas, and brain metastases. We also present a comparative view of AA metabolite profiles in apparently nonpathological brain tissue, considered as the "normal" counterpart for gliomas. Correlations between prostanooid production and other biochemical and proliferative characteristics of each tumor are currently under investigation.

MATERIALS AND METHODS

Standards. PGF2α, PGF2α-D4, PGE2, PGE2-D4, PGD2, 6-keto-PGF1α, 6-keto-PGF1α-D4, and TXB2 were kindly supplied by Dr. J. Pike of The Upjohn Co. (Kalamazoo, MI). TXB2-D8 was a kind gift of Dr. G. Galli (Università degli Studi di Milano, Milan, Italy).

Clinical Samples. Specimens of intracranial tumors (33 gliomas, 32 meningiomas, and six metastases) were collected during surgery. Specimens of brain tissue (n = 26) were collected from some of these patients who underwent lobectomy as part of a correct surgical treatment. We will hereafter use the term "normal brain" to indicate a specimen which: (a) has been collected from tumor-bearing patients in an area apart from the tumor; (b) has been proven free of tumoral cells by histological examination of a section immediately adjacent to that used for AA metabolism.

Ex Vivo Metabolism of Endogenous AA. The ex vivo evaluation of endogenous AA cyclooxygenase metabolism has been described in previous papers (24, 25). Briefly, samples (0.1–2 g) were rapidly frozen in liquid nitrogen at the time of surgery and stored (−80°C) until analysis. The frozen samples were directly homogenized in 10 volumes of 50 mM phosphate buffer (pH 7.4). The homogenates were incubated for 15 min at 37°C, then spun at 15,000 × g for 45 min. The samples were maintained at 4°C during homogenization and centrifugation. The supernatants were kept at −20°C until analyzed. This procedure allows the ex vivo metabolism of endogenous AA to be reproduced metabolite profiles as reported previously (24, 25).

Quantitation of AA Metabolites by HRGC-MS. The five stable metabolites of AA (PGF2α, PGE2, PGD2, 6-keto-PGF1α, and TXB2) were assayed by HRGC-MS and quantified by stable isotope dilution assay. Supernatants were spiked with deuterium-labeled internal standards (PGF2α-D4, PGE2-D4, 6-keto-PGF1α-D4, and TXB2-D8, 30 ng each; PGE2-D4 was used as internal standard for PGD2). Samples were acidified (pH 3.5) with 1 N HCl and applied to a Baker C18 SPE column (J. T. Baker Chemicals, Phillipsburg, NJ) preswashed with methanol and 1 mM HCl. After washing with water and petroleum ether (10 ml each), the column was eluted with methyl formate (1.5 ml). The eluate was dried under an air flow and then derivatized to give the pentafluorobenzyl ester derivatives of AA metabolites and their deuterated analogues. The reaction was carried out by adding 30 μl of a mixture of pentafluorobenzyl bromide (Fluka, Buchs, Switzerland) and acetoneitrile...
(1:2, v/v) and 10 µl disopropylethylamine (Fluka, Buchs, Switzerland) to the residue and heating at 40°C for 5 min. The sample was thoroughly dried and the procedure repeated. The dried residue was then resuspended in chloroform and applied to a Silica Bond-Elut column (Analytichem International, Harbor City, CA), prewashed with chloroform. After washing with chloroform (10 ml), the column was eluted with 1.5 ml chloroform:methanol (9:1, v/v). The eluate was taken to dryness under an air stream.

AA metabolites and their deuterated analogues were converted to the methyloxime, trimethylsilyl ether derivatives with 50 µl bis(trimethylsilyl) trifluoroacetamide (Fluka, Buchs, Switzerland) (15 min at 60°C). AA metabolites were analyzed by HRGC-MS in the selected ion-monitoring mode. All samples were analyzed in the EI mode, and some selected samples were also analyzed in the NICI mode to further confirm the identity of the compounds. A fully computerized VG TS-250 mass spectrometer equipped with a Hewlett-Packard 5890 gas chromatograph was used. EI-MS operating conditions were: electron energy, 22.5 eV; ionizer temperature, 200°C. For EI-selected ion monitoring the following ions were recorded: m/z 589 for PGF₂α and m/z 593 for PGF₂α-D₄; m/z 461 for PGE₂ and m/z 465 for PGE₂-D₄; m/z 544 for PGD₂ and 6-keto-PGF₁α, and m/z 548 for 6-keto-PGF₁α-D₄; m/z 301 for TXB₂ and m/z 304 for TXB₂-D₄. For NICI-selected ion monitoring, carboxylate anions (M-181) were recorded. HRGC operating conditions were: 25 m CP Sil 5 CB (Chrompack, The Netherlands) fused silica capillary column (0.32-mm I.D., 0.13-µm film thickness), oven temperature programming 160-300°C (1°C isothermal, then 15°C/min), splitless injection mode, helium as carrier gas.

