Measurement of arachidonate and its metabolites extracted from human normal and malignant gastrointestinal tissues

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SUMMARY This is the first report of human gastrointestinal arachidonate and prostanoids measured quantitatively by gas chromatography-mass spectrometry (GC-MS) in extracts of human cancers and macroscopically normal tissues from the stomach and colon. There were µg/g amounts of arachidonate, and the particularly high yield from the tumours may explain why they usually produce more prostaglandins than the normal tissues in which they arise. There was only a small conversion of the arachidonate into prostanoids. 6-Keto-PGF₁α was the most abundant metabolite measured, particularly in the tumour extracts, with smaller amounts of prostaglandins E₂, F₂α and D₂.

Previous qualitative gas chromatography-mass spectrometry (GC-MS)¹ has shown that extracts of human homogenised gastrointestinal tissues contain arachidonate, 6-keto-PGF₁α, thromboxane B₂, 12-hydroxy-eicosatetraenoate (12-HETE) and usually PGD₂, PGE₂ and PGF₂α. Prostaglandin-like substances extracted from various human gastrointestinal tissues have been measured by bioassay²⁻⁴ and radioimmunoassay⁵,⁶ but these have problems of specificity. We now report quantitative measurements of PGD₂, PGE₂, PGF₂α, 6-keto-PGF₁α and free arachidonate by selective ion monitoring GC-MS of extracted human normal and malignant gastrointestinal tissues.

Methods

SPECIMENS
Gastrointestinal tissues were obtained at operation for various malignant or benign disorders, and reached the laboratory within one hour of removal. There were 55 tissues from the stomach and colon, from 23 patients who, as far as we could ascertain, had not recently taken any drugs known to affect prostaglandin synthesis. Tumour, and/or macroscopically normal tissue taken at least 5 cm from the site of any abnormality, were cleared of mesentery and fat, and the mucosal layers separated from the muscle. The fresh muscle, mucosa and/or tumour were cut into small pieces (approximately 2 mm³) and washed in Krebs solution. Weighed portions were homogenised (0·1 g tissue/ml; 30 s; Silverson homogeniser) at room temperature in Krebs solution, and the individual homogenates were extracted for prostaglandins using chloroform/formic acid.⁷⁻⁸

The methods for gas chromatography-mass spectrometry (GC-MS) were as reported previously.¹ In brief, the dried extract obtained as described above was dissolved in dichloromethane. An aliquot was taken for GC-MS and further purified by LH20 column chromatography; non-polar impurities were eluted with dichloromethane, and the eicosanoids were eluted with methanol which was then evaporated. Deuterated standards were added, and each dried methanol extract was dissolved in double-distilled water acidified to pH 3 with hydrochloric acid. Each solution was percolated through an Amberlite column which was then washed with distilled water, and the eicosanoids were eluted with...
methanol. The concentrated methanolic extracts were co-chromatographed simultaneously with authentic standards on silica gel TLC plates using the F6 solvent system of Andersen.\(^1\)

Chemical derivatives were made by forming O-methyloximes, which were converted first into methyl esters and then into trimethylsilyl ethers by heating with N,N-bis (trimethylsilyl)-trifluoroacetamide (BSTFA; Sigma), as described previously.\(^1\)

Samples were analysed by selective ion monitoring using a Riber 10-10C mass spectrometer interfaced with a Jirdel 31 gas chromatograph equipped with a 12.5 metre fused SE30 silica capillary column (Hewlett Packard). Helium 2 ml/min was used as the carrier gas with a column temperature of 210–260°C. The mass spectrometer was operated at 70eV electron energy and an electron multiplier setting of 2200V.

Quantitative selective ion monitoring of derivatised PGD\(_2\) (assayed after conversion to PGF\(_{2\alpha}\)), PGE\(_2\), PGF\(_{2\alpha}\), 6-keto-PGF\(_{1\alpha}\) and arachidonate was carried out on the extracted samples with added deuterated standards. Prostanoid standards were obtained from the Upjohn Company, Michigan, USA. The chloroform extraction method gives recoveries of at least 70\% with PGE and PGF compounds;\(^6\) subsequent recoveries were about 60–80\%.

