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. PGF<sub>2α</sub>, PGE<sub>2</sub>, PGD<sub>2</sub>, 6-keto-PGF<sub>1α</sub>, (the hydrolysis product of PGF<sub>2α</sub>) and TXB<sub>2</sub> (the hydrolysis product of TXA<sub>2</sub>) were determined by high-resolution gas chromatography-mass spectrometry after ex vivo metabolism of endogenous arachidonic acid. Prostanoid profiles (relative abundance of each metabolite) were different for gliomas and meningiomas, but similar for gliomas and their nontumoral counterpart, "normal" brain. Mean overall prostanoid production was significantly higher in gliomas (539 ± 95) and meningiomas (523 ± 69) than in "normal" brain (198 ± 23). Prostanoid synthesis significantly increased with anaplastic grade (glioblastomas > anaplastic astrocytomas > slow-growing astrocytomas > "normal" brain), while profiles did not substantially change (TXB<sub>2</sub> was the most and 6-keto-PGF<sub>1α</sub>, the least abundant product). Meningioma profiles 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 TXB<sub>2</sub> and PGE<sub>2</sub> and very low PGD<sub>2</sub> synthesis.

INTRODUCTION

Several experimental and human tumors synthesize prostanoids (1-5), which can be increasingly produced during tumor development (6-9). These cyclooxygenase metabolites of AA<sup>3</sup> (PGF<sub>2α</sub>, PGE<sub>2</sub>, PGD<sub>2</sub>, PGF<sub>1α</sub>, and TXA<sub>2</sub>) 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 prostanoids. In human cancer, high content or synthesis capacity of selected prostanoids 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 prostanoid 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 prostanoid production by human intracranial tumors. Elevated amounts of prostanoids, especially TXB<sub>2</sub> (the hydrolysis product of TXA<sub>2</sub>) and PGE<sub>2</sub>, 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 prostanoid 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 meningiomas, meningiomas, and brain metastases. We also present a comparative view of AA metabolic profiles in apparently nonpathological brain tissue, considered as the "normal" counterpart for gliomas. Correlations between prostanoid production and other biochemical and proliferative characteristics of each tumor are currently under investigation.

MATERIALS AND METHODS

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1 The abbreviations used are: AA, arachidonic acid; PG, prostaglandin; TX, thromboxane; PGF<sub>2α</sub>, PGE<sub>2</sub>, PGD<sub>2</sub>, 6-keto-PGF<sub>1α</sub>; PGE<sub>2</sub>-D<sub>4</sub>, PGD<sub>2</sub>-D<sub>4</sub>, 6-keto-PGF<sub>1α</sub>-D<sub>4</sub>, and TXB<sub>2</sub> were kindly supplied by Dr. J. Pike of The Upjohn Co. (Kalamazoo, MI). TXB<sub>2</sub>-D<sub>8</sub> 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 give reproducible metabolic profiles as reported previously (24, 25).

Quantitation of AA Metabolites by HRGC-MS. The five stable metabolites of AA (PGF<sub>2α</sub>, PGE<sub>2</sub>, PGD<sub>2</sub>, 6-keto-PGF<sub>1α</sub>, and TXB<sub>2</sub>) were assayed by HRGC-MS and quantified by stable isotope dilution assay. Supernatants were spiked with deuterium-labeled internal standards (PGF<sub>2α</sub>-D<sub>4</sub>, PGE<sub>2</sub>-D<sub>4</sub>, 6-keto-PGF<sub>1α</sub>-D<sub>4</sub>, and TXB<sub>2</sub>-D<sub>8</sub>). Samples were acidified (pH 3.5) with 1 N HCl and applied to a Baker 150-SPE column (J. T. Baker Chemicals, Phillipsburg, NJ) prewashed 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 pentfluorobenzyl ester derivatives of AA metabolites and their deuterated analogues. The reaction was carried out by adding 30 µl of a mixture of pentfluorobenzyl bromide (Fluka, Buchs, Switzerland) and acetonitrile...
(1:2, v/v) and 10 μl diisopropylethylamine (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 methyloxime hydrochloride (Pierce Chemicals, Rockford, IL) (30 min at 60°C) followed by 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; 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-D8. For NICI-selected ion monitoring, carboxylate anions (M-anions) 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 prostanooid 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 prostanooids has already been seen in a number of human tumors (22, 26–29).

When different histological subgroups of gliomas were examined (Table 1), prostanooid production was found to increase with anaplastic grade (from “normal” brain to glioblastomas). Glioblastomas synthesized significantly more prostanooids 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 prostanooid 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 prostanooid 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 prostanooid 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 prostanooid 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 prostanooid 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 prostanooids 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 prostanooids 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...
Table 1  AA metabolites in "normal" brain and gliomas

<table>
<thead>
<tr>
<th>Histology</th>
<th>TXB₂</th>
<th>PGE₂</th>
<th>PGD₂</th>
<th>6-keto-PGF₁₀</th>
<th>PGF₁₀</th>
<th>Total AA metabolites</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Normal&quot; brain (n = 26)</td>
<td>87 ± 4</td>
<td>25 ± 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</td>
<td>172 ± 84</td>
<td>99 ± 31</td>
<td>45 ± 15</td>
<td>137 ± 29</td>
<td>768 ± 196</td>
</tr>
</tbody>
</table>

* Mean ± SE; Data were analyzed by ANOVA. Statistical differences were determined by Tukey's multiple comparison test.

Table 2  AA metabolites in meningiomas

<table>
<thead>
<tr>
<th>Histological subgroups</th>
<th>TXB₂</th>
<th>PGE₂</th>
<th>PGD₂</th>
<th>6-keto-PGF₁₀</th>
<th>PGF₁₀</th>
<th>Total AA metabolites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meningothelial (n = 11)</td>
<td>122 ± 37</td>
<td>93 ± 23</td>
<td>105 ± 31</td>
<td>58 ± 29</td>
<td>66 ± 16</td>
<td>444 ± 126</td>
</tr>
<tr>
<td>Fibroblastic (n = 9)</td>
<td>177 ± 47</td>
<td>85 ± 19</td>
<td>102 ± 42</td>
<td>105 ± 34</td>
<td>57 ± 7</td>
<td>527 ± 113</td>
</tr>
<tr>
<td>Transitional (n = 4)</td>
<td>155 ± 68</td>
<td>146 ± 67</td>
<td>93 ± 30</td>
<td>98 ± 28</td>
<td>79 ± 33</td>
<td>574 ± 214</td>
</tr>
<tr>
<td>Psammomatous (n = 4)</td>
<td>318 ± 102</td>
<td>229 ± 78</td>
<td>81 ± 43</td>
<td>118 ± 55</td>
<td>92 ± 32</td>
<td>837 ± 244</td>
</tr>
<tr>
<td>Anaplastic (n = 4)</td>
<td>81 ± 45</td>
<td>161 ± 82</td>
<td>52 ± 17</td>
<td>33 ± 14</td>
<td>40 ± 13</td>
<td>367 ± 134</td>
</tr>
</tbody>
</table>

* Mean ± SE; Data were analyzed by ANOVA. Statistical differences were determined by Tukey's multiple comparison test.

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REFERENCES


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