Results are expressed as AA metabolite synthesis potential (ng/g) and profile (percentage of each metabolite formed).

RESULTS AND DISCUSSION

Cyclooxygenase AA metabolism (overall and relative prostanoid production) by human intracranial tumors and "normal" brain tissue (a definition is given under "Materials and Methods") is shown in Fig. 1. AA metabolism by "normal" brain showed a characteristic profile, with a net prevalence of TXB₂ and low levels of 6-keto-PGF₁α (the hydrolysis product of PGI₂), as preliminarily reported with fewer specimens from glioma-bearing patients (24). In the present set of data we obtained similar profiles with "normal" brain from patients bearing gliomas, meningiomas, and metastases.

Glioma profiles were strikingly similar to those of their nonpathological counterpart, i.e., "normal" brain. This might indicate that the pattern of AA metabolism in tumoral cells is strongly influenced by the enzymatic endowment of the tissue from which they originate, rather than being specific to neoplastic cells in general. Mean synthesis capacity varied widely in different specimens of gliomas, possibly being influenced by factors such as tumor size, karyotypic heterogeneity, presence of necrosis, edema, or hemorrhage. Nevertheless, average prostanoid production (539 ± 95 ng/g) was significantly higher than in "normal" brain tissue (198 ± 23 ng/g). The increased capacity of neoplastic cells to produce prostanoids has already been seen in a number of human tumors (22, 26-29).

When different histological subgroups of gliomas were examined (Table 1), prostanoid production was found to increase with anaplastic grade (from "normal" brain to glioblastomas). Glioblastomas synthesized significantly more prostanoid than slow-growing astrocytomas and "normal" brain. Some changes in the proportions of each metabolite were noted when anaplastic features differed, although the overall profile was not substantially altered (Table 1). TXB₂ and 6-keto-PGF₁α were, respectively, the most and least abundant products, regardless of the grade of anaplasia. These findings confirm those reported earlier with a smaller number of cases (24). However, this set of data did not confirm the previous observation that the increased prostanoid synthesis in glioblastomas seemed to be preferentially directed toward TXB₂.

The AA metabolic profile in meningiomas as a class was markedly different from that of gliomas (Fig. 1). PGE₂, PGD₂, and 6-keto-PGF₁α, relative amounts were higher (P < 0.01, t test) in meningiomas than in gliomas, while the opposite was found for TXB₂ (P < 0.01, t test). Table 2 shows prostanoid production in different histological subclasses of meningiomas. Generally, absolute and relative synthesis of each product were similar for all subclasses but for anaplastic meningiomas, which tended to synthesize lower amounts of TXB₂ (statistically significant difference only versus psammomatous meningiomas). The small number of observations does not allow any speculation on a possible biological significance of the different prostanoid profile of anaplastic meningiomas. We could not compare meningiomas with "normal" tissue as we did for gliomas, since for ethical reasons it was not available.

The biological relevance of increased prostanoid production by tumors has not been clearly established. However, ongoing studies by our groups preliminarily show that in gliomas as well as in meningiomas the overall capacity of prostanoid synthesis correlates directly with the proliferative capacity of the tumor. Moreover, as a consequence of an increased AA metabolism, certain tumors could synthesize abnormal amounts of selected prostanoids which might facilitate or impair the growth and dissemination of the tumor itself or affect the host's defense mechanisms (1–5). In this respect, it is interesting to note some relative proportions of prostanoids found in the rapidly growing infiltrating gliomas compared to the slow-growing benign meningiomas. In fact, gliomas synthesize higher relative proportions of TXB₂ and lower relative amounts of 6-keto-PGF₁α and PGD₂ than meningiomas. TXA₂ (the active unstable precursor of TXB₂) has been shown in some experimental studies to favor tumor growth and spread (14, 30, 31), while the opposite has been reported for PGD₂ (32–35) and PGI₂ (the active unstable
We also examined six cases of brain metastases (three lung carcinomas, one breast carcinoma, one gastric carcinoma, one melanoma). Prostanoid synthesis capacity and profiles varied even in metastases of similar origin (lung carcinomas). Nevertheless, common features of the five carcinoma metastases were high TXB\(_2\) synthesis (range, 98–318 ng/g; mean ± SE, 188 ± 36 ng/g) and low PGD\(_2\) production (range, 11–42 ng/g; mean ± SE, 20 ± 6). Other prostanoids were formed in variable amounts, with an overall synthesis ranging from 167 to 2163 ng/g, the average profile being TXB\(_2\) > PGE\(_2\) > PGF\(_2\alpha\) > 6-keto-PGF\(_{1\alpha}\) > PGD\(_2\). The single case of melanoma metastasis had very low AA metabolism (total prostanoid synthesis 43 ng/g) and low PGD\(_2\) production (range, 11–42 ng/g; mean ± SE, 15 ± 3); however, in other experimental models, straightforward effects of these metabolites could not be demonstrated (7, 15, 16).