**STATISTICAL ANALYSIS**

Results, uncorrected for losses, are expressed as medians with semiquartile ranges in parentheses. Statistical evaluation of the amounts of prostanoids from the same tissue was by the Wilcoxon’s matched pairs test, and comparisons between different tissues were by the Mann-Whitney U-test.

**Results**

**STOMACH**

The gastric tissues were obtained from 10 patients (six men, four women) who underwent surgery for malignant (eight) or benign (two) disease. With the malignant specimens both tumour and macroscopically normal tissues were available in four cases, two of eight provided only tumour, and the other two provided only 'normal' tissues. The tumour extracts contained the greatest amounts of prostanoids. In all normal and malignant tissue extracts the eicosanoid present in the highest amounts was arachidonate (\(\mu g/g\) range). The prostanoids were present in ng/g amounts, with more 6-keto-PGF\(_{1\alpha}\) than PGE\(_2\) (except for the higher amount from one tumour), PGD\(_2\) and PGF\(_{2\alpha}\) (Table 1). The tumours usually yielded more of each substance compared with the mucosa.

**LARGE BOWEL**

The specimens of large bowel from 13 patients included five rectal tumours with macroscopically normal sigmoid colonic tissue, three tumours plus normal tissues from the ascending colon, and five samples of macroscopically normal tissues from sigmoid colons removed for carcinoma (no tumour was provided). These specimens are dealt with together because the findings from the two regions were mainly similar.

In colon tumour extracts, arachidonate was always present in the greatest amounts (\(\mu g/g\)). The prostanoids were present in ng/g amounts, and there was always more 6-keto-PGF\(_{1\alpha}\) than the others (Table 2).

In comparison with the mucosa, all tumours of the large bowel yielded more arachidonate/g tissue. Amounts of PGE\(_2\) were higher in six of seven tumour extracts than in the corresponding mucosa (p<0-05), and the overall comparison including unpaired tissues was also p<0-05. The tumour yields were greater than from mucosa in five of seven cases with PGD\(_2\) and five of seven cases with PGF\(_{2\alpha}\); of the two exceptions, one tumour yielded less of both prostanoids. When paired and unpaired tissues were considered together, the tumour and mucosal amounts were similar (Table 2). Although the overall statistical comparison for 6-keto-PGF\(_{1\alpha}\), including data from unpaired tissues was p<0-05, only three of

<table>
<thead>
<tr>
<th></th>
<th>PGD(_2)</th>
<th>PGE(_2)</th>
<th>PGF(_{2\alpha})</th>
<th>6-keto-PGF(_{1\alpha})</th>
<th>Arachidonate</th>
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<tbody>
<tr>
<td>Muscle</td>
<td>12 (8–63) n=7</td>
<td>47 (24–66) n=6</td>
<td>16 (5–43) n=7</td>
<td>200 (120–290) n=4</td>
<td>34000 (26000–40000) n=7</td>
</tr>
<tr>
<td>Mucosa</td>
<td>11 (7–68) n=7*</td>
<td>18 (11–23) n=8*</td>
<td>38 (12–55) n=8*</td>
<td>100 (59–250) n=7</td>
<td>33000 (24000–54000) n=8</td>
</tr>
<tr>
<td>Tumour</td>
<td>140 (11–290) n=6*</td>
<td>300 (140–480) n=6*</td>
<td>100 (15–140) n=6*</td>
<td>960 (570–1570) n=6*</td>
<td>124000 (98000–158000) n=68</td>
</tr>
</tbody>
</table>

The results are ng/g shown as median with semiquartile ranges in parentheses. Comparison between 6-keto-PGF\(_{1\alpha}\), and other prostanoids within each specimen (Wilcoxon’s test): *p<0-05; tp<0-01. Since this paired test requires at least six specimens, p values have not been calculated for the muscle. With the Mann-Whitney U-test, however, the differences between 6-keto-PGF\(_{1\alpha}\) and the other prostanoids are p<0-01. Tumour vs mucosa for each prostanoid: tp<0-01; arachidonate tp<0-002 (Mann-Whitney U-test). There were no significant differences in prostanoid content of muscle compared with mucosa (p>0-1). Only seven specimens of muscle were available. Where the numbers of muscle and mucosa are less than seven and eight respectively, the samples were lost.
seven colon tumours yielded more 6-keto-PGF$_{1α}$ than the corresponding normal mucosa.

**Discussion**

Our previous results showed that homogenisation in Krebs solution increases the formation of prostaglandins, presumably because arachidonate is released during tissue disruption. The quantitative measurements of PGD$_2$, PGE$_2$, PGF$_{2α}$, 6-keto-PGF$_{1α}$ and arachidonate by GC-MS selective ion monitoring show that the extracted homogenates of macroscopically normal gastrointestinal tissues and tumours contained predominantly arachidonate, with 6-keto-PGF$_{1α}$ as the most abundant metabolite. We did not, however, determine the losses in the individual samples, and although this seems unlikely to be an important factor it might distort the relative amounts.

Using radioimmunoassay of human gastric mucosal incubates, Jeremy et al. also found that the prostaglandin yield was 6-keto-PGF$_{1α}$, PGE$_2$, PGF$_{2α}$, and Whittle and Salmon found a slightly greater production of 6-keto-PGF$_{1α}$ than of PGE$_2$. In contrast, Peskar et al. obtained more PGE$_2$ than 6-keto-PGF$_{1α}$ and endogenous substrate, and Ahlquist et al. found that the prostaglandin formation from [14C]-arachidonic acid by human fundic mucosa was PGE$_2$ > PGD$_2$ > PGF$_{2α}$ > 6-keto-PGF$_{1α}$, TXB$_2$.

With regard to the colon, Boughton-Smith et al. homogenised five specimens of human colonic mucosa and then incubated them with exogenous [14C]-arachidonic acid. Their yields were PGE$_2$ > PGF$_{2α}$ > PGD$_2$ > TXB$_2$ > 6-keto-PGF$_{1α}$, in contrast with our finding of most 6-keto-PGF$_{1α}$.

The reasons for different prostaglandin yields obtained by the various groups is likely to be partly methodological, but there are other factors such as losses during extraction and preparation for assay, and variables such as each patient’s age, sex, disease, and medication.

Exact measurements of prostaglandin formation may well be of value in studying aspects of physiology and pathology, provided that the samples are obtained, extracted and assayed under standardised conditions. There is little merit, however, in making comparisons when this is not the case, and we must also bear in mind that different cell types in the tissues, including blood elements, contribute to the yield. Nevertheless, it is important to know what types of prostanoids can be produced by tissues, and whether their amounts might be sufficient to affect the biological activity. Our results with gas chromatography – mass spectrometry, a method without the specificity problems of other assays, suggests that this may be so for various prostanoids.

With regard to gastrointestinal malignancy, previous work has shown that human colonic cancer cells released more PGE-like substance than did normal cells. Gastrointestinal tumours often, but not always, produced more PG-LM than normal mucosa, as judged by bioassay on rat gastric fundus, and many other cancers produce raised amounts of prostaglandins (see refs). In the present experiments, extracts of large bowel tumours usually contained more prostaglandins than did those of the associated normal mucosa, and gastric carcinomas always did so. The reason why tumours form more prostaglandins than the normal tissue in which they arise has received little study. Hong et al. found that transformed 3T3 fibroblasts had a raised amount of prostaglandin synthetase. Our results indicate that the reason may be the greater amount of arachidonate. We do not know yet if this results from increased activity of enzymes such as phospholipase A$_2$ that release the arachidonate, or whether more precursor is present in the cancer cells.

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References