Table 1  AA metabolites in “normal” brain and gliomas

<table>
<thead>
<tr>
<th>Histology</th>
<th>TXB(_2) (ng/g)</th>
<th>PGE(_2) (ng/g)</th>
<th>PGD(_2) (ng/g)</th>
<th>6-keto-PGF(_{1\alpha}) (ng/g)</th>
<th>PGF(_2\alpha) (ng/g)</th>
<th>Total AA metabolites (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Normal” brain (n = 26)</td>
<td>87 ± 14(^d)</td>
<td>24 ± 2</td>
<td>29 ± 4</td>
<td>7 ± 1</td>
<td>51 ± 6</td>
<td>198 ± 23</td>
</tr>
<tr>
<td>Slow-growing astrocytomas (n = 8)</td>
<td>174 ± 35</td>
<td>19 ± 4</td>
<td>22 ± 8</td>
<td>4 ± 0,5</td>
<td>33 ± 11</td>
<td>253 ± 52</td>
</tr>
<tr>
<td>Anaplastic astrocytomas (n = 11)</td>
<td>182 ± 35</td>
<td>73 ± 22</td>
<td>56 ± 14</td>
<td>16 ± 5</td>
<td>69 ± 12</td>
<td>455 ± 93</td>
</tr>
<tr>
<td>Glioblastomas (n = 14)</td>
<td>314 ± 72(^a)</td>
<td>172 ± 84(^a)</td>
<td>99 ± 31(^f)</td>
<td>45 ± 15(^a)</td>
<td>137 ± 29(^a)</td>
<td>768 ± 196(^a)</td>
</tr>
</tbody>
</table>

* A definition for “normal” brain is given under “Materials and Methods.”

\(^a\) Numbers in parentheses, mean relative amount.

\(^d\) Mean ± SE. Data were analyzed by ANOVA. Statistical differences were determined by Tukey’s multiple comparison test.

\(^f\) \(P < 0.01\) versus slow-growing astrocytomas.

\(^g\) \(P < 0.05\) versus “normal” brain.

\(^h\) Numbers in parentheses, mean relative amount.

\(^\circ\) \(P < 0.05\) versus slow-growing astrocytomas.

\(^\circ\) \(P < 0.05\) versus “normal” brain.

Our preliminary observation of high TXB\(_2\) and PGE\(_2\) and low PGD\(_2\) synthesis in carcinoma metastases, although limited to a small number of cases, merits further investigation to establish whether the potential of cells to metastasize to the brain can be influenced by their capacity to synthesize selected AA metabolites.

Although our measurements describe the potential capacity of tumors to synthesize prostanoids, recent findings suggest that these indirect measurements may reflect a real in vivo situation. In patients with ovarian cancer, a strong correlation has in fact been noted between urinary levels and in vitro tumor production of 6-keto-PGF\(_{1\alpha}\) (21). Preliminary data from our laboratory show increased urinary levels of thromboxanes in mice bearing the thromboxane-producing M5076 ovarian reticulosarcoma (7). If consistent shifts in urinary prostanoid profiles could be demonstrated in patients with different tumors with well-characterized metabolic profiles, this might provide a new tool for diagnosis and monitoring of malignancy.

ACKNOWLEDGMENTS

We thank Judith Baggott and the staff of the Gustavus A. Pfeiffer Memorial Library who helped prepare the manuscript.

REFERENCES


PROSTANOID SYNTHESIS BY HUMAN BRAIN TUMORS


Prostaglandin and Thromboxane Synthesis by Human Intracranial Tumors

Maria Grazia Castelli, Chiara Chiabrando, Roberto Fanelli, et al.


Updated version

Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/49/6/1505

E-mail alerts

Